Образац **4.**

Факултет Фармацеутски

УНИВЕРЗИТЕТ У БЕОГРАДУ

01 број
(Број захтева)

Већу научних области медицинских наука
(Назив већа научне области коме се захтев упућује)

20.06.2024.
(Датум)

З А Х Т Е В

за давање сагласности на одлуке о усвајању извештаја Комисије за оцену докторске дисертације и о именовању комисије за одбрану

Молимо да, сходно члану 47. ст. 5. тач. 4. Статута Универзитета у Београду ("Гласник Универзитет", број 186/15- пречишћени текст и 189/16), дате сагласност на одлуку о усвајању извештаја Комисије за оцену докторске дисертације:

КАНДИДАТ **ЋИРИЋ (РАДОВАН) АНА**
(име, име једног од родитеља и презиме)

студент докторских студија на студијском програму Фармацеутске науке

пријавио је докторску дисертацију под називом:

„Развој микрочестица на бази полиелектролитних комплекса хитозана и ксантан гуме као потенцијалних носача са продуженим ослобађањем за пероралну примену ибупрофена и есцина“

из научне области: ФАРМАЦЕУТСКА ТЕХНОЛОГИЈА

Универзитет је дана 01.06.2021.године својим актом под бр. 02-01 број 61206-1292/4-21 дао сагласност на предлог теме докторске дисертацијекоја је гласила:

„Развој микрочестица на бази полиелектролитних комплекса хитозана и ксантан гуме као потенцијалних носача са продуженим ослобађањем за пероралну примену ибупрофена и есцина“

Име и презиме ментора : Проф др. Љиљана Ђекић, редовни професор, Универзитет у Београду – Фармацеутски факултет;

Комисија за оцену докторске дисертације именована је на седници одржаној 07.03.2024.године одлуком факултета под бр. 01 бр.504/2 , у саставу:

Име и презиме члана комисије	звање	научна област	Установа у којој је запослен

1. Др сци. Јелена Ђуриш, редовни професор, Универзитет у Београду – Фармацеутски факултет
2. Др сци. Ђорђе Медаревић, виши научни сарадник, Универзитет у Београду – Фармацеутски факултет
3. Др сци. Лидија Петровић, редовни професор, Универзитет у Новом Саду – Технолошки факултет Нови Сад
4. Др сци. Александар Рашковић, редовни професор, Универзитет у Новом Саду – Медицински факултет Нови Сад
5. Др сци. Теодора Јанковић, научни саветник, Институт за проучавање лековитог биља „Др Јосиф Панчић“

Напомена**:** уколико је члан Комисије у пензији навести датум пензионисања.

Датум стављања извештаја Комисије и докторске дисертације на увид јавности: 16.05.2024.године.

Наставно-научно веће факултета усвојило је извештај Комисије за оцену докторске дисертације наседници одржаној дана 20.06.2024.године .

Комисија за одбрану докторске дисертације именована је на седници одржаној 07.03.2024.године

одлуком факултета под бр. 01 број 504/2, у саставу:

Име и презиме члана комисије	звање	научна област	Установа у којој је запослен

1. Др сци. Јелена Ђуриш, редовни професор, Универзитет у Београду – Фармацеутски факултет
2. Др сци. Ђорђе Медаревић, виши научни сарадник, Универзитет у Београду – Фармацеутски факултет
3. Др сци. Лидија Петровић, редовни професор, Универзитет у Новом Саду – Технолошки факултет Нови Сад
4. Др сци. Александар Рашковић, редовни професор, Универзитет у Новом Саду – Медицински факултет Нови Сад
5. Др сци. Теодора Јанковић, научни саветник, Институт за проучавање лековитог биља „Др Јосиф Панчић“

Напомена: уколико је члан Комисије у пензији навести датум пензионисања.

ДЕКАН ФАКУЛТЕТА

Прилози:

1. Одлука Наставно-научног већа о усвајању извештаја Комисије за оцену докторске дисертације и одлука о именовању Комисије за одбрану докторске дисертације

2. Извештај Комисије о оцени докторске дисертације

3. Примедбе на извештај Комисије о оцени докторске дисертације (уколико их је било) и мишљење Комисије о примедбама

<u>Напомена:</u> Факултет доставља Универзитету захтев са прилозима у електронској форми и у једном писаном примерку за архиву Универзитета

УНИВЕРЗИТЕТ У БЕОГРАДУ
ФАРМАЦЕУТСКИ ФАКУЛТЕТ
11000 - БЕОГРАД
Ул. Војводе Степе 450.
01. број_______
20.06.2024. године

На основу члана 28. Статута и предлога Комисије за последипломске студије, Наставно-научно веће Универзитета у Београду – Фармацеутског факултета на седници одржаној 20.06.2024. године, донело је

О Д Л У К У

ПРИХВАТА СЕ позитиван извештај Комисије за оцену и одбрану завршене докторске дисертације, кандидата **маг.фармације Ане Ћирић** под насловом: **„Развој микрочестица на бази полиелектролитних комплекса хитозана и ксантан гуме као потенцијалних носача са продуженим ослобађањем за пероралну примену ибупрофена и есцина“** и упућује Већу научних области медицинских наука на усвајање, а по добијеној писаној сагласности одобрава јавна одбрана пред Комисијом у саставу:

1. Др сци. Јелена Ђуриш, редовни професор, Универзитет у Београду – Фармацеутски факултет
2. Др сци. Ђорђе Медаревић, виши научни сарадник, Универзитет у Београду – Фармацеутски факултет
3. Др сци. Лидија Петровић, редовни професор, Универзитет у Новом Саду – Технолошки факултет Нови Сад
4. Др сци. Александар Рашковић, редовни професор, Универзитет у Новом Саду – Медицински факултет Нови Сад
5. Др сци. Теодора Јанковић, научни саветник, Институт за проучавање лековитог биља „Др Јосиф Панчић“

Универзитет је дана 01.06.2021.године својим актом бр.: 02-01 бр: 61206-1292/4-21 дао сагласност на предлог теме докторске дисертације.

Кандидат маг. фарм. Ана Ћирић, објавила је резултате из ове докторске дисертације у три рада категорије М21а у међународним часописима са СЦИ листе:

1. **Ćirić A**, Milinković Budinčić J, Medarević Đ, Dobričić V, Rmandić M, Barudžija T, Malenović A, Petrović L, Djekic Lj. 2022. Evaluation of chitosan/xanthan gum polyelectrolyte complexes potential for pH-dependent oral delivery of escin. International Journal of Biological Macromolecules, 221, 48–60. doi: 10.1016/j.ijbiomac.2022.08.190
   IF (2022) = 8,2; Polymer Science (5/86) M21a
2. **Ćirić A**, Medarević Đ, Čalija B, Dobričić V, Rmandić M, Barudžija T, Malenović A, Djekic L. 2021. Effect of ibuprofen entrapment procedure on physicochemical and controlled drug release performances of chitosan/xanthan gum polyelectrolyte complexes. International Journal of Biological Macromolecules, 167, 547–558. doi: 10.1016/j.ijbiomac.2020.11.201
   IF (2021) = 8,025; Polymer Science (6/90) M21a

3. **Ćirić A**, Medarević Đ, Čalija B, Dobričić V, Mitrić M, Djekic L. 2020. Study of chitosan/xanthan gum polyelectrolyte complexes formation, solid state and influence on ibuprofen release kinetics. International Journal of Biological Macromolecules, 148, 942–955. doi: 10.1016/j.ijbiomac.2020.01.138
   IF (2020) = 6,953; Polymer Science (6/91) M21a

Одлуку доставити: именованој, Универзитету, члановима комисије, декану, секретару,продекану за последипломске студије, ментору (Проф др. Љиљани Ђекић), Одсеку за наставу и студентска питања, Одсеку за правне и опште послове, пословном секретару и архиви.

ПРЕДСЕДНИК
НАСТАВНО-НАУЧНОГ ВЕЋА
ФАРМАЦЕУТСКОГ
ФАКУЛТЕТА

Проф. др Слађана Шобајић

УНИВЕРЗИТЕТ У БЕОГРАДУ - ФАРМАЦЕУТСКИ ФАКУЛТЕТ

НАСТАВНО - НАУЧНОМ ВЕЋУ

КОМИСИЈИ ЗА ПОСЛЕДИПЛОМСКУ НАСТАВУ - ДОКТОРСКЕ СТУДИЈЕ

Предмет: Извештај Комисије за оцену и одбрану завршене докторске дисертације кандидата магистра фармације Ане Ћирић

На седници Наставно-научног већа Универзитета у Београду – Фармацеутског факултета, одржаној 7. 3. 2024. године, Одлуком број 504/2 именовани су чланови Комисије за оцену и одбрану завршене докторске дисертације, кандидата маг. фарм. Ане Ћирић, под насловом:

„Развој микрочестица на бази полиелектролитних комплекса хитозана и ксантан гуме као потенцијалних носача са продуженим ослобађањем за пероралну примену ибупрофена и есцина“

Комисија у саставу:

1. **Др сц. Јелена Ђуриш, редовни професор,** Универзитет у Београду – Фармацеутски факултет
2. **Др сц. Ђорђе Медаревић, виши научни сарадник,** Универзитет у Београду – Фармацеутски факултет
3. **Др сц. Лидија Петровић, редовни професор,** Универзитет у Новом Саду – Технолошки факултет Нови Сад
4. **Др сц. Александар Рашковић, редовни професор,** Универзитет у Новом Саду – Медицински факултет Нови Сад
5. **Др сц. Теодора Јанковић, научни саветник,** Институт за проучавање лековитог биља „Др Јосиф Панчић“

прегледала је приложену докторску дисертацију и подноси Наставно-научном већу Универзитета у Београду – Фармацеутског факултета следећи извештај.

ИЗВЕШТАЈ

1. ПРИКАЗ САДРЖАЈА ДОКТОРСКЕ ДИСЕРТАЦИЈЕ

Докторска дисертација маг. фарм. Ане Ћирић, под називом: „Развој микрочестица на бази полиелектролитних комплекса хитозана и ксантан гуме као потенцијалних носача са продуженим ослобађањем за пероралну примену ибупрофена и есцина“ подељена је у шест целина (1. Увод, 2. Циљ истраживања, 3. Експериментални део, 4. Резултати и дискусија, 5. Закључак, 6. Литература) и написана на 174 стране стандардног формата (проред 1; фонт *Times New Roman* – 12). Поред наведених тематских целина дисертација укључује сажетак на српском и енглеском језику, садржај, прилоге (списак радова и саопштења који чине део докторске дисертације и биографију кандидата), као и изјаве о ауторству, истоветности штампане и електронске верзије и коришћењу докторске дисертације. Дисертација је написана јасним и прегледним стилом и садржи 51 слику, 40 табела и 354 литературна навода.

Увод дисертације је систематичан преглед литературних података од значаја за предмет проучавања дисертације и постављање циљева истраживања. У поглављу 1.1. описани су значај, примена, предности и изазови микроинкапсулације активних фармацеутских супстанци, као једног од савремених приступа формулације фармацеутских препарата. У поглављу 1.2. разматрани су полиелектролитни комплекси (ПЕК-ови) на бази биокомпатибилних полимера као потенцијални носачи активних супстанци, са освртом на њихово добијање, својства и карактеризацију. Такође, коментарисане су полазне супстанце и типови ПЕК-ова, као и најчешће примењиване методе за добијање микрочестица на бази ПЕК-ова. Поглавље 1.3 садржи податке о добијању полисахарида хитозана, најчешће разматране катјонске компоненте ПЕК. Приказани су подаци о својствима хитозана који потврђују његов потенцијал за употребу као фармацеутског ексципијенса, али и ограничења његове самосталне употребе у развоју носача активних супстанци за перорални пут примене, због чега је неопходно комбиновати га са другим полимерима. Поглавље 1.4. је преглед до сада описаних носача на бази ПЕК-ова хитозана и различитих анјонских полиелектролита. Обухвата литературне наводе о способности носача на бази ових ПЕК-ова да постигну жељену кинетику ослобађања и побољшају биолошку расположивост инкорпорираних модел активних супстанци након пероралне примене. У поглављу 1.5. систематизовани су литературни подаци о ксантан гуми (добијање, физичко-хемијска својства и употреба у формулацији фармацеутских препарата) који указују на погодност и значај њеног комбиновања са хитозаном. Поглавље 1.6. описује ПЕК-ове на бази хитозана и ксантан гуме и развој носача активних супстанци на бази ових ПЕК-ова. У оквиру овог поглавља размотрен је утицај услова припреме ПЕК-ова ова два полиелектролита на добијање носача са жељеним карактеристикама, односно дефинисани су параметри чији утицај на формирање и својства ПЕК-ова хитозана и ксантан гуме као носача активних фармацеутских супстанци нису довољно испитани. Поглавље 1.7 садржи преглед података о фармаколошким, физичко-хемијским и биофармацеутским својствима ибупрофена, који је у овом истраживању коришћен као модел активна супстанца која има ограничену и *pH*-зависну растворљивост у води. Ово поглавље приказује и различите технолошке приступе предложене ради превазилажења недостатака примене конвенционалних фармацеутских облика са ибупрофеном, са посебним освртом на развој носача на бази полимера. У поглављу 1.8. описан је есцин, који је у овој дисертацији употребљен као хидросолубилна модел активна супстанца. Описана су физичко-хемијска и фармаколошка својства есцина и изазови његове пероралне примене.

Циљеви су дефинисани као општи и специфични (у склопу три фазе истраживања). Општи циљ био је развој микрочестица на бази ПЕК-ова хитозана и ксантан гуме са карактеристикама носача са продуженим ослобађањем за пероралну примену ибупрофена и комерцијално доступног есцина. Специфични циљеви у оквиру прве фазе подразумевали су проучавање процеса формирања плацебо ПЕК-ова хитозана и ксантан гуме, као и детаљну процену утицаја формулационих параметара на физичко-хемијске особине ПЕК-ова и микрочестица добијених након сушења плацебо ПЕК-ова под амбијенталним условима. Додатни циљ у оквиру прве фазе био је испитивање утицаја плацебо микрочестица на кинетику ослобађања ибупрофена из физичких смеша микрочестица и активне супстанце пуњених у тврде капсуле. У оквиру друге фазе специфични циљеви били су процена утицаја поступака додавања ибупрофена на својства микрочестица на бази ПЕК-ова хитозана и ксантан гуме, проучавање значаја масеног односа хитозан/ксантан гума на формирање ПЕК-ова, физичко-хемијска, фармацеутско-технолошка и биофармацеутска својства добијених микрочестица. Спроведено је и истраживање процеса формирања ПЕК-ова, као и карактеризација микрочестица са различитим масеним уделима есцина, као и поређење својстава микрочестица добијених сушењем распршивањем и микрочестица еквивалентног састава које су сушене под амбијенталним условима. Специфични циљеви треће фазе обухватили су процену биолошке расположивости, антиноцицептивне и антиедематозне активности микроинкапсулираних модел активних супстанци у микрочестице на бази ПЕК-ова хитозана и ксантан гуме, као и процену њихове нешкодљивости код понављане пероралне примене на пацовима или мишевима.

Експериментални део је обухватио опис коришћених материјала и примењене методологије. Најпре су наведени сви материјали коришћени у експерименталном раду, описано поступање са експерименталним животињама и наведени бројеви дозвола за рад (Етичке комисије за заштиту добробити огледних животиња Универзитета у Новом Саду, бр. одобрења 04-81/123 и Министарства пољопривреде, шумарства и водопривреде Републике Србије, бр. одобрења 323-07-13890/2020-05). У оквиру методологије <u>прве фазе</u> детаљно су описани поступци припреме водених раствора хитозана и ксантан гуме, као и процедура припреме плацебо ПЕК-ова. Описано је и праћење формирања плацебо ПЕК-ова мерењем трансмитанце, електричне проводљивости, *pH* и реолошких параметара. Затим је описан поступак добијања плацебо микрочестица након сушења ПЕК-ова под амбијенталним условима. У даљем опису метода карактеризације микрочестица описан је поступак одређивања приноса, садржаја резидуалне влаге (гравиметријском методом), микроскопске анализе на оптичком и скенирајућем електронском микроскопу (*SEM*), проточности (применом индиректне методе), инфрацрвене спектроскопије са Фуријеовом трансформацијом (*FT-IR*), дифракције X-зрака на узорку прашка (*PXRD*) и диференцијалне скенирајуће калориметрије (*DSC*). Такође, детаљно је описан поступак испитивања способности рехидратације плацебо микрочестица. Коначно, у склопу прве фазе је описана метода за испитивање утицаја плацебо микрочестица на кинетику ослобађања ибупрофена из њихових физичких смеша пуњених у тврде капсуле (на апаратури са ротирајућим лопатицама). Експериментални рад <u>друге фазе</u> био је подељен у четири потфазе како би се остварили сви постављени специфични циљеви. У *првој потфази* најпре је описана припрема ПЕК-ова са ибупрофеном различитим поступцима додавања активне супстанце и наведено да је праћење формирања ових ПЕК-ова обухватило мерење трансмитанце, електричне проводљивости, *pH* и реолошких параметара на начин описан у методологији прве фазе. Наведено је да је карактеризација микрочестица са ибупрофеном добијених након сушења ПЕК-ова под амбијенталним условима (описано у склопу прве фазе) обухватила: одређивање приноса, ефикасности инкорпорирања и садржаја ибупрофена, садржаја резидуалне влаге (Карл-Фишер титрација), морфолошку анализу (оптичка микроскопија) и процену проточности, *FT-IR* спектроскопију, *PXRD*, *DSC*, процену способности рехидратације и испитивање утицаја инкорпорирања ибупрофена у микрочестице на кинетику ослобађања ове активне супстанце. Пошто су сличне методе карактеризације примењене у овој потфази истраживања у односу на прву фазу експерименталног рада, у склопу описа методологије описане су разлике у експерименталним процедурама, а које су се односиле на одређивање садржаја резидуалне влаге и испитивање утицаја инкорпорирања ибупрофена у микрочестице на кинетику ослобађања ове активне супстанце. У *другој потфази* испитиван је утицај масеног односа хитозана и ксантан гуме на формирање ПЕК-ова и на својства микрочестица добијених након сушења ових ПЕК-ова под амбијенталним условима применом идентичне методологије која је описана код прве потфазе друге фазе истраживања. У оквиру *треће потфазе* описана је припрема ПЕК-ова са различитим масеним уделима есцина и наведено да је праћење формирања ових ПЕК-ова (мерење трансмитанце, проводљивости, *pH* и реолошких параметара) спроведено према протоколима описаним у методологији прве фазе експерименталног рада. Затим су наведене методе карактеризације микрочестица са есцином добијених након сушења ПЕК-ова под амбијенталним условима, у складу са протоколима сушења и карактеризације који су описани у претходним фазама експерименталног рада. У овој потфази детаљно су описани поступци за квантификацију есцина *HPLC* методом, испитивање способности *pH*-зависне рехидратације микрочестица на бази ПЕК-ова хитозан/ксантан гума и испитивање утицаја инкорпорирања ибупрофена и есцина у микрочестице на бази ових ПЕК-ова на могућност постизања *pH*-зависног ослобађања активних супстанци, које је праћено у апаратури са ротирајућом корпицом. У оквиру *четврте потфазе* детаљно је описан поступак сушења распршивањем за добијање микрочестица и наведене су методе карактеризације микрочестица (одређивање приноса, ефикасности инкорпорирања, садржаја модел активне супстанце и садржаја резидуалне влаге, *SEM*, процена проточности, *FT-IR* спектроскопија, *PXRD*, *DSC*, процена способности *pH*-зависне рехидратације и постизања *pH*-зависног ослобађања микроинкапсулираних модел активних супстанци), уз назнаку да су спроведене према процедурама описаним у претходним фазама истраживања. У оквиру <u>треће фазе</u> експерименталног рада (*in vivo* студија) описана је припрема третмана коришћених у истраживању. Затим је описана метода за испитивање утицаја микроинкапсулације ибупрофена и есцина на њихову биолошку расположивост након пероралне примене код пацова. Такође, описан је протокол за испитивање нешкодљивости микрочестица на бази ПЕК-ова хитозан/ксантан гума након њихове понављане четрнаестодневне пероралне примене на пацовима. Дефинисани су и протоколи за процену утицаја микроинкапсулације обе модел активне супстанце на њихову антиедематозну активност (на пацовима) и антиноцицептивну активност (на мишевима). На крају је дефинисан поступак статистичке обраде резултата *in vivo* студије.

Резултати и дискусија су прегледно приказани у три целине које одговарају дефинисаним фазама експерименталног рада, при чему је друга целина подељена у четири потфазе. Резултати докторске дисертације су приказани јасно и систематично, текстуално и на 43 слике и 38 табела. Дискусија је обухватила анализу и разматрање приказаних резултата са критичким освртом на резултате сродних истраживања.

Закључак докторске дисертације садржи концизно приказане закључке свих фаза и потфаза истраживања, а који су проистекли из постављених циљева. На крају је наведен и општи закључак и научни допринос докторске дисертације.

У оквиру **Литературе** наведено је 354 референци коришћених у докторској дисертацији које су цитиране Харвардским стилом.

Прилози садрже списак радова и саопштења који чине део докторске дисертације и кратку биографију кандидата.

2. ОПИС ПОСТИГНУТИХ РЕЗУЛТАТА

У оквиру ове докторске дисертације утврђени су оптимални параметри за припрему микрочестица на бази ПЕК-ова хитозана и ксантан гуме као носача са продуженим и *pH*-зависним ослобађањем за пероралну примену ибупрофена и есцина. Такође, утврђен је значајан утицај микроинкапсулације обе модел активне супстанце на одређене фармакокинетичке параметре, као и на антиедематозну и антиноцицептивну активност. Показана је и нешкодљивост микрочестица након понављане четрнаестодневне примене.

Резултати **прве фазе** показали су да на растворљивост хитозана утичу *pH* и тип киселине коришћене као средства за подешавање *pH*, да се боље раствара у разблаженим растворима органских киселина и да је оптимално средство за подешавање *pH* које резултује најбољим растварањем хитозана сирћетна киселина. Мерењем трансмитанце, електричне проводљивости, *pH* и реолошких параметара током формирања плацебо ПЕК-ова (при масеном односу хитозан/ксантан гума 1:1) показано је да се најекстензивније интеракције између полимера у њима остварују коришћењем сирћетне киселине као средства за подешавање *pH* и при *pH* 3,6 или 4,6, када је постигнуто потпуно растварање хитозана. Током сушења уочен је брз развој микроорганизама у ПЕК-овима који су садржали млечну киселину као средство за подешавање *pH*, па ови ПЕК-ови нису разматрани у даљем истраживању. Показано је да су плацебо микрочестице добијене након сушења ПЕК-ова под амбијенталнм условима имале релативно низак принос (18,02 – 40,88%), зависан од *pH* и типа киселине коришћене за подешавање *pH*, али и релативно високе вредности садржаја резидуалне влаге (~10%). Резултати *FT-IR* спектроскопије показали су да су у свим припремљеним плацебо микрочестицама (са различитим киселинама и при различитим *pH*) остварене искључиво нековалентне интеракције између хитозана и ксантан гуме, али да су оне биле најекстензивније када је *pH* подешавана на 3,6 или 4,6 сирћетном киселином. *PXRD* и *DSC* показале су некристалну природу плацебо микрочестица. Показана је и способност свих плацебо микрочестица да у контакту са фосфатним пуфером pH 7,2 потпуно рехидратишу након 10 *h*, мада је брзина рехидратације зависила од *pH* (најбрже су рехидратисале микрочестице на бази ПЕК чија је *pH* подешена на 5,6, а најспорије оне на бази ПЕК-ова чија је *pH* била 3,6). Разликама у брзини рехидратације ових микрочестица објашњене су разлике у кинетици ослобађања ибупрофена из физичких смеша са њима и утврђени су следећи параметри за припрему плацебо микрочестица са највећим потенцијалом за постизање продуженог ослобађања ибупрофена: *pH* 4,6 и 5,6 и сирћетна киселина као средство за подешавање *pH* раствора хитозана, па су само ови параметри разматрани у даљем истраживању.

У оквиру **прве потфазе друге фазе** истраживања мерењем трансмитанце, електричне проводљивости, *pH* и реолошких параметара показано је да се ПЕК-ови хитозан/ксантан гума (при масеном односу полимера 1:1) могу припремити и у присуству слабо растворне модел активне супстанце, ибупрофена (при масеном односу ибупрофена и укупног садржаја полимера 1:1), да се екстензивније интеракције између компонената ПЕК остварују када се ибупрофен додаје током формирања ПЕК (у поређењу са додавањем активне супстанце након комплексирања полимера), нарочито када је за припрему ПЕК коришћен раствор хитозана *pH* 4,6 (у поређењу са раствором чија је *pH* 5,6). Присуство ибупрофена у ПЕК-овима резултовало је и повећањем приноса (48,04 – 54,14%) и смањењем садржаја резидуалне влаге (3,27 – 5,80%) у микрочестицама са ибупрофеном у поређењу са плацебо микрочестицама (прва фаза истраживања). Резултати *FT-IR* спектроскопије указали су да су у микрочестицама са ибупрофеном интеракције између свих компонената ПЕК-ова нековалентне природе, а да су најекстензивније одржане у микрочестицама на бази ПЕК-а припремљеног са раствором хитозана *pH* 4,6 када је ибупрофен додат током формирања ПЕК-а. *PXRD* и *DSC* показале су кристалну природу микрочестица са ибупрофеном, али и делимично нарушавање кристалне решетке ибупрофена у односу на чисту супстанцу. Уочен је значајан утицај поступка инкорпорирања ибупрофена и *pH* раствора хитозана на рехидратациону способност микрочестица. Најспорије су рехидратисале микрочестице на бази ПЕК-а са најекстензивнијим интеракцијама (припремљен са раствором хитозана *pH* 4,6 када је ибупрофен додат током формирања ПЕК-а). Показано је продужено ослобађање микроинкапсулираног ибупрофена, са око 60% ослобођене активне супстанце током 12 *h* из свих микрочестица, али је утврђено да на кинетику ослобађања микроинкапсулираног ибупрофена у већој мери утиче *pH* раствора хитозана коришћеног за припрему ПЕК-ова него поступак инкорпорирања активне супстанце. Такође, показано је да се најбоља контрола ослобађања микроинкапсулираног ибупрофена постиже из микрочестица на бази ПЕК-а припремљеног са раствором хитозана *pH* 4,6 када је ибупрофен додат током формирања ПЕК-а, па су наведени *pH* и поступак додавања активне супстанце одабрани као оптимални и једини су разматрани у даљем истраживању.

Резултати мерења трансмитанце, електричне проводљивости, *pH* и реолошких параметара током **друге потфазе друге фазе истраживања** показали су да ПЕК-ови хитозан/ксантан гума могу бити формирани при масеном уделу ксантан гуме који је два или три пута већи од масеног удела хитозана (масени однос хитозан/ксантан гума 1:2 или 1:3), како у присуству ибупрофена (ПЕК-ови са ибупрофеном), тако и у одсуству модел активне супстанце (плацебо ПЕК-ови). Утврђено је да се степен интеракција у овим ПЕК-овима није значајно променио када је масени удео ксантан гуме био дупло већи од удела хитозана, али са додатним повећањем удела ксантан гуме у ПЕК-у, при односу 1:3, долазило је до слабљења интеракција. Показано је и да повећање масеног удела ксантан гуме у ПЕК-овима резултује извесним повећањем приноса микрочестица, али и повећањем садржаја резидуалне влаге. *FT-IR* спектроскопија је и код ових микрочестица показала искључиво нековалентне интеракције, као и екстензивније интеракције при масеном односу полимера 1:2. Да је ибупрофен у микрочестице инкорпориран у кристалном облику показано је *PXRD* и *DSC* анализом, али је уочено и делимично нарушавање кристалне решетке микроинкапсулиране активне супстанце. И плацебо микрочестице и микрочестице са ибупрофеном са масеним односима хитозан/ксантан гума 1:2 и 1:3 имале су способност да потпуно рехидратишу у фосфатном пуферу pH 7,2 након 12 *h*, али је спорија рехидратација због екстензивнијих интеракција уочена при односу 1:2 код оба типа микрочестица. Из микрочестица са ибупрофеном при масеном односу полимера 1:2 ослобођено је 58,04% ибупрофена током 12 *h*, а у случају микрочестица са односом полимера 1:3 ослобођено је 67,60% активне супстанце, мада је модел-независна анализа показала да су профили ослобађања слични. Закључено је да микрочестице са ибупрофеном на бази ПЕК-а добијеног мешањем хитозана раствореног на *pH* 4,6 и двоструко веће количине ксантан гуме има најбољи однос степена интеракција, приноса, контроле и кинетике ослобађања модел активне супстанце, тако да је само масени однос полимера 1:2 разматран у даљем истраживању.

У оквиру **треће потфазе друге фазе истраживања** мерењем трансмитанце, електричне проводљивости, *pH* и реолошких параметара утврђено је да се ПЕК-ови хитозан/ксантан гума (однос полимера 1:2) могу формирати и у присуству хидросолубилне модел активне супстанце, есцина, при различитим масеним односима есцина и укупног садржаја полимера (1:1, 1:2 и 1:4). Резултати нису указали на значајне разлике у екстензивности интеракција између компонената ПЕК-ова при различитим масеним уделима есцина, али је установљено да су међумолекулске интеракције биле знатно екстензивније у ПЕК-овима са хидросолубилним есцином у поређењу са ПЕК-овима са слабо растворним ибупрофеном, а нарочито у поређењу са плацебо ПЕК-овима са истим масеним односом полимера. Уочен је много већи утицај масеног удела есцина на својства микрочестица са есцином добијених након сушења ПЕК-ова под амбијенталним условима. Пораст масеног удела есцина резултовао је повећањем приноса микрочестица и ефикасности инкорпорирања активне супстанце. Ипак, примећено је да је присуство ове хидросолубилне супстанце у ПЕК-овима (без обзира на масени удео) резултовало нешто већим садржајем резидуалне влаге у микрочестицама (8,41 – 9,74%) у поређењу са присуством ибупрофена (претходна потфазе истраживања). *FT-IR* спектроскопијом је и код микрочестица са есцином утврђено постојање искључиво нековалентних интеракција између компонената ПЕК-ова, а утврђене су екстензивније међумолекулске интеракције при највећем масеном уделу есцина. *PXRD* и *DSC* анализа показале су да је есцин задржао своју аморфну природу након инкорпорирања у микрочестице. Показано је да су све испитиване микрочестице теже и спорије рехидратисале у медијуму *pH* 1,2 у односу на медијум *pH* 7,4, и да су показале значајан потенцијал за *pH*-зависну рехидратацију. За микроинкапсулирани ибупрофен је утврђено да је знатно мања количина активне супстанце ослобођена у медијуму *pH* 1,2 током 3 *h* (1,70%) у поређењу са медијумом *pH* 7,4 током 9 *h* (26,06%). Оптимално *pH*-зависно ослобађање микроинкапсулираног есцина је постигнуто из микрочестица са масеним односом есцина и укупног садржаја полимера 1:1 (4,30% при *pH* 1,2 током 3 *h* и 37,93% при *pH* 7,4 током 9 *h*). Показано је да микроинкапсулација ибупрофена и есцина може обезбедити продужено и одложено (*pH*-зависно) ослобађање активне супстанце.

Резултати **четврте потфазе друге фазе** истраживања показали су да се сушењем распршивањем могу припремити микрочестице на бази ПЕК-ова хитозан/ксантан гума са и без активне супстанце. Међутим, утврђено је да се овом методом сушења добијају значајно нижи приноси микрочестица (7,37 – 27,18%), као и вишеструко мања ефикасност инкорпорирања и садржај инкорпорираних активних супстанци. Уочено је да са повећањем екстензивностиа међумолекулских интеракција у ПЕК-овима који се подвргавају сушењу опадају вредности наведених параметара. *FT-IR* спектроскопија показала је знатно слабије међумолекулске интеракције у микрочестицама добијеним сушењем распршивањем у поређењу са микрочестицама еквивалентног састава које су добијене сушењем под амбијенталним условима. *PXRD* и *DSC* анализа показале су да се сушењем распршивањем постиже потпуна аморфизација ибупрофена, док есцин и у микрочестицама добијеним овом методом сушења задржава аморфни карактер. Показано је да микрочестице добијене сушењем распршивањем подједнако добро рехидратишу и у медијуму *pH* 1,2 и у медијуму *pH* 7,4. Сушењем распршивањем добијене су микрочестице са ибупрофеном које могу обезбедити жељени профил ослобађања активне супстанце (4,91% при *pH* 1,2 током 3 *h* и 25,64% при *pH* 7,4 током 9 *h*), док у случају есцина нису добијене микрочестице које могу обезбедити *pH*-зависно ослобађање активне супстанце. Због већег потенцијала да омогуће *pH*-зависно ослобађање активних супстанци, бољег приноса и ефикасности инкорпорирања активних супстанци, оптималним носачима су сматране микрочестице са ибупрофеном и микрочестице са есцином добијене сушењем под амбијенталним условима, те су оне укључене у *in vivo* студију.

У оквиру **треће фазе** истраживања (*in vivo* студија) показано је да је инкорпорирање обе модел активне супстанце у микрочестице на бази ПЕК-ова хитозан/ксантан гума значајно утицало на поједине фармакокинетичке параметре. У случају ибупрофена примећено је да је максимална концентрација у серуму (C_{max}) била око 3 пута већа након пероралне примене микрочестица у поређењу са суспензијом ибупрофена. Показано је и да се микроинкапсулацијом ибупрофена постиже статистички значајно продужење полувремена елиминације ($t_{1/2}$) у односу на интравенску примену раствора ибупрофена. Ипак, биолошка расположивост ибупрофена није се значајно разликовала након интравенске примене раствора ове активне супстанце и пероралне примене суспензије и микрочестица са ибупрофеном. У случају есцина примећен је утицај микроинкапсулације на вредност C_{max} (повећање око 2,5 пута) и на биолошку расположивост након пероралне примене (повећање око 1,3 пута) у односу на раствор есцина. Проценом нешкодљивости микрочестица након четрнаестодневне пероралне примене нису уочени статистички значајни порасти концентрација триглицерида, холестерола и показатеља функције бубрега (креатинин, мокраћна киселина и уреа), као ни повећана активност трансаминаза јетре у односу на контролну групу, на основу чега је закључено да се микрочестице са ибупрофеном и микрочестице са есцином на бази ПЕК-ова хитозан/ксантан гума могу сматрати нешкодљивим носачима. Значајан антиедематозни ефекат микроинкапсулираног ибупрофена и микроинкапсулираног есцина, сличан ефекту дексаметазона, уочен је 4 до 6 *h* након пероралне примене.

Показано је и да микроинкапсулирани есцин бољи антиедематозни ефекат остварује при дози активне супстанце 10 *mg/kg* у поређењу са 20 *mg/kg* (ефекат није дозно зависан). Проценом антиноцицептивног ефекта показано је да се 30 *min* након пероралне примене постиже статистички значајан антиноцицептивни ефекат микроинкапсулираних активних супстанци у поређењу са контролном групом и да се овај ефекат одржава током 8 *h*. Најснажнији антиноцицептивни ефекат микроинкапсулираног ибупрофена, сличан ефекту морфина, уочен је 1 *h* након примене, а трајао је 4 *h*. Микроинкапсулирани есцин (10 *mg/kg*) имао је ефикасност сличну морфину током 8 *h*, а максимални ефекат уочен је 2 *h* након примене. Показано је да антиноцицептивни ефекат микроинкапсулираног есцина није дозно зависан.

3. УПОРЕДНА АНАЛИЗА СА РЕЗУЛТАТИМА ИЗ ЛИТЕРАТУРЕ

Микроинкапсулација је један од савремених приступа формулацији фармацеутских препарата којим се може значајно утицати на стабилност, безбедност, терапијску ефикасност и постизање контролисаног ослобађања активних супстанци (1,2). Микрочестице као носачи активних фармацеутских супстанци најчешће се састоје од једног или комбинације више полимера синтетског или природног порекла (3). Природни полимери све више добијају предност над синтетским јер обезбеђују одрживост у развоју лекова (енгл. *sustainable drug development*), због доступности у великим количинама из обновљивих извора, биокомпатибилности и биоразградивости (4). Приликом формулисања микрочестица на бази полимера врло често се прибегава њиховом комбиновању како би се превазишли недостаци употребе појединачних полимера. Пажњу истраживача привлачи приступ који се базира на формирању ПЕК-ова који се доминантно заснивају на јонским интеракцијама између полимера, због једноставности припреме и избегавања употребе потенцијално токсичних реагенаса (5). Најчешће се као катјонска компонента ПЕК-ова користи хитозан, као природни, биокомпатибилни и биоразградиви полимер (6). До сада су испитивани различити природни полимери као анјонске компоненте ПЕК-ова, а један од најперспективнијих је ксантан гума (7). Иако се неколико истраживачких група бавило развојем носача активних супстанци на бази ПЕК-ова хитозана и ксантан гуме (7,8), бројни аспекти њихове припреме и употребе остали су недовољно разјашњени.

У **првој фази** ове докторске дисертације мерењем трансмитанце је показано да се хитозан најбоље раствара у присуству сирћетне киселине при *pH* у опсегу 3,6 – 4,6. Наведено запажање је у сагласности са литературним подацима да се хитозан боље раствара у растворима органских киселина у поређењу са растворима неорганских киселина (9). Праћењем формирања плацебо ПЕК-ова између хитозана и ксантан гуме при масеном односу полимера 1:1 потврђено је да се комплексирање ова два полимера успешно одвија мешањем раствора у којима је концентрација оба полимера 0,65% (7). У овој докторској дисертацији је први пут систематично размотрен комбиновани утицај *pH* и врсте средства за подешавање *pH* на екстензивност међумолекулских интеракција између хитозана и ксантан гуме у ПЕК-овима. Мерењем трансмитанце и привидног вискозитета, у склопу реолошке карактеризације, утврђено је да су најекстензивније интеракције између хитозана и ксантан гуме биле у ПЕК-овима припремљеним са сирћетном киселином и при *pH* раствора хитозана 3,6 и 4,6, када је најбоља растворљивост хитозана. У литератури се наводи да се највеће вредности привидних вискозитета повезују са најекстензивнијим интеракцијама у систему (10). Анализом *FT-IR* спектара плацебо микрочестица показано је да су између хитозана и ксантан гуме успостављене искључиво нековалентне интеракције, а пошто је познато да је интензитет пикова на спектрима у негативној корелацији са интензитетом интеракција у систему (8), показано је да је највећи степен интеракција између хитозана и ксантан гуме у ПЕК-овима припремљеним у присуству сирћетне киселине при *pH* 3,6 или 4,6 одржан и у сувом стању, након добијања микрочестица. Доказане разлике у обиму интеракција између хитозана и ксантан гуме у ПЕК-овима припремљеним при различитим *pH* и коришћењем различитих средстава за подешавање *pH* утицале су на брзину рехидратације од њих добијених плацебо микрочестица, као и на кинетику ослобађања ибупрофена из физичких смеша са овим микрочестицама у фосфатном пуферу pH 7,2. У ранијим истраживањима се такође наводи да су рехидратација и капацитет бубрења носача на бази ПЕК-ова хитозан/ксантан гума у фосфатном пуферу pH 7,2 засновани на одбијању негативно наелектрисаних ланаца ксантан гуме и да најспорије рехидратишу они са најекстензивнијим интеракцијама између полимера (11). Поред тога, са порастом иницијалне *pH* раствора хитозана (од 3,6 до 5,6) долазило је до повећања утицаја добијених плацебо микрочестица на брзину ослобађања ибупрофена из физичких смеша и закључено је да се микрочестице на бази ПЕК-ова припремљених са растворима хитозана *pH* 4,6 и 5,6, која је подешена сирћетном киселином, могу сматрати оптималним за постизање продуженог ослобађања ибупрофена.

Резултати **друге фазе** истраживања обухватају измерене вредности трансмитанце, електричне проводљивости и реолошких параметара, на основу којих је показано да се ПЕК-ови хитозан/ксантан гума при масеном односу полимера 1:1 могу формирати и у присуству ибупрофена, при чему је масени однос ибупрофена и укупног садржаја полимера износио 1:1. Највеће вредости привидног вискозитета ПЕК-ова припремљених под различитим условима (*pH* раствора хитозана 4,6 или 5,6 и додавање ибупрофена током или након комплексирања полимера) измерене су када је ибупрофен додаван током формирања комплекса и коришћењем раствора хитозана *pH* 4,6. Веће вредности привидних вискозитета могу бити повезане са екстензивнијим интеракцијама, што доводи до формирања система са боље организованом структуром (10). Зато су се као оптимални услови припреме ПЕК-ова са ибупрофеном издвојили *pH* раствора хитозана 4,6 (подешена сирћетном киселином) и додавање ибупрофена током формирања комплекса. Садржај резидуалне влаге у микрочестицама са ибупрофеном био је мањи у поређењу са садржајем влаге у плацебо микрочестицама испитаним у првој фази, на основу чега је закључено да инкорпорирање активне супстанце у ПЕК-ове може бити погодно са аспекта микробиолошке стабилности (12). Анализом *FT-IR* спектара микрочестица са ибупрофеном доказано је учешће ове модел активне супстанце у успостављању јонских интеракција у ПЕК-овима, јер се у литератури може наћи потврда да јонске интеракције у систему резултују померањем таласних бројева ка већим вредностима у поређењу са чистом супстанцом (13). Пошто је интензитет пикова на спектрима у негативној корелацији са интензитетом интеракција у систему (8), потврђене су најекстензивније интеракције у микрочестицама на бази ПЕК-а припремљеног са раствором хитозана *pH* 4,6 додавањем ибупрофена током његовог формирања. *PXRD* дифрактограм чистог ибупрофена потврдио је кристалну структуру ове активне супстанце, а добијене 2θ вредности биле су у сагласности са резултатима других истраживачких група (14,15). Дифрактограми микрочестица са ибупрофеном показали су пикове на сличним вредностима 2θ, што је објашњено инкорпорирањем кристалног облика активне супстанце. Ипак, интензитети пикова на дифрактограмима микрочестица са ибупрофеном били су значајно мањи у поређењу са чистим ибупрофеном, што је објашњено делимичним нарушавањем кристалне решетке ибупрофена услед успостављања интеракција са хитозаном и ксантан гумом. И друге истраживачке групе повезују смањење интензитета пикова са нарушавањем кристалне решетке ибупрофена (14). Ослобађање микроинкапсулираног ибупрофена било је продужено током 12 *h* и пратило је *Korsmeyer-Peppas*-ов модел, који је према литературним наводима типичан за носаче на бази полимера са високом способношћу бубрења (16). Поред тога, вредности дифузионих експонената за *Korsmeyer-Peppas*-ов модел (*n*) биле су близу 1 што је значило да се ослобађање ибупрофена из микрочестица може објаснити кинетиком нултог реда са великим степеном поузданости (17). Због тога је количина ибупрофена ослобађана из свих микрочестица у јединици времена скоро константна. На основу вредности коефицијента детерминације (R^2) за нулти ред закључено је да је најбоља контрола ослобађања ибупрофена постигнута из микрочестица на бази ПЕК-а припремљеног са раствором хитозана *pH* 4,6 додавањем активне супстанце у току формирања комплекса. У наставку друге фазе испитана је могућност формирања плацебо ПЕК-ова и ПЕК-ова са ибупрофеном при масеним односима хитозан/ксантан гума 1:2 и 1:3. На основу резултата реолошких мерења и става да је већи привидни вискозитет повезан са екстензивнијим интеракцијама у систему (8), показано је да масени однос хитозан/ксантан гума 1:2 резултује већим степеном међумолекулских интеракција у поређењу са односом 1:3. Смањење привидног вискозитета при односу 1:3 приписано је екстензивнијим нејонским интеракцијама између самих молекула ксантан гуме и смањењу броја молекула доступних за ступање у јонске интеракције са хитозаном. Узимајући у обзир чињеницу да су јонске интеракције јаче у односу на нејонске (18), које су се у већој мери јављале само при односу 1:3, већи привидни вискозитет измерен за ПЕК-ове при масеном односу полимера 1:2 у поређењу са односом 1:3 био је очекиван. Изглед *FT-IR* спектара потврђује слабљење међумолекулских интеракција у ПЕК-овима са повећањем масеног удела ксантан гуме и у сувом стању. Слабијим и мање обимним интеракцијама при масеном односу 1:3 објашњена је нешто већа количина ослобођеног ибупрофена из микрочестица са наведеним односом полимера (око 70%) у поређењу са микрочестицама где је однос полимера 1:2 (око 60%). Поред тога, *n* вредности близу 1 у *Korsmeyer-Peppas*-овом моделу потврдиле су да се ослобађање ибупрофена из оба типа микрочестица може објаснити кинетиком нултог реда са високим степеном поузданости (17), а вредности R^2 указују да се боља контрола ослобађања постиже при масеном односу полимера 1:2. У наставку друге фазе показано је да се ПЕК-ови хитозан/ксантан гума могу формирати при различитим масеним односима есцина, хидросолубилне модел активне супстанце (19) и укупног садржаја полимера (1:1, 1:2 и 1:4). Примећена је блага тенденција повећања привидног вискозитета са повећањем удела есцина у ПЕК-у. *Ceschan* и сарадници наводе да повећање вискозитета може бити последица већег садржаја чврстих састојака (честица) у узорку (20). Овим је образложен најбољи принос, ефикасност инкорпорирања есцина и највећи обим интеракција (показан анализом *FT-IR* спектара) у микрочестицама са масеним односом есцин/полимери 1:1. Примећен је значајан утицај *pH* медијума на рехидратацију свих испитиваних микрочестица на бази ПЕК-ова хитозан/ксантан гума добијених сушењем ПЕК-ова под амбијенталним условима. Бржа и потпунија рехидратација уочена је при *pH* 7,4 у поређењу са *pH* 1,2, што је објашњено вишком полианјона ксантан гуме у структури ПЕК-ова од којих су микрочестице добијене (масени однос хитозан/ксантан гума 1:2). Слична запажања имали су *Malik* и сарадници за хидрогелове на бази ПЕК-ова ова два полимера као потенцијалне носаче ацикловира (21). Резултати испитивања *pH*-зависног ослобађања су показали да се знатно мања количина микроинкапсулираног ибупрофена ослобађа у медијуму *pH* 1,2 током 3 *h* (1,70%) у поређењу са медијумом *pH* 7,4 у којем се током наредних 9 *h* ослобађа 26,06% ибупрофена. Ово је од посебног значаја због доказаног иритационог потенцијала перорално примењеног ибупрофена на слузницу желуца (22). Микроинкапсулацијом ибупрофена потенцијално се може постићи његово ослобађање у танком цреву, што би могло смањити иритацију желуца. Есцин такође може да доведе до иритације слузокоже и успореног пражњења желуца након пероралне примене (23), и пожељно је инкорпорирати га у носаче који би обезбедили његово *pH*-зависно ослобађање у цревима (24). За испитиване микрочестице *pH*-зависно ослобађање микроинкапсулираног есцина било је најизраженије за масени однос есцин/полимери 1:1 (4,30% активне супстанце ослобођено при *pH* 1,2 после 3 *h*, а преосталих 37,93% при *pH* 7,4 током наредних 9 *h*), па су сматране најперспективнијим носачем за постизање *pH*-зависног ослобађања есцина након пероралне примене. У последњем делу друге фазе истраживања показано је да се микрочестице на бази ПЕК-ова хитозан/ксантан гума као носачи ибупрофена и есцина могу добити сушењем ових ПЕК-ова распршивањем. Ипак, због релативно јаких међумолекулских интеракција у ПЕК-овима који су сушени добијени су ниски приноси, ефикасност инкорпорирања и садржај активних супстанци у њима. Како наводе *Sosnik* и *Seremeta* (25), принос код сушења распршивањем под лабораторијским условима није висок. Они наводе и да је највећи вискозитет узорка који обезбеђује задовољавајуће приносе након сушења распршивањем 300 mPa·s, а ПЕК-ови припремани у овом истраживању имали су веће привидне вискозитете. Поређењем *FT-IR* спектара утврђено је да су интензитети карактеристичних пикова микрочестица добијених сушењем распршивањем били већи у поређењу са оним код микрочестица добијених сушењем под амбијенталним условима, што потврђује слабије међумолекулске интеракције (8,13), па је закључено да се интегритет ПЕК-ова делимично нарушава током припреме за сушење и током самог процеса сушења распршивањем. Проценом способности *pH*-зависне рехидратације микрочестица добијених сушењем распршивањем претпостављено је да оне имају значајно мањи потенцијал за постизање *pH*-зависног ослобађања у односу на микрочестице добијене сушењем под амбијенталним условима. У случају ибупрофена показано је да се његовим инкорпорирањем у микрочестице добијене обема методама сушења може постићи *pH*-зависно ослобађање, што је важно са аспекта безбедности примене (22). Ипак, предност се може дати микрочестицама добијеним сушењем под амбијентлним условима, због знатно већег приноса, ефикасности инкорпорирања и садржаја ибупрофена. Сушењем распршивањем нису добијене микрочестице са есцином које омогућују његово *pH*-зависно ослобађање, што може бити неповољно са аспекта подношљивости након пероралне примене (23,24). Микрочестице са ибупрофеном, односно есцином, добијене сушењем под амбијенталним условима су укључене у *in vivo* студију.

У оквиру **треће фазе** показано је да се микроинкапсулацијом ибупрофена постиже повећање C_{max} након пероралне примене микрочестица у поређењу са суспензијом ибупрофена. T_{max} није се значајно разликовало између ове две групе. Показано је и да се микроинкапсулацијом ибупрофена постиже статистички значајно продужење $t_{1/2}$ у односу на интравенску примену раствора ибупрофена, што није уочено након пероралне примене суспензије ибупрофена. Апсолутна биолошка расположивост ибупрофена није се значајно разликовала након интравенске примене раствора ове активне супстанце и пероралне примене суспензије и микрочестица са ибупрофеном, што указује на готово потпуну ресорпцију ибупрофена и у складу је са литературним наводима да се након пероралне примене различитих формулација ибупрофена може постићи апсолутна биолошка расположивост (26). *Yang* и сарадници (27) показали су да се веће вредности C_{max}, а мање T_{max} могу очекивати након пероралне примене формулација са продуженим ослобађањем ибупрофена. У случају есцина T_{max} било је значајно смањено након примене микрочестица у поређењу са чистом супстанцом, док је за C_{max} уочено статистички значајно повећање. Узимајући у обзир литературне наводе да есцин успорава пражњење желуца (23), може се закључити да се његовим инкорпорирањем у микрочестице може ограничити овај ефекат. Показано је и повећање биолошке расположивости перорално примењеног микроинкапсулираног есцина у односу на раствор есцина. До сада није испитиван утицај инкорпорирања есцина у различите носаче на биолошку расположивост након пероралне примене, али се наводи да перорална примена чистог есцина резултује ниском биорасположивошћу (24). *Wu* и сарадници (28–30) су пријавили знатно мању биолошку расположивост појединачних изомера есцина у односу на биорасположивост микроинкапсулираног есцина у утврђену у овом истраживању. Они су уочили и да се пероралном применом смеше изомера есцина постиже већа укупна биолошка расположивост, што иде у прилог резултатима ове студије, јер је комерцијално доступни есцин коришћен у овом истраживању смеша више изомера (19). На бољу биолошку расположивост може утицати аморфни карактер микроинкапсулираног есцина, јер кристална форма има јако ниску биолошку расположивост (24). Објављене су студије које наводе да ПЕК-ови на бази хитозана могу побољшати биолошку расположивост различитих активних супстанци (31). Проценом антиедематозног и антиноцицептивног ефекта у овој дисертацији показано је да микроинкапсулирани есцин боље ефекте остварује када је доза активне супстанце 10 *mg/kg* у поређењу са 20 *mg/kg*, што је повезано са успореним желудачним пражњењем при већој дози активне супстанце и последично смањеном апсорпцијом (32).

Генерално гледано, део резултата докторске дисертације маг. фарм. Ане Ћирић у сагласности је са подацима из литературе. То се пре свега односи на опште резултате и запажања који се односе на ПЕК-ове као носаче активних фармацеутских супстанци, као и на ибупрофен и есцин као модел активне супстанце. С обзиром на то да су у овој докторској дисертацији први пут разматрани ПЕК-ови хитозана и ксантан гуме као потенцијални носачи за пероралну примену ибупрофена и есцина, већина резултата и специфичних запажања није могла бити проверена у доступним литературним изворима и сматра се оригиналним доприносом истраживачког рада кандидата.

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4. ОБЈАВЉЕНИ И САОПШТЕНИ РЕЗУЛТАТИ КОЈИ ЧИНЕ ДЕО ДИСЕРТАЦИЈЕ

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1. Ćirić A, Milinković Budinčić J, Petrović L, Đekić Lj. 2022. Characterization of escin-loaded chitosan/xanthan-based polyelectrolyte complexes for pH-driven oral drug delivery. 2nd International Conference on Advanced Production and Processing (ICAPP 2022), Novi Sad, Serbia, 20–22 October 2022. (NP–P5).

2. Ćirić A, Milinković Budinčić J, Petrović L, Đekić Lj. 2022. Interactions in escin-loaded chitosan/xanthan-based polyelectrolyte complexes: evaluation of drug content and drying method impact. 2nd International Conference on Advanced Production and Processing (ICAPP 2022), Novi Sad, Serbia, 20–22 October 2022. (NP–P24).

3. Ćirić A, Mitrić M, Dobričić V, Đekić Lj. 2019. Influence of drying method on chitosan/xanthan polyelectrolyte complex characteristics. 1st International Conference on Advanced Production and Processing (ICAPP 2019), Novi Sad, Serbia, 10–11 October 2019. (NP–P7)

4. Ćirić A, Đekić Lj. 2019. *In vitro* characterization of rehydration process and dissolution of ibuprofen from chitosan/xanthan polyelectrolyte complex based drug delivery systems. 1st International Conference on Advanced Production and Processing (ICAPP 2019), Novi Sad, Serbia, 10–11 October 2019. (NP–P8)

5. Ćirić A, Đekić Lj. 2019. Razvoj nosača tipa polielektrolitnih kompleksa hitozan/ksantan:

karakterizacija procesa rehidratacije *in vitro*/Development of drug delivery systems based on chitosan/xanthan polyelectrolyte complexes: *in vitro* characterization of rehydration process. 3rd Congress of Pharmacists of Montenegro with international Participation, Budva, Montenegro, 9–12 May 2019. (Oral presentation)

Саопштења са скупова националног значаја штампана у изводу (М64):

1. Ćirić A, Đekić Lj. 2022. Fizičko-hemijska karakterizacija formiranja polielektrolitnih kompleksa hitozan/ksantan guma kao nosača ibuprofena/Physicochemical characterization of the formation of chitosan/xanthan gum-based polyelectrolyte complexes as ibuprofen carriers. Arhiv za farmaciju 72(4) Suplement, 239–240. VIII Kongres farmaceuta Srbije sa međunarodnim učešćem, Beograd, Srbija, 12–15. oktobar 2022. (YOP 17).

2. Ćirić A, Đekić Lj. 2018. Ispitivanje uticaja hidrogela tipa polielektrolitnog kompleksa hitozan/ksantan na *in vitro* kinetiku oslobađanja ibuprofena/Investigation of the influence of polyelectrolyte complex chitosan/xanthan hydrogels on the *in vitro* release kinetics of ibuprofen. Arhiv za farmaciju, 68(3) Suplement, 613–614. VII Kongres farmaceuta Srbije sa međunarodnim učešćem, Beograd, Srbija, 10–14. oktobar 2018. (FTK–P27)

5. ЗАКЉУЧАК – ОБРАЗЛОЖЕЊЕ НАУЧНОГ ДОПРИНОСА ДОКТОРСКЕ ДИСЕРТАЦИЈЕ

Највећим научним доприносом ове докторске дисертације може се сматрати детаљна и систематична анализа утицаја услова припреме микрочестица на бази ПЕК-ова хитозан/ксантан гума на њихова својства као потенцијалних носача ибупрофена и есцина, модел активних супстанци различитих биофармацеутских карактеристика, као и процена утицаја микроинкапсулације ових активних супстанци на биолошку расположивост након пероралне примене и постизање одговарајућих фармаколошких ефеката, уз добијање нешкодљивих носача. Ово је од посебне важности због препознате одрживе и еколошки прихватљиве производње хитозана и ксантан гуме као основних градивних компонената ПЕК-ова који се израђују под благим реакционим условима, без штетног утицаја на животну средину. Резултати приказани у овој дисертацији говоре у прилог нешкодљивости микрочестица на бази ПЕК-ова као потенцијалних носача активних супстанци који би тек требало да нађу своје место у формулацији различитих фармацеутских облика за пероралну примену.

6. ПРОВЕРА ОРИГИНАЛНОСТИ ДОКТОРСКЕ ДИСЕРТАЦИЈЕ

На основу извештаја о провери оригиналности докторске дисертације коришћењем програма *iThenticate* регистровано подударање текста износи 11%. Овај степен подударности последица је претходно публикованих резултата истраживања докторанда, цитата, личних имена, општих места и података, што је у складу са чланом 9. Правилника.

На основу свега изнетог, а у складу са чланом 8. став 2. Правилника о поступку провере оригиналности докторских дисертација које се бране на Универзитету у Београду, изјављујемо да извештај указује на оригиналност докторске дисертације, те се прописани поступак припреме за њену одбрану може наставити.

18. априла 2024. године

Ментор:

Др сц. Љиљана Ђекић, редовни професор
Универзитет у Београду – Фармацеутски факултет

7. ПРЕДЛОГ КОМИСИЈЕ ЗА ОЦЕНУ ЗАВРШЕНЕ ДОКТОРСКЕ ДИСЕРТАЦИЈЕ

На основу изложеног, Комисија закључује да докторска дисертација кандидаткиње маг. фарм. Ане Ћирић, чија је израда одобрена на седници Већа научних области медицинских наука Универзитета у Београду (Одлука бр. 61206-1292/4-21 од 1. 6. 2021. године), задовољава критеријуме оригиналног научног дела. Кандидаткиња је успешно реализовала постављене циљеве истраживања, а резултати приказани у овој докторској дисертацији представљају оригинално и самостално научно дело са значајним научним доприносом у области фармацеутске технологије. Резултати докторске дисертације су публиковани у: једном поглављу у књизи међународног значаја (М14), три рада у међународним часописима изузетних вредности (М21а), три рада у врхунским часописима националног значаја (М51), као и у оквиру два саопштења са међународних скупова штампана у целини (М33), пет саопштења са међународних скупова штампаних у изводу (М34) и два саопштења са скупова националног значаја штампана у изводу (М64).

Комисија у наведеном саставу позитивно оцењује докторску дисертацију маг. фарм. Ане Ћирић под називом „**Развој микрочестица на бази полиелектролитних комплекса хитозана и ксантан гуме као потенцијалних носача са продуженим ослобађањем за пероралну примену ибупрофена и есцина**“ и предлаже Наставно-научном већу Фармацеутског факултета, Универзитета у Београду да прихвати овај Извештај о израђеној докторској дисертацији и упути га Већу научних области медицинских наука ради добијања сагласности за јавну одбрану докторске дисертације.

Комисија за оцену и одбрану завршене докторске дисертације

____
Др сц. Јелена Ђуриш, редовни професор
Универзитет у Београду – Фармацеутски факултет

____
Др сц. Ђорђе Медаревић, виши научни сарадник
Универзитет у Београду – Фармацеутски факултет

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Др сц. Лидија Петровић, редовни професор
Универзитет у Новом Саду – Технолошки факултет Нови Сад

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Др сц. Александар Рашковић, редовни професор
Универзитет у Новом Саду – Медицински факултет Нови Сад

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Др сц. Теодора Јанковић, научни саветник
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Razvoj mikročestica na bazi polielektrolitnih kompleksa hitozana i ksantan gume kao potencijalnih nosača sa produženim oslobađanjem za peroralnu primenu ibuprofena i escina

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Effect of ibuprofen entrapment procedure on physicochemical and controlled drug release performances of chitosan/xanthan gum polyelectrolyte complexes

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ABSTRACT

The effect of the entrapment procedure of a poorly water soluble drug (ibuprofen) on physicochemical and drug release performances of chitosan/xanthan polyelectrolyte complexes (PECs) was investigated to achieve controlled drug release as the ultimate goal. The formation of PECs for two drug entrapment procedures (before or after the mixing of polymers) at pH 4.6 and 5.6 and three chitosan-to-xanthan mass ratios (1:1, 1:2 and 1:3) was observed by continuous decrease in conductivity during the PECs formation and increased apparent viscosity and hysteresis values. The most extensive crosslinking was observed with ibuprofen added before the PECs formation at pH 4.6 and chitosan-to-xanthan mass ratio 1:1. The PECs prepared at polymers' mass ratios 1:2 and 1:3 had higher yield and drug entrapment efficiency. DSC and FT-IR analysis confirmed ibuprofen entrapment in PECs and the partial disruption of its crystallinity. All ibuprofen release profiles were similar, with 60–70% of drug released after 12 h, mainly by diffusion, but erosion and polymer chain relaxation were also included. Potentially optimal can be considered the PEC prepared at pH 4.6, ibuprofen entrapped before the mixing of polymers at chitosan-to-xanthan mass ratio 1:2, which provided controlled drug release by *zero-order* kinetics, high yield, and drug entrapment efficiency.

1. Introduction

Ibuprofen, a propionic acid derivative, is a widely used non-steroidal anti-inflammatory drug (NSAID) for the treatment of pain, fever, symptoms of rheumatoid arthritis, and osteoarthritis [1]. It is a colourless to white needle-like (acicular) crystalline powder with poor dissolution characteristics [2]. Ibuprofen is a weakly acidic (pKa 4.5–4.6) and hydrophobic molecule [3]. The aqueous solubility of ibuprofen is pH-dependent and extremely low in acidic media (46 μg/ml at pH 1.5 and 25 °C), but increases in neutral and alkaline aqueous media (>300 μg/ml at pH above 7 at 25 °C). The most commonly used oral dose is 200–600 mg every 6 h. The bioavailability of ibuprofen after oral administration is approximately 100%. According to high permeability and pH-dependent solubility, ibuprofen is classified in BCS class 2 [4]. The required single dose for therapeutic effect in adults would be approximately 20–30 mg since the onset of analgesia occurs at plasma concentrations between 6.8 and 10.1 mg/l. Nevertheless, the common doses are more than 10 times higher due to the first-pass metabolism (the peak plasma level of ibuprofen from a 400 mg tablet lies between 17 and 36 mg/l) [5–7]. For all those reasons and its short half-life (~2 h), ibuprofen is a good candidate for the development of controlled-release formulations for oral administration [8]. Among recent approaches to control ibuprofen release, the combination with polymers and their conjugates stands out [2,5,9,10], however, zero order kinetics, which is a prerequisite for the drug release at a constant rate over a relatively long period of time, has not been achieved. Some conjugates can also lead to the modification of crystal properties of ibuprofen resulting in improved dissolution characteristics [10,11].

Mixing of the aqueous solutions of oppositely charged polymers can lead to the spontaneous formation of PECs which can be used as controlled-release drug carriers [12–21]. Several chitosan-based PECs have been described as carriers for the extended release of ibuprofen, prepared with carboxymethylated psyllium arabinoxylan [22], poly(sodium-4-styrene)sulphonate and bovine serum albumin [5], and

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xanthan gum [23]. Chitosan is a cationic biopolymer containing randomly distributed β-1,4-linked glucosamine and *N*-acetyl-D-glucosamine units. At pH values lower than its pKa (6.3) [24], chitosan amino groups are ionized and can interact with anionic polymers forming PECs [25,26]. Formulations of oral drug delivery systems which allow controlled drug release require the resistance both to enzyme degradation and the impact of pH gradient (1–7). Chitosan is soluble under acid conditions [27], which restrict its applicability. To improve chitosan properties for controlled drug delivery, it is often combined with oppositely charged polymers, including xanthan gum [23]. Xanthan gum is the extracellular polysaccharide obtained from *Xanthomonas campestris*, composed of glucose, mannose, glucuronic acid, acetate, and pyruvate, with pKa 3.1 [28]. Xanthan gum cannot form a rigid gel by itself but can form a gel when mixed with chitosan [29]. Its pH-sensitive swelling can enable the controlled release of entrapped drugs [16,30,31]. Our previous study demonstrated the possibility of extended ibuprofen release from hard capsules filled with physical mixtures of ibuprofen and chitosan/xanthan gum PECs prepared using 2 different pH adjusting agents (hydrochloric and acetic acid) and 3 different pH values (3.6, 4.6 and 5.6). The pH values between 4.6 and 5.6 adjusted with acetic acid were the most appropriate for the formulation of chitosan/xanthan gum PECs as carriers for extended ibuprofen release [23]. However, in order to characterize the overall potential of PECs as carriers for controlled drug release, it is necessary to consider the influence of drug entrapment procedure on the physicochemical characteristics of PECs and drug release kinetics. Various methods for the drug substance entrapment into PEC-based carriers have been described in the literature, such as diffusion of the drug substance from solution into PEC hydrogel [32,33], dissolution or dispersion of drug substance in polymer solutions that constituted PEC [34,35], or the use of co-solvents in the entrapment of hydrophobic drugs into hydrophilic carriers [36]. It is known that ibuprofen is a negatively charged compound when dissolved in aqueous media due to its carboxylic group [9,37], so it can easily interact electrostatically with positively charged compounds, such as chitosan. Moreover, Lowe et al. [38] suggested that the release of hydrophobic ibuprofen was slower from a strongly aggregated and heterogeneous network than from a more homogeneous one. In addition, Argin-Soysal et al. [13] demonstrated that the chitosan/xanthan gum crosslinking density was less dependent on chitosan solution concentration than xanthan gum solution concentration and chitosan solution pH. However, the influence of drug entrapment procedure and the mass ratio of polymers in PECs on the characteristics of this type of carrier for controlled drug release have not been clarified so far.

This study aimed to evaluate the influence of ibuprofen entrapment procedure into the chitosan/xanthan gum PECs, at different polymer mass ratios, on polymers crosslinking and drug release kinetics.

2. Materials and methods

2.1. Materials

The cationic component of PECs was chitosan (Sigma Aldrich, USA) of medium molecular weight (190,000–310,000 g mol^{-1}) with 75–85% deacetylation degree and viscosity of 200–800 cps (1% w/w solution in 1% w/w acetic acid). Xanthan gum (Jungbunzlauer, Switzerland) of pharmaceutical and food grade, used as the anionic component of PECs, was a kind gift by Inexall Company d.o.o. – Subotica (Serbia). The viscosity of the xanthan gum solution (1% w/w in 1% w/w KCl solution, Brookfield LVTD, spindle 4, 60 rpm at 25 °C) is 1300–1700 mPa·s. Ibuprofen, racemic (BASF, Germany), used as model drug substance, was a gift by Galenika a.d. – Belgrade (Serbia). All the other chemicals (acetic acid (Fisher Scientific, UK), sodium hydroxide (Sigma Aldrich, USA), potassium dihydrogen phosphate (Centrohem, Serbia), methanol (Sigma Aldrich, USA)) were of analytical grade. All substances were used as received without further purification. Purified water (Ph. Eur. 10.0) was used throughout the study.

2.2. Methods

2.2.1. Preparation of chitosan solutions

Two chitosan solutions at a concentration of 0.65% w/v in purified water were prepared by mixing under ambient conditions using the mechanical stirrer (laboratory propeller mixer RZR 2020, Heidolph, Germany) for 24 h at 500 rpm. The solutions differed in the term of pH value. The pH was adjusted up to 4.6 or 5.6 using the acetic acid solution (1.5% v/v) and 0.2 M sodium hydroxide solution, where necessary. The pH was checked on the HI 8417 pH meter (Hanna Instruments, USA), previously calibrated with the standard buffer solutions of pH 4.0 and 7.0. The prepared chitosan solutions were stored at 5 ± 3 °C (in the refrigerator) for 24 h to achieve complete hydration of the polymer.

2.2.2. Transparency of chitosan solutions

The transmittance (%T) of the prepared chitosan solutions was measured on the Evolution 300 spectrophotometer (Thermo Scientific, USA) at 600 nm in order to check their transparency. The acetic acid aqueous solution in the concentration used for the preparation of the corresponding chitosan solution was set as 100% %T. The complete dissolution of chitosan was considered when %T was higher than 90%, as previously reported by Kiechel and Schauer [39].

2.2.3. Preparation of xanthan gum solution and ibuprofen dispersion in xanthan gum solution

The xanthan gum solution at a concentration of 0.65% w/v was prepared under ambient conditions by its dissolution in purified water using the laboratory propeller mixer RZR 2020 at 500 rpm. The dissolution process was carried out until the homogeneous solution was obtained. Additionally, to prepare the drug dispersion in the polymer solution, ibuprofen was added into the xanthan gum solution. The concentration of the drug in the dispersion was 1.3% w/v, 0.975% w/v or 0.867% w/v for the preparation of PECs at chitosan-to-xanthan gum mass ratios 1:1, 1:2 and 1:3, respectively, to achieve the same drug-to-polymers mass ratio (1:1) in final PECs. The mixing was continued as previously described until the homogeneous dispersions were obtained. The pH values of the prepared xanthan gum solution and ibuprofen dispersion in xanthan gum solution were checked on calibrated HI 8417 pH meter. After preparation, xanthan gum solution and ibuprofen dispersion in xanthan gum solution were stored at 5 ± 3 °C (in the refrigerator) for 24 h to allow complete hydration of the polymer.

2.2.4. Preparation of PEC dispersions and ibuprofen entrapment

In the first phase of the study, four PEC dispersions with entrapped ibuprofen were prepared under ambient conditions by mixing the corresponding chitosan solution (pH 4.6 or 5.6) with the xanthan gum solution or ibuprofen dispersion in xanthan gum solution on the mechanical propeller mixer RZR 2020 for 24 h at 800 rpm. The chitosan-to-xanthan gum mass ratio was 1:1 and the drug-to-polymers mass ratio was always 1:1. The dispersions differed in the initial pH of chitosan solution (4.6 or 5.6) and the ibuprofen entrapment procedure (before or after the complexation of chitosan and xanthan gum). Two PEC dispersions were prepared with the xanthan gum solution, in accordance with the procedure recently proposed by Ćirić et al. [23], and ibuprofen was added after the formation of the complex. The dispersions were first washed with purified water and the excess of liquid was removed by filtration. Then, ibuprofen was added to the PECs. These PECs were signed with the initial pH of chitosan solution with attached "A" and chitosan-to-xanthan gum mass ratio (*i.e.*, 4.6A_1:1 and 5.6A_1:1). The other two PECs were prepared by mixing the chitosan solutions with ibuprofen dispersion in xanthan gum solution. The dispersion of ibuprofen in xanthan gum solution was briefly mixed on the propeller mixer immediately before the formation of PECs to ensure its homogeneity. These PECs were signed as "B" samples (*i.e.*, 4.6B_1:1 and 5.6B_1:1).

During the study, based on the characterization results, it was determined at which pH and ibuprofen entrapment procedure the most favourable interactions between polymers and entrapment efficiencies were achieved. For optimal pH and drug entrapment procedure, the influence of the chitosan-to-xanthan gum mass ratio in PEC was examined. For that reason, four additional PEC dispersions were prepared at chitosan-to-xanthan gum mass ratios 1:2 and 1:3 (two with ibuprofen (4.6B_1:2 and 4.6B_1:3), and two without the drug). The drug-to-polymers mass ratio was unchanged (1:1). All prepared PECs were stood for 24 h at 5 ± 3 °C (in the refrigerator) prior to further characterization.

2.2.5. Characterization of crosslinking density in dispersions of PECs with ibuprofen

In order to evaluate the influence of ibuprofen entrapment procedure on crosslinking density (at different pH of chitosan solutions and chitosan-to-xanthan gum mass ratios), characterization of dispersions of PECs was performed in accordance with the methodology previously described by Ćirić et al. [23] for the drug-free chitosan/xanthan gum PECs. Conductivity was monitored during the preparation of PECs. Initial conductivity of the chitosan solutions, as well as after each 10% of the total amount of the xanthan gum solution or ibuprofen dispersion in xanthan gum solution added to the corresponding chitosan solution and 5 min of mixing, was measured using the CDM 230 conductivity meter (Radiometer, Denmark). The conductivity meter was calibrated before measurements using 0.01 M potassium chloride solution. The measurements were done in triplicate and the results shown as mean ± standard deviation (S.D.). Also, pH was checked at the beginning and the end of the formation of PECs. For each polymer solution, ibuprofen dispersion in xanthan gum solution, and obtained semisolid PEC, pH was measured using HI 8417 pH meter, calibrated as previously described. The measurements were done in triplicate and the results shown as mean ± S.D. Dispersions of formed PECs were rinsed with purified water and excess liquid was eliminated by filtration until PECs of semisolid consistency were obtained. Afterwards, the rheological behaviour of PECs was characterized on the Rheolab MC 120 rheometer (Paar Physica, Austria) coupled with the rotating cylinder measuring device (Z3 DIN) at 20 ± 0.2 °C. The flow curves were constructed using a controlled shear rate (CSR) procedure. The shear rate first increased from 0 s^{-1} to 100 s^{-1} and then decreased back to 0 s^{-1}, with each stage lasting for 400 s. The measurements were done in triplicate and the results shown as mean ± S.D.

2.2.6. Drying of ibuprofen loaded PECs

Thin layers of all semisolid PECs were poured into Petri dishes and dried under ambient conditions (at 20 ± 3 °C). The PECs were dried until the films of constant mass were obtained. The films were then ground and sieved through the sieve with a mesh size of 355 μm (Ph. Eur. 10.0) to remove large particles from the powder. The obtained powders were stored in a desiccator and used for solid-state characterization.

2.2.7. Characterization of ibuprofen loaded PECs in solid state

Characterization of solid ibuprofen loaded PECs included: determination of yield, ibuprofen entrapment efficiency (*%EE*) and drug loading (*%DL*), residual moisture content, and light microscopy. In order to elucidate the interactions between the polymers and ibuprofen in the solid PECs, DSC, FT-IR and PXRD analysis were performed. Moreover, to consider the chitosan/xanthan gum crosslinking density, the rheological behaviour of the rehydrated PECs was investigated.

2.2.7.1. Determination of yield, %EE and %DL. After grinding and sieving, the mass of solid ibuprofen loaded PECs was measured on AE 240 single pan balance (Sartorius, Germany) and the yield was calculated as shown in Eq. (1):

$$\text{Yield}\ (\%) = \text{weight of dried PEC (g)}/[\text{weight of chitosan (g)} + \text{weight of xanthan gum (g)} + \text{weight of ibuprofen (g)}] \cdot 100 \quad (1)$$

The mass of chitosan, xanthan gum, and ibuprofen used for the preparation of PECs was defined as 100%.

%EE and *%DL* were determined according to the method described by Kulkarni et al. [16]. To determine the ibuprofen *%EE* and drug loading (*%DL*), approximately 20 mg of each ground and sieved PEC was measured on AE 240 single pan balance and dissolved in 100 ml of medium consisting methanol and phosphate buffer pH 7.2 in 80:20 volume ratio by sonication on Sonorex RK1024 ultrasonic bath (Bandelin electronic GmbH & Co. KG, Germany) at 20 ± 3 °C during 30 min for complete extraction of the drug from the polymer matrix. The medium consisting of methanol and phosphate buffer pH 7.2 in 80:20 volume ratio was chosen for %EE and %DL determination since it dissolves ibuprofen rapidly, while both chitosan and xanthan gum remain insoluble [10,40]. After sonication, each sample was filtered through a 0.45 μm MF-Millipore® membrane filter (Millipore Corporation, Bedford, USA). The concentration of ibuprofen was determined on the Evolution 300 spectrophotometer at the wavelength of maximum absorption of the drug (224 nm). The solution of corresponding drug-free PEC in the medium, prepared by applying the same protocol, was used as blank. The test was performed in triplicate for each sample and the results are shown as the mean ± S.D.

%EE was calculated as shown in Eq. (2):

$$\%EE = \text{actual amount of ibuprofen in PEC (g)}/\text{theoretical amount of ibuprofen in PEC (g)} \times 100 \quad (2)$$

%DL was calculated as shown in Eq. (3):

$$\%DL = \text{actual amount of ibuprofen (g)}/\text{measured weight of the dried PEC (g)} \times 100 \quad (3)$$

2.2.7.2. Light microscopy. The dispersions of the ground and sieved PECs in silicone oil were examined by the Olympus BX51P polarizing microscope (Olympus, Japan) with cellSens Entry software Version 1.14 (Olympus, Japan). The ImageJ software, version 1.52a (National Institutes of Health, USA) was used for the estimation of particle size and shape. One hundred particles were randomly selected from each sample. The length and the width of the particles were measured, and the results were shown as mean, minimum, and maximum for both parameters. The shape of particles was estimated by calculating the elongation ratio (*ER*) for all PECs and ibuprofen itself. *ER* is a ratio of the length and the width of the particle (Eq. (4)):

$$ER = \text{length}\ (\mu\text{m})/\text{width}\ (\mu\text{m}) \quad (4)$$

Particles with $ER < 1.15$ were considered spherical [14]. The calculated *ER* was shown as *ER* for the mean particle size (ER_{mean}), *ER* for the smallest particle (ER_{min}), and ER for the largest particle (ER_{max}) for each investigated sample.

2.2.7.3. Determination of residual moisture content. The residual moisture content determination (by Karl Fischer coulometric titration method) was performed on 831 KF Coulometer (Metrohm Switzerland). Hydranal-Coulomat AG (Fluka, Germany) was used as the titration reagent. Before the titration, validation of the method was performed, examining its specificity, linearity, precision, and accuracy (details shown in the Supplementary material). The titration was performed in triplicate for each sample and the results are shown as the mean ± S.D.

2.2.7.4. DSC analysis. The samples were accurately weighed (5–10 mg) and crimped in a standard 40 μl aluminium pan. Then, the samples were heated from 25 °C to 360 °C at a heating rate of 10 °C/min, under a constant nitrogen flow rate of 50 ml/min on the DSC 1 instrument (Mettler Toledo, Switzerland). The empty sealed pan was used as a reference.

2.2.7.5. FT-IR analysis. The attenuated total reflectance (ATR) FT-IR spectra of solid ibuprofen loaded PECs were obtained on a Nicolet iS10 FT-IR Spectrometer (Thermo Fisher Scientific, USA). The wavelength range was between 4000 and 400 cm^{-1} with resolution 4, and 16 scans per sample.

2.2.7.6. PXRD analysis. PXRD analysis was performed on Bruker D8 advance diffractometer (Bruker, Germany) in Bragg-Brentano θ-2θ geometry, using Ge-crystal primary monochromator (Johanson type) that generates CuKα1 radiation ($\lambda = 1.541$ Å). Diffraction data were obtained in the 2θ range from 5 to 45° with a scan step of 0.05° and a holding time of 12 s [23].

2.2.7.7. Rheological characterization of rehydrated ibuprofen loaded PECs. Samples for rheological measurements were prepared by dispersing the solid ibuprofen loaded PECs in phosphate buffer pH 7.2 (USP) at a concentration of 2% w/v, as previously proposed [23]. Briefly, the dispersion process was carried out on a Shaking Water Bath LSB18 Aqua Pro (Grant, UK) for 12 h at 37 ± 1 °C and agitation rate of 100 rpm. Continuous rheological characterization was performed at 37 ± 0.2 °C on a rheometer, Rheolab MC 120, coupled with the rotating cylinder measuring device Z3 DIN. The CSR procedure was performed by increasing a shear rate from 0 s^{-1} to 100 s^{-1} and back to 0 s^{-1} (each stage lasting for 400 s).

2.2.8. In vitro ibuprofen release testing

In vitro drug release test was performed in order to evaluate drug release kinetics from the investigated PECs as well as to compare ibuprofen release profiles from the PECs prepared by different ibuprofen entrapment procedure. Dried ibuprofen loaded PECs were filled into hard hydroxypropyl methylcellulose (HPMC) capsules size 0 (Fagron, Greece) in an amount comprising 100 mg of ibuprofen. The *in vitro* release of ibuprofen was investigated on the rotating paddle apparatus, DT70 (Erweka, Germany), at 50 rpm, using 900 ml of the medium (phosphate buffer pH 7.2 (USP)), at 37 ± 1 °C. The small amounts (~4 ml) of the medium were sampled during 12 h at fixed time intervals (15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 600 and 720 min), and immediately replaced by an equal amount of fresh buffer solution. Each sample was filtered through a 0.45 μm MF-Millipore® membrane filter. The amount of released ibuprofen was determined on Evolution 300 spectrophotometer at the wavelength of maximum absorption of the drug (221 nm). The test was performed in triplicate for each sample and the results are shown as the mean of the released amount of the drug substance ± S.D. The obtained data of ibuprofen release was fitted into mathematical models using the DDSolver Excel Add-In [41]. The applied mathematical models (*zero-order* and *Korsmeyer-Peppas* kinetics) are shown by Eqs. (5) and (6):

$$\textit{Zeroorder}\ \text{kinetics}: Q = k_0 \cdot t \tag{5}$$

$$\textit{Korsmeyer–Peppas}\ \text{kinetics}: Q = k \cdot t^n \tag{6}$$

Q represents the amount of the drug released after time t, n the diffusion exponent which indicates the mechanism of drug release, and k_0 and k the release rate constants for *zero-order* and *Korsmeyer-Peppas* kinetic models, respectively.

The model-independent approach (calculation of similarity (f_2) and difference (f_1) factors) was used for the comparison of drug release profiles. The experimental results were also compared using DDSolver Excel Add-In and Eqs. (7) and (8):

$$f_1 = \Sigma_{(t=1-n)}\ |R_t - T_t| / \Sigma_{(t=1-n)}\ R_t \cdot 100 \tag{7}$$

$$f_2 = 50 \log \left\{ \left[1 + 1/n \cdot \Sigma_{(t=1-n)}\ (R_t - T_t)^2 \right]^{-0.5} \cdot 100 \right\} \tag{8}$$

n is the number of samples, R_t the released amount of the drug (%) after the time t (reference/sample 1), and T_t released amount of the drug (%) after the time t (test/sample 2).

For $50 < f_2 < 100$ and/or $0 < f_1 < 15$ the ibuprofen release profiles can be considered similar [41,42].

3. Results and discussion

The prepared PECs, after washing and removing the excess liquid by filtration, had the consistency of semisolid hydrogels (PEC hydrogels). Their conductivity, pH, and rheological measurements were measured in order to elucidate the interactions between the polymers and the drug in aqueous media during the formation of PECs. Moreover, characterization of PECs in solid state intended to investigate the types of interactions between the polymers and ibuprofen, and their influence on *in vitro* drug release kinetics.

3.1. Formation of ibuprofen loaded PECs

Chitosan solutions at pH 4.6 and 5.6 were used for the preparation of PECs. The %T of chitosan solutions was measured to evaluate its solubility at investigated pH values. It was observed that chitosan solutions prepared at pH 4.6 were clearer than those prepared at pH 5.6. The measured values of %T were in accordance with this observation: the %T value of chitosan solution at pH 4.6 was 98.26% ± 0.66%, while the %T of chitosan solution at pH 5.6 was 58.27% ± 15.15%. Furuike et al. [43] showed that the %T value is directly related to the solubility. Higher %T value at pH 4.6 confirmed the higher solubility of chitosan at lower pH values. The chitosan solution at pH 5.6 likely comprised the less hydrated polymer and impeded the free transmission of the light through the system, resulting in a significantly lower %T value.

Chitosan solutions were combined with xanthan gum solution (with or without ibuprofen) in order to obtain PECs. The PECs were characterized during their formation. The results of conductivity (σ) measurements during the PECs formation are shown in Fig. 1 while the pH values of each PEC hydrogel are shown in Table 1.

The initial conductivity values of chitosan solutions at different pH values were compared firstly. The conductivity of the chitosan solution at pH 4.6 was higher than at pH 5.6. The obtained result indicated that at

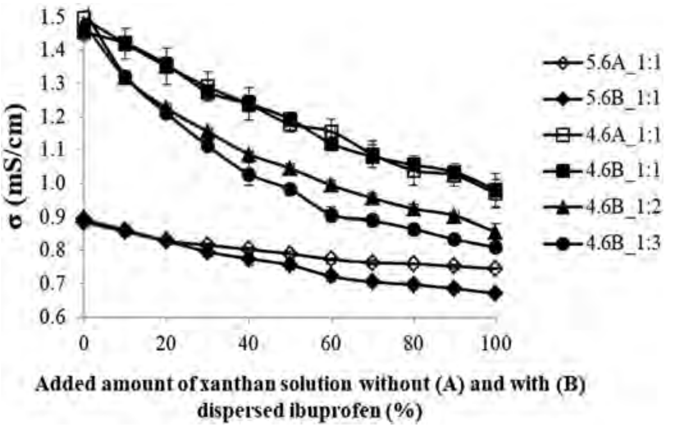

Fig. 1. Conductivity during the formation of ibuprofen loaded PECs.

Table 1
The pH values of ibuprofen loaded PEC hydrogels.

PEC hydrogel	pH value ± S.D.
5.6A_1:1	5.32 ± 0.05
5.6B_1:1	5.11 ± 0.04
4.6A_1:1	4.33 ± 0.05
4.6B_1:1	4.49 ± 0.05
4.6B_1:2	4.23 ± 0.04
4.6B_1:3	4.33 ± 0.02

pH 4.6 there were a larger number of dissolved free charge carriers in the solution and that their mobility was higher [44]. That was in accordance with the measured %T values. The conductivity of xanthan gum solution was 0.657 ± 0.031 mS/cm and pH 6.85 ± 0.05. For ibuprofen dispersion in xanthan gum solution, the conductivity was 0.551 ± 0.012 mS/cm and pH 5.25 ± 0.03. The dispersion of ibuprofen in xanthan gum solution lowered the conductivity and pH value. The reduction of pH after the addition of ibuprofen in the xanthan gum solution could be the consequence of the weak acidic properties of the drug [2,4,9]. On the other hand, non-covalent interactions (*e.g.*, hydrogen bonds, ion-dipole, dipole-dipole, van der Waals interactions) between ibuprofen and xanthan gum were likely established in the dispersion, leading to the decrease in free charge mobility in the system and the decrease in conductivity.

The continuous reduction in conductivity was observed during the addition of the drug-free xanthan gum solution or ibuprofen dispersion in xanthan gum solution into the corresponding chitosan solution, confirming the formation of PECs at both pH values and polymer mass ratio 1:1. The decrease in conductivity value was due to the decrease in the amount and mobility of free charge, which indicated the establishment of ionic interactions between the polymers [23,45,46]. The mixing of chitosan solutions with ibuprofen dispersion in xanthan gum solution did not show any significant difference in comparison with the mixing with the drug-free xanthan gum solution. For 4.6A_1:1, the conductivity decreased from 1.495 ± 0.050 mS/cm to 0.969 ± 0.044 mS/cm, for 4.6B_1:1 from 1.454 ± 0.027 mS/cm to 0.980 ± 0.051 mS/cm. For 5.6A_1:1 decrease was from 0.885 ± 0.016 mS/cm to 0.745 ± 0.001 mS/cm and for 5.6B_1:1 from 0.892 ± 0.013 mS/cm to 0.671 ± 0.005 mS/cm. Ibuprofen, a weak acid, was expected to be more soluble at pH 5.6 (˃50%), in comparison to pH 4.6 (~50%). At pH 5.6 ibuprofen ionization was less suppressed, so it was more incorporated into the PECs for both procedures, but there was generally less crosslinking between the polymers because chitosan was less dissolved (*i.e.*, hydrated and ionized) at pH 5.6. The conductivity of PECs lowered with an increase of pH from 4.6 to 5.6, so it could be assumed that a slightly higher amount of dissolved ibuprofen at pH 5.6 did not influence significantly the number of free ions in the system. For these samples the effects of pH and polymer charge were dominant.

Previous results have shown that PECs were formed at both pH values and independently of the presence of ibuprofen, *i.e.*, that ibuprofen can be added to the xanthan gum solution before the formation of PECs, which shortened the number of steps in the PEC formation process. The elimination of the subsequent addition of ibuprofen to the formed PECs was an advantage of this entrapment procedure. At pH 4.6 there was more dissolved chitosan, which opened the possibility to increase the mass fraction of xanthan gum. Therefore, the chitosan-to-xanthan gum mass ratios of 1:2 and 1:3 in PECs obtained by entrapment procedure B were additionally examined. The decrease in conductivity with an increase in the xanthan gum relative content in PECs was confirmed by a decrease in the free charge concentration and mobility originating from the positively charged amino groups of dissolved chitosan, indicating that the chitosan and xanthan gum complexation also occurred when xanthan gum was in significant excess (at chitosan-to-xanthan gum mass ratio up to 1:3). For 4.6B_1:2 the conductivity decreased from 1.478 ± 0.009 mS/cm to 0.855 ± 0.027 mS/cm, and for 4.6B_1:3 from 1.519 ± 0.008 mS/cm to 0.810 ± 0.009 mS/cm.

After the addition of the xanthan gum solution or the dispersion of ibuprofen in xanthan gum solution into the corresponding chitosan solution, a slight decrease in pH in the obtained PECs was observed (Table 1). This decrease can be explained by the ionization of entrapped ibuprofen to hydrogen and carboxylate ions [9,37]. The poor effect of the complexation of polymers on the pH value of PEC hydrogels may be the consequence of the high buffering capacity of the samples due to the presence of acetic acid [23]. The addition of xanthan gum solution, with or without ibuprofen, did not significantly affect the initial pH of the chitosan solution, even at high xanthan gum relative content. That was expected, since xanthan gum is a weak acid, and the acidic pH suppresses its ionization. This effect was more pronounced at pH 4.6.

In order to determine the flow behaviour and to elucidate the strength and extent of interactions between the polymers and ibuprofen in the prepared PEC hydrogels, rheological characterization was performed. The minimal and the maximal apparent viscosity as well as the hysteresis area values of PEC hydrogels are shown in Table 2. The investigated systems showed pseudo-plastic flow behaviour with thixotropy. Thixotropy was estimated by calculating the hysteresis area (H).

The PECs prepared at chitosan-to-xanthan gum mass ratio 1:1 and pH 4.6 had higher apparent viscosity values compared to those prepared at pH 5.6 at the same polymer mass ratio. Higher apparent viscosity and hysteresis values of PEC hydrogels correspond with higher strength and extent of interactions (crosslinking) between the polymers, or polymers and ibuprofen, leading to more structured systems [23,47]. The thixotropy is an indicator of the sample structure rupture during the force application and its ability to re-establish after the termination of the application of force [48]. That ability of PEC hydrogels was the consequence of reversible non-covalent interactions between the polymers, as also demonstrated in some previous studies [23,29,48]. The ibuprofen entrapment procedure had a more significant effect on interaction strength and extent at pH 4.6 and chitosan-to-xanthan gum mass ratio 1:1. The maximum apparent viscosity and hysteresis were significantly higher for the sample prepared by procedure B. Stronger and more extensive interactions were observed when ibuprofen was added prior to the formation of PECs by its dispersion in xanthan gum solution. That was one of the reasons why only this entrapment procedure was further considered at different chitosan-to-xanthan gum mass ratios.

When comparing the ibuprofen loaded PECs prepared by entrapment procedure B, the highest η_{max} at initial pH 4.6 of 2.50 ± 0.20 Pa·s had 4.6B_1:1, the medium, 2.18 ± 0.04 Pa·s, had 4.6B_1:2, and the lowest, 1.09 ± 0.15 Pa·s, had 4.6B_1:3. The obtained results could be explained by an increase in the xanthan gum content. Stronger and more extensive interactions between the constituents of PECs were established at the chitosan-to-xanthan gum mass ratio 1:1. The η_{max} decrease with an increase in the relative content of xanthan gum could be the consequence of more extensive interactions among the molecules themselves due to the high proportion of hydrophilic functional groups in its structure. That made it possible to establish hydrogen bonds between xanthan gum molecules, thereby reducing the number of molecules that could form ionic bonds with the oppositely charged

Table 2
Maximal apparent viscosity (η_{max} at 22.2 s^{-1}), minimal apparent viscosity (η_{min} at 100 s^{-1}) and hysteresis area (H) of ibuprofen loaded PEC hydrogels.

PEC hydrogel	η_{max} ± S.D. (Pa·s)	η_{min} ± S.D. (Pa·s)	H ± S.D. (Pa/s)
5.6A_1:1	1.15 ± 0.09	0.15 ± 0.01	756.27 ± 71.86
5.6B_1:1	1.22 ± 0.06	0.28 ± 0.01	525.05 ± 32.16
4.6A_1:1	2.16 ± 0.13	0.69 ± 0.04	68.20 ± 47.39
4.6B_1:1	2.50 ± 0.20	0.63 ± 0.02	1021.49 ± 384.24
4.6B_1:2	2.18 ± 0.04	0.62 ± 0.04	786.39 ± 207.56
4.6B_1:3	1.09 ± 0.15	0.24 ± 0.01	438.74 ± 85.35

chitosan. The higher chitosan-to-xanthan gum mass ratio (1:1) reduced the possibility for the formation of non-ionic interactions between xanthan gum molecules themselves. These interactions are weaker relative to the ionic ones [49] and were established when one of the polymers was in excess. Also, at the chitosan-to-xanthan gum mass ratio 1:3, the entrapment of ibuprofen led to a weakening of the interactions in the PEC, probably due to the high amount of negative charge originating both from the xanthan gum and ibuprofen. In this case, the amount of chitosan was insufficient to maintain the highly ordered structure observed at the polymers' mass ratio 1:1 and 1:2.

The apparent viscosity for the sample 4.6A_1:1, obtained by the entrapment of ibuprofen after the complexation of chitosan and xanthan gum, was 2.16 ± 0.13 Pa·s. When compared this value with the apparent viscosity values of the samples prepared by entrapment procedure B, at pH 4.6 and all chitosan-to-xanthan gum mass ratios (1:1, 1:2 and 1:3) it can be assumed that stronger and more exhaustive interactions between chitosan, xanthan gum, and ibuprofen could be established when ibuprofen was added into the system before the PECs formation. The ionization of ibuprofen is suppressed, but in procedure B, it was already partially bound to xanthan gum, which affected its participation in the crosslinking of polymers during the formation of PECs. With an increase in the relative content of xanthan gum, there were actually fewer interactions between the polymers that are characteristic for PECs, so the viscosity and hysteresis decreased. In samples prepared at pH 5.6, there was generally less crosslinking between the polymers (and therefore lower apparent viscosities), but ibuprofen ionization was also less suppressed, so it probably had a greater effect on the crosslinking. Therefore, these samples had higher H for both entrapment procedures. Ibuprofen, a low molecular weight substance, led to the formation of “bridges” between chitosan and xanthan gum and, therefore, to an increase in H of PECs.

3.2. Characteristics of dried ibuprofen loaded PECs

3.2.1. Yield, %EE, %DL and residual moisture content

The yield of dried drug loaded PECs, ibuprofen *%EE*, and *%DL* as well as the residual moisture content in dried PECs are shown in Table 3.

The yield was higher for the samples prepared at pH 4.6 and ibuprofen entrapment procedure B at all chitosan-to-xanthan gum mass ratios compared to those prepared at pH 5.6 and compared to 4.6A_1:1. Also, the yield of these samples increased with increasing xanthan gum content, 61.61% ± 4.72% for 4.6B_1:2 and 64.13% ± 5.98% 4.6B_1:3. These results confirmed the continuous complexation of chitosan and xanthan gum even in high excess of xanthan gum. During the formation of PECs with a higher relative content of xanthan gum, almost the entire amount of positively charged chitosan interacted with negatively charged ibuprofen and xanthan gum, but also the excess molecules of xanthan gum were bound to each other by weak hydrogen bonds, resulting in a slightly higher yield. However, at 1:2 and 1:3 chitosan-to-xanthan gum mass ratios, the total amount of the PEC was lower so the overall interaction strength and apparent viscosity were lower. The equal amount of both polymers at mass ratio 1:1 led to the formation of a denser network between chitosan and xanthan gum, resulting in higher apparent viscosity, but without the excess of xanthan gum and with subsequent lower yields.

%EE values were similar to yield values, so it can be assumed that the losses of chitosan, xanthan gum, and ibuprofen during the preparation, drying, grinding and sieving were uniform due to the interactions between all PEC components. The reason for relatively high ibuprofen *%EE* may be its low solubility at acidic pH values. Therefore, ibuprofen got physically entrapped into the PECs. The content of ibuprofen (*%DL*) was approximately 50% in all the samples, which was expected since the mass ratio of polymers and ibuprofen during the PECs formation was always 1:1. This mass ratio was chosen due to the results of our previous study, showing that the relative content of ibuprofen in the mixture of more than 50% significantly reduced the effect of PECs on its release [23]. However, *%DL* slightly decreased with an increase in the relative content of xanthan gum. This could be explained by the lower content of positively charged amino groups of chitosan available for the establishment of ionic interactions with negatively charged carboxyl groups in ibuprofen [14,50,51].

In general, the moisture content was well below 10% in all samples. The residual moisture content was higher at pH 5.6 (4.92% ± 0.04% (5.6A_1:1) and 5.80% ± 0.13% (5.6B_1:1)) than at pH 4.6 (3.27% ± 0.06% (4.6A_1:1) and 4.63% ± 0.06% (4.6B_1:1)). At pH 5.6 chitosan is less soluble and less available for interactions with xanthan gum. Due to its high hygroscopicity and low extent of interaction with poorly dissolved chitosan at pH 5.6, xanthan gum molecules could bind higher amounts of water. Also, in samples prepared at pH 4.6 and entrapment procedure B a continuous increase in residual moisture content was observed with an increase in the content of xanthan gum, a highly hygroscopic polymer, from 4.63% ± 0.06% in 4.6B_1:1, *via* 4.99% ± 0.10% in 4.6B_1:2, to 5.22% ± 0.06% in 4.6 B_1:3. The moisture content in all ibuprofen-loaded PECs was even lower than moisture content in the drug-free chitosan/xanthan gum PECs (10.08–10.97%) [23], so the drug entrapment during the PECs formation may be favourable over the preparation of physical mixtures of ibuprofen and PECs, primarily in terms of microbial stability. Das et al. [14] came to the same conclusion for zinc/pectin/chitosan composite particles for resveratrol delivery to the colon.

3.2.2. Shape and size of the PEC particles

The films obtained by drying the ibuprofen loaded PEC hydrogels were ground into powders and sieved through a sieve of mesh size 355 μm to remove coarse particles. Photomicrographs of dried PECs after grinding and sieving are shown in Fig. 2, while particle size and shape data are shown in Table 4.

The ibuprofen particles were generally of regular, quadrangular to needle-like shape (ER 2.29–3.13), as expected for crystalline substances. Their length ranged from 22.44 μm to 258.50 μm. Particles of all PECs were irregular in shape, mostly needle-shaped and quadrangular (ER 1.25–2.30). However, PECs had the shape closer to spherical, which may be reflected in the crystallinity of ibuprofen entrapped into the chitosan/xanthan gum complexes. Therefore, their length and width were measured, and the mean, minimum, and maximum values of these parameters were presented in Table 4. A very wide range of their dimensions was observed. All PECs had a high fraction of particles below

Table 3
Yield, *%EE*, *%DL* and residual moisture content of dried ibuprofen loaded PECs.

PEC	Yield ± S.D. (%)	*%EE* ± S.D.	*%DL* ± S.D.	Moisture content (%)
5.6A_1:1	48.26 ± 2.94	49.19 ± 2.50	51.01 ± 2.43	4.92 ± 0.04
5.6B_1:1	48.11 ± 3.29	49.19 ± 2.48	51.19 ± 2.10	5.80 ± 0.13
4.6A_1:1	48.04 ± 2.08	53.19 ± 3.07	55.39 ± 3.09	3.27 ± 0.06
4.6B_1:1	54.14 ± 3.14	59.05 ± 3.14	54.54 ± 0.27	4.63 ± 0.06
4.6B_1:2	61.61 ± 4.72	61.24 ± 5.64	51.29 ± 2.15	4.99 ± 0.10
4.6B_1:3	64.13 ± 5.98	61.99 ± 10.39	49.03 ± 3.17	5.22 ± 0.06

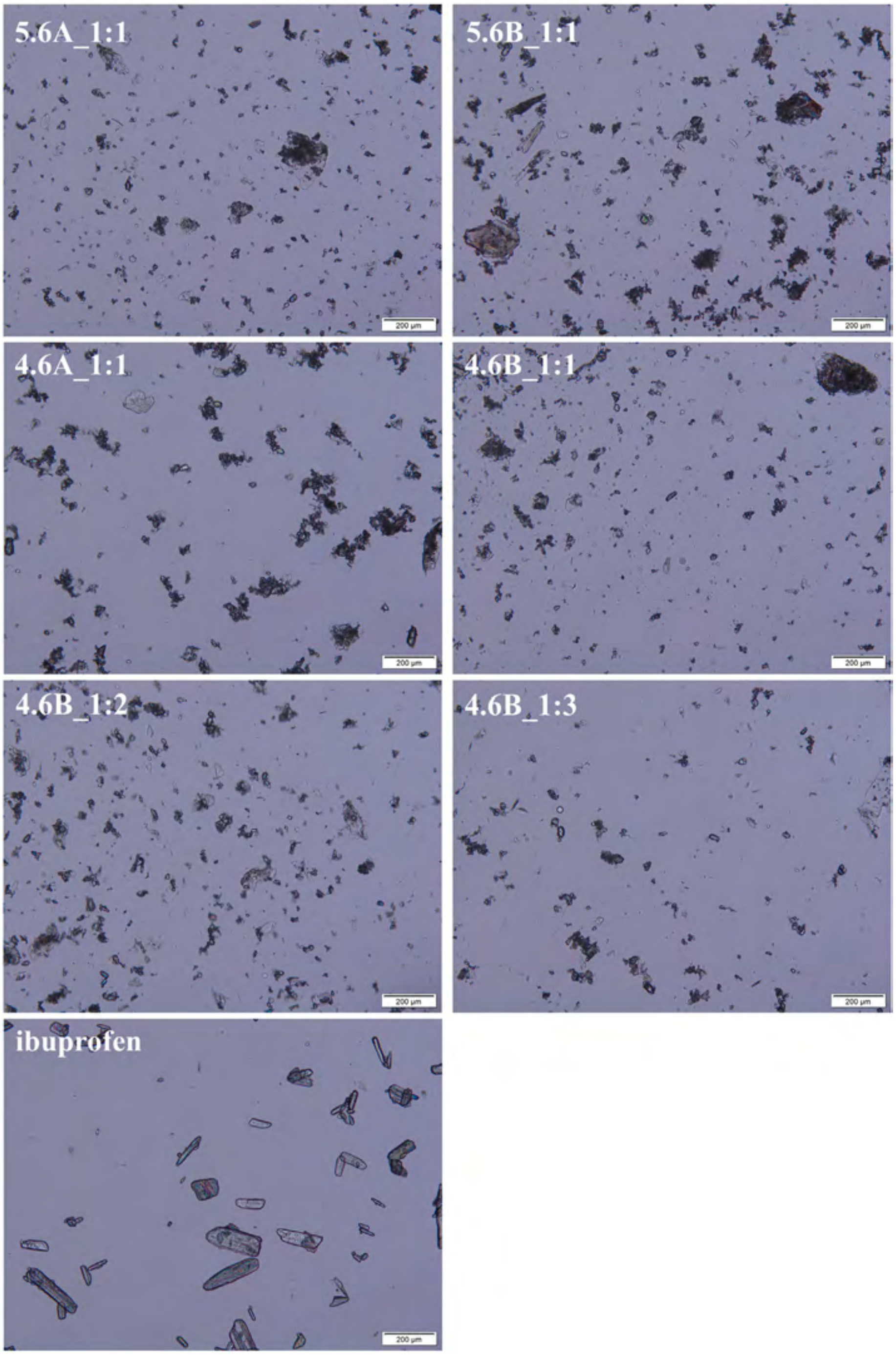

Fig. 2. Photomicrographs of ibuprofen and dried ibuprofen loaded PECs after grinding and sieving (magnification 100).

Table 4
Size of ibuprofen and PEC particles: length (mean, minimum, maximum), width (mean, minimum, maximum) and ER (mean, minimum, maximum).

Sample	Length			Width			ER		
	Mean	Min	Max	Mean	Min	Max	ER_{mean}	ER_{min}	ER_{max}
Ibuprofen	84.78	22.44	258.50	30.76	9.81	82.62	2.76	2.29	3.13
5.6A_1:1	66.13	29.43	252.11	37.55	12.17	201.76	1.76	2.42	1.25
5.6B_1:1	80.53	38.10	218.30	38.60	16.55	152.38	2.09	2.30	1.43
4.6A_1:1	89.71	25.81	246.18	49.30	12.17	133.44	1.82	2.12	1.84
4.6B_1:1	67.58	25.67	284.48	39.16	13.61	155.77	1.72	1.89	1.83
4.6B_1:2	66.26	25.09	178.95	41.91	13.88	128.85	1.58	1.81	1.39
4.6B_1:3	52.95	14.65	141.73	31.91	10.15	67.80	1.66	1.44	2.09

100 μm, with the greater fraction of small particles in PECs prepared at an initial pH 5.6. The interactions of chitosan and xanthan gum in these PECs were weaker so they were less resistant to grinding. Also, the addition of ibuprofen prior to the complexation of chitosan and xanthan gum led to the interference in their complexation, which may explain the higher fraction of fine particles in samples B compared to samples A within the same initial pH. An increase in the relative content of xanthan gum in PECs prepared at pH 4.6, using ibuprofen entrapment procedure B, led to a low decrease in particle size due to the lower interaction strength and extent and lower resistance to the crushing.

3.2.3. Evaluation and interpretation of DSC curves

Thermograms obtained by DSC analysis of the dry ibuprofen loaded PECs and pure ibuprofen are shown in Fig. 3a.

The ibuprofen thermogram showed a sharp and narrow endothermal peak at 78.13 °C (with onset at 76.30 °C and endset at 81.55 °C) representing the melting temperature [52], which proved its crystalline structure, as also reported by Hussain et al. [8]. The endothermal peaks of the PEC samples were shifted to lower values (75.76 °C for 5.6A_1:1, 75.30 °C for 5.6B_1:1, 76.36 °C for 4.6A_1:1, 76.29 °C for 4.6B_1:1, 76.01 °C for 4.6B_1:2, and 76.18 °C for 4.6B_1:3) than 78.13 °C, found for pure ibuprofen. DSC analysis of ibuprofen loaded PECs could reflect its entrapment as well as a partial disruption of its crystalline structure. The lower melting temperature (approximately 2 °C) of the entrapped ibuprofen and the broader ranges of the melting temperatures (70.55 °C–81.59 °C) indicated a partial collapse of its crystalline structure due to dissolution, and particularly due to the interactions with chitosan and xanthan gum. Also, the decrease in the melting enthalpy from 117.17 J/g for pure ibuprofen to the values in the range of 57.49 J/g to 89.93 J/g for ibuprofen loaded PECs confirmed the disruption of its crystal lattice and was in accordance with published results of other research groups [8,10,18]. The absence of wide endothermal peaks at 100 °C–110 °C in ibuprofen loaded PECs can be explained by their low residual moisture content, as well as the high content of ibuprofen in the samples. In addition, exothermal changes at temperatures between 275 °C and 285 °C were associated with sample degradation. Finally, the absence of new peaks, except those characteristic for chitosan, xanthan gum and ibuprofen, confirmed the assumption that entrapped drug interacted physically with functional groups of chitosan and xanthan gum, and that no chemical reactions occurred during the PECs formation. Lower enthalpies of PECs prepared at pH 5.6 (57.49 J/g for 5.6A_1:1 and 67.07 J/g for 5.6B_1:1) confirmed the lower strength and extent of interactions in these samples. The ibuprofen entrapment procedure did not have a significant influence on the thermal characteristics of PECs prepared at pH 4.6 and chitosan-to-xanthan gum mass ratio 1:1. On the other hand, at pH 5.6, higher enthalpy was observed for 5.6B_1:1, which was expected given that the interactions in this sample were stronger and more extensive in comparison to the sample 5.6A_1:1. Also, the increase in xanthan gum relative content in PECs prepared at pH 4.6 and entrapment procedure B, resulted in the decrease in the enthalpies (85.21 J/g for 4.6B_1:1, 72.73 J/g for 4.6B_1:2 and 64.79 J/g for 4.6B_1:3), reflecting the lower interaction extent at higher relative content of xanthan gum.

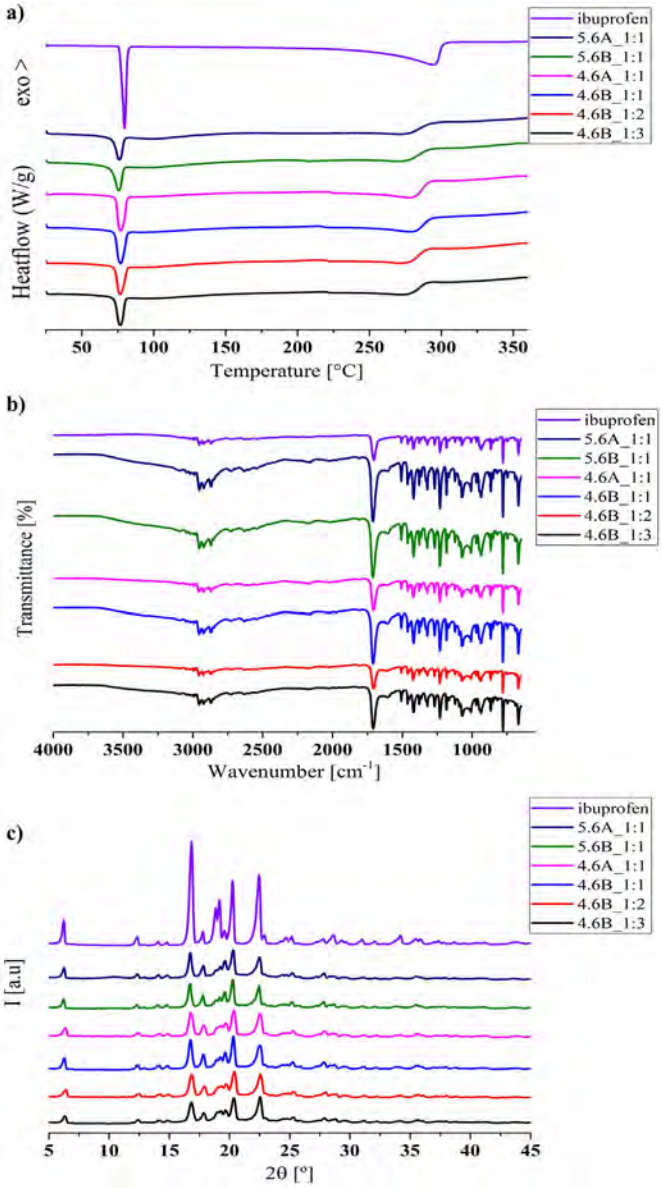

Fig. 3. DSC thermograms (a), FT-IR spectra (b) and PXRD spectra (c) of pure ibuprofen and dry ibuprofen loaded PECs.

3.2.4. Evaluation and interpretation of FT-IR spectra

Fig. 3b shows the FT-IR spectra of pure ibuprofen and solid ibuprofen loaded PECs. The peak at 1704.32 cm^{-1} in the pure ibuprofen spectrum corresponds to the non-ionized carboxylic group in its structure [8,9]. This peak with lower shifts to higher values (1705.41–1710.20 cm^{-1}) was also observed in all solid ibuprofen loaded PECs. The shifting in wavenumbers and the higher peak intensity in PECs in comparison to pure ibuprofen strongly supported the assumption based on the DSC results that the crystal lattice of ibuprofen partially disrupted due to its entrapment into PECs. Due to the organization of ibuprofen molecules in the crystal lattice, the characteristic peaks in its FT-IR spectrum are not of high intensity. The peak intensity is often correlated with the extent of interactions in the system, *i.e.*, the peak intensity increases with a decrease in interaction extent, and *vice versa* [23]. After the disruption of the crystal lattice and the “release” of characteristic groups, an increase in peak intensity in all solid ibuprofen loaded PECs was noticed. In PECs prepared at pH 5.6, the disruption of the crystal lattice was likely due to the dissolution and ionization of ibuprofen (leading also to the shifting of wavenumbers to higher values), which was maintained even after drying [8]. In 4.6B_1:1 due to the high relative amount of positively charged chitosan and its interactions with ibuprofen during the formation of the complex, the crystal lattice was disrupted. Also, owing to the same mass fraction of chitosan and xanthan gum, a significant amount of amide groups in acetylated units of chitosan (characteristic peak at 1700–1650 cm^{-1}) was present in samples 5.6A_1:1, 5.6B_1:1 and 4.6B_1:1, leading to the amplification of peak intensity [5,9,23]. On the other hand, when chitosan-to-xanthan gum mass ratio was 1:2 and 1:3, as well as in the sample 4.6A_1:1, the lower peak intensity may be a consequence of lower interaction extent between ibuprofen with chitosan due to lower chitosan content, lower extent of ibuprofen crystallinity disruption, and also lower amide concentrations in complexes. In 4.6A_1:1, the lower extent of ibuprofen crystal lattice disruption was a consequence of its subsequent addition to the complex, after the establishment of interactions between chitosan and xanthan gum. Due to the high content of ibuprofen (~50%) in PECs and the multitude of peaks characteristic for its structure, they largely cover the peaks characteristic for chitosan and xanthan gum. Other peaks in the ibuprofen spectrum are visualised around 3000–2850 cm^{-1} (characteristic for –C–H stretching); 1510–1500 cm^{-1} (characteristic for aromatic ring vibration); 1420–670 cm^{-1} (characteristic for primary alcohols, including –O–H bend, and –C–O stretch) [9,10] and were also present in solid ibuprofen loaded PECs spectra. In our previous study [23], we showed that the absence of peaks characteristic for the primary amino group of chitosan (1655–1300 cm^{-1}) in PECs is the evidence of the establishment of ionic interactions between chitosan and xanthan gum, which was confirmed in this study. The only difference is that, in this case, chitosan could interact both with xanthan gum and ibuprofen. Also, peaks at 1604.26 cm^{-1} and 1402.16 cm^{-1} characteristic for asymmetric and symmetric vibrations of carboxylates in the structure of xanthan gum and/or ibuprofen, respectively, were not observed, which further confirms the establishment of ionic interactions between the ionized groups of PEC components. Moreover, the absence of new peaks, except those characteristic for chitosan, xanthan gum and ibuprofen, confirmed that no chemical reactions occurred during the PECs formation.

3.2.5. Evaluation and interpretation of PXRD spectra

Fig. 3c shows the PXRD spectra of pure ibuprofen and solid ibuprofen loaded PECs. Peaks characteristic for amorphous chitosan and semi-crystalline xanthan gum [23] were covered by many peaks typical for crystalline ibuprofen. The spectrum of pure ibuprofen, with the peak of highest intensity (I) at $2\theta = 16.00°$ and other sharp peaks of high intensity at 2θ values of 6.25, 12.35, 17.80, 18.85, 19.15, 20.25, and 22.45°, demonstrated its crystalline structure, as previously reported by various groups of authors [53–55]. PXRD spectra of solid ibuprofen loaded PECs showed peaks at similar 2θ values, which finally confirmed the entrapment of crystalline form of drug substance into PECs [53]. Nevertheless, the intensity of peaks corresponding to solid ibuprofen loaded PECs was significantly lower compared to pure ibuprofen, which could be the consequence of partial disruption of ibuprofen crystal lattice due to the establishment of interactions with chitosan and xanthan gum [55,56], as previously supposed. Consistent with previous observations of Abioye et al. [57], it could be assumed that interactions between ibuprofen and polymers, especially chitosan, were based on hydrogen bonds and led to partial amorphization of the drug.

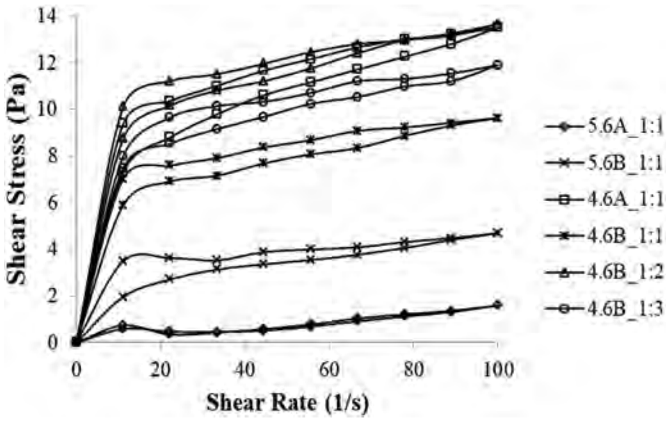

Fig. 4. Flow curves of ibuprofen loaded PECs rehydrated in phosphate buffer pH 7.2 (the standard deviation values were less than 10% of the mean values and the corresponding deviation bars were omitted for clarity).

Table 5
Maximal apparent viscosity (η_{max}) (at 22.2 s^{-1}), minimal apparent viscosity (η_{min}) (at 100 s^{-1}), and hysteresis area (H) for PECs rehydrated in phosphate buffer pH 7.2.

PEC	η_{max} ± S.D. (Pa·s)	η_{min} ± S.D. (Pa·s)	H ± S.D. (Pa/s)
5.6A_1:1	0.02 ± 0.00	0.01 ± 0.00	8.63 ± 3.04
5.6B_1:1	0.16 ± 0.01	0.05 ± 0.00	30.66 ± 4.55
4.6A_1:1	0.47 ± 0.00	0.13 ± 0.00	86.63 ± 3.26
4.6B_1:1	0.34 ± 0.03	0.10 ± 0.01	22.10 ± 4.94
4.6B_1:2	0.50 ± 0.00	0.14 ± 0.00	74.68 ± 21.42
4.6B_1:3	0.45 ± 0.03	0.12 ± 0.01	51.59 ± 11.28

3.2.6. Rehydration ability of dried PECs

Flow curves of ibuprofen loaded PECs after rehydration are shown in Fig. 4, while the apparent viscosity and H values are presented in Table 5.

Flow curves of dispersions obtained by the rehydration of dried PECs in phosphate buffer pH 7.2 also showed pseudo-plastic flow behaviour with thixotropy. Thixotropy was estimated by calculating the H values of dispersions.

The results of the rheological characterization of dispersions obtained by rehydration of PECs were consistent with the results of the rheological characterization of PEC hydrogels. In phosphate buffer pH 7.2, carboxylic groups of xanthan gum and ibuprofen were completely deprotonated and negatively charged, which resulted in a high amount of water bound and lower apparent viscosity values. At pH 7.2 chitosan amino groups are expected to be less than 50% protonated (as observed at pH above 6.3) [58]. Therefore, chitosan chains were less available for interactions with surrounding xanthan gum chains and ibuprofen *via* ionic and hydrogen bonds, in comparison with PEC hydrogels before drying. Thus, at pH 7.2, ibuprofen is expected to dissolve in the buffer to a higher extent and the complexes were partially degraded. In comparison with PECs prepared at pH 5.6, the apparent viscosities were higher for PECs prepared at lower pH (*i.e.*, 4.6A_1:1, 4.6B_1:1, 4.6B_1:2 and 4.6B_1:3), so these samples had lower rehydration ability (water binding capacity) due to stronger interactions between polymers and ibuprofen. Generally, it is considered that stronger interactions lead to less swelling ability [17,19,25,59]. In 4.6B group of samples the highest η_{max} had 4.6B_1:2 (0.50 ± 0.00 Pa·s) and the lowest 4.6B_1:1 (0.34 ± 0.03 Pa·s). PECs with a lower extent of interactions between the polymers and ibuprofen rehydrated more readily and had more extensive interactions with the buffer.

A difference was observed between 4.6A_1:1 and the group of 4.6B samples. In 4.6A_1:1, ibuprofen was added after the formation of PEC and was not part of the internal bonds, so after the addition of buffer it dissolved, and the PEC remained less hydrated and had a higher H. In the group of 4.6B samples, ibuprofen was a part of the bonds in the PECs. It dissolved in the buffer and for that reason, the bonds in the PECs were probably easier to break, so they hydrated better and had lower H compared to 4.6A_1:1. In 4.6B samples, there was no clear correlation between H and the mass fraction of xanthan gum, because it was necessary to break the chitosan-xanthan gum bonds and break the bonds between the molecules of xanthan gum itself during the rehydration. In 4.6B_1:1 the bonds were between chitosan and xanthan gum, in 4.6B_1:2 between the PEC and xanthan gum (in excess), and in 4.6B_1:3 the dominant were the bonds between xanthan gum molecules themselves and their dissolution in the buffer.

3.3. In vitro ibuprofen release

Fig. 5 shows the ibuprofen release profiles from the hard capsules filled with PECs prepared at different pH values, entrapment procedures, and chitosan-to-xanthan gum mass ratios.

Ibuprofen release profiles showed its extended release from all PECs during 12 h (Table 6). Ibuprofen release profiles did not show the significant difference (basing on the similarity factor values, Table 7), so it can

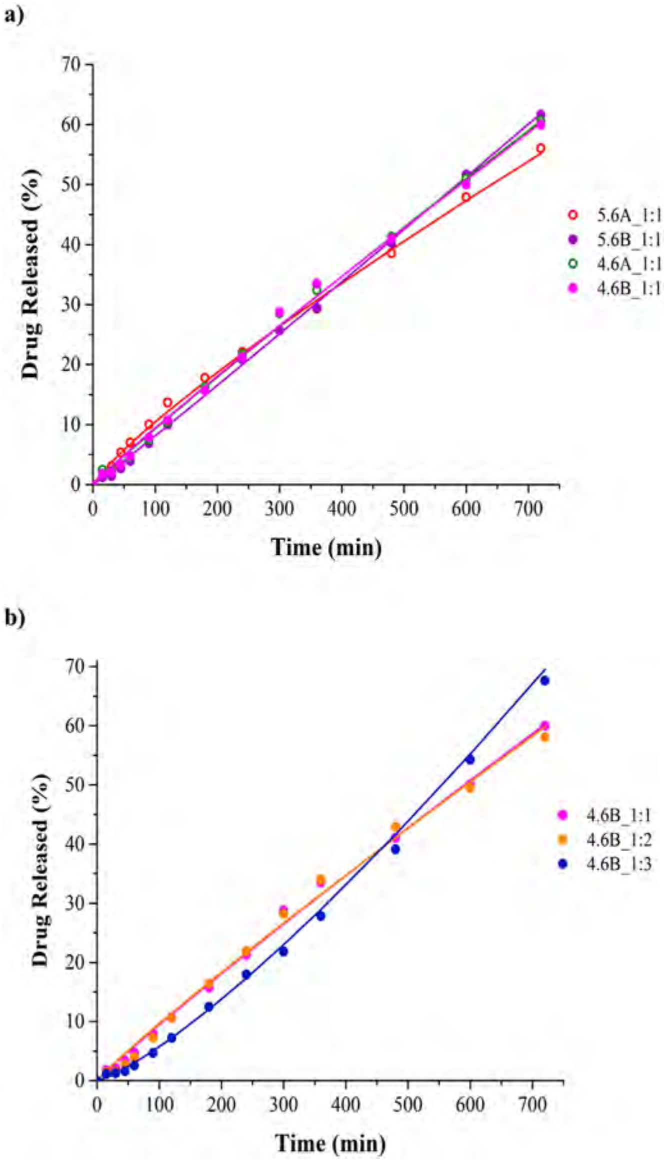

Fig. 5. Ibuprofen release profiles from the hard capsules filled with the ibuprofen loaded PECs in phosphate buffer pH 7.2 and their *Korsmeyer-Peppas* fitting curves: the influence of pH and drug entrapment procedure (a), and the influence of chitosan-to-xanthan gum mass ratio (b) (the standard deviation values were omitted for clarity).

be assumed that the pH, entrapment procedure, and chitosan-to-xanthan gum mass ratio did not influence significantly the drug release from PECs filled in hard HPMC capsules.

Table 6 shows the released amounts of ibuprofen, correlation coefficients of mathematical models of ibuprofen release kinetics, and *Korsmeyer-Peppas* diffusion exponents (n).

The drug release from all the samples followed the *Korsmeyer-Peppas* model, typical for polymer-based carriers with high swelling ability, as previously was reported [23]. Based on the n values, it can be assumed that the dominant mechanism of ibuprofen release was the diffusion of the drug after the swelling of PECs. Also, the erosion and polymer chain relaxation mechanisms were expected [20]. Moreover, high values of the correlation coefficient for *zero-order* kinetics (>0.98) indicated that the rate of ibuprofen release was independent of its concentration, so a constant amount of drug was released per unit time: 0.078 mg/min from 5.6A_1:1, 0.086 mg/min from 5.6B_1:1, 0.084 mg/min from 4.6A_1:1, 0.083 mg/min from 4.6B_1:1, 0.081 mg/min from 4.6B_1:2, and 0.094 mg/min from 4.6B_1:3. In addition, n values close to 1 in the *Korsmeyer-Peppas* model confirmed that the release of ibuprofen could be explained by *zero-order* kinetics model with a high degree of reliability [21]. The entrapment of ibuprofen in PECs enabled a higher control of drug release with the almost constant ibuprofen release rate. This assumption is supported by the high value of the correlation coefficients for the *zero-order* kinetics. Our previous study [23] showed that physical mixtures of ibuprofen with chitosan/xanthan gum PECs can enable extended ibuprofen release for up to 10 h, however, the release was not predominantly controlled by the carrier, but mechanisms that fit into *first order*, *Higuchi* and *Korsmeyer-Peppas* ($n \le 0.636$) kinetics were involved, depending on the composition and PECS preparation procedure.

All the pairs of ibuprofen release profiles can be considered similar based on their similarity factors. Although, the obtained values of f_1 and f_2 allowed the estimation which parameter (pH, entrapment procedure, or chitosan-to-xanthan gum mass ratio) varied during the preparation of PECs was the cause of the greatest variations between the samples (Table 7).

The model-independent analysis was used to examine the similarity of ibuprofen release profiles from the investigated PECs. The lower the values of f_1 and the higher values of f_2 were, the greater was the similarity between the pair of ibuprofen release profiles, and *vice versa*. The difference factor values $>$ 15 for ibuprofen release profiles from 4.6B_1:3 and other PECs (except 5.6B_1:1) indicated that the xanthan gum mass content triple of chitosan's was the source of the most significant variations between the samples. Values of f_1 about 10–11 and f_2 about 77–78 showed that the pH of chitosan solution (in addition to the polymer mass ratio 1:3 at pH 4.6) had a greater effect on ibuprofen release than the entrapment procedure.

Controlled release by *zero-order* kinetics was achieved for 4.6A_1:1, 4.6B_1:1, and 4.6B_1:2. Deviations from this model occurred for samples prepared at pH 5.6, and with increasing xanthan gum content at pH 4.6 (for 4.6B_1:3). Therefore, the ibuprofen entrapment procedure significantly affected the physicochemical properties of PECs, but the pH and chitosan-to-xanthan gum mass ratio were of greater importance for drug release kinetics.

4. Conclusions

PECs were formed at both investigated pH values (4.6 and 5.6) and both ibuprofen entrapment procedures at chitosan-to-xanthan gum mass ratio 1:1. The entrapment procedure B (when ibuprofen was added prior to the formation of the complex) at pH 4.6 was more suitable for the preparation of PECs with a higher extent of crosslinking compared to those prepared at pH 5.6 at polymer mass ratio 1:1. However, with an increase in the relative content of xanthan gum, at chitosan-to-xanthan gum mass ratios 1:2 and 1:3, the overall crosslinking extent between the polymers decreased due to decreased amount of the PECs in a mixture with the excess of xanthan gum. However, an increase in relative xanthan gum content led to an increase in yield and *%EE*. DSC and FT-IR analysis confirmed the entrapment of

Table 6
Released amounts of ibuprofen from HPMC hard capsules filled with ibuprofen loaded PECs, models of ibuprofen release kinetics with corresponding correlation coefficients (R^2) and *Korsmeyer-Peppas* diffusion exponents (n).

PEC	Released amount of ibuprofen ± S.D. (%)	R^2		n
		Zero order	*Korsmeyer-Peppas*	
5.6A_1:1	56.03 ± 2.83	0.9860	**0.9978**	0.844
5.6B_1:1	61.63 ± 6.92	0.9986	**0.9987**	1.029
4.6A_1:1	60.54 ± 1.45	0.9969	**0.9976**	0.950
4.6B_1:1	59.97 ± 4.72	0.9954	**0.9965**	0.939
4.6B_1:2	58.04 ± 6.19	0.9915	**0.9931**	0.924
4.6B_1:3	67.60 ± 4.41	0.9804	**0.9990**	1.264

Table 7
Difference (f_1) and similarity (f_2) factors for tested pairs of ibuprofen release profiles.

Profiles compared	f_1	f_2
4.6A_1:1 *vs.* 4.6B_1:1	2.08	97.17
4.6A_1:1 *vs.* 4.6B_1:2	4.02	91.25
4.6A_1:1 *vs.* 4.6B_1:3	15.39	70.26
4.6A_1:1 *vs.* 5.6A_1:1	10.32	78.12
4.6A_1:1 *vs.* 5.6B_1:1	5.14	88.61
4.6B_1:1 *vs.* 4.6B_1:2	3.45	93.51
4.6B_1:1 *vs.* 4.6B_1:3	16.04	68.98
4.6B_1:1 *vs.* 5.6A_1:1	10.51	78.46
4.6B_1:1 *vs.* 5.6B_1:1	5.73	85.75
4.6B_1:2 *vs.* 4.6B_1:3	17.12	66.93
4.6B_1:2 *vs.* 5.6A_1:1	10.90	77.46
4.6B_1:2 *vs.* 5.6B_1:1	6.90	82.32
4.6B_1:3 *vs.* 5.6A_1:1	20.17	63.97
4.6B_1:3 *vs.* 5.6B_1:1	10.67	76.93
5.6A_1:1 *vs.* 5.6B_1:1	10.73	76.66

ibuprofen in the crystalline form, but with partial disruption of the crystalline lattice due to its interactions with the polymers. The high content of ibuprofen in PECs (~50%) slowed their rehydration and hydrogel formation upon contact with the aqueous medium (phosphate buffer pH 7.2). Slow rehydration and swelling of PECs resulted in controlled release of up to 67.60% ± 4.41% ibuprofen for at least 12 h, which can be promising to reduce the frequency of daily administration and increase compliance, particularly in chronic therapy of inflammatory conditions. All ibuprofen release profiles were considered similar. The drug release kinetics was mainly affected by pH and chitosan-to-xanthan gum mass ratio, while the ibuprofen entrapment procedure significantly affected the physicochemical properties of PECs. Considering the yield and *%EE*, together with the controlled drug release kinetics, the PEC 4.6B_1:2 can be considered the most promising carrier among the investigated PECs.

CRediT authorship contribution statement

Dr Ljiljana Djekic, Associate Professor, have made contributions to the setting of the hypothesis and the aim of research, conception and design of the study, and in the overall data analysis. Ana Ćirić, PhD student, was involved in the preparation of the PECs and in all aspects of their characterization of aqueous dispersions as well as in solid state. Additionally, Dr Đorđe Medarević was involved in light microscopy examination of the dried PECs. Dr Bojan Čalija was performed DSC analysis of the dried PECs. Dr Vladimir Dobričić was performed FT-IR analysis of the dried PECs. Tanja Barudžija, Research Associate, was performed PXRD analysis of the dried PECs and pure ibuprofen. Milena Rmandić, PhD student, and Dr Anđelija Malenović, Professor, are responsible for performing and validation of coulometric Karl-Fischer (cKF) method. All the authors have made contributions in interpretation of the obtained results as well as in the article revision process.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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Evaluation of chitosan/xanthan gum polyelectrolyte complexes potential for pH-dependent oral delivery of escin

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ABSTRACT

Escin is an amphiphilic and weakly acidic drug that oral administration may lead to the irritation of gastric mucosa. The entrapment of escin into chitosan (CH)/xanthan gum (XG)-based polyelectrolyte complexes (PECs) can facilitate controlled drug release which may be beneficial for the reduction of its side effects. This study aimed to investigate the influence of escin content and drying method on the formation, physicochemical, and controlled, pH-dependent drug release properties of CH/XG-based PECs. Measurements of transmittance, conductivity, and rheological characterization confirmed the formation of CH/XG-based PECs with escin entrapped at escin-to-polymers mass ratios 1:1, 1:2, and 1:4. Ambient-dried PECs had higher yield, entrapment efficiency, and escin content in comparison with spray-dried ones. FT-IR spectra confirmed the interactions between CH, XG, and escin, which were stronger in ambient-dried PECs. PXRD and DSC analyses showed the amorphous escin character in all dry PECs, regardless of the drying method. The most promising controlled and pH-dependent *in vitro* escin release was from the ambient-dried PEC at the escin-to-polymers mass ratio of 1:1. For that reason and due to the highest yield and entrapment efficiency, this carrier has the potential to prevent the irritation of gastric mucosa after oral administration of escin.

1. Introduction

Escin is a vasoprotective used in the treatment of chronic venous disease, available on the world market in the form of gastro-resistant tablets containing 20 mg or 40 mg of the drug [1]. The use of gastro-resistant dosage forms is recommended due to the ability of escin to irritate gastric mucosa and slow or even prevent gastric emptying [2]. It would be optimal to formulate an oral drug carrier from which escin would not be released under the acidic environment of the stomach, while the entire amount of the drug would be released under a neutral or weakly alkaline environment of the small intestine. Chitosan/xanthan gum-based polyelectrolyte complexes (PECs) can be evaluated as carriers for pH-dependent release of escin after its oral administration to achieve gastric protection from its side effects [1]. PECs formed by establishing electrostatic interactions between oppositely charged polymers are considered attractive drug carriers for different routes of administration. Chitosan (CH) is a natural, biocompatible, biodegradable, and non-toxic polymer (LD50 is 16 g/kg body weight) [3]. The positive charge of CH chains enables its interaction with negatively charged (anionic) polymers, such as xanthan gum. Xanthan gum (XG) is a biocompatible, biodegradable, non-toxic, and low-cost anionic polysaccharide, extensively used in the food and pharmaceutical industry [4]. CH/XG-based PECs are considered suitable carriers for both hydrophilic [5,6] and hydrophobic [7–9] low- and high-dose drugs. It has

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been shown that the preparation conditions of PECs (polymers' concentration, polymers' mass ratio, polymers' mixing order, pH at which PEC is formed, type of pH adjusting agent, *etc.*), as well as the drug entrapment procedure, can affect the drug release profile (immediate, sustained, or pH-dependent) [5,6,8–13]. Some research groups formulated CH/XG-based PECs with the pH-dependent release of hydrophobic (*e.g.*, glipizide) [8] and hydrophilic (*e.g.*, isosorbide mononitrate) [5] drugs. A small amount of these drugs was released at acidic pH (1.0–2.0) and significantly higher at neutral to weakly alkaline pH values (6.8–7.4). This could be important in the case of drugs unstable at low pH values prevailing in the stomach, as well as in those that can irritate the gastric mucosa [14]. Due to our best knowledge, PEC-based carriers for pH-dependent release of escin have not been investigated yet. Escin is a saponoside with aglycone and glycone parts in its structure. The aglycone of escin is a mixture of isomers comprising protoescigenin esterified with acetic acid at position C22 (β-escin) or C28 (cryptoescin), and at the C21 position with angelic acid or tiglic acid. The glycone of these isomeric molecules is a trisaccharide formed from one molecule of glucuronic acid and two molecules of glucose. The molecular formula of all escin isomers is $C_{55}H_{86}O_{24}$ [15], and their molecular weight is 1131.269 gmol^{-1} [16]. Escin is a weak acid with the carboxylic group located in the glycone part of the molecule (pKa ~ 4.7) [17]. Due to the presence of hydrophobic (aglycone) and hydrophilic (glycone) parts in the molecule, escin has an amphiphilic character [18]. Indena, the largest manufacturer of commercially available escin, states that its solubility in water at 20 °C and pH 7.2 is 178 g/l [19]. It has been stressed that the aqueous solubility of escin can be significantly increased by an increase in pH [17]. Amphiphilic character and pH-dependent solubility may affect its ability to form complexes with polymers such as CH and XG which also contain both hydrophobic and hydrophilic domains by establishing electrostatic, hydrogen, and hydrophobic interactions [3,18,20,21]. The strength and extent of these interactions may affect drug release properties.

To develop solid dosage forms for oral administration, PECs can be subjected to various drying methods, *e.g.*, drying under ambient conditions [9,10,13], in a vacuum [22], drying at elevated temperatures in ovens [23], freeze-drying (lyophilization) [24,25], spray-drying [7,26], *etc.* Ambient drying is a simple method that can be used at a laboratory level to obtain PECs in the form of powders with high yield, drug entrapment efficiency, and controlled drug release properties, as has been shown in our previous studies [9,27]. On the other hand, spray drying is a convenient method both for the laboratory preparation and the industrial production of powders which can be used for the preparation of different solid dosage forms, such as tablets and capsules [28]. The choice of drying method can significantly affect the characteristics of PECs as drug carriers [27]. Detailed characterization of PECs prepared by different drying methods could indicate the optimal drying parameters to obtain PECs with the highest yield, drug entrapment efficiency, and best drug release control. Our previous studies have shown that solid CH/XG-based PECs (in the form of powders) can be obtained both by drying under ambient conditions and spray drying. Moreover, these studies confirmed that these PECs can be considered suitable carriers for the pH-dependent release of the hydrophobic drug ibuprofen [9,27]. Although PEC formulations with both hydrophobic (*e.g.*, rifampicin [12] and quercetin [7]) and hydrophilic (*e.g.*, doxycycline hyclate and levofloxacin [29]) drugs are already described, CH/XG-based PECs obtained by spray drying have been investigated scarcely as drug carriers.

The effect of amphiphilic drugs, such as escin, on the formation and properties of PECs, has not been elucidated yet. Also, the impact of the drying method on the properties of escin-loaded CH/XG-based PECs is completely unexplored. Therefore, this study aimed to investigate the influence of escin content and drying method on the formation of CH/XG-based PECs, their physicochemical properties, and the ability to ensure the pH-dependent release of the entrapped escin.

2. Materials and methods

2.1. Materials

Medium molecular weight CH (190000–310000 gmol^{-1}) (Sigma Aldrich, USA; CAS Number 9012-76-4, Lot Number STBJ3281), with 85 % deacetylation degree, and viscosity of 331 cps (1 % *w/w* solution in 1 % *w/w* acetic acid) was used as the cationic component of PECs. XG (Jungbunzlauer, Switzerland; CAS Number 11138-66-2) of pharmaceutical and food grade, the anionic component of PECs, was a kind gift by Inexall Company d.o.o. – Subotica (Serbia). The specified viscosity of the XG solution (1 % *w/w* in 1 % *w/w* KCl solution, Brookfield LVTD, spindle 4, 60 rpm at 25 °C) is 1300–1700 mPa·s. A model drug substance, escin free acid (CAS Number 6805-41-0), was a gift by Indena – Milan (Italy). All the other chemicals: purified water, acetic acid, sodium acetate, sodium hydroxide, potassium dihydrogen phosphate, methanol, trifluoroacetic acid, and acetonitrile (Ph. Eur. 10.0) were of HPLC grade. All substances were used as received without further purification.

2.2. Methods

The methodology (*i.e.*, preparation of the solutions of polymers and PEC dispersions, escin entrapment, drying procedures, and characterization of escin-loaded PEC dispersions and dried PECs) was closely related to approaches established in our previous studies for CH/XG-based PECs without and with ibuprofen as a model drug [9,10,27].

2.2.1. Preparation of chitosan solution

CH solution at a concentration of 0.65 % *w/v* in purified water was prepared under ambient conditions by mixing on the laboratory propeller mixer RZR 2020 (Heidolph, Germany) at 500 rpm. The pH was adjusted to 4.6 using the acetic acid solution (1.5 % *v/v*) and checked on the HI 8417 pH meter (Hanna Instruments, USA), previously calibrated with the standard buffer solutions of pH 7.0 and 4.0. The prepared CH solution was stored at 5 ± 3 °C (in the refrigerator) for 24 h to achieve complete hydration and solubilization of the polymer.

2.2.2. Preparation of xanthan gum solutions with escin

XG solution at a concentration of 0.65 % *w/v* was prepared under ambient conditions by dissolving the polymer in purified water using the laboratory propeller mixer RZR 2020 at 500 rpm. Then, escin was added to the XG solution and the stirring was continued. Three different XG solutions with escin, which varied in escin concentration, were prepared. The concentration of the drug was 0.975 % *w/v*, 0.487 % *w/v*, or 0.244 % *w/v* in order to achieve the escin-to-polymers mass ratio of 1:1, 1:2, and 1:4, respectively, in the final PECs. Prepared XG solutions with escin were then stored at 5 ± 3 °C (in the refrigerator) for 24 h to allow complete hydration of the polymer and the establishment of possible interactions between the drug and XG. After 24 h, pH values of XG solutions with escin were checked on the calibrated HI 8417 pH meter.

2.2.3. Preparation of escin-loaded PECs

Three different escin-loaded PEC dispersions were prepared using the complex coacervation method by mixing the CH solution with double the amount of a corresponding XG solution with escin. Therefore, CH-to-XG mass ratio was always 1:2 in final PECs, since our previous study showed the best release control of the entrapped drug, ibuprofen, from PECs prepared at polymers' mass ratio of 1:2 [9]. Prepared escin-loaded PEC dispersions differed in the escin-to-polymers mass ratio *i.e.*, the mass ratio of escin relative to the total amount of polymers (CH and XG) used for the preparation of PECs. These mass ratios were 1:1, 1:2, and 1:4, and they were obtained when XG solutions with escin at a drug concentration of 0.975 % *w/v*, 0.487 % *w/v*, and 0.244 % *w/v*, respectively, were mixed with the adequate amount of CH solution. Based on the escin-to-polymers mass ratio, the expected content of the drug in prepared PECs after drying was 50.0 %, 33.3 %, and 20.0 % for escin-to-polymers mass ratios of 1:1, 1:2, and 1:4, respectively. Formed PEC dispersions, labeled as E1:1, E1:2, and E1:4 due to escin-to-polymers mass ratios, were stood for 24 h at 5 ± 3 °C (in the refrigerator) prior to further characterization to enable the formation of possible interactions between polymers and escin. Each PEC dispersion consisted of two phases, a liquid phase (supernatant) and a phase consisting of the coacervate, *i.e.*, PEC itself. Coacetvates had the characteristics of hydrogels, which is why they were labeled as PEC hydrogels.

2.2.4. *Characterization of PEC dispersions*

Characterization of PEC dispersions included the measurement of transmittance, conductivity, pH, and rheological characterization during the PECs formation.

2.2.4.1. Measurement of transmittance during PEC formation. The transmittance of CH solution and XG solutions with escin was measured just before their mixing. Also, the transmittance was measured during the formation of escin-loaded PEC dispersions, while adding the XG solution with escin in portions of 10 % of their total volume in the CH solution. Measurements were performed on an Evolution 300 spectrophotometer (Thermo Scientific, USA) at a wavelength of 600 nm. For each sample, the measurement was repeated three times, and the results are presented as mean ± standard deviation (σ).

2.2.4.2. Conductivity and pH measurements. Conductivity was monitored before and during the preparation of PECs. Initial conductivity of the CH solution was measured, as well as after each 10 % of the total volume of XG solution with escin added to the CH solution and 5 min of stirring. Measurements were performed on the CDM 230 conductivity meter (Radiometer, Denmark) calibrated using 0.01 M potassium chloride solution. Also, pH was checked at the beginning and the end of the formation of escin-loaded PECs using the calibrated HI 8417 pH meter. All measurements were done under ambient conditions, in triplicate, and the results are shown as mean ± σ.

2.2.4.3. Rheological characterization. Rheological measurements included the characterization of CH solution, XG solutions with escin, escin-loaded PEC hydrogels, and supernatants separated from escin-loaded PEC hydrogels formed in the prepared dispersions. The supernatants were separated from the escin-loaded PEC hydrogels by filtration. Rheological characterization was performed on a rotational and oscillatory rheometer Rheolab MC 120 (Paar Physica, Austria), using the rotating cylinder measuring device Z2 DIN (45 mm) in the case of polymer solutions and supernatants, and Z3 DIN (25 mm) in the case of PEC hydrogels. The measurements were performed at a temperature of 20 ± 0.1 °C. The shear rate increased linearly from 0 to 100 s^{-1} and then decreased from 100 to 0 s^{-1}. Each phase lasted 400 s. For each sample, the measurement was repeated three times, and the results are presented as the mean ± σ.

2.2.5. *Drying of escin-loaded PECs*

The prepared escin-loaded PEC hydrogels were dried using two different drying methods, ambient drying and spray drying, as described for drug-free and ibuprofen-loaded PECs in our previous studies [9,10,27].

For ambient drying, escin-loaded PEC hydrogels were first rinsed with acetate buffer solution pH 4.6 to remove excess CH, XG, and escin that have not participated in the formation of the PEC hydrogel network and not to disturb the formed interactions in the complexes. Thin layers of rinsed hydrogels were poured into Petri dishes and dried to obtain dry films of constant mass. The films were then ground using an electric kitchen mill and sieved through a 355 μm sieve (Ph. Eur. 10.0) to obtain PECs in powder form. After grinding and sieving, the prepared samples were stored in a desiccator until further testing. The samples prepared by ambient drying (AD) were labeled as E1:1_AD, E1:2_AD, and E1:4_AD.

Before spray drying, escin-loaded PEC hydrogels were first rinsed with acetate buffer solution to remove excess CH, XG, and escin. Then, the hydrogels were redispersed in the same buffer solution and the obtained dispersions were subjected to rotor-stator homogenization on T25 digital Ultra-Turrax® (IKA, Germany) for 5 min at 17500 rpm, as proposed by Bigucci et al. [30] for the preparation of CH/pectin-based PECs for spray-drying. Redispersion was done to obtain the samples suitable for spray drying (which can be difficult due to their high apparent viscosities) without disturbing the total content of solid constituents (CH, XG, and escin) in spray-dried PECs in comparison with ambient-dried ones. The redispersed and homogenized escin-loaded PEC hydrogels were then spray-dried on a Mini Spray Dryer 190 (Büchi, Switzerland) using a 0.5 mm diameter nozzle, to obtain PECs in the form of powder. The inlet temperature was kept at 140 °C, the outlet at 100 °C, and the feeding rate of PEC dispersions was 3 ml/min. Spray-dried (SD) PECs were labeled as E1:1_SD, E1:2_SD, and E1:4_SD. The prepared samples were stored in a desiccator until further testing.

2.2.6. *Characterization of escin-loaded PECs in solid state*

Characterization of solid escin-loaded PECs included determination of yield, escin entrapment efficiency (%EE), drug loading (%DL), residual moisture content, scanning electron microscopy (SEM), and powder flowability. To elucidate the type of interactions between the polymers and escin in the solid PECs, Fourier-transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD), and differential scanning calorimetry (DSC) analyses were performed.

2.2.6.1. Determination of yield, %EE and %DL. After drying, the weight of all escin-loaded PECs was measured on the analytical balance AE 240 (Sartorius, Germany). The yield was calculated based on the known amounts of CH, XG, and escin used to prepare the PECs, which represented 100 %. These values were compared with the measured amounts of dry PECs (Eq. (1)):

$$\text{Yield } (\%) = weight\ of\ dried\ PEC\ (g)/[weight\ of\ CH\ (g) + weight\ of\ XG\ (g) + weight\ of\ escin\ (g)]\cdot 100 \tag{1}$$

To determine the %EE and escin content (%DL), 10 mg of each dry escin-loaded PEC was measured and dissolved in 10 ml of a medium consisting of methanol and phosphate buffer pH 7.4, in a volume ratio of 80:20, on an ultrasonic bath Sonorex RK1024 (Bandelin electronic, Germany), at 20 ± 2 °C for 30 min. The sonication led to the complete extraction (*i.e.*, solubilization) of the entrapped escin. The amount of escin in PECs was determined by the HPLC method using Dionex Ultimate 3000 system (Thermo Fisher Scientific, Germany) equipped with Dionex Ultimate 3000 quaternary pump, autosampler, DAD detector, and the column Zorbax Extend C18 (150 mm × 4.6 mm, 5 μm particle size). Gradient elution was applied (Table 1). The column temperature was adjusted to 25 °C and the flow rate was 1 ml/min. The detection of escin was performed at 210 nm.

%EE was calculated using the following equation (Eq. (2)):

$$\%\text{EE} = actual\ amount\ of\ escin\ in\ PEC\ (g)/theoretical\ amount\ of\ escin\ in\ PEC\ (g)\cdot 100 \tag{2}$$

%DL was calculated as shown in the equation (Eq. (3)):

Table 1
Gradient elution program used for the quantification of escin.

Time (min)	0.05 % trifluoroacetic acid (%)	Acetonitrile (%)	Flow rate (ml/min)
0.0	90	10	1
0.5	90	10	1
5.0	20	80	1
5.1	90	10	1
9.0	90	10	1

$$\%\mathrm{DL} = actual\ amount\ of\ escin\ in\ PEC\ (g) / measured\ weight\ of\ the\ dried\ PEC\ (g) \cdot 100 \quad (3)$$

For each sample, the measurement was repeated three times, and the results are presented as the mean ± σ.

2.2.6.2. Determination of residual moisture content. The residual moisture content determination in dry escin-loaded PECs was carried out by the validated Karl Fischer coulometric titration method [9]. Measurements were performed on the 831 KF Coulometer (Metrohm Switzerland). Hydranal-Coulomat AG (Fluka, Germany) was used as the titration reagent. The titration was performed in triplicate for each sample and the results are shown as the mean ± σ.

2.2.6.3. SEM. Before SEM analysis, the SCD 005 Cool Sputter Coater (Bal-Tec, Germany) was used to apply a thin conductive layer of gold on dry escin-loaded PECs. The micrographs were recorded in a high vacuum (5 kV) using a scanning electron microscope JSM-6390LV (JEOL, Japan). The ImageJ software, version 1.52a (National Institutes of Health, USA), was used for the estimation of particle size and shape. One hundred particles were randomly selected from each sample and their dimensions were measured. The dimensions of ambient-dried particles were expressed as the mean, maximum, and minimum of their length and width, while the dimensions of spray-dried particles were expressed as mean diameter ± σ. The particle shape of dried PECs was estimated by calculating the elongation ratio (ER). ER represents the ratio of the length and width of the particle (Eq. (4)):

$$\mathrm{ER} = length\ (\mu m)/width\ (\mu m) \quad (4)$$

Particles with ER = 1 can be considered perfectly spherical, with $1 < \mathrm{ER} < 1.15$ spherical, and with $\mathrm{ER} > 1.15$ non-spherical [31]. The calculated ER values were shown as ER for the mean particle size, $\mathrm{ER}_{\mathrm{mean}}$ (for ambient-dried and spray-dried PECs), ER for the smallest particle, $\mathrm{ER}_{\mathrm{min}}$ (for ambient-dried PECs), and ER for the largest particle, $\mathrm{ER}_{\mathrm{max}}$ (for ambient-dried PECs).

2.2.6.4. Determination of powder flowabilty. The powder flowability was determined using the indirect method, described in Ph. Eur. 10.0, based on the calculated values of the *Hausner* ratio and the compressibility index (*Carr* index). Bulk (ρ_b) and tapped (ρ_t) density were calculated by pouring a known mass of each dry escin-loaded PEC into a measuring cylinder (ρ_b), and then, after tapping the samples 1250 times (ρ_t) on a StaV 2003 volumeter (J. Engelsmann AG, Germany). The *Hausner* ratio and the *Carr* index were calculated using the following equations (Eqs. (5) and (6)):

$$\text{Hausner ratio} = \rho_t/\rho_b \quad (5)$$

$$\text{Carr index } (\%) = (\rho_t - \rho_b)/\rho_t \cdot 100 \quad (6)$$

2.2.6.5. FT-IR analysis. The attenuated total reflectance (ATR) FT-IR spectra of dried escin-loaded PECs were recorded on a Nicolet iS10 FT-IR Spectrometer (Thermo Fisher Scientific, USA). The wavelength ranged between 4000 and 400 cm^{-1} (resolution 4 cm^{-1} and 16 scans per sample).

2.2.6.6. PXRD analysis. PXRD analysis of dry escin-loaded PECs was performed on Bruker D8 advance diffractometer (Bruker, Germany) in Bragg-Brentano θ-2θ geometry, using Ge-crystal primary monochromator (Johanson type) that generates CuKα1 radiation ($\lambda = 1.541$ Å). Diffraction data were obtained in the 2θ range from 5 to 45° with a scan step of 0.05° and a holding time of 12 s.

2.2.6.7. DSC analysis. All dry escin-loaded PECs were accurately weighed in an amount of 5–10 mg and then crimped into standard aluminum pans (40 μl). The samples were heated from 25 to 360 °C (heating rate 10 °C/min), under a constant nitrogen flow (50 ml/min) on the DSC 1 instrument (Mettler Toledo, Switzerland). The empty sealed pan was used as a reference.

2.2.7. In vitro escin release testing

Dry escin-loaded PECs were filled into hard hypromellose capsules size 0 (Fagron, Greece) in an amount corresponding to 100 mg of escin. It is known that 100–150 mg of escin daily in a sustained-release dosage form can be used for the symptomatic treatment of patients with chronic venous insufficiency [32]. The *in vitro* release of escin was tested on a rotating basket apparatus Erweka DT 600 (Erweka, Germany), at 37 ± 0.5 °C and under stirring at 100 rpm. The dissolution medium during the first 3 h was 500 ml of 0.1 M hydrochloric acid (pH 1.2). 500 ml of phosphate buffer pH 7.4 (USP) was used from the 3rd hour until the end of the assay (next 9 h). The sampling of 4 ml of medium was performed after 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 600, and 720 min. Each sample was filtered through a 0.45 μm MF-Millipore® membrane filter. The concentration of escin was determined using the previously described HPLC method. The test was performed in triplicate for each sample and the results are shown as the mean of the released amount of escin ± σ. Mathematical models for *zero-order*, *first-order*, *Higuchi*, and *Korsmeyer-Peppas* kinetics were applied to the results. Data were processed using the DDSolver Excel Add-In [33].

In addition, the model-independent approach was used to compare the drug dissolution profiles. The similarity (f_2) factor was also calculated using the DDSolver Excel Add-In [33].

3. Results and discussion

The CH solution was a pale yellow clear liquid. On the other hand, XG solutions with escin and escin-loaded PEC hydrogels were homogeneous mixtures of liquid or semi-solid consistency, respectively, white in color and opalescent, with clearly visible foam formed on the surface, probably due to the surface activity of escin [15]. The supernatants were transparent to slightly turbid pale yellow liquids.

3.1. Formation of escin-loaded PECs

The transmittance value of the CH solution was high (99.60 ± 2.88 %) due to its high solubility at pH 4.6. The transmittance of the opalescent drug-free XG solution was 27.67 ± 0.75 %. The addition of escin into the XG solution led to a decrease in transmittance. Transmittance values of XG solutions with escin were 2.11 ± 0.54 % at escin concentration of 0.975 % *w/v*, 4.07 ± 0.21 % at escin concentration of 0.487 % *w/v*, and 2.17 ± 0.21 % at escin concentration of 0.244 % *w/v* since these samples were also opalescent. No correlation was observed between drug concentration and measured transmittance. The lower transmittance of XG solutions with escin in comparison with the drug-free XG solution is probably due to the establishment of interactions between escin and XG [34]. Both escin and XG have hydrophilic and hydrophobic domains in their structures, so transmittance could be reduced after the establishment of hydrogen bonds and hydrophobic interactions [18,20,21]. During the formation of escin-loaded PECs by adding the corresponding XG solution with escin into the CH solution, the transmittance of PEC dispersions continuously decreased to very low values: 0.12 ± 0.01 % for E1:1, 0.14 ± 0.00 % for E1:2, and 0.11 ± 0.03 % for E1:4 (Fig. 1A).

Lower transmittances measured for PEC dispersions compared to the transmittances of CH solution and XG solutions with escin confirmed the formation of complexes. Lower transmittance values (*i.e.*, lower transparency) in PECs could be associated with intermolecular interactions established in complexes [34]. No significant differences in transmittance were observed in final PEC dispersions at different escin-to-polymers mass ratios. The transmittance of PEC hydrogels could not be measured due to their thick consistency.

For purified water used for the preparation of PECs, a conductivity of

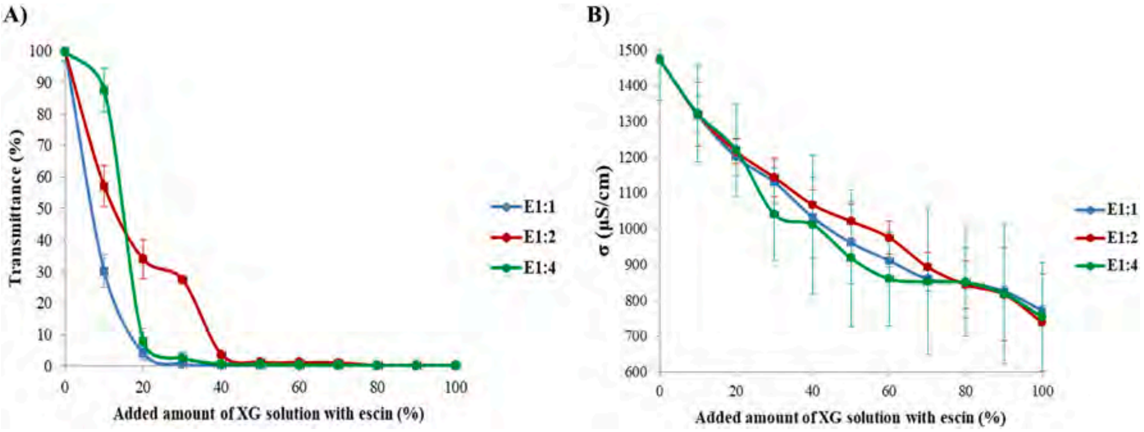

Fig. 1. Transmittance (A) and conductivity (B) during the formation of escin-loaded PECs.

3 ± 0 µS/cm was measured. When escin was dissolved in purified water, the conductivity increased. At an escin concentration of 0.975 % *w/v*, a conductivity of 330 ± 7 µS/cm was measured, at a concentration of 0.487 % *w/v* it was 218 ± 1 µS/cm, and at a concentration of 0.244 % *w/v* 142 ± 4 µS/cm. This increase in conductivity can be explained by escin's acidic properties and its dissociation to hydrogen (H^+) and carboxylate ions. Higher conductivity values were measured at higher concentrations of escin. For drug-free XG solution, the conductivity of 657 ± 31 µS/cm was reported in our previous research [9]. Significantly higher conductivity of XG solution at a concentration of 0.65 % *w/v* compared to escin solutions at different concentrations (0.244–0.975 % *w/v*) reflected a different nature of these substances. Since XG is a high molecular weight polyelectrolyte (polyacid), at similar concentrations it has more carboxylic groups available for dissociation and release of H^+ ions in comparison with escin, as well as of counter-ions such as Na^+, K^+, and Ca^{2+} [4]. H^+ and metal cations are more mobile compared to the carboxylate anions of XG and escin and therefore affect the conductivity of the samples to a greater extent [35]. Measured conductivity of XG solutions with escin was 536 ± 54 µS/cm at drug concentration of 0.975 % *w/v*, 558 ± 46 µS/cm at a concentration of 0.487 % *w/v*, and 517 ± 86 µS/cm at a concentration of 0.244 % *w/v*. Measured values showed no significant differences at different escin concentrations. The lower conductivity of XG solutions with escin compared to the conductivity of drug-free XG solution can be attributed to the interactions established between XG and escin, as previously mentioned. The conductivity of the CH solution was 1473 ± 113 µS/cm. A continuous decrease in conductivity from the initial value measured for CH solution was observed during the formation of PECs, *i.e.*, during the addition of a corresponding XG solution with escin into CH solution (Fig. 1B). That confirmed the establishment of typical interactions between the components of PECs. For E1:1 dispersion the final conductivity of 771 ± 14 µS/cm was measured, for E1:2 it was 738 ± 135 µS/cm, and for E1:4 it was 753 ± 153 µS/cm. No significant differences in the conductivity of escin-loaded PEC dispersions were observed at different escin-to-polymers mass ratios. Also, a constant decrease in conductivity when mixing CH solution with XG solutions with escin (without reaching a plateau, and subsequent increase in conductivity) could indicate that after the formation of PECs, positively charged CH remained in excess. This assumption was confirmed by the rheological characterization of the supernatants. The trend of decrease in conductivity was also observed in our previous study during the preparation of drug-free and ibuprofen-loaded PECs. For drug-free PEC final conductivity of PEC dispersion was 872 ± 12 µS/cm and for ibuprofen-loaded 855 ± 27 µS/cm [9]. Conductivity values of escin-loaded PEC dispersions were lower compared to the previously reported conductivity of drug-free and ibuprofen-loaded PEC dispersions. Lower conductivity value can be explained by a higher degree of interactions in the sample since it is known that only free and mobile ions are responsible for the conductivity of the system [9,36]. Therefore, it can be assumed that stronger and more extensive interactions between CH and XG were established in the presence of both drugs. Also, it can be expected that amphiphilic escin can interact more extensively with CH and XG during the PECs formation than hydrophobic ibuprofen.

Measured pH values of XG solutions with escin, escin-loaded PEC dispersions and PEC hydrogels are shown in Table 2. For the drug-free XG solution pH of 6.85 ± 0.05 was reported in our previous study [9]. It can be seen that the addition of escin into the XG solution led to a significant lowering of pH compared to the drug-free XG solution, probably due to escin's weak acid properties [17]. With the increase of escin concentration, a decrease in pH was observed, *i.e.*, the lowest pH was measured for XG solution with escin at the escin concentration of 0.975 % *w/v* and the highest for the concentration of 0.244 % *w/v*. Also, pH values of all XG solutions with escin were lower in comparison with the pH of the ibuprofen dispersion in XG solution (pH of 5.25 ± 0.03 at ibuprofen concentration of 0.975 % *w/v*) [9]. This can be the consequence of the higher aqueous solubility of escin compared to ibuprofen, rather than of their mutually similar pKa values (4.7 for escin and 4.5–4.6 for ibuprofen) [17,37]. After mixing the CH solution with the corresponding XG solution with escin, the pH of the obtained escin-loaded PEC dispersions was similar to the pH of the CH solution, probably due to the buffering capacity of CH itself [38] and acetic acid used to adjust its pH [10]. Therefore, it can be assumed that escin in the prepared PEC dispersions was present equally in the uncharged (free acid) and charged (carboxylate anion) forms, as well as ibuprofen in previously prepared PECs. Moreover, escin-loaded PEC hydrogels had lower pH values than the corresponding PEC dispersions. This result can be explained by the entrapment of escin within the 3D hydrogel network and its higher concentration in comparison with PEC dispersions.

To evaluate the strength and extent of interactions between CH, XG, and escin during and after the preparation of PECs, rheological characterization was performed for polymers' solutions, escin-loaded PEC hydrogels, and their corresponding supernatants. CH solution had a Newtonian type of flow and dynamic viscosity of 0.09 ± 0.01 Pa·s. Derkach et al. [39] have shown that aqueous solutions of CH at concentrations up to 0.8 % are Newtonian fluids. On the other hand, drug-free XG solution and XG solutions with escin were pseudoplastic systems

Table 2
pH of XG solutions with escin, escin-loaded PEC dispersions and PEC hydrogels.

PEC	pH value ± σ		
	XG solution with escin	PEC dispersion	PEC hydrogel
E1:1	3.97 ± 0.08	4.45 ± 0.27	3.92 ± 0.05
E1:2	4.27 ± 0.07	4.59 ± 0.22	4.16 ± 0.15
E1:4	4.60 ± 0.06	4.67 ± 0.23	4.28 ± 0.23

with thixotropy. Thixotropy was estimated by calculating the hysteresis area (H). For drug-free XG solution, H value of 5.32 ± 0.26 Pa/s, maximum apparent viscosity (η_{max}) of 0.20 ± 0.00 Pa·s, and minimum apparent viscosity (η_{min}) of 0.07 ± 0.00 Pa·s were measured. The addition of escin to the XG solution at different concentrations did not significantly affect the H values (4.67 ± 2.12 Pa/s at escin concentration of 0.975 % *w/v*, 2.75 ± 1.87 Pa/s at a concentration of 0.487 % *w/v*, and 1.94 ± 1.27 Pa/s at a concentration of 0.244 % *w/v*) and η_{max} (0.28 ± 0.01 Pa·s at escin concentration of 0.975 % *w/v*, 0.21 ± 0.00 Pa·s at a concentration of 0.487 % *w/v*, and 0.20 ± 0.00 Pa·s at a concentration of 0.244 % *w/v*). A slightly higher value of η_{max} for XG solution with the highest escin concentration may be associated with a higher content of solid constituents in the sample [40]. The η_{min} of 0.07 ± 0.00 Pa·s was measured for all XG solutions with escin.

The escin-loaded PEC hydrogels showed pseudoplastic flow behavior with thixotropy. The H values, η_{max}, and η_{min} of escin-loaded PEC hydrogels are shown in Table 3. The thixotropy of all escin-loaded PEC hydrogels was similar. This result indicated that all hydrogels can recover their structure after the removal of shear stress in the almost same manner [9,10,41]. Measured apparent viscosities for PEC hydrogels were higher in comparison with those of individual polymers' solutions used for their preparation. Kim et al. [22] showed that the increase in apparent viscosities of the formed PECs in comparison with viscosities of the individual polymers' solutions confirms the establishment of interactions between them and the formation of complexes. The values of apparent viscosities of PEC hydrogels reflected the strength and extent of interactions between the components of the complexes [9,10,39]. By comparison of their η_{max} values, no significant differences were observed between escin-loaded PECs. However, a slight tendency of apparent viscosity to increase with an increase in the escin-to-polymers mass ratio can be observed, which can be explained by the higher content of solid constituents [40]. In addition, η_{max} values of all escin-loaded PEC hydrogels were higher than the η_{max} values of drug-free and ibuprofen-loaded PEC hydrogels (at the same CH-to-XG mass ratio and ibuprofen-to-polymers mass ratio of 1:1) reported in our previous study [9]. That was an additional indicator of stronger and more extensive interactions in CH/XG-based PECs after the entrapment of amphiphilic drug escin in comparison to hydrophobic ibuprofen and drug-free PECs.

The analysis of the supernatants showed their Newtonian behavior with a dynamic viscosity of 0.04 ± 0.00 Pa·s for E1:1 and 0.02 ± 0.00 Pa·s for E1:2 and E1:4. Lower dynamic viscosity values of supernatants compared to the viscosity of CH solution before its complexation can be the consequence of its participation in the formation of PECs, as reported in the literature for the PEC formation between gellan gum and cationic guar gum [34]. For that reason, it may be assumed that supernatants were mainly composed of excess of dissolved CH, with the possible presence of smaller amounts of XG and escin that did not participate in the formation of the complexes.

3.2. Characteristics of dry escin-loaded PECs

PECs obtained by both drying methods were fine white powders, with spray-dried samples being significantly more voluminous than ambient-dried ones.

Table 3

Hysteresis areas (H), maximal apparent viscosity (η_{max} at 22.2 s^{-1}), and minimal apparent viscosity (η_{min} at 100 s^{-1}) of escin-loaded PEC hydrogels.

Escin-loaded PEC hydrogel	H ± σ (Pa/s)	η_{max} ± σ (Pa·s)	η_{min} ± σ (Pa·s)
E1:1	889.40 ± 100.23	3.36 ± 0.51	0.67 ± 0.25
E1:2	810.13 ± 244.49	3.26 ± 0.81	0.57 ± 0.17
E1:4	781.84 ± 164.79	3.00 ± 0.23	0.69 ± 0.23

3.2.1. Yield, %EE, %DL and residual moisture content

The yield, %EE, %DL, and the residual moisture content of ambient-dried and spray-dried escin-loaded PECs are shown in Table 4.

It was observed that the yields of ambient-dried samples were significantly higher compared to the spray-dried samples even though the total content of solid constituents was equal in PEC hydrogels and PEC hydrogels redispersed in acetate buffer, respectively, at the same escin-to-polymers mass ratio before drying. As reported by Sosnik & Seremeta [28], the yield of spray drying at a laboratory scale is not optimal (usually 20–70 %) due to the loss of the product on the walls of the drying chamber. Significant losses of escin-loaded PECs were visually observed on the spray-dryer walls. Moreover, described escin-loaded PEC hydrogels have a significantly higher apparent viscosity than 0.3 Pa·s, reported to be the highest viscosity that ensures satisfactory yields after spray drying (50 % or more) at the laboratory scale. In ambient-dried samples, the highest yield was observed for E1:1_AD and the lowest for E1:4_AD. The highest yield in these samples can be correlated with the highest content of dispersed escin at escin-to-polymers mass ratio of 1:1 and lower losses of PEC components during their preparation. On the other hand, in spray-dried samples the lowest yield was observed for E1:1_SD and the highest for E1:4_SD, so for this drying method, the higher content of dispersed escin resulted in a lower yield. Ceschan et al. [40] reported that drug carriers with higher content of dispersed solids result in higher viscosities. When the apparent viscosity increases the spray drying performance decreases due to the sample sticking onto the surface of the chamber of spray dryers. Rheological characterization of escin-loaded PEC hydrogels also showed a slight tendency for apparent viscosity to increase with increased dispersed escin content. A similar influence of the drying method on the yield was recently reported for ambient-dried and spray-dried drug-free and ibuprofen-loaded PECs [27]. So, it can be assumed that spray drying is a less suitable drying method in comparison with ambient drying to obtain satisfactory yields of PECs even after the redispersion of PEC hydrogels. Systems with high viscosities such as PECs need the optimization of the spray drying method to obtain higher yields.

Generally higher %EE values were observed in ambient-dried PECs in comparison with the spray-dried ones. The highest value of %EE had E1:1_AD and the lowest E1:1_SD. As in the case of the yield of the samples, this finding can be explained by the content of dispersed escin in complexes. Therefore, in ambient-dried PECs, %EE was higher at higher contents of dispersed escin, while in spray-dried PECs the opposite trend could be observed (*i.e.*, the higher the content of dispersed escin the lower %EE values were obtained). The obtained %DL values of ambient-dried samples were expected and in accordance with the theoretical content of escin in PECs calculated *via* the escin-to-polymers mass ratio (50 % for E1:1, 33.33 % for E1:2, and 20 % for E1:4). On the other hand, the %DL of spray-dried complexes did not follow any trend established so far, and the obtained values were low due to the significant loss of PECs during the drying process.

In all dry escin-loaded PECs, the residual moisture content varied between 6.26 % and 9.74 %. The measured moisture content was lower than 10 %, which can be considered convenient for their microbiological

Table 4

Yield, %EE, %DL and residual moisture content of dried escin-loaded PECs.

PEC	Yield ± σ (%)	%EE ± σ	%DL ± σ	Moisture content ± σ (%)
E1:1_AD	66.13 ± 12.00	64.12 ± 1.10	50.29 ± 0.86	8.41 ± 0.09
E1:2_AD	54.25 ± 5.15	37.41 ± 4.90	34.49 ± 4.51	8.45 ± 0.16
E1:4_AD	50.88 ± 2.71	15.79 ± 0.17	15.52 ± 0.17	9.74 ± 0.24
E1:1_SD	7.37 ± 0.38	1.15 ± 0.43	7.81 ± 2.94	6.26 ± 0.10
E1:2_SD	17.52 ± 0.61	4.27 ± 0.34	8.13 ± 0.65	8.33 ± 0.04
E1:4_SD	26.36 ± 0.59	4.67 ± 0.67	3.55 ± 0.51	6.67 ± 0.16

stability [9]. Although spray drying implied higher temperature and more controlled drying conditions in comparison with ambient drying, the residual moisture content in the samples obtained by different drying methods did not differ much. This can be due to the presence of XG, a polymer with pronounced hydrophilic and hygroscopic properties [42]. XG can retain high amounts of water in samples even after drying or processing at elevated temperatures [43]. Within the samples obtained by the same drying method, the residual moisture content decreased with decreasing polymers' and increasing escin content. It can be assumed that besides polymers content, escin also may interfere with water to squeeze it out of the complex. A similar observation was reported by Das et al. [31] for resveratrol in CH/pectin-based PECs. When comparing escin-loaded PECs with drug-free and ibuprofen-loaded PECs obtained by the same drying methods [9,10,27], it can be concluded that the entrapment of either escin or ibuprofen leads to a decrease in residual moisture content in the investigated PECs. Moreover, higher values were observed in the case of amphiphilic escin compared to hydrophobic ibuprofen [27]. It can be assumed that hydrophobic drugs hinder the incorporation of water in the structure of CH/XG-based PECs to a greater extent than drugs with a more pronounced hydrophilic character. It can be concluded that the residual moisture content has not been affected by the drying procedure, while the content and physicochemical properties of entrapped drugs had an important effect.

3.2.2. Shape and size of the escin-loaded PEC particles

Photomicrographs of ambient-dried and spray-dried escin-loaded PECs are shown in Fig. 2, while particle size and shape data are shown in Table 5. Microparticles of ambient-dried PECs were irregular in shape (ER 1.52–1.97), from approximately spherical, through plate- and needle-like to polygonal, with a wide size distribution. Spray-dried microparticles were significantly smaller and with shapes closer to spherical (ER 1.18–1.25). Most of the spray-dried microparticles had cavities and irregularities on their surface which contributed to the ER values slightly higher than 1.15. These irregularities are likely the consequence of the spray-drying conditions. The 3D network of PEC hydrogels abruptly lost water and collapsed during the spray-drying procedure [29]. Ambient-dried PECs lost the water gradually and spontaneously, so the obtained microparticles did not have any irregularities on the surface.

Micrographs in Fig. 2 and particle dimensions shown in Table 5 can indicate that the particles of ambient-dried PECs were significantly larger than those obtained by spray drying. For ambient-dried PECs, the smallest particles were observed for E1:1_AD, while the particle size for E1:2_AD and E1:4_AD was approximately the same. It can be assumed that with the increase in the drug content in the PEC, their crushing becomes easier because the relative content of polymers was lower [27]. In the spray-dried complexes, the particle size was always below 10 μm and no significant difference has been observed at different escin-to-polymers mass ratios. Since the spray drying conditions (inlet drying temperature, flow rate of PECs, and nozzle diameter) were strictly controlled, it was expected to obtain finer and more uniform particles in comparison with ambient-dried ones. The particles of pure escin had a shape similar to escin-loaded spray-dried PECs with some irregularities and cavities on the surface. The mean diameter of the particles of pure escin was higher than the mean diameters of escin-loaded PECs, and their particle size distribution was wide.

3.2.3. Powder flowability

Table 6 shows the values of bulk (ρ_b) and tapped (ρ_t) density, the *Carr* index, and the *Hausner* ratio of dry escin-loaded PECs, as well as descriptive terms of flowability based on the calculated values. ρ_b and ρ_t values were significantly higher for ambient-dried PECs compared to spray-dried ones. These values are in agreement with the results for the escin content (%DL) in PECs and show that the density of powders also depended on the content of solid constituents of the samples. At significantly higher content of solids in ambient-dried PECs, they had higher values of ρ_b and ρ_t, while spray-dried PECs were more voluminous. All dried PECs had similar values of *Carr* index and *Hausner* ratio and their flowability was characterized as poor (E1:1_AD, E1:2_AD, E1:1_SD) or very poor (E1:4_AD, E1:2_SD, E1:4_SD). It can be concluded that the drying method did not affect significantly the flowability of escin-loaded PECs, although ambient-dried samples had slightly better flowability at the escin-to-polymers ratio of 1:1 and 1:2. At the escin-to-polymers ratio of 1:4, the spray-dried PEC had better flowability. This may be due to their moisture content since the lower moisture content can be related to the better flowability of powders [44]. Significantly smaller particles of spray-dried PECs in comparison with the ambient-dried ones also may be the reason for their poorer flowability despite a particle shape closer to spherical [45].

3.2.4. FT-IR, PXRD, and DSC analyses

Fig. 3 shows the FT-IR spectra of pure escin and dry escin-loaded PECs. The spectrum of pure escin had characteristic peaks at 3390.74 cm^{-1} (–O–H stretching vibration), 2925.23 cm^{-1} (–C–H stretching vibration), 1715.59 cm^{-1} (–C=O stretching vibration), 1372.10 cm^{-1}, 1261.59 cm^{-1}, and 1158.29 cm^{-1} (–C–C– and –C–O– skeleton vibrations), 1072.86 cm^{-1} and 1023.64 cm^{-1} (–C–H bending vibrations). The obtained results were in accordance with the results published by Tomečková et al. [46] for commercially available pure escin isolated from horse chestnut seeds. Spectra of ambient-dried and spray-dried escin-loaded PECs showed the same peaks as pure escin with minor shiftings to lower values, probably due to the establishment of characteristic (electrostatic, hydrogen bonds, and hydrophobic) interactions with polymers in PECs [34]. In escin-loaded PECs, this primarily refers to peaks at wavenumbers from approximately 1700 to 1100 cm^{-1} that are characteristic of functional groups (amino, carboxyl, and hydroxyl groups) responsible for establishing interactions between escin, CH, and XG [9,10,46]. The intensity of peaks in FT-IR spectra can be related to the extent and strength of interactions in complexes. Higher intensity of peaks (*i.e.*, lower values of transmittance) is a consequence of weaker intermolecular interactions [9–11]. Based on the FT-IR spectra, it can be concluded that stronger intermolecular interactions were present in the ambient-dried PECs compared to those obtained by spray-drying. The weakening of interactions between CH, XG, and escin in spray-dried PECs can be explained by their homogenization on a rotor-stator homogenizer and the spray drying process itself. When comparing ambient-dried samples, FT-IR spectra indicated the strongest interactions in E1:1_AD, then in E1:2_AD, and the weakest in E1:4_AD. This trend was in accordance with the yield and %EE. FT-IR analysis confirmed that higher content of solid constituents in PECs resulted in stronger and more extensive interactions between CH, XG, and escin. An inconsistent trend of interaction strength was observed in spray-dried escin-loaded PECs. The strongest interactions were observed in E1:4_SD, then in E1:1_SD, and the weakest in E1:2_SD. When comparing PECs prepared at the same escin-to-polymers mass ratio, but dried using different methods, it can be seen that at mass ratios of 1:1 and 1:2 stronger interactions were detected in ambient-dried samples, while at a mass ratio of 1:4 they were similar. These results confirmed that the drying method had the greatest impact on the intensity of interactions in PECs in the solid state.

Fig. 4 shows PXRD diffractograms of pure escin, ambient-dried, and spray-dried escin-loaded PECs. It can be seen from the diffractogram that pure escin is an amorphous powder that has one wide peak that starts at approximately 10° and ends at approximately 20°, while its maximum was at 13.75°. The obtained values were similar to those reported by Tomečková et al. [46]. Ambient-dried escin-loaded PECs were also amorphous powders. For E1:1_AD, two broad peaks were observed and almost merged into one. The beginning was at about 10°, the end at about 30°, and the maximums at 15.25 and 20.3°. The diffractogram of E1:2_AD was similar in appearance - two wide peaks almost merged into one whose beginning was at approximately 10°, ending at about 30°, and maximums at 15.25 and 20.5°. E1:4_AD had one wide peak with a

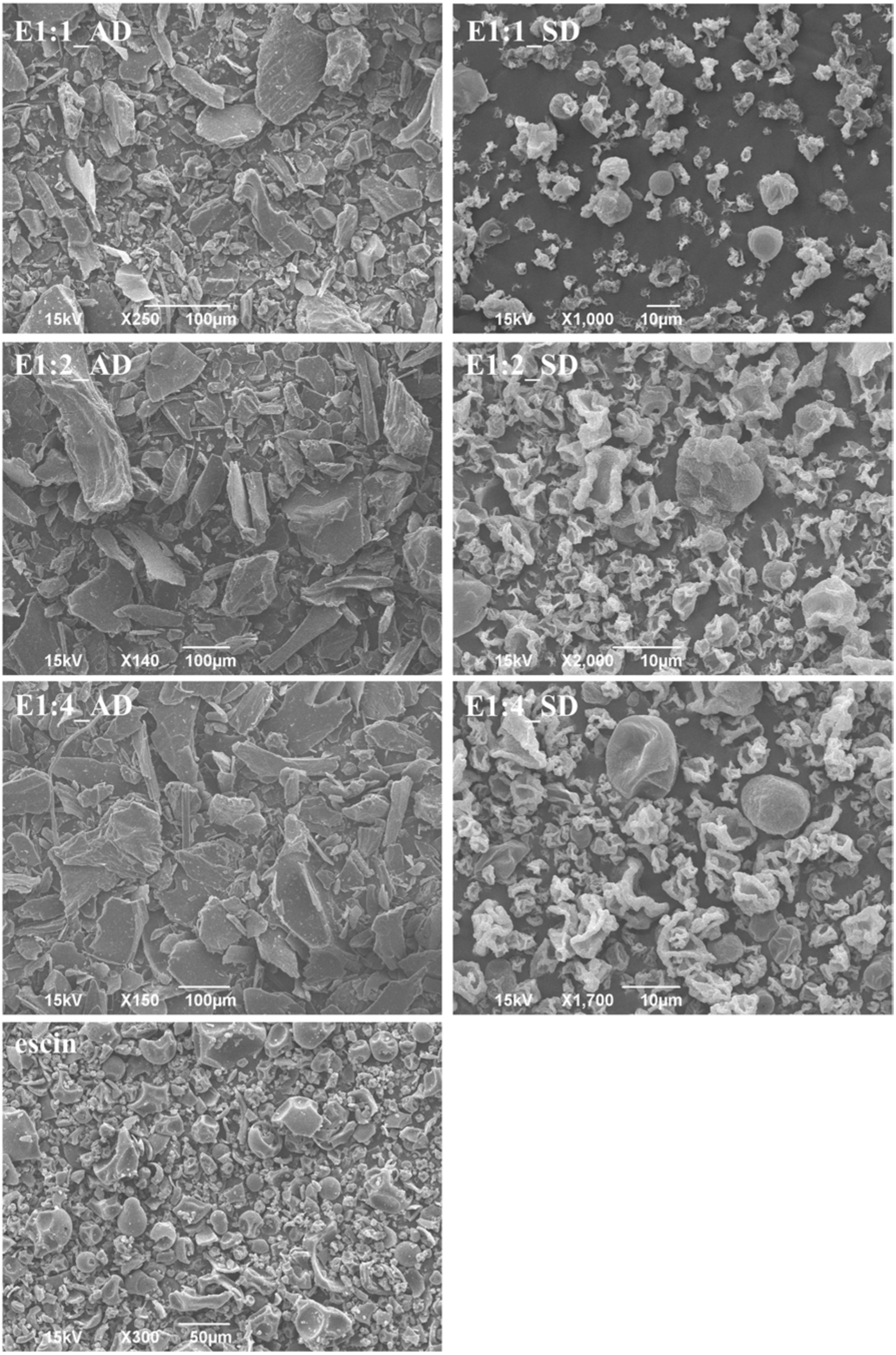

Fig. 2. SEM micrographs of pure escin, ambient-dried, and spray-dried escin-loaded PECs.

Table 5
Dimensions and ER values of pure escin, ambient-dried, and spray-dried escin-loaded PECs.

Sample	Length (µm)			Width (µm)			Diameter ± σ (µm)	ER		
	mean	max	min	mean	max	min		mean	max	min
E1:1_AD	32.45	140.70	7.45	21.35	93.84	4.04	N/A	1.52	1.50	1.84
E1:2_AD	78.87	342.37	19.81	39.98	131.35	11.38	N/A	1.97	2.83	1.74
E1:4_AD	74.10	262.05	20.05	40.40	144.56	7.66	N/A	1.83	1.81	2.62
E1:1_SD	N/A	N/A	N/A	N/A	N/A	N/A	6.11 ± 2.54	1.23	N/A	N/A
E1:2_SD	N/A	N/A	N/A	N/A	N/A	N/A	4.42 ± 2.03	1.25	N/A	N/A
E1:4_SD	N/A	N/A	N/A	N/A	N/A	N/A	5.81 ± 2.43	1.18	N/A	N/A
escin	N/A	N/A	N/A	N/A	N/A	N/A	25.71 ± 16.73	1.21	N/A	N/A

Table 6
ρ_b, ρ_t, *Carr* index, *Hausner* ratio and flowability of ambient-dried and spray-dried escin-loaded-PECs.

Sample	$\rho_b \pm \sigma$ (g/ml)	$\rho_t \pm \sigma$ (g/ml)	Carr index (%)	Hausner ratio	Flowability
E1:1_AD	0.47 ± 0.01	0.65 ± 0.01	27.51	1.38	Poor
E1:2_AD	0.41 ± 0.01	0.60 ± 0.01	30.99	1.45	Poor
E1:4_AD	0.33 ± 0.01	0.50 ± 0.01	33.99	1.51	Very poor
E1:1_SD	0.09 ± 0.00	0.13 ± 0.00	30.99	1.45	Poor
E1:2_SD	0.07 ± 0.00	0.11 ± 0.00	33.00	1.49	Very poor
E1:4_SD	0.11 ± 0.01	0.16 ± 0.01	31.97	1.47	Very poor

start at approximately 10°, an end at approximately 30°, and a maximum at 20.2°. The peak at 15.25° observed in E1:1_AD and E1:2_AD was due to the high escin content in these samples. The shift of the peak maximum to a higher value can be explained by the establishment of interactions of escin with CH and XG. The escin-derived peak was not observed in E1:4_AD, probably due to its low content in this PEC. Peaks in ambient-dried escin-loaded PECs between 20° and 20.5° were due to the presence of CH and XG in their structure [10]. Escin retained its amorphous structure after the preparation of PECs and their drying under ambient conditions. On the other hand, several characteristic peaks were observed for E1:1_SD, E1:2_SD, and E1:4_SD showing their semi-crystalline structure. The peak of the highest intensity was the one at approximately 9° while other peaks were observed at 17.95, 19.35, 24.8, 27.35, 31.15, 32.35, and 36.2°. Peaks at 9 and 19.35° originated from CH and XG [10]. The semi-crystalline structure of spray-dried PECs could also be due to the weakening of interactions between CH, XG, and escin in complexes and easier detection of semi-crystalline XG on the diffractometer. On the other hand, stronger intermolecular interactions in ambient-dried PECs led to its amorphization. This assumption can be supported by the fact that almost identical values were observed for drug-free PECs [27]. Escin-derived peaks were not observed in spray-dried PECs, probably due to its low content in these complexes.

Fig. 5 shows DSC thermograms of pure escin and dry escin-loaded PECs. The thermogram of pure escin showed a low-intensity endothermic peak with a maximum at 91.16 °C, associated with the evaporation of water from the substance (the manufacturer states that the moisture content is ≤5 % [47]). Carlotti et al. [48] indicated 222 °C as the melting temperature of the crystalline escin. Based on the shape of the escin thermogram and the absence of a sharp peak related to the melting of the crystalline drug, its amorphous structure can be reaffirmed. Two exothermic peaks, both of very low intensity, with maximums at 206.96 and 228.28 °C, were associated with escin degradation which is in accordance with the results obtained by the manufacturer [19] and Djekic et al. [49] for the same model substance. In all investigated PECs, both ambient-dried and spray-dried, escin retained its amorphous character during the preparation, since the thermograms did not show peaks that indicate the presence of crystalline structures. These thermograms showed broad endothermic peaks with maximums close to 100 °C (106.09 °C for E1:1_AD, 96.75 °C for E1:2_AD, 107.11 °C for E1:4_AD, 85.48 °C for E1:1_SD, 93.00 °C for E1:2_SD, and 101.47 °C for E1:4_SD) associated with residual moisture evaporation. The intensity of

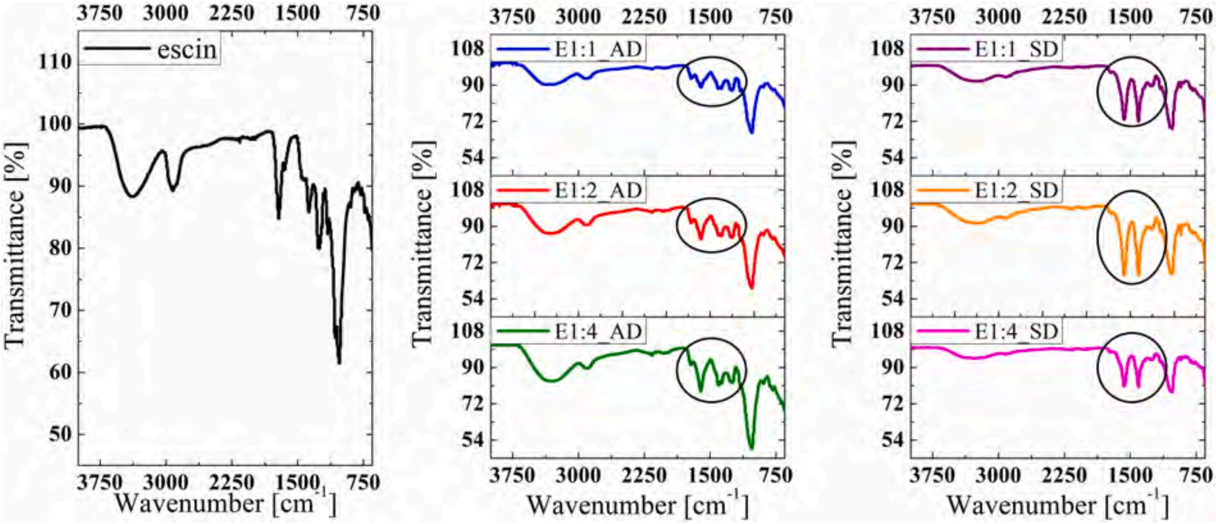

Fig. 3. FT-IR spectra of pure escin, ambient-dried, and spray-dried escin-loaded PECs.

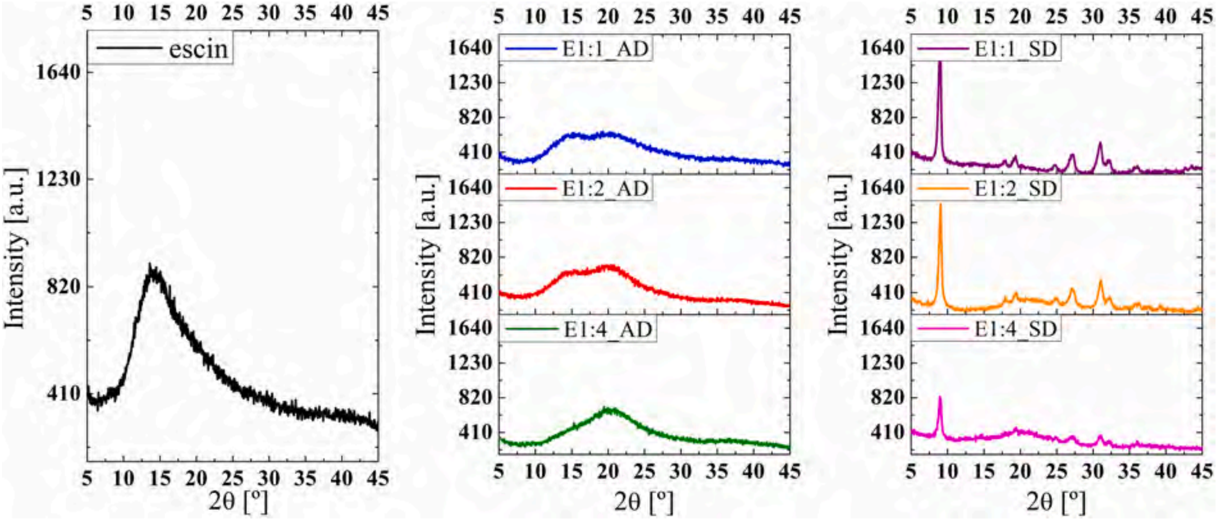

Fig. 4. PXRD diffractograms of pure escin, ambient-dried, and spray-dried escin-loaded PECs.

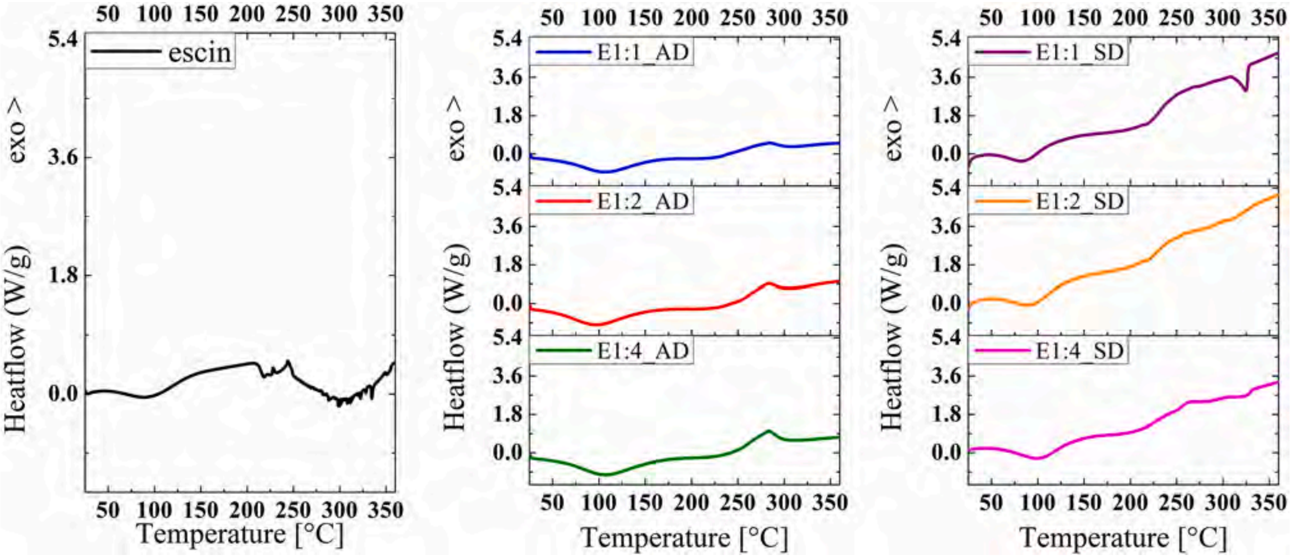

Fig. 5. DSC thermograms of pure escin, ambient-dried, and spray-dried escin-loaded PECs.

these peaks was higher compared to the peak of pure escin considering that the content of residual moisture in dry PECs was higher compared to the pure substance. Exothermic peaks between 225 and 325 °C were associated with the degradation of escin and complexes [10,49]. Peaks around 225 °C in PECs, originating from the entrapped escin, were of lower intensity compared to the pure substance due to its content in the complexes (3.55–50.29 %). An endothermic peak at 325.18 °C was observed in the thermogram of E1:1_SD which can be related to the breakdown of weak electrostatic interactions between complex components. The mentioned peak was observed only in this sample probably due to the initially highest content of solid constituents (CH, XG, and escin) in PEC dispersion E1:1. The spray drying of this dispersion led to the highest degree of weakening of interactions compared to E1:2 and E1:4, which was also reflected in the yield and % EE values of E1:1_SD.

3.3. *In vitro escin release*

Fig. 6 shows release profiles of escin from hard capsules filled with ambient-dried and spray-dried escin-loaded PECs. Solutions at pH values of 1.2 (0.1 M hydrochloric acid) and 7.4 (phosphate buffer) were used as typical dissolution media for *in vitro* oral drug release testing representing physiological pH in the stomach and intestine, respectively.

In vitro release profiles of escin showed its prolonged release during 12 h from all samples. The highest amount of escin (77.93 ± 3.98 %) was released from E1:1_SD and the lowest (28.04 ± 2.13 %) from E1:2_AD. When comparing PECs with the same escin-to-polymers mass ratio, it can be seen that higher amounts of the entrapped drug were released from spray-dried PECs. This can be explained by weaker interactions between escin and polymers in these samples compared to ambient-dried ones. The smaller particle size of spray-dried PECs can also contribute to this result due to their greater surface in contact with dissolution media. In addition, it can be assumed that in spray-dried PEC particles the drug could be rinsed from their surface, while in ambient-dried PECs escin was entrapped into the polymer network from where it had to diffuse after the swelling of the complex and the relaxation of polymers' chains. Within the samples obtained by the same drying method, the highest amount of escin was released from PECs in which the escin-to-polymers mass ratio was 1:1 (42.23 ± 3.02 % from E1:1_AD and 77.93 ± 3.98 % from E1:1_SD), then 1:4 (35.82 ± 12.26 % from E1:4_AD and 45.43 ± 12.88 % from E1:4_SD), and the lowest at a ratio of

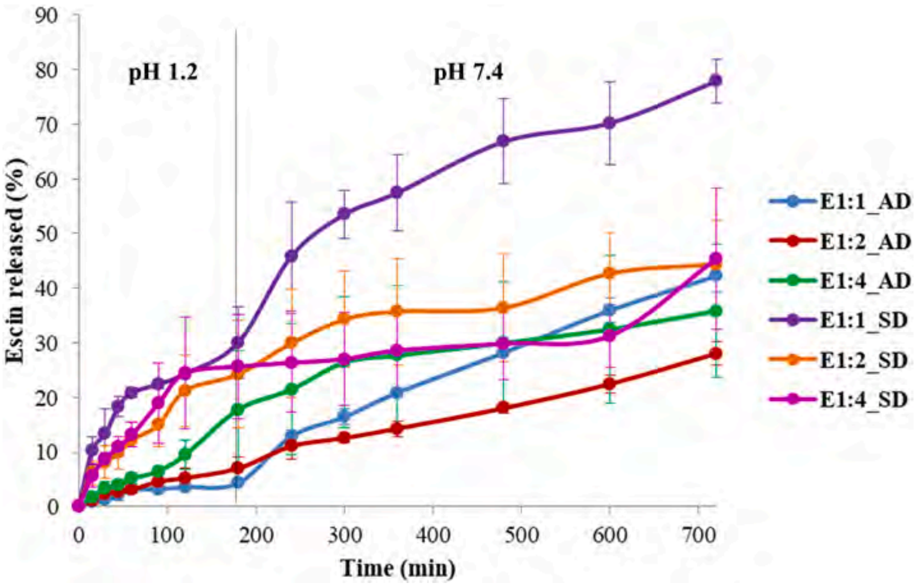

Fig. 6. *In vitro* dissolution profiles of escin from ambient-dried and spray-dried escin-loaded PECs.

1:2 (28.04 ± 2.13 % from E1:2_AD and 44.39 ± 7.94 % from E1:2_SD). It is known that escin can lead to the irritation of the gastric mucosa after oral administration, so the formulation of carriers that would provide its pH-dependent release in the lower parts of the GIT is desirable [1,2]. The pH-dependent profile of escin release was most pronounced for E1:1_AD from which 4.30 % of the drug was released at pH 1.2 after 3 h and the remaining 37.93 % at pH 7.4 over the next 9 h. Also, from E1:2_AD and E1:1_SD a lower amount of escin was released at pH 1.2, while from E1:4_AD approximately the same amount of the drug was released at both pH values. In contrast, E1:2_SD and E1:4_SD released greater amounts of escin dissolved at pH 1.2, which may be considered unfavorable for its oral administration. Therefore, it could be assumed that E1:1_AD was the most promising to achieve pH-dependent release of escin upon oral administration.

Table 7 shows the models of escin release kinetics with corresponding coefficients of determination (R^2) and *Korsmeyer-Peppas* diffusion exponents (n).

Based on the values of the coefficients of determination it can be seen that escin release followed *Korsmeyer-Peppas* kinetics in most cases. The exceptions were E1:2_SD and E1:4_SD from which escin release was best described by the *Higuchi* model. As shown in numerous studies, *Korsmeyer-Peppas* release kinetics is common for swellable drug carriers, such as carriers based on hydrophilic polymers [9,10,50]. Also, based on the n values for the *Korsmeyer-Peppas* model, mechanisms of drug release could be set up. Values of n close to 1 indicate controlled and constant release of the drug regardless of its content in the carrier [51]. So, the best release control of the entrapped escin was in the case of E1:1_AD and E1:2_AD. This is supported by high R^2 values for *zero-order* kinetics in these two samples. From E1:4_AD and E1:1_SD, escin release followed a non-Fickian diffusion mechanism, while Fickian diffusion was a characteristic of E1:2_SD and E1:4_SD.

Table 7
Models of escin release kinetics with corresponding coefficients of determination (R^2) and *Korsmeyer-Peppas* diffusion exponents (n).

Sample	R^2				n
	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
E1:1_AD	0.9783	0.9587	0.7684	**0.9868**	1.182
E1:2_AD	0.9902	0.9934	0.8799	**0.9944**	0.894
E1:4_AD	0.8674	0.9266	0.9261	**0.9493**	0.659
E1:1_SD	0.8055	0.9622	0.9758	**0.9778**	0.568
E1:2_SD	0.6665	0.8171	**0.9799**	0.9726	0.491
E1:4_SD	0.4376	0.5830	**0.9071**	0.8954	0.409

Calculated f_2 values (Table 8) showed that E1:1_AD and E1:2_AD, E1:1_AD and E1:4_AD, E1:2_AD and E1:4_AD, E1:4_AD and E1:2_SD, E1:4_AD and E1:4_SD can be considered similar, as well as E1:2_SD and E1:4_SD. Escin release profiles from all ambient-dried PECs can be considered similar. In these samples, the escin-to-polymers mass ratio had a low impact on drug release. On the contrary, the escin-to-polymers mass ratio had a more significant impact on drug release from spray-dried PECs. Also, it can be concluded that the drying process had a greater impact on the release of the entrapped escin than the escin-to-polymers mass ratio. Moreover, a greater influence of the drying method on the release of escin was noted compared to ibuprofen. Also, larger amounts of amphiphilic escin (42.23 ± 3.02 % from ambient-dried and 77.93 ± 3.98 % from spray-dried PEC) were released from CH/XG-based PECs compared to the hydrophobic ibuprofen (27.76 ± 0.65 % from ambient-dried and 30.55 ± 4.36 % from spray-dried PEC) (Ćirić et al., 2022), likely due to the higher solubility of escin in aqueous media.

4. Conclusions

The measurements of transmittance, conductivity, and rheological

Table 8
Similarity (f_2) factors for tested pairs of escin release profiles.

Profiles compared	f_2
E1:1_AD *vs.* E1:2_AD	**59.66**
E1:1_AD *vs.* E1:4_AD	**60.55**
E1:1_AD *vs.* E1:1_SD	20.60
E1:1_AD *vs.* E1:2_SD	45.96
E1:1_AD *vs.* E1:4_SD	47.26
E1:2_AD *vs.* E1:4_AD	**54.33**
E1:2_AD *vs.* E1:1_SD	25.08
E1:2_AD *vs.* E1:2_SD	41.13
E1:2_AD *vs.* E1:4_SD	44.45
E1:4_AD *vs.* E1:1_SD	31.01
E1:4_AD *vs.* E1:2_SD	**55.91**
E1:4_AD *vs.* E1:4_SD	**57.03**
E1:1_SD *vs.* E1:2_SD	38.04
E1:1_SD *vs.* E1:4_SD	34.09
E1:2_SD *vs.* E1:4_SD	**65.55**

characterization confirmed the formation of CH/XG-based PECs with escin entrapped at different escin-to-polymers mass ratios (1:1, 1:2, and 1:4). Two drying methods of PECs, ambient drying and spray drying, were compared. Higher yield, entrapment efficiency, and escin content were observed in ambient-dried PECs. Although spray drying gave smaller particles of a more regular shape, it was recognized the need for the optimization of the spray drying procedure to obtain PECs with desired characteristics. The flowability of all dry PECs was poor or very poor. FT-IR spectra confirmed the presence of characteristic interactions between complex components in dry PECs, with stronger interactions observed in ambient-dried ones. PXRD and DSC analyses indicated the amorphous escin structure in all dry PECs regardless of the drying method. An *in vitro* study of escin release showed its prolonged release during 12 h from all dried PECs. However, spray-dried PECs could not enable the aimed pH-dependent release of the entrapped drug with low amounts released at pH 1.2 and higher at pH 7.4. The best control of pH-dependent escin release was observed for E1:1_AD. For that reason and due to the highest yield and entrapment efficiency, E1:1_AD can be considered the most promising carrier with the potential to prevent the risk of irritation of gastric mucosa as a major side effect of orally administered escin.

CRediT authorship contribution statement

Ana Ćirić: Conceptualization, Methodology, Investigation, Writing - original draft, Visualization, Data curation. **Jelena Milinković Budinčić:** Methodology, Formal analysis, Writing - original draft. **Đorđe Medarević:** Methodology, Formal analysis, Writing - original draft. **Vladimir Dobričić:** Methodology, Formal analysis, Writing - original draft. **Milena Rmandić:** Methodology, Formal analysis, Writing - original draft. **Tanja Barudžija:** Methodology, Formal analysis, Writing - original draft. **Anđelija Malenović:** Resources. Writing - review & editing. **Lidija Petrović:** Resources. Writing - review & editing. **Ljiljana Djekic:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Study of chitosan/xanthan gum polyelectrolyte complexes formation, solid state and influence on ibuprofen release kinetics

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in vitro drug release

ABSTRACT

This study investigated the combined influence of pH adjusting agent type (hydrochloric, acetic or lactic acid) and initial pH value (3.6, 4.6, and 5.6) on formation of biocompatible chitosan/xanthan polyelectrolyte complexes (PECs), their characteristics in solid state and influence on *in vitro* ibuprofen release kinetics. Conductivity measurements and rheological characterization revealed generally higher extent of ionic interactions in PEC dispersions comprising acetic acid and at pH 3.6. Acid type and pH affected significantly the yield and particle size (100–250 μm) of the dried PECs. Differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FT-IR), and powder X-ray diffraction (PXRD) analysis of the solid PECs confirmed exclusively physical (ionic, hydrogen bonds) interactions between chitosan and xanthan gum. PECs prepared with acetic acid at pH 4.6 and 5.6 had enhanced rehydration ability in phosphate buffer pH 7.2, and at PEC-to-drug mass ratio up to 1:2, enabled extended ibuprofen release from hard capsules during 10 h.

1. Introduction

Many novel drug delivery systems are based on a combination of two or more polymers, covalently or non-covalently cross-linked [1–3]. Due to toxicity of cross-linkers often used in covalent cross-linking (such as glutaraldehyde, diglycidylether, diisocyanate, diacrylate), non-covalent cross-linking between biocompatible polymers is considered more acceptable [4–6]. Drug delivery systems based on biocompatible polyelectrolyte complexes (PECs) could be formed by mixing the dispersions of cationic polymers (*e.g.*, chitosan, poly-L-lysine, polyethyleneimine, polyamidoamine) [7–9] and anionic polymers (*e.g.*, xanthan gum, alginates, carrageenan, pectin, hyaluronic acid, gum kondagogu, gelatine, polyglutamic acid, polyacrylic acid, polymethacrylate copolymers) [10–15], when oppositely charged polymers establish mutual electrostatic (ionic) interactions. PECs formulated with chitosan and xanthan gum are considered particularly promising oral drug delivery carriers due to nontoxicity, nonimmunogenicity, biodegradability, low-cost production, and renewability of these natural polysaccharides [16–18]. Chitosan is a linear polysaccharide derived by the exhaustive chemical or enzymatic deacetylation of chitin from the exoskeleton of crustaceans and insects [19,20]. It consists of randomly distributed β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units. At acidic pH values, the primary amino groups of chitosan (pKa 6.2–7.0) can be protonated allowing increase in aqueous solubility of this polycationic polymer [20–23]. Therefore, chitosan itself is not suitable for formulation of extended release drug delivery systems due to its rapid dissolution in acidic environment of the stomach. Xanthan gum is a highly hydrophilic natural heteropolysaccharide produced by the bacteria *Xanthomonas campestris* [24]. The primary structure of this polymer consists of five repetitive sugar units: two β-D-glucose units in the basic chain, and two mannose units and one glucuronic acid in the side chain. The side chains contain pyruvic acid residues that make xanthan gum a natural anionic polyelectrolyte (pKa 3.1) [25]. Thus, xanthan gum is considered a suitable candidate for combining with chitosan and development of PECs drug carriers. PECs based on chitosan and xanthan gum already demonstrated improved drug solubility and/or permeability, pH-sensitivity, and controlled (extended or delayed) drug release [26–30]. The ionic interactions in PECs are considered strong, but reversible. Many formulation parameters and preparation conditions may affect degree of ionization, charge density and distribution, and flexibility of polymer chains, and thus strength and extent of ionic interactions and drug delivery potential of PECs. The formation of PECs and precipitation of PEC coacervates takes place at pH values near pKa values of the polymers [1]. The study of Goñi et al. [31] recognised the importance of pH and type of pH adjusting agent on PECs formation. However, studies

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investigating the PECs prepared with different acids and at different pH values have not been conducted systematically, particularly the combined effect of type of acid and pH value of chitosan solutions, on formation, solid state characteristics and drug release kinetics of the chitosan/xanthan gum PECs. In response to these knowledge gaps, the present study expands on the previous research, analysing the effect of mentioned formulation parameters on characteristics of the PECs, both during their formation (in aqueous solutions) and after drying, as well as their capacity for rehydration in a suitable *in vitro* drug release medium.

Ibuprofen ((2*RS*)-2-[4-(2-Methylpropyl) phenyl] propanoic acid) is a non-selective reversible inhibitor of the cyclooxygenase enzymes (COX-1 and COX-2), which are responsible for the production of prostaglandins (PGE_2 and PGI_2), thus providing analgesic and anti-inflammatory effects [32]. Due to its well-known efficiency and fewer side effects in comparison to other non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen is one of the most frequently used NSAID for the treatment of inflammation and pain related with rheumatoid arthritis, osteoarthritis, dysmenorrhea, trauma and surgery [32–34], and it is recommended by the World Health Organization for treating fever in children [35]. However, this drug is a weak acid (pKa 4.5–4.6) with low aqueous solubility and high permeability, so it is classified in the group 2 of the Biopharmaceutical Classification System (BCS) [36,37]. It has short half-life ($t_{1/2}$ ~2 h) and requires frequent oral administration of immediate release dosage forms which can lead to severe adverse effects such as gastrointestinal bleeding and ulceration [32–34]. Although few research groups have investigated the influence of chitosan-based PECs on biopharmaceutical properties of ibuprofen to improve its oral bioavailability [38] or to achieve colon-targeted drug delivery [39], in these studies as anionic components of PECs were used synthetic non-biodegradable polymers, such as methoxy poly(ethylene glycol) methacrylate-co-poly(methylacrylic acid) [38] and poly(ethyl acrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride) [39]. There are no PECs based on chitosan/xanthan gum for oral delivery of ibuprofen described in the literature. Such PECs can be investigated as potential biocompatible carriers for extended release of ibuprofen with the aim of reducing the frequency of administration.

The aim of this study was to investigate the influence of formulation factors (type of pH adjusting agent and pH value) on the formation of chitosan/xanthan PECs and their characteristics in aqueous dispersions as well as in solid state. Additional goal was to analyse the influence of the PECs on *in vitro* release kinetics of a model drug substance ibuprofen, in a solid dosage form (hard capsule) for oral administration.

2. Materials and methods

2.1. Materials

Medium molecular weight (190000–310,000 $gmol^{-1}$) chitosan (Sigma Aldrich, USA) with a degree of deacetylation of 75–85% and viscosity of 200–800 cps in concentration of 1% w/w in 1% w/w acetic acid, was used as the cationic component of PECs. Xanthan gum (Jungbunzlauer, Switzerland) was of pharmaceutical and food grade and used as the anionic component of PECs. The viscosity of the xanthan gum solution 1% w/w in 1% w/w KCl solution (Brookfield LVTD, spindle 4, 60 rpm at 25 °C) is 1300–1700 mPa·s. Ibuprofen ((2*RS*)-2-[4-(2-Methylpropyl)phenyl]propanoic acid) (BASF, Germany) was a gift by Galenika a.d. - Belgrade (Serbia). All other chemicals and reagents (hydrochloric acid (Sigma Aldrich, USA), acetic acid (Fisher Scientific, UK), lactic acid (2-hydroxypropanoic acid) (Fagron, Inc., USA), sodium hydroxide (Sigma Aldrich, USA), potassium dihydrogen phosphate (Centrohem, Serbia)) were of analytical grade. All substances were used as received without further purification. Purified water (Ph. Eur. 9.0) was used throughout the study.

2.2. Methods

2.2.1. Preparation of chitosan solutions

Nine solutions of chitosan at a concentration of 0.65% w/v in purified water were prepared under ambient conditions. The solutions differed in terms of the acid used as well as the pH value. The pH was adjusted up to 3.6, 4.6, or 5.6, by addition of hydrochloric acid (0.2 M), acetic acid (1.5% v/v), or lactic acid solution (1% v/v). The final pH adjustment of the solutions was achieved by adding 0.2 M sodium hydroxide solution where necessary. The pH value was checked by the pH meter HI 9321 (Hanna Instruments, USA). Prior to the measurements of the pH value, the apparatus was calibrated with the standard buffer solutions of pH 4.0 and 7.0. During the preparation, the chitosan solutions were mixed on the mechanical stirrer (laboratory propeller mixer Heidolph RZR 2020, Heidolph, Germany) for 24 h at 500 rpm, until homogeneous solutions were obtained. The prepared chitosan solutions were stored at 5 ± 3 °C (in a refrigerator) for 24 h prior to the preparation of PECs, in order to achieve complete hydration of the polymer.

2.2.2. Preparation of xanthan gum solution

The xanthan gum solution was prepared at a concentration of 0.65% w/v by dissolving the polymer in purified water under ambient conditions. The dissolution process was carried out on the laboratory propeller mixer Heidolph RZR 2020 (Heidolph, Germany) at 500 rpm, until the homogeneous solution was obtained. After preparation, the xanthan gum solution was stored at 5 ± 3 °C (in a refrigerator) for 24 h, to allow hydration of the polymer, before the preparation of PECs.

2.2.3. Preparation of PEC dispersions

Nine PEC dispersions were prepared under ambient conditions by combining the corresponding nine chitosan solutions with the xanthan gum solution. Each chitosan solution was mixed with the xanthan gum solution at a volume ratio 1:1 on the mechanical stirrer (propeller mixer Heidolph RZR 2020, Heidolph, Germany) for 24 h at 800 rpm. In 200 ml of the chitosan solution, 200 ml of the xanthan solution was added in portions of 20 ml. The prepared PEC dispersions were named in accordance with the acid and the initial pH value of the chitosan solution used as: H3.6, H4.6, and H5.6 (for the PECs prepared with hydrochloric acid); A3.6, A4.6, and A5.6 (for the PECs prepared with acetic acid); L3.6, L4.6, and L5.6 (for the PECs prepared with lactic acid). The prepared PEC dispersions were stand for 24 h at 5 ± 3 °C (in a refrigerator) prior further characterization.

2.2.4. Characterization of PEC dispersions

In order to confirm the formation of PECs based on ionic interactions between the polymers, conductivity was monitored to characterize the charge variations during the mixing of the polymer solutions. Also, pH of the obtained PEC dispersions was checked, and their rheological behaviour was characterized.

2.2.4.1. Conductivity monitoring during PECs formation. Initial conductivity of the chitosan solutions was measured using the conductivity meter CDM 230 (Radiometer, Denmark), under ambient conditions. Also, after each added amount of 20 ml of the xanthan gum solution to 200 ml of each chitosan solution, and 5 min of mixing, conductivity of the dispersion was measured. The conductivity meter was calibrated before measurements using 0.01 M potassium chloride solution.

2.2.4.2. pH measurement of PEC dispersions. For each PEC dispersion, after the addition of the total amount of xanthan gum solution to the chitosan solution, pH was measured using pH meter HI 9321 (Hanna Instruments, USA), previously calibrated with the standard buffer solutions of pH 4.0 and 7.0.

2.2.4.3. Rheological characterization of PEC dispersions. Prior the rheological characterization, the prepared PEC dispersions were washed with purified water. The excess liquid was removed by filtration and PECs with a consistency of semisolid hydrogels were obtained. Measurements were carried out on a rotational and oscillatory rheometer Rheolab MC 120 (PaarPhysica, Austria) coupled with the rotating cylinder measuring device Z3 DIN (25 mm) at 20 ± 0.1 °C. The controlled shear rate (CSR) procedure was applied for flow curve construction by increasing the shear rate from 0 to 100 s^{-1} and back to 0 s^{-1}, with each stage lasting for 400 s. In order to elucidate the interactions between the polymers in the PEC dispersions, for comparison, the rheological characterization was also performed under the same conditions in the solutions of the individual polymers used for PECs preparation.

2.2.5. Drying of PEC dispersions

Thin layers of the semisolid PECs were poured into Petri dishes and dried under ambient conditions (at 20 ± 3 °C) until the mass of the dry films was constant. The dry films were grinded and the resulting powders passed through the sieve with a mesh size of 355 μm (Ph. Eur. 9.0) to remove large pieces of the grinded film from the particles. The dried grinded PEC samples were stored in the desiccator and used for solid state characterization.

2.2.6. Characterization of dried PECs

Characterization of the dried (solid state) PECs included: determination of yield and moisture content, light microscopy, scanning electron microscopy (SEM), differential scanning calorimetry (DSC) analysis, Fourier-transform infrared spectroscopy (FT-IR) analysis, and powder X-ray diffraction (PXRD) analysis. In order to elucidate the interactions between the polymers in the dried PECs and compare with the pure polymers, SEM, DSC, FT-IR, and PXRD analysis were performed also for pure chitosan, xanthan gum, and their physical mixture. Moreover, rheological behaviour of the PEC dispersions after rehydration, was investigated.

2.2.6.1. Yield of dried PECs. After preparation, drying, grinding and sieving, the mass of dried PECs was measured on AE 240 single pan balance (Sartorius, Germany) and the yield was calculated as shown in the Eq. (1):

$$\begin{aligned}\text{Yield }(\%) = {} & \text{weight of dried PEC (g)} \\ & /[\text{weight of chitosan (g)} + \text{weight of xanthan gum (g)}] \\ & \cdot 100\end{aligned} \tag{1}$$

The total weight of chitosan and xanthan gum used for the preparation of PECs was defined as 100%.

2.2.6.2. Moisture content in dried PECs. The residual moisture content in dried PECs was determined gravimetrically using the LJ16 halogen moisture analyser (Mettler Toledo, Switzerland) by heating the samples up to 105 °C until constant mass was reached.

2.2.6.3. Light microscopy. The dispersions of the grinded and sieved dried PECs in silicone oil were examined by the Olympus BX51P polarizing microscope (Olympus, Japan) with cellSens Entry software. In accordance with the irregular shape of the plate particles, their maximum length is considered.

2.2.6.4. SEM. A thin conductive layer of gold was applied to the dried PEC samples using SCD 005 Cool Sputter Coater (Bal-Tec, Germany) and photomicrographs were recorded in high vacuum at 5 kV using a scanning electron microscope JSM-6390LV (JEOL, Japan).

2.2.6.5. DSC analysis. The DSC analysis was carried out using the DSC 1 instrument (Mettler Toledo, Switzerland). Accurately weighted (5–10 mg) samples were crimped in a standard 40 μl aluminium pan and heated from 25 °C to 360 °C at a heating rate of 10 °C/min, under a constant nitrogen flow rate of 50 ml/min. The empty sealed pan was used as a reference.

2.2.6.6. FT-IR analysis. The attenuated total reflectance (ATR) FT-IR spectra were recorded using a Nicolet iS10 FT-IR Spectrometer (Thermo Fisher Scientific, USA) in the wavelength range between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.2.6.7. PXRD analysis. PXRD analysis was carried out on Bruker D8 advance diffractometer (Bruker, Karlsruhe, Germany) in Bragg–Brentano θ–2θ geometry, using Ge-crystal primary monochromator (Johanson type) that generates CuKα1 radiation ($\lambda = 1.541$ Å). Diffraction data were recorded in the 2θ range from 5 to 45° with a scan step of 0.05° and holding time of 12 s.

2.2.6.8. Rheological characterization of dried PECs after rehydration. Samples for rheological measurements were prepared by dispersing the dried PECs in phosphate buffer pH 7.2 (USP) and at a concentration of 2% w/v which corresponded with the concentration of PECs in the dispersions before drying. Dispersion process was carried out on a Shaking Water Bath LSB18 Aqua Pro (Grant, UK) for 10 h at 37 ± 1 °C and agitation rate of 100 rpm. Continuous rheological characterization was performed at 37 ± 0.1 °C on a rotational and oscillatory rheometer, Rheolab MC 120 (Paar Physica, Austria), coupled with the rotating cylinder measuring device Z3 DIN (25 mm). The CSR procedure was performed by increasing a shear rate from 0 to 100 s^{-1} and back to 0 s^{-1} (each stage lasting for 400 s).

2.2.7. In vitro ibuprofen release testing

To evaluate the influence of the PECs as well as the PEC-to-drug mass ratio on ibuprofen release kinetics, physical mixtures of the dried PECs with ibuprofen were prepared (without solvent) at two mass ratios (1:1 and 1:2). Additionally, for comparison, physical mixtures of the pure polymers (chitosan or xanthan gum) or chitosan+xanthan gum physical mixture (at a mass ratio 1:1), with ibuprofen, were prepared at two mass ratios (1:1 and 1:2) and investigated under the same conditions. The mixtures were filled into hard gelatine capsules size 1 (Capsugel®, USA) in an amount comprising 100 mg of ibuprofen, which corresponds to the lowest strength of this drug in commercially available solid dosage forms for the oral administration as well as the usual single therapeutic dose for paediatric administration [40]. The *in vitro* release of ibuprofen was investigated in the rotating paddle apparatus, Erweka DT70 (Erweka, Germany), at 50 rpm, using 900 ml of the compendial medium for ibuprofen solid dosage forms (phosphate buffer pH 7.2 (USP)), at 37 ± 1 °C. The small volumes (roughly 4 ml) of the acceptor medium were sampled during the period of 10 h at fixed time intervals (15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540 and 600 min), and immediately replaced by equal amount of fresh buffer solution. The concentration of ibuprofen in the collected samples was determined on Evolution 300 spectrophotometer (Thermo Scientific, USA) at the wavelength of maximum absorption of the drug (221 nm). The test was performed in triplicate for each sample and the results are shown as the mean of the released amount of the drug substance ± standard deviation (S.D.). Mathematical models applied to the obtained data were *zero order*, *first order*, *Higuchi* and *Korsmeyer-Peppas* kinetics are shown by the Eqs. (2)–(5):

Model	Equation	
Zero order kinetics	$Q = k_0 \cdot t$	(2)
First order kinetics	$\ln M = \ln M_0 - k_1 \cdot t$	(3)
Higuchi kinetics	$Q = k_H \cdot t^{1/2}$	(4)
Korsmeyer-Peppas kinetics	$Q = k \cdot t^n$	(5)

where M is non-dissolved amount of the drug after time t, M_0 non dissolved amount of the drug at the beginning of the dissolution process,

Q is the amount of the drug dissolved after time t, n is diffusion exponent indicative of the mechanism of transport of drug, and k_0, k_1, k_H and k are release rate constants for *zero order*, *first order*, *Higuchi* and *Korsmeyer-Peppas* kinetic models, respectively.

Fitting the data into these mathematical models was performed by using the software DDSolver developed by Zhang et al. [41].

Additionally, mutual comparison of the drug release profiles was carried out by model-independent approach. Similarity (f_2) and difference (f_1) factors were used for the comparison of profiles (Eqs. (6) and (7)) [42]:

$$f_1 = \frac{\sum_{t=1}^{n} \mid R_t - T_t \mid}{\sum_{t=1}^{n} R_t} \cdot 100 \tag{6}$$

$$f_2 = 50\ \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0,5} \cdot 100\right\} \tag{7}$$

where n represents number of samples, R_t is released amount of the drug (%) in time point t (reference/sample 1), and T_t is released amount of the drug (%) in time point t (test/sample 2).

The investigated release profiles can be considered similar if $50 < f_2 < 100$ and $0 < f_1 < 15$ [42].

3. Results and discussion

The prepared PECs were characterized during their formation as well as in solid state. Moreover, the solid PECs were combined with ibuprofen and filled into the hard capsules for *in vitro* drug release evaluation.

3.1. Formation of the PECs in the mixtures of the solutions of chitosan and xanthan gum

The appearance of the solutions of chitosan and xanthan gum was checked just before they were used for preparation of the PEC dispersions. All solutions were homogeneous opalescent viscous liquids. It was observed that acetic acid as a pH adjusting agent produced less opalescent chitosan solutions compared with those prepared with hydrochloric acid, due to higher solubility of this polymer in solutions of organic acids than in inorganic acids [43]. Solutions prepared with lactic acid were also more opalescent than those prepared with acetic acid, but less opalescent compared to chitosan solutions prepared with hydrochloric acid. Moreover, the appearance of foam on the surface of the chitosan solutions prepared with lactic acid was noted, which did not disappear even 24 h after the completion of the mixing. For all acids, the chitosan solutions at pH 3.6 were less opalescent than at pH 4.6 and 5.6. This observation was related with the decrease of solubility of chitosan with the increase of pH value and thus increase in ionic strength of the dispersing media, as also reported by Singla and Chawla [44].

The prepared PECs were opalescent semisolid hydrogels. In case of lactic acid, microbiological contamination with visible fungal growth in the PEC hydrogels took place within 48 h, even though they were kept in the refrigerator. For that reason lactic acid was not considered suitable for pH adjustment of chitosan dispersions and the corresponding PECs (L3.6, L4.6, and L5.6) were excluded from the study.

The obtained curves of conductivity as a function of the volume of the xanthan gum solution added during formation of the PECs containing hydrochloric acid and acetic acid are presented in Fig. 1a.

The initial conductivity values of the starting chitosan solutions were first compared. For solutions prepared with hydrochloric acid, the initial conductivity was observed to decrease with increasing pH. The highest initial value of conductivity in chitosan solution at pH 3.6 could be explained by hydrochloric acid's complete dissociation to hydrogen and chloride ions in aqueous solution. That resulted in a higher amount of free charge originating from both ions. For two chitosan solutions with higher pH (4.6 and 5.6), probably the larger amount of hydrogen ions protonated the amino groups of chitosan, while free chloride ions were predominantly responsible for the conductivity, thus the number of free charge carriers was decreased. Very recently, Wu and Zhang [45] came up with similar conclusions for conductivity of chitosan solutions in hydrochloric acid. In case of the chitosan solutions comprising acetic acid, the highest initial conductivity was at pH 4.6, while it was significantly lower at pH 3.6 and the lowest at pH 5.6. In this case, the low conductivity at pH 3.6 could be explained by suppression of dissociation of weak acetic acid by increasing its concentration [46], thus decreasing the amount of hydrogen and acetate ions as charge carriers.

In all cases the continuous reduction in conductivity, along with the increase in pH, was observed during the addition of the xanthan gum solution into the chitosan solutions. In PECs prepared with hydrochloric acid the conductivity value decreased from 1.991 mS/cm (before the addition of the xanthan gum solution) to 1.053 mS/cm (after the addition of the complete amount of the xanthan gum dispersion) for H3.6. Also, the conductivity decreased from 1.604 mS/cm to 0.937 mS/cm for H4.6, while the minimal effect upon the addition of xanthan gum solution on the conductivity decrease was observed for H5.6 (from 0.699 mS/cm to 0.662 mS/cm). In PECs prepared with acetic acid, the decrease in conductivity was in a relatively narrow range from 1.456 mS/cm to 1.169 mS/cm for A3.6, and from 1.412 mS/cm to 0.936 mS/cm for A5.6. The highest initial conductivity value and decrease (from 2.227 mS/cm to 1.462 mS/cm) were observed for A4.6. The conductivity measurements

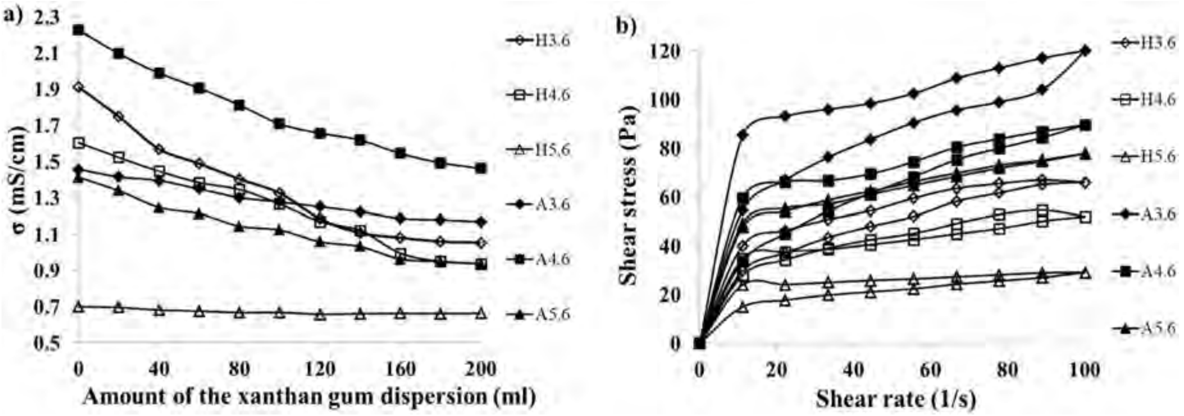

Fig. 1. Characteristics of PEC dispersions containing hydrochloric acid or acetic acid: a) conductivity of the PEC dispersions as a function of the volume of the xanthan gum solution added into the chitosan solutions; b) hysteresis loops of the PEC dispersions (the standard deviation values were <10% of the mean values and the corresponding deviation bars were omitted for clarity).

were carried out without the addition of salts (counter-ions), thus the decrease in the conductivity values during the addition of xanthan gum solution in the chitosan solution could be explained by reduction in the amount of free charge due to the establishment of ion interactions between the polymers, that was also reported by Acar and Tulun [47], Dautzenberg and Karibyants [48] and Tennouga et al. [49]. Also, as suggested by De Robertis et al. [2], the first step in PECs formation is the establishment of Coulomb forces between oppositely charged polymers, the second step is the formation of new connections to achieve appropriate spatial conformation of the complex, while the third step involves the stabilization of the resulting PEC, primarily by the establishment of hydrophobic interactions between polymer chains.

For all PEC dispersions, the final pH value was increased relative to the initial values in the corresponding chitosan solution (Table 1).

An increase in the pH value after the addition of the entire amount of xanthan gum solution was expected since the pH of the xanthan gum solution was 6.09. In addition, an increase in total volume resulted in decrease in the concentration of hydrogen ions.

The pH increase during the PECs formation was more noticeable when hydrochloric acid was used as pH adjusting agent, likely because of the lower buffer capacity of the investigated dispersions in comparison with those prepared with acetic acid. For PEC formation, both polymers, chitosan and xanthan gum, should be ionized. As already demonstrated, the pH value of the reaction medium should be between the pKa values of both polymers (*i.e.*, between 3.1 and 6.5) [21,25]. The final pH values in all PEC dispersions were in the range from 3.78–5.90 (Table 1), so this request for PECs formation was fulfilled in all cases.

In order to elucidate the strength and extent of interactions in the prepared PECs, rheological characterization was performed. Upward and downward flow curves of the PEC dispersions are shown in Fig. 1b. The hysteresis loop between the flow curves for the investigated PECs showed pseudo-plastic flow behaviour with thixotropy. Thixotropy was estimated by calculating the hysteresis area. The values of maximal apparent viscosity (η_{max}), minimal apparent viscosity (η_{min}) and hysteresis area (H) for the PEC dispersions are shown in Table 2.

Additionally, rheological behaviour of the individual polymer solutions was characterized. The xanthan gum solution showed pseudo-plastic flow behaviour with η_{max} value of 0.449 Pa·s and η_{min} value of 0.13 Pa·s. All chitosan solutions have also showed pseudo-plastic behaviour with thixotropy with η_{max} values from 0.0459 Pa·s (for chitosan solution prepared with acetic acid at pH 3.6) to 0.0629 Pa·s (for chitosan solution prepared with hydrochloric acid at pH 5.6) and η_{min} values from 0.0124 Pa·s (for chitosan solution prepared with acetic acid at pH 3.6) to 0.0354 Pa·s (for chitosan solution prepared with hydrochloric acid at pH 5.6). The chitosan solution prepared with the strong hydrochloric acid had also higher values of hysteresis area (23.91–982.82 Pa/s) compared to those prepared with weak acetic acid (10.43–161.45 Pa/s). This result was in accordance with previous observation that chitosan dissolves better in acetic acid compared to hydrochloric acid solutions. Due to the higher solubility of chitosan in acetic acid, the extent of interactions between the chains of this polymer itself and the hysteresis area were lower than in the solutions with hydrochloric acid. This could indicate that chitosan in acetic acid solution were probably more accessible to interactions with xanthan gum than chitosan in hydrochloric acid solutions.

It has been observed that all PEC dispersions had higher values of apparent viscosities in comparison to the solutions of individual polymers. Also, the thixotropy of the PEC dispersions was significantly higher in comparison to individual polymers solutions. These results clearly confirmed the establishment of interactions between the polymers in PECs. Higher apparent viscosity and hysteresis area values of PECs correspond with higher strength of ionic interactions between the polymers and more structured systems. As the shear stress increases, the non-covalent polymer interactions break progressively, while the polymer chains remain intact. As shown by pronounced hysteresis, the bonds between the polymers can re-establish, leading to the neglectable permanent deformation [28,50–52].

Table 1
Values of pH of the PECs dispersions prepared with hydrochloric acid and acetic acid.

PEC dispersions containing hydrochloric acid	pH	PEC dispersions containing acetic acid	pH
H3.6	4.54	A3.6	3.78
H4.6	5.05	A4.6	4.76
H5.6	5.90	A5.6	5.70

Table 2
Maximal apparent viscosity (η_{max} at 22.2 s^{-1}), minimal apparent viscosity (η_{min} at 100 s^{-1}) and hysteresis area (H) for the PEC dispersions prepared with hydrochloric and acetic acid.

PEC dispersion	η_{max} (Pa·s)	η_{min} (Pa·s)	H (Pa/s)
H3.6	2.08 ± 0.04	0.660 ± 0.021	562.39
H4.6	1.69 ± 0.02	0.519 ± 0.036	117.78
H5.6	1.09 ± 0.01	0.291 ± 0.025	412.88
A3.6	4.04 ± 0.03	1.200 ± 0.043	1464.86
A4.6	2.98 ± 0.03	0.896 ± 0.037	951.42
A5.6	2.40 ± 0.05	0.778 ± 0.020	78.42

The results in Table 2 showed that the PECs prepared with acetic acid had higher apparent viscosities than ones prepared with hydrochloric acid. That indicated the higher interaction strength in PEC dispersions with acetic acid, which was expected from the results of the rheological characterization of chitosan solutions.

For both acids was observed the decrease of apparent viscosities values of the PECs, with increase of pH (Table 2). That indicated that in both cases there was a decrease in the strength of the interactions between chitosan and xanthan gum, with increasing pH. Hysteresis area values for the PECs prepared with acetic acid ranged from 78.42 Pa/s to 1464.86 Pa/s (Table 2). Lower variations of the hysteresis area values between 117.78 Pa/s and 562.39 Pa/s were observed for the PEC dispersions prepared with hydrochloric acid. Therefore, the obtained results clearly indicated that the strength and extent of interactions in the investigated PECs were significantly influenced by pH and acid selection. The highest interaction extent between chitosan and xanthan gum in the investigated PEC dispersions was noticed in A3.6, and the lowest in H5.6.

3.2. Characteristics of the PECs in solid state

When the PEC semisolid hydrogels were dried, the transparent xerogel films were obtained. Precipitation of chitosan was observed in H5.6, while this phenomenon was not noted in other PECs comprising hydrochloric acid as well as in the PECs prepared with acetic acid (Fig. A.1). The results of conductometric and rheological characterization indicated the weakest interactions between chitosan and xanthan gum in this sample. Also, the final pH value of H5.6 dispersion, after the addition of the xanthan gum solution, was 5.90. This value was near the pKa value of chitosan, so the cationic charge of chitosan probably decreased, which resulted in its precipitation during drying process.

The yield values and residual moisture content of the dried PECs are shown in Table 3.

Table 3
Yield values and residual moisture content of the dried PECs.

PEC	Yield (%)	Moisture content (%)
H3.6	23.26 ± 3.32	10.43
H4.6	22.10 ± 4.84	10.14
H5.6	39.75 ± 6.07	10.08
A3.6	37.44 ± 3.21	10.50
A4.6	33.19 ± 2.76	10.97
A5.6	34.62 ± 2.94	10.76

The yield of PECs prepared with acetic acid was 33.19–37.44%. The yield of H3.6 and H4.6 was significantly lower. The yield of H5.6 was the highest, however, the precipitation of chitosan in this PEC was noticed, which increased the results relative to the actual value of the yield. Therefore, the yield in the presence of acetic acid may be considered higher than when hydrochloric acid is used as the pH adjusting agent, again in accordance with the higher extent of interactions between chitosan and xanthan gum in the presence of acetic acid observed during the formation of the PECs. For the same reason, the pH had a lower impact on the yield of the samples with acetic acid. The moisture content of roughly 10% was observed in all PECs. It was ascribed to the passive drying process under ambient conditions as well as generally high hygroscopicity of PECs [53–55]. However, moisture content in the formulated PECs was significantly lower than near 20% in PECs dried at 38 °C for 18 h reported by De Morais Lima et al. [56]. The lower moisture content could be important for better microbiological stability of the dried PECs.

The films were grinded into powders and sieved through a sieve of mesh size 355 μm to remove coarse particles. In Fig. 2 are shown micrographs of the investigated grinded samples by using the light polarizing microscope. The largest particle length was 326 μm for A3.6, 329 μm for A4.6 (Fig. A.2), and 172 μm for A5.6 (Fig. A.3). The largest particle length of H3.6 was 355 μm, of H4.6 219 μm, and of H5.6 125 μm. The observed maximum particle length differences could be related to the different resistance among the PECs to grinding. Different resistance to grinding could be the consequence of differences in strength and extent of ionic interactions between the polymers, as demonstrated during the rheological characterization of the PEC dispersions. The higher extent

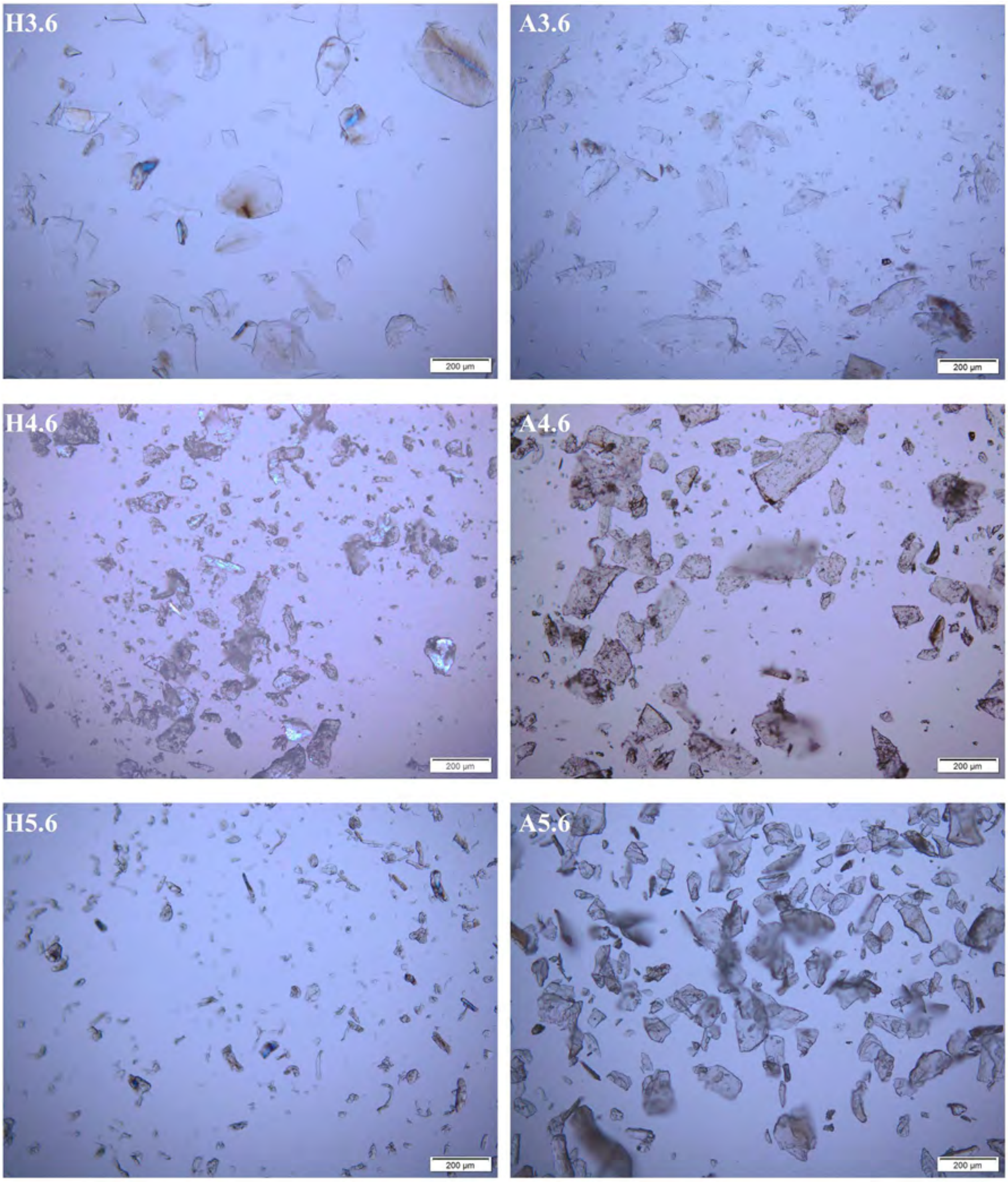

Fig. 2. Light microscopy micrographs of the dried PECs.

of interactions in PECs A3.6, A4.6, and H3.6 most likely provided greater cohesiveness after drying and higher resistance during grinding, thus these samples contained even the largest particles that could pass through the sieve 355. In PECs A5.6 and H4.6, a lower extent of interactions between the polymers was observed, which probably resulted in lower cohesiveness after drying, and the lower maximum particle length was obtained during grinding. In H5.6, where the least cohesiveness was expected, the smallest maximum particle length (up to 125 μm) was obtained after grinding.

Fig. 3 shows SEM photomicrographs of chitosan, xanthan gum, their physical mixture and the dried PECs.

The obtained photomicrographs showed flaky shape of chitosan particles and oval shape of xanthan particles, which can also be visualised on the photomicrograph of their physical mixture. On the other hand, all PECs had plate shape particles with the compact nonporous structure comprising collapsed planar sheets (Fig. 3). The observed structure of the PECs can be explained by the "plane by plane" superficial water evaporation and the collapse of the PECs during the drying process, as recently was suggested by Conzatti et al. [57] for alginate/chitosan PECs that were dried with hot air at 50 °C.

The DSC thermograms of chitosan, xanthan gum, their physical mixture and PECs are shown in Fig. 4a.

Scans of both polymers showed a broad endothermal peak with the maximum at 105.97 °C for chitosan and 112.36 °C for xanthan gum. Also, the polymers showed a broad exothermal peak with the maximum at 302.56 °C (chitosan) and 283.86 °C (xanthan gum). As expected, the physical mixture of the polymers showed also a broad endothermal peak (with the maximum at 109.09 °C) and a broad exothermal peak (with the maximum at 301.22 °C). The PECs prepared with hydrochloric acid showed the endothermal peak with the maximum at 112.16 °C (H3.6), 116.41 °C (H4.6), and 112.83 °C (H5.6), and the exothermal peak with the maximum at 284.77 °C (H3.6), 284.79 °C (H4.6), and 284.5 °C (H5.6). The PECs prepared with acetic acid showed the endothermal peak with the maximum at 111.17 °C (A3.6), 109.83 °C (A4.6) and 110.17 °C (A5.6), and the exothermal peak with the maximum at 285.85 °C (A3.6), 281.17 °C (A4.6 and A5.6). All endothermal peaks of the PECs were related to residual moisture evaporation. Therefore, the DSC analysis coincides with the results of the moisture content measurement. The exothermal peaks were related to degradation of polymers, their physical mixture as well as PECs. Noncrystalline structure of all investigated samples (PECs, chitosan, xanthan and their physical mixture) was also confirmed, due to the absence of sharp peaks (characteristic for crystalline materials) in the thermograms. Glass transition temperatures of all investigated samples found in literature were in the range from 100 to 120 °C [27]. We assume that glass transition temperatures of chitosan, xanthan gum, their physical mixture, and PECs, were covered by peaks corresponding to water evaporation. No additional peaks were observed in the thermograms of the investigated PECs, different from the pure polymers and their physical mixture, thus the DSC analysis confirmed the formation of

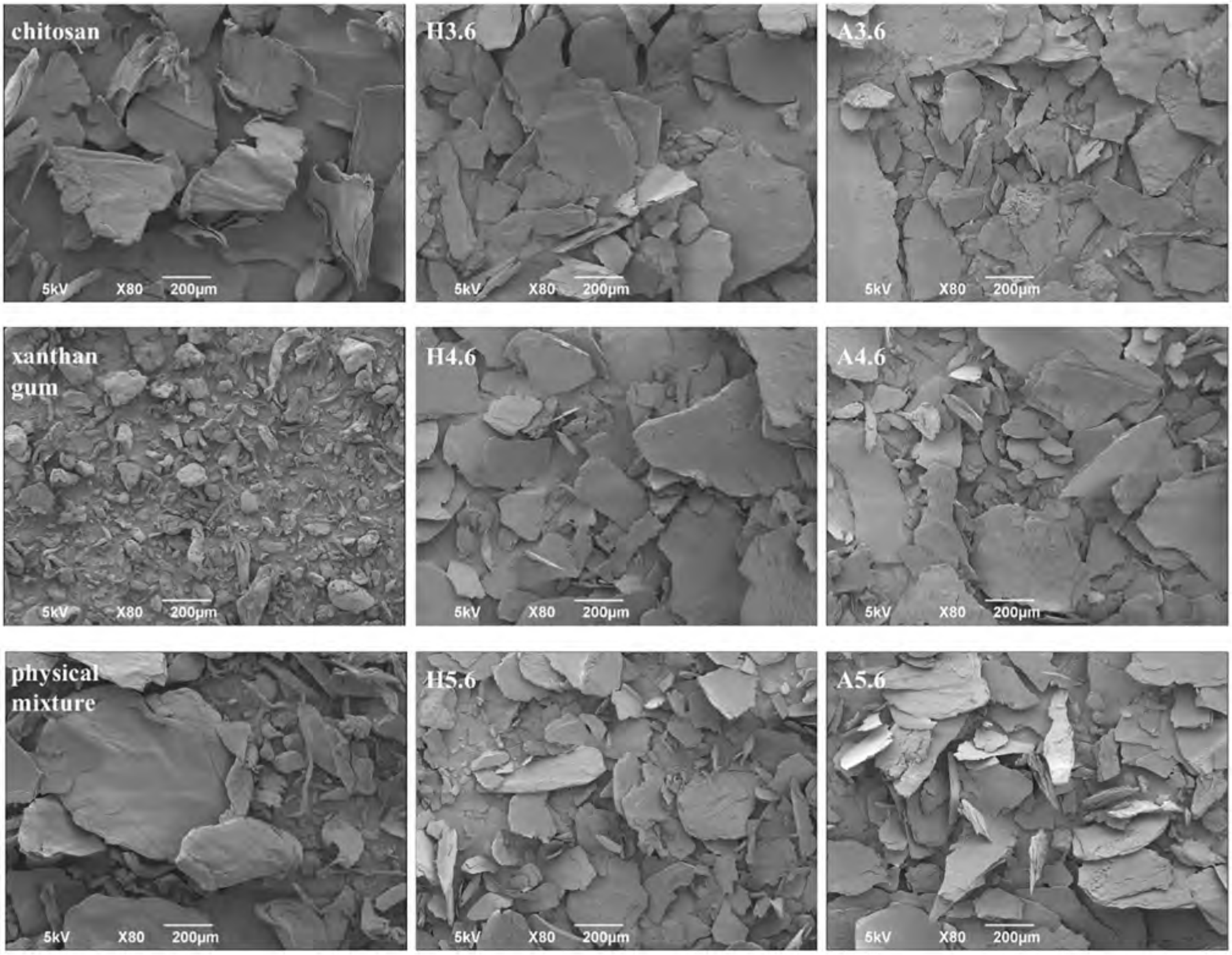

Fig. 3. SEM photomicrographs of chitosan, xanthan gum, chitosan/xanthan physical mixture and the dried PECs.

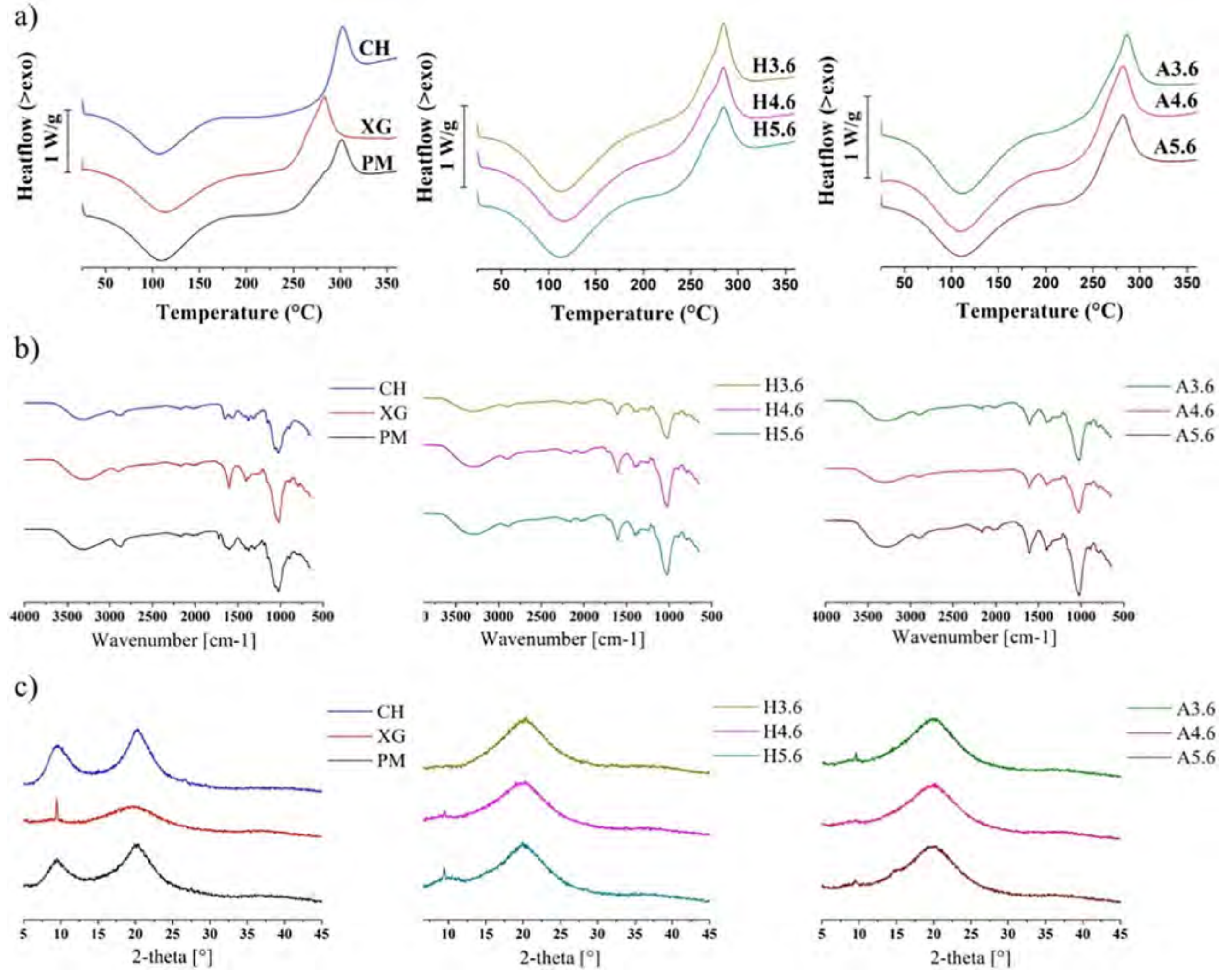

Fig. 4. Characterization of chitosan, xanthan gum, chitosan/xanthan physical mixture and the PECs in solid state: a) DSC thermograms, b) FT-IR spectra, and c) PXRD spectra.

PECs for both acids and the three pH values based exclusively on physical (ionic, hydrogen bonds) interactions between chitosan and xanthan.

Fig. 4b shows FT-IR spectra of the investigated dried PECs as well as chitosan and xanthan gum powders and the physical mixture chitosan/xanthan gum at mass ratio 1:1. Obtained FT-IR spectra of the dried PECs, chitosan, xanthan gum, and their physical mixture showed absorption bands around 3300 cm^{-1}, typical for –O-H stretching vibrations, and around 2900 cm^{-1}, which is common for –C-H stretching vibrations. The spectra of the dried PECs showed the absence of absorption bands characteristic for amines in the structure of chitosan: 1652.23 cm^{-1} for –C=O stretching vibrations (amide I), 1564.43 cm^{-1} for –N-H bending vibration (amide II), 1374.98 cm^{-1} and 1312.84 cm^{-1} for amide III and minor shifting of absorption bands of carboxylic groups in the structure of xanthan: 1604.26 cm^{-1} (asymmetrical vibrations of carboxylic group) and 1402.16 cm^{-1} (symmetrical vibrations of carboxylic group), that could be the consequence of ionic interactions between the polymers. The absence of new absorption bands, different from the spectra of the individual polymers, confirmed that no chemical reactions occurred between the polymers *i.e.*, only physical interactions were established in the investigated PECs. It was considered that the intensity of peaks could be correlated to ionic interactions intensity. Lower peak intensity (higher transmittance value) was detected when a higher percentage of characteristic functional groups of chitosan and xanthan gum interacted with each other (functional groups were not free to absorb the IR signal, but the established interactions led to a decrease in absorbance, *i.e.*, an increase in the transmittance value), as also previously reported by Dehghan and Kazi [27] and Popa et al. [29]. Therefore, the highest degree of interactions was observed between chitosan and xanthan in dry PECs A3.6, A4.6, and H3.6.

Fig. 4c shows the PXRD spectra of the investigated dried PECs, chitosan and xanthan gum powders and the chitosan/xanthan physical mixture in polymer mass ratio 1:1. The diffraction pattern of chitosan showed two broad peaks at $2\theta = 9.57°$ and 20.33° indicating its amorphous structure. In the diffraction spectra of xanthan gum one sharp peak at $2\theta = 9.48°$ and one broad peak at 19.83° demonstrated the semi-crystalline structure of xanthan gum. The diffraction patterns of the pure polymers coincided well with the results of Chen et al. [58], Lankalapalli and Kolapalli [59] and Minkal et al. [60]. The diffraction pattern of the physical mixture showed the spectra similar with chitosan, probably due to the overlapping of the sharp xanthan gum peak with the wide peak that originates from chitosan at similar 2θ values. X-ray diffraction spectra of PECs indicated semi-crystalline structure (for A3.6, A5.6, H4.6, and H5.6) with a sharp peak around 9.5° and a broad peak around 20°, or amorphous structure (for A4.6 and H3.6), with one broad peak around 20°. The lower intensity of sharp peaks or their absence in the PECs compared to the xanthan gum diffractogram, indicated the ionic interaction formation between protonated amino and deprotonated carboxyl group. The obtained results confirmed the formation of PECs with different degrees of crystallinity depending on the choice of pH value and pH adjusting agent used for their preparation.

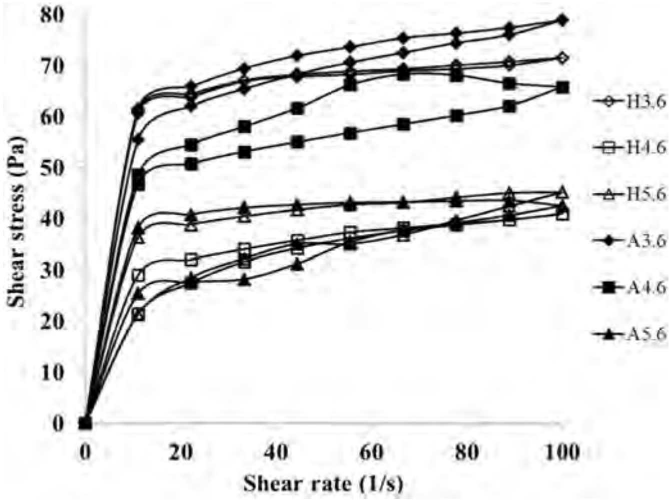

Fig. 5. Hysteresis loops of the rehydrated PECs in phosphate buffer pH 7.2 (the standard deviation values were <10% of the mean values and the corresponding deviation bars were omitted for clarity).

The dried PECs were completely rehydrated in phosphate buffer pH 7.2 after 10 h. Hysteresis loops for the investigated rehydrated PECs are shown in Fig. 5. Table 4 shows the values for η_{min}, η_{max} and H.

The hydrogels obtained by rehydration of the dried PECs were also pseudo-plastic non-Newtonian systems with thixotropy. Changes in the pH of the dispersions could potentially influence the establishment and strength of ionic interactions between chitosan and xanthan gum. In weak alkali (phosphate buffer), carboxyl groups of xanthan gum are completely deprotonated and negatively charged. Negative charge of xanthan gum and the repulsion of polymer chains resulted in high amount of water bounded, while chitosan was partially positively charged and it was expected that >50% of amino groups are protonated at pH values below 6.3. Therefore, chitosan chains are available for enhanced interactions with surrounding xanthan gum chains by ionic and/or hydrogen bonds [26]. When PECs rehydrated in phosphate buffer, the apparent viscosity was also higher for PECs comprising acetic acid as well as at lower pH. However, the opposite impact of pH on thixotropy has been noticed (Table 4) (*i.e.*, the H values were higher as pH of the PECs increased). With both acids the increase in swelling ability of PECs has been noticed with increasing initial pH. Since previous solid state characterization of PECs showed the strongest interactions in A3.6 and H3.6, it was expected that PECs with stronger interactions between chitosan and xanthan gum had lower rehydration capacity. These PECs interact less with the buffer, so the smaller is the total extent of interactions in the dispersion. PECs with lower extent of interactions between the polymers (especially H5.6 and A5.6), rehydrate more readily and have a higher extent of interactions with the buffer as well as the higher corresponding H value. The observed significant difference in phosphate buffer rehydration capacity of the investigated PECs, may affect the release kinetics of the drug substance.

Table 4
Maximal apparent viscosity (η_{max}) (at 22.2 s^{-1}), minimal apparent viscosity (η_{min}) (at 100 s^{-1}), and hysteresis area (H) for the investigated PECs rehydrated in phosphate buffer pH 7.2.

PEC	η_{max} (Pa·s)	η_{min} (Pa·s)	H (Pa/s)
H3.6	2.90 ± 0.03	0.715 ± 0.026	81.18
H4.6	1.24 ± 0.01	0.409 ± 0.018	197.65
H5.6	1.28 ± 0.02	0.446 ± 0.019	684.18
A3.6	2.96 ± 0.04	0.789 ± 0.035	300.29
A4.6	2.45 ± 0.03	0.658 ± 0.028	515.15
A5.6	1.24 ± 0.01	0.419 ± 0.013	800.37

3.3. *In vitro* ibuprofen release

Fig. 6 shows drug release profiles of ibuprofen from the hard capsules filled with the mixtures of the investigated PECs (prepared with both hydrochloric and acetic acid), the pure polymers or their physical mixture (1:1), with the drug substance in mass ratios 1:1 and 1:2.

Model dependent analysis of the obtained ibuprofen release profiles was used to elucidate the impact of the PECs on the drug release kinetics (Table B.1).

In the samples comprising pure chitosan, at both mass ratios, immediate drug release was observed. For mass ratio 1:1100% of ibuprofen was released after 30 min (Fig. 6). For mass ratio 1:2 the active substance was completely released in first 15 min (Fig. 6). The results demonstrated that the drug release rate was dependent only on ibuprofen concentration, and increased as the drug content increased, while the presence of chitosan did not affect this process. The later observation was related with the fact that the polymer is not protonated at pH 7.2.

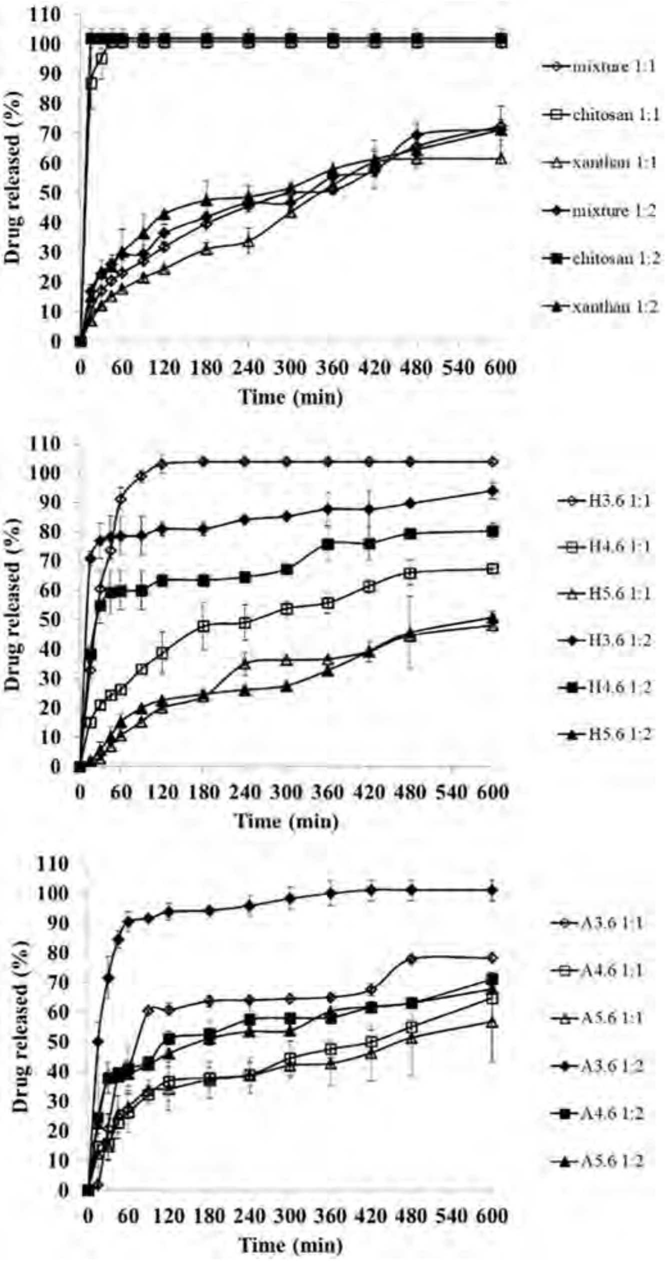

Fig. 6. Ibuprofen release profiles from the hard capsules filled with the drug combined with: chitosan, xanthan gum, chitosan/xanthan gum physical mixture, and the PECs.

For the capsules comprising ibuprofen and pure xanthan gum, extended drug release has been noticed at both mass ratios. At 1:1 mass ratio 17.56% of ibuprofen was released after 60 min, and 61.39% after 10 h. The drug release followed the *Korsmeyer-Peppas* model, which is typical for polymer-based carriers with high swelling ability. Considering n value (Table B.1), it was assumed that mechanism of ibuprofen release was a combination of swelling, erosion and diffusion ($0.5 < n < 1$) [30,61,62]. However, when ibuprofen content prevailed (at mass ratio 1:2) 30.05% of ibuprofen was released after 60 min and 70.99% after 10 h, with $n < 0.5$, thus a modified Fickian diffusion mechanism was assumed (*i.e.*, the influence of the polymer was suppressed).

The presence of physical mixture also enabled extended release of ibuprofen. After 10 h 72.36% (mass ratio 1:1) and 71.17% (mass ratio 1:2) ibuprofen was released. Higher amounts of drug released in the presence of chitosan/xanthan gum physical mixture, compared to pure xanthan, could be explained by influence of chitosan, which has disintegrant properties [63]. Moreover, for mass ratio 1:1, ibuprofen release followed *Higuchi's* kinetic which describes the release of the drug by the Fickian diffusion process through the hydrated and swollen polymer matrix in an aqueous medium during the test [62]. In case of mass ratio 1:2 ibuprofen release followed *Korsmeyer-Peppas* kinetics with $n < 0.5$ (Table B.1), indicating modification of the Fickian diffusion mechanism when the drug fraction was higher in comparison with the mixture of two polymers.

In presence of H3.6 after 60 min was released 90.99% of ibuprofen (at mass ratio 1:1) and 78.51% (at mass ratio 1:2). For mass ratio 1:1 ibuprofen release followed the *first order* kinetics where the drug release mechanism depends on its concentration as well as the presence of the PEC, while at mass ratio 1:2 the drug release followed the *Korsmeyer-Peppas* model with n value near zero *i.e.*, the PEC has only small influence on ibuprofen release. H3.6 rehydrates the fastest, swells and disperses in the medium, with minimal or no limitation on the release rate and dissolution of ibuprofen. For other PECs prepared with hydrochloric acid, amount of ibuprofen released after 10 h was 67.26% (H4.6; mass ratio 1:1), 80.17% (H4.6; mass ratio 1:2), 48% (H5.6; mass ratio 1:1), 50.67% (H5.6; mass ratio 1:2), and all followed the *Korsmeyer-Peppas* model as carriers with high swelling ability. At both mass ratios n value of H4.6 was <0.5, while for H5.6 was ($0.5 < n < 1$). The change in the release kinetics model and the decrease in the amount of released ibuprofen in these PECs could be associated with differences in their thixotropy after rehydration in phosphate buffer, observed during rheological characterization (Table 4). The increase in the extent of interactions in rehydrated PECs in the H3.6 < H4.6 < H5.6 series has led to an increase in the effect of PEC on the release mechanism and the reduction in the ibuprofen release rate.

In acetic acid based PECs, extended release of ibuprofen was achieved in all cases except with A3.6 at mass ratio 1:2 where immediate release with 90.55% of the drug released after 60 min was observed. Amount of ibuprofen released after 10 h from other PECs was: 78.18% (A3.6, mass ratio 1:1), 64.84% (A4.6, mass ratio 1:1), 71.10% (A4.6, mass ratio 1:2), 56.84% (A5.6; mass ratio 1:1), 67.75% (A5.6, mass ratio 1:2). Release of ibuprofen followed *Korsmeyer-Peppas* release kinetics with $n < 0.5$ which was related with modified Fickian diffusion. The values of the diffusion coefficient n were similar which indicated that the PECs prepared with acetic acid extend the release and ibuprofen dissolution rate. The rate of release and the amount of released ibuprofen were to a lesser extent influenced by the PEC-to-ibuprofen mass ratio compared to PECs prepared with hydrochloric acid. Only A4.6 and A5.6 could be considered as extended release carriers for ibuprofen release was unaffected significantly by the PEC-to-ibuprofen mass ratio.

Model independent analysis (calculating the values of difference factor (f_1) and similarity factor (f_2) for each tested pair of the release profiles) was used to examine difference and similarity of ibuprofen release profiles in presence of the investigated PECs (Table B.2). The investigated release profiles can be considered similar if $50 < f_2 < 100$ and $0 < f_1 < 15$ [42]. Ibuprofen release profiles with H4.6 and A4.6 at mass ratio 1:1 and H3.6 and A3.6 at mass ratio 1:2 could be considered similar. Results indicated that choice of the acid for PECs preparation, particularly at pH 3.6 and 5.6 (mass ratio 1:1) as well as at pH 4.6 and 5.6 (mass ratio 1:2) influence significantly the drug dissolution rate. PECs prepared with hydrochloric acid were significantly different at different pH, for both mass ratios with ibuprofen. Drug release profiles in presence of A4.6 and A5.6 were considered similar at both mass ratios. This observation indicated less variations of drug release when acetic acid was used as a pH adjusting agent at pH 4.6 and 5.6. Also, the effect of drug substance content was not significant.

4. Conclusions

Conductometric and rheological analysis have demonstrated the interactions between chitosan and xanthan gum during the formation of PECs and the significant influence of type and acid concentration on their extent and strength. With acetic acid, chitosan was better dissolved, and upon the addition of xanthan gum, the interactions between the two polymers were stronger and more extensive. For both acids, at pH 3.6 the interactions were strongest and most abundant, while their strength and extent decrease with increasing pH in aqueous dispersions as well as in solid state. Physical (ionic, hydrogen bonds) interactions between the polymers and semi-crystalline or amorphous structure have been proven in dried PECs. The swelling ability in phosphate buffer pH 7.2 increased significantly with increasing initial pH from 3.6 to 5.6, for both acids. PEC H3.6 has the lowest swelling capacity and did not affect significantly the release profile of ibuprofen. Increasing the pH, particularly in acetic acid-based PECs, increased the swelling capacity of PEC in phosphate buffer and the degree of hindrance of ibuprofen release and dissolution. PECs A4.6 and A5.6 demonstrated the best capacity to extend the release of ibuprofen from hard gelatine capsules for 10 h at PEC-to-drug mass ratio up to 1:2, *i.e.*, the drug release was controlled by these PECs even when the content of the drug substance was twice that of the carrier. The study demonstrated that the selection of the optimal acid and initial pH value enabled design of PECs with characteristics favourable to control ibuprofen release rate and thus promising for treatment of acute fever in paediatric patients and chronic pain.

CRediT authorship contribution statement

Ana Ćirić: Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft.**Đorđe Medarević:**Methodology, Formal analysis, Writing - original draft.**Bojan Čalija:**Methodology, Formal analysis, Writing - original draft.**Vladimir Dobričić:** Methodology, Formal analysis, Writing - original draft.**Miodrag Mitrić:** Methodology, Formal analysis, Writing - original draft.**Ljiljana Djekic:** Conceptualization, Methodology, Formal analysis, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

None.

Appendix A

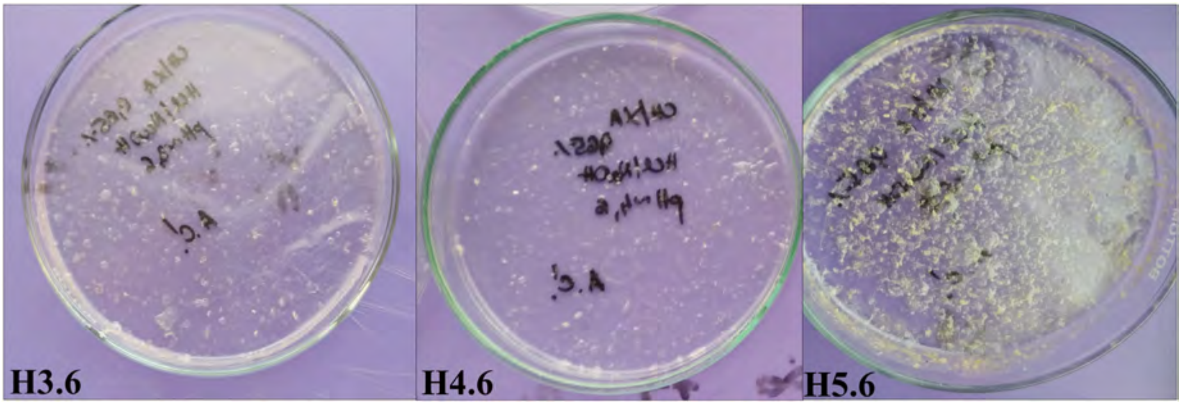

Fig. A.1. Appearance of films formed by drying of the PEC dispersions prepared with hydrochloric acid.

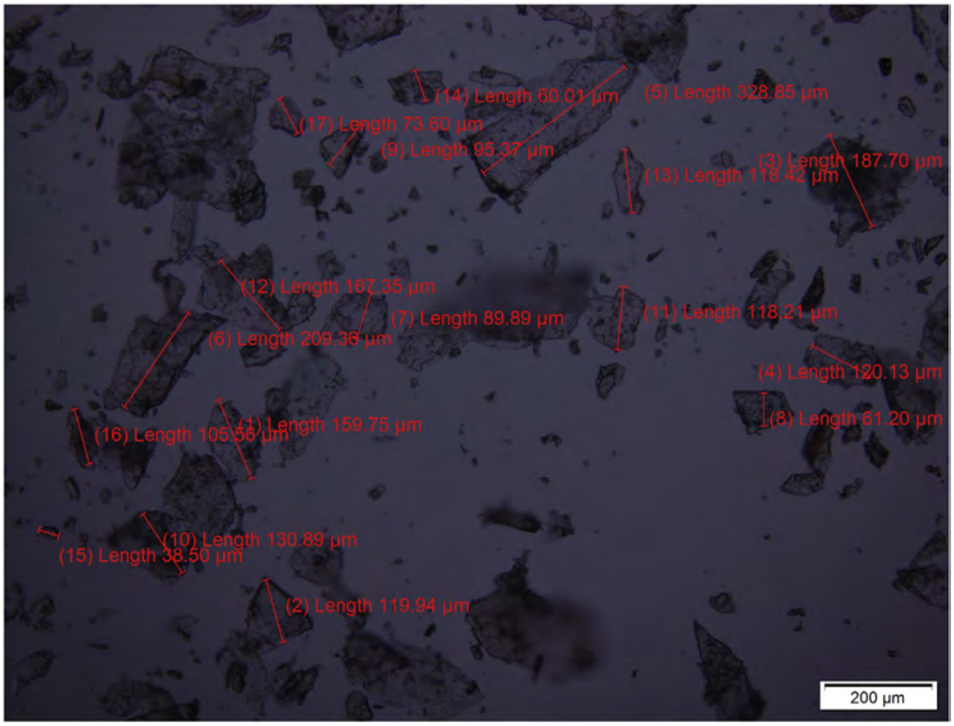

Fig. A.2. Light microscopy micrographs of the dried PEC A4.6.

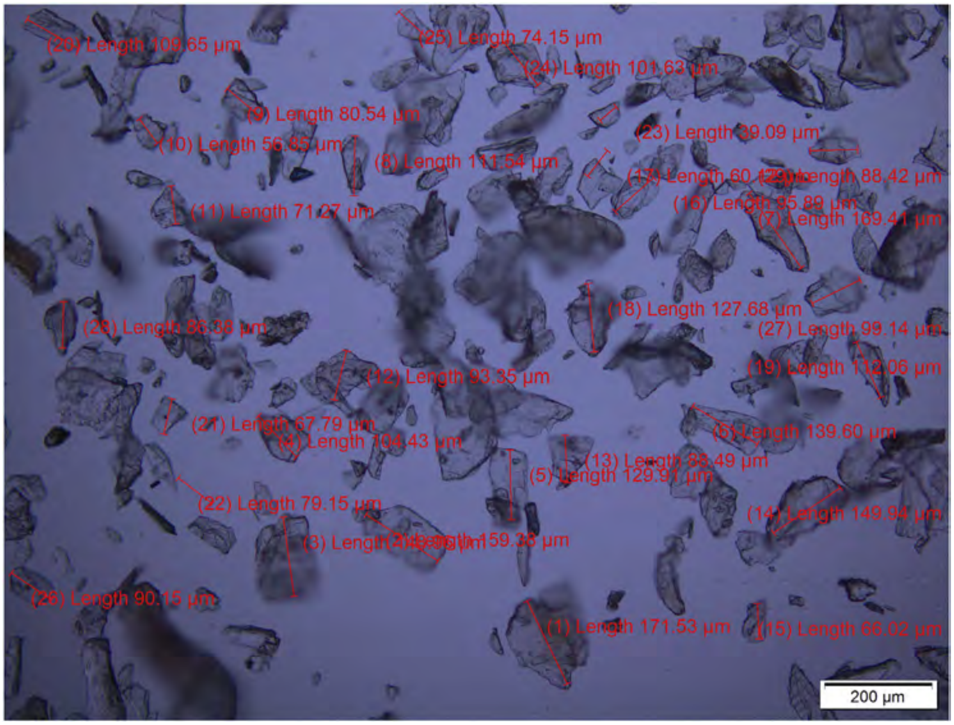

Fig. A.3. Light microscopy micrographs of the dried PEC A5.6.

Table B.1
Summary of best-fit models of ibuprofen release kinetics with corresponding correlation coefficient (R^2), release rate constant (k), and Korsmeyer-Peppas diffusion exponent (n) (where applicable).

Polymer/PEC-to-ibuprofen mass ratio	Polymer/PEC	Best-fit model	R^2	k	n[a]
1:1	Chitosan	Immediate release	n.a.	n.a.	n.a.
	Xanthan	Korsmeyer-Peppas	0.9727	1.457	0.598
	Mixture	Higuchi	0.9949	2.892	n.a.
	H3.6	First order	0.9841	0.032	n.a.
	H4.6	Korsmeyer-Peppas	0.9886	5.642	0.394
	H5.6	Korsmeyer-Peppas	0.9616	0.877	0.636
	A3.6	Korsmeyer-Peppas	0.7866	10.133	0.328
	A4.6	Korsmeyer-Peppas	0.9649	5.223	0.382
	A5.6	Korsmeyer-Peppas	0.9413	6.464	0.333
1:2	Chitosan	Immediate release	n.a.	n.a.	n.a.
	Xanthan	Korsmeyer-Peppas	0.9862	6.540	0.371
	Mixture	Korsmeyer-Peppas	0.9697	5.159	0.406
	H3.6	Korsmeyer-Peppas	0.9212	60.000	0.064
	H4.6	Korsmeyer-Peppas	0.8793	30.533	0.149
	H5.6	Korsmeyer-Peppas	0.9544	1.079	0.596
	A3.6	Immediate release	0.9864	0.042	n.a.
	A4.6	Korsmeyer-Peppas	0.9563	16.245	0.225
	A5.6	Korsmeyer-Peppas	0.9609	14.830	0.236

a

n.a. – not applicable.

Table B.2
Difference factor (f_1) and similarity factor (f_2) for tested pairs of ibuprofen release profiles.

PEC-to-ibuprofen mass ratio	Profiles compared	f_1	f_2
1:1	H3.6 *vs.* H4.6	52.90	15.10
	H3.6 *vs.* H5.6	72.89	8.41
	H4.6 *vs.* H5.6	42.45	36.64
	A3.6 *vs.* A4.6	34.39	35.31
	A3.6 *vs.* A5.6	37.05	33.66
	A4.6 *vs.* A5.6	7.38	72.70
	H3.6 *vs.* A3.6	40.65	21.22
	H4.6 *vs.* A4.6	12.75	57.71

(continued on next page)

Table B.2 (*continued*)

PEC-to-ibuprofen mass ratio	Profiles compared	*f₁*	*f₂*
	H5.6 *vs.* A5.6	44.32	45.92
1:2	H3.6 *vs.* H4.6	21.60	36.50
	H3.6 *vs.* H5.6	69.98	11.69
	H4.6 *vs.* H5.6	61.72	19.73
	A3.6 *vs.* A4.6	43.78	19.86
	A3.6 *vs.* A5.6	45.68	18.94
	A4.6 *vs.* A5.6	4.13	77.50
	H3.6 *vs.* A3.6	14.29	45.32
	H4.6 *vs.* A4.6	21.53	41.93
	H5.6 *vs.* A5.6	98.07	30.40

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Biocompatible non-covalent complexes of chitosan and different polymers: characteristics and application in drug delivery

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Abstract

The formulation of biocompatible drug carriers based on cationic biopolymer chitosan and natural or synthetic polymers represents an important research interest. Therefore, this review aims to perceive their potential in drug delivery. The most investigated chitosan-based polymer blends are polyelectrolyte complexes (PECs) obtained by establishing ionic interactions with biocompatible polyanions as alginates, pectin, xanthan gum, carrageenan, carboxymethylcellulose, and collagen. Depending on the preparation conditions, PECs could be prepared in versatile forms including membranes/films, hydrogel beads, nanoparticles, and microparticles, to achieve controlled (e.g., extended, delayed, colon-specific and pH-dependent) drug delivery. PECs can encapsulate hydrophilic and lipophilic drug substances with different molecular weights. Drug encapsulation allows the preservation of their structure, activity, improvement in absorption efficiency, reduction in adverse effects and long-term stability *in vitro* and *in vivo*. The biocompatible structures as non-covalent chitosan-based complexes could be formed also by establishing hydrogen bonds, for example with poly(vinyl alcohol). The swelling of these complexes is not pH-dependent and encapsulated drug substances are often released by already known types of diffusion. Moreover, grafted chitosan derivatives (e.g., carboxymethyl chitosan, trimethyl chitosan, acrylated chitosan) are synthesized to improve water solubility at a wide pH range and enhance the encapsulation capacity of promising PEC-based drug carriers.

Keywords: chitosan, polyelectrolyte complex, biocompatibility, drug carriers, controlled drug delivery

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1. Introduction

Numerous drug carriers are based on the combination of polymers, which may be covalently or non-covalently linked. Due to the toxicity of the reagents used for covalent bonding, current research interest is focused on polymer blends with non-covalent (ionic, hydrogen, hydrophobic) interactions between polymer chains (1).

Chitosan is the most commonly used natural cationic polymer obtained by deacetylation of chitin. Chitosan contains N-acetyl-D-glucosamine and D-glucosamine units with one amino and two hydroxyl groups in each glycoside unit (Fig. 1). At media pHs under chitosan pKa value, due to the protonation of amino groups, chitosan becomes positively charged which increases its solubility. The properties of chitosan as mucoadhesion, controlled drug delivery, *in situ* gelling, permeability enhancement, colon-specific drug delivery, inhibition of efflux pumps, etc. make it a suitable excipient for development of various pharmaceutical formulations (2–4). Chitosan *per se* is not convenient in designing the controlled-release drug delivery systems because it dissolves rapidly in the stomach at lower pH values. That is why it is often combined with anionic polymers (5–7).

The oppositely charged polyelectrolytes stay interconnected by establishing electrostatic (ionic) interactions whereby they form polyelectrolyte complexes (PECs) (Fig. 1) (1, 8). Ionic interactions are strong but reversible and their formation is a common approach to evade the use of potentially toxic cross-linkers. The reaction takes place at pH values close to the pKa values of the polyelectrolytes that form PEC. Upon the formation of ionic interactions, new bonds between the polymers are created and the proper spatial conformation is achieved, followed by the stabilization of the obtained PEC by establishing the hydrophobic bonds between polymer chains (8). During the PEC formation, polymers can form coacervates as a consequence of precipitation due to the strong interactions between oppositely charged polyions (5, 9). Precipitation can be prevented by the addition of salts, e.g., sodium chloride. The presence of ions reduces the attractive forces between the oppositely charged polymers, prevents phase separation, and ensures the formation of viscous and homogeneous gels (1). PEC-based carriers are principally considered biocompatible and well-tolerated. Moreover, they may be sensitive to changes in pH and/or ionic strength. The formation and stability of PECs depend on numerous factors, such are the ionization degree of the polymers, the charge distribution along the polymer chains, the concentration of polymers, polymer ratio in the mixture, the mixing order of polymers' dispersions (i.e., the addition of the dispersion of anionic polymer into the dispersion of cationic polymer, or *vice versa*), the polymer molecular weight, temperature, ionic strength, pH of the reaction medium. It is important to stress that stoichiometric PECs always have an even amount of positive and negative charge, resulting in zero net charge, and their water solubility is poor. However, with the addition of one polymer in excess to the polymer mixture, the water-soluble non-stoichiometric PECs are formed (5, 10–12) (Fig. 1). More recently, numerous PECs based on polysaccharides and proteins have been formulated as carriers for drug delivery, DNA delivery, or tissue engineering (2, 4, 7). Many different polyanions have been used for the formation of PECs with chitosan, such as natural polymers and their semi-synthetic derivatives (e.g., alginates (13–18), pectin (19–23), xanthan gum (11, 12, 24–27), carrageenan (28–30), carboxymethylcellulose (31, 32), collagen (25)), synthetic polymers and tripolyphosphate (TPP) (33) (Tab. I). Chitosan-based PECs can be obtained by various methods and therefore be of different shapes/geometries, including membranes/films (34–36), hydrogel beads (24, 37, 38), nanoparticles and microparticles (14, 15, 33, 35, 39–41) (Fig. 1). Since chitosan biocompatibility is retained after the formation of PECs, the application and safety of chitosan-based PECs are dependent on the used polyanions (7). The important characteristic of PECs is their sensitivity to pH changes. In the acidic environment, the polyanion is neutralized and amino groups of chitosan are protonated. The repulsion of positively charged chitosan chains in the presence of media leads to the swelling of the formed gel. In the alkaline media, the swelling occurs in the same manner, but it is induced by the repulsion of negative charge of the polyanion (11). As PECs swelling depends on numerous factors, modification of drug release from the carrier could be achieved (1).

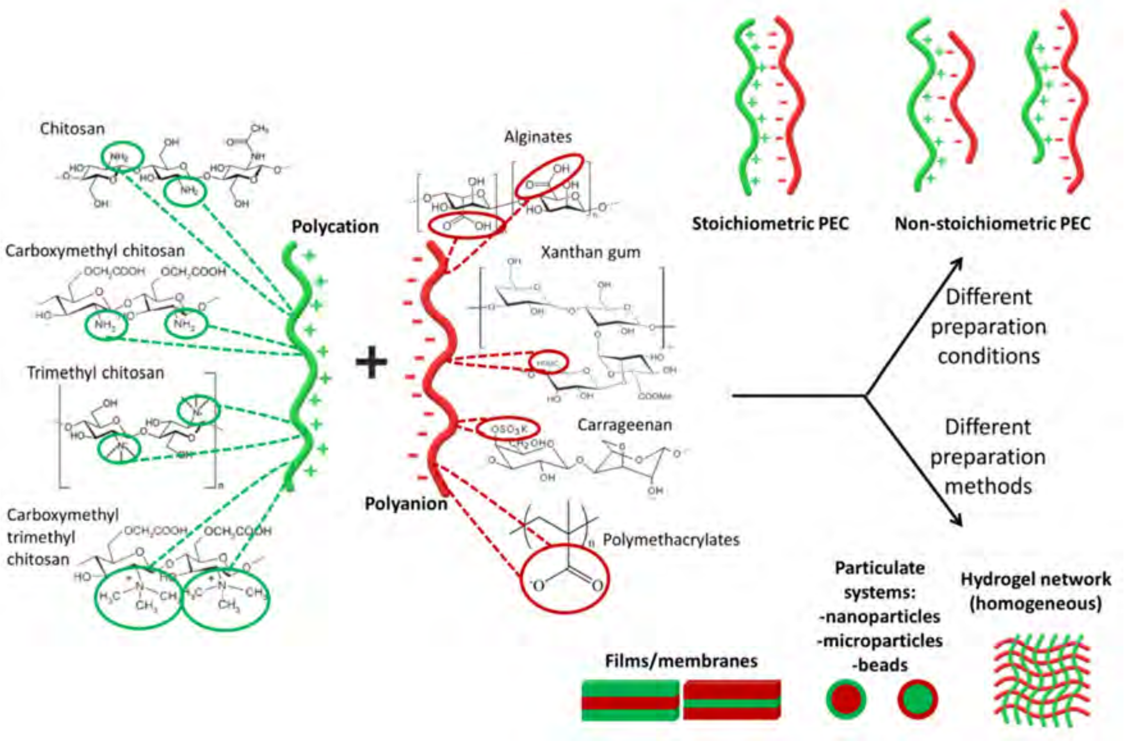

Figure 1. Schematic presentation of the formation and structure of different drug carriers obtained from chitosan-based PEC .

Slika 1. Shematski prikaz formiranja i strukture različitih vrsta nosača lekovitih supstanci dobijenih od PEK na bazi hitozana.

Additionally, complexes similar to PECs can be formed by combining chitosan and polyvinyl alcohol (PVA), based on hydrogen bonds between the hydroxyl groups of PVA and the hydroxyl or amino groups of chitosan. These carriers are also biocompatible and have a potential for use in biomedicine. Complexes prepared by the autoclaving method can be used as scaffolds in cell culture, while complexes prepared by freeze/thaw method can be used as drug carriers (1).

The limitation of the use of chitosan in drug delivery is its insolubility at pH values higher than 6.5. Besides, chitosan *per se* has low drug entrapment capacity, limited ability to control drug release, and modest mucoadhesive properties. To overcome these limitations, chemically modified chitosan (grafted chitosan) have been synthesized and used in the formulation of drug delivery systems (1, 42, 43).

2. PECs of chitosan and natural polymers

Many natural polymers (polysaccharides), owing to their biocompatibility and low toxicity, have been used in the formulation of chitosan-based PECs. The most commonly used anionic polysaccharide for the preparation of chitosan-based PEC drug carriers is alginate. Among other natural polyanions often used in the formulation of chitosan-based PECs are pectin, xanthan gum, carrageenan, hyaluronic acid, and gellan gum (7) (Tab. I). There are several other natural polysaccharides (and their semi-synthetic derivatives) that can form PECs with chitosan. Although they have not been extensively investigated as drug carriers so far, many of them have a certain potential: for colon-specific drug delivery (44, 45); gum kondagogu for improved oral delivery of diclofenac-sodium (46); carboxymethylated psyllium arabinoxylan for prolonged release of ibuprofen (47); ulvan for the preparation of matrices for enzyme-induced biomimetic bone mineralization (48); carboxymethyl gum katira for the prolonged of ofloxacin release (49); curdlan sulphate for controlled and pH-dependent zidovudine release (50); carboxylic curdlan for sustained 5-fluorouracil delivery (51); carboxymethyl starch for extended paracetamol release (52).

2.1. Chitosan/alginate-based PECs

Alginates are natural linear and biodegradable polysaccharides containing 1,4-linked β-D-mannuronic and α-L-guluronic acid in different ratios (Fig. 1). Alginates are obtained by extraction from brown seaweed, *Laminaria hyperborea*, *Ascophyllum nodosum*, or *Macrocystis pyrifera*. Negatively charged carboxylate groups of mannuronic and guluronic acid in alginates can establish ionic interactions with positively charged amino groups of chitosan and form PECs (1, 5, 7, 53). Alginates are the most investigated anionic polyelectrolytes used for the formation of PECs with chitosan due to their biodegradability and biocompatibility (18, 37, 54, 55).

Even under mild reaction conditions, alginates can easily interact with divalent cations such as Ca^{2+} (the most commonly used), Sr^{2+}, or Zn^{2+}, which act as cross-linkers between the functional groups of alginate chains. Gelling occurs due to the formation of insoluble alginates when divalent cations form the bonds mostly between guluronic acid residues of alginate chains, which leads to the formation of a three-dimensional network (the "egg-box" structure). However, monovalent cations and Mg^{2+} ions cannot act as cross-linkers between alginate chains and gelation does not occur (53, 56, 57). Two methods (external and internal) of alginate gelation with polyvalent cations are described by Chan *et al.* (58). In the external gelation method, an alginate/drug mixture is added directly to the calcium salt (e.g., calcium chloride) solution dropwise. In the internal gelation method, the mixture of alginate and drug substance is pre-mixed with insoluble calcium salt (e.g., calcium carbonate), and the mixture is then added to the acidified oily phase, resulting in the release of Ca^{2+} ions which interact with alginate. The alginate/Ca^{2+} gels obtained by described methods often have a loose structure, which leads to the loss of drug substances throughout the gelling process, mostly water-soluble ones.

The chitosan/alginate-based PECs were prepared by various methods and in various forms, including nanoparticles, microparticles, beads/hydrogels, filaments/fibers, and 3D matrices. When compared with alginate gels obtained by calcium ions crosslinking or chitosan gels, PECs possessed significantly improved physicochemical properties (2, 7).

Drug carriers based on chitosan/alginate PECs are generally in the form of particles (nanoparticles, microparticles, beads), which depends on the preparation conditions, as well as the formulation itself. In order to prepare chitosan/alginate-based PECs in the form of particles, the external gelation method is commonly used. The preparation process can be carried out as a one-step and a two-step process. In a one-step process, alginate dispersion is slowly added to the dispersion of chitosan with or without Ca^{2+} ions, drop by drop. In a two-step process, an alginate-calcium gel is formed first and then added to the chitosan dispersion to form a PEC membrane around the alginate-calcium gel. Using both methods, particles of various sizes, from nanoparticles to beads of several hundred μm in diameter can be obtained, depending on the concentration of polymers and Ca^{2+} ions, polymers' molecular weight, and the charge density (7, 55, 59, 60). Many studies involve an additional step following the two-step procedure. It leads to an increase in the strength of the PEC membrane by adding TPP, polyanion that achieves electrostatic interactions with chitosan (61).

The net charge and polymers' molecular weight are considered the most important parameters that influence the physicochemical properties of chitosan/alginate-based PECs. To obtain the particles with a small diameter, one polyelectrolyte should be in excess (55, 60).

Wong *et al.* (61) formulated hydrogel beads based on chitosan/alginate PEC using the one-step process with the addition of TPP to increase the mechanical strength of the chitosan coating of beads and prevent their adhesion during drying (Tab. I). High concentrations (3% alginate, 4% calcium ions, and 0.5% chitosan) were used, which led to the preparation of beads with a large diameter (1.7 – 1.9 mm). Microwave irradiation was applied during the preparation, and sulfathiazole was selected as the model drug for encapsulation into beads. Microwave irradiation did not affect the chemical stability of sulfathiazole, but significantly extended its release in comparison with non-irradiated beads. The results showed that microwave irradiation can be used in the formulation of solid dosage forms for controlled drug delivery. Numerous studies have shown that different drying processes have a strong effect on particle morphology and drug release profiles from chitosan/alginate PEC-based particles. Air-dried beads retained a spherical shape and sponge-like structure with a smooth surface. Lyophilized beads lost a spherical shape and had a rough surface. Considering these structural differences, lyophilized beads had a higher swelling capacity, whereas air-dried beads showed delayed release of the drug in the simulated gastrointestinal tract. Given the possibility of industrial production and application in drug delivery, air-dried beads may have better characteristics than freeze-dried beads (37, 62).

Table I Examples of different chitosan-based PECs and their performances as drug delivery carriers.

Tabela I Primeri različitih PEK na bazi hitozana i njihova svojstva kao nosača lekovitih supstanci.

Polyanion	Drug	PEC type/ dosage form	Preparation method	Use/route of administration	Effect on drug release	Ref.
Alginate + TPP	Sulphathiazole	Hydrogel beads (chitosan-coated)	Extrusion method + drying at 40 °C + microwave irradiation	n.d. (oral*)	Controlled release of drug (in comparison with non-irradiated beads)	(61)
Alginate	Vancomycin	Microparticles (alginate-coated)	Coacervation method + air-drying or lyophilization	Injectable drug delivery system	Controlled (prolonged) release during 3 weeks	(15)
Alginate	Insulin	Microparticles (chitosan-coated)	Membrane emulsification method + lyophilization	Oral	*In vitro*: prolonged release during 14 days, pH-dependent release *In vivo* (in rats): hypoglycemic effect during 60 h	(63)
Alginate	Ketotifen-fumarate	Membrane/film (matrix type)	Casting method + air-drying	Transdermal	Enhanced drug permeability through the skin	(16)
Alginate + PGA	Piroxicam	Composite hydrogel	Semi-dissolution/ acidification/ sol-gel transition method	Oral	pH-dependent, prolonged (up to 8 h) and colon-specific drug delivery; reduced GIT irritation	(9)

Polyanion	Drug	PEC type/ dosage form	Preparation method	Use/route of administration	Effect on drug release	Ref.
HA + TPP	Dexamethasone – sodium phosphate	Nanoparticles (HA-coated)	Ionic gelation method + lyophilization	Ocular (topical)	*In vitro*: sustained drug release during 12 h	(69)
HA + TPP	Dexamethasone – sodium phosphate	Nanoparticles (HA-coated)	Ionic gelation method + lyophilization	Ocular (topical)	*In vivo* (in rabbits): prolonged retention in the eye, adequate drug concentrations in aqueous humor up to 24 h	(70)
HA	Vancomycin, insulin	Bulk PEC/nasal insert	Complex coacervation method + lyophilization	Nasal (systemic effect)	Prolonged and pH-dependent release of both drugs during 6 h	(71)
Pectin (amide)	Indomethacin, sulfamethoxazole	Hydrogel beads (chitosan-coated)	Extrusion method + drying at 50 °C	n.d. (oral*)	pH-dependent and prolonged drug release (up to 5 h)	(81)
Pectin	Resveratrol	Nanoparticles (chitosan-coated)	Extrusion method	n.d. (oral*)	Colon-specific drug release	(23)
Pectin	Mesalamine, curcumin, progesterone	Thermoreversible hydrogels	Complex coacervation method	n.d. (oral*)	Sustained release of all 3 drugs during 24 h at physiological conditions	(22)
κ-carrageenan	Ovoalbumin	Nanoparticles (chitosan-coated)/implants	Ionic gelation method + lyophilization	Implantation into the affected area	Controlled drug release up to 3 weeks	(86)
κ-carrageenan + hydroxyapatite	Ciprofloxacin	Hydrogel nanocomposites	Mixing of polymer solutions + lyophilization	n.d.	Sustained drug release during 120 h	(30)
XG	Glipizide	Floating mucoadhesive beads (chitosan-coated)	Complex coacervation method + air-drying	Oral	Controlled (sustained) and pH-dependent drug release during 24 h	(24)
XG	Chlorohexidine	Microspheres	Complex coacervation method + lyophilization	Periodontal injection	Prolonged drug release up to 7 days	(26)
XG	Ibuprofen	Bulk PEC	Complex coacervation method + air-drying	Oral	Extended drug release up to 10 h	(12)
GG	Ketoconazole	Nanoparticles (chitosan-coated)	Electrostatic complexation method + lyophilization	n.d. (topical*)	Sustained drug delivery; significantly higher antifungal activity against *Aspergillus niger* compared to pure drug	(91)

Polyanion	**Drug**	**PEC type/ dosage form**	**Preparation method**	**Use/route of administration**	**Effect on drug release**	**Ref.**
CMC	Vancomycin	Microparticles (chitosan-coated)	Complex coacervation method + spray-drying	n.d. (oral*)	Colon-specific drug delivery; prevention of drug degradation at low pH and the presence of pepsin	(32)
Poly acrylic acid	Teophylline	Bulk PEC/matrix tablet	Complex coacervation method + lyophilization	n.d. (oral*)	pH-dependent and prolonged drug delivery during 12 h	(93)
Eudragit® S-100	Curcumin	Microparticles (Eudragit®-coated)	Emulsion cross-linking + solvent evaporation method + drying at 50 °C	n.d. (oral*)	*In vitro*: Controlled drug release up to 12 h and prevention of premature release *In vivo* (in mice): colon-specific drug delivery	(97)

n.d. – not defined; *conclusion based on experimental conditions (data) in the study; Abbreviations: CMC – carboxymethylcellulose; GG – gellan gum; GIT – gastrointestinal tract; HA – hyaluronic acid; PEC – polyelectrolyte complex; PGA – polyglutamic acid; TPP – tripolyphosphate; XG – xanthan gum.

In another study chitosan/alginate PEC-based microparticles with vancomycin were prepared by the coacervation method and dried by lyophilization or air-dried. Lyophilized microparticles showed the best control of drug release with the average release of 22 µg/day during 14 days. With an increase in alginate concentration, the vancomycin release rate did not increase. Drug release followed Peppas-Sahlin kinetics, with Fickian diffusion and case II relaxation release mechanisms (15) (Tab. I).

Encapsulation of macromolecular drug substances into PEC-based carriers with alginate aims at preserving their structure and biological activity, protect them from negative environmental factors, and improve absorption efficiency. To examine the encapsulation of proteins and their release, chitosan/alginate-based PECs are often prepared by the two-step process in a form of microparticles or beads. The delivery of insulin encapsulated into chitosan/alginate PEC-based microparticles after oral administration has been tested *in vivo* in rats (63) (Tab. I). It has been shown that encapsulated insulin was well protected from the effect of the gastrointestinal tract and easily absorbed *in vivo*. The effect of insulin released from microparticles was conserved during more than 60 h, implying its long-term stability *in vivo*. Lefnaoui *et al.* (16) developed chitosan/alginate PEC-based matrix-type films for transdermal delivery of ketotifen fumarate (Tab. I). Films with different PEC compositions were prepared at various chitosan-to-sodium alginate ratios by the film casting method. Propylene glycol was used as a film plasticizer, and Tween® 80 and Span® 20 as permeation enhancers.

The obtained results revealed no significant interaction between ketotifen and polymers. It was observed that when Tween® 80 was used as a permeability enhancer, an adequate drug release profile was obtained (with 99.88% of the drug released) and 2.121 mg/cm^2 of ketotifen permeated through rat abdominal skin after 24 h. The optimal formulation had a permeability coefficient of 14.00 ± 0.001 cmh^{-1} and a lag time of 0.35 ± 0.02 h.

Recently, many other chitosan/alginate-based PECs with the addition of the third component were formulated. For example, chitosan/polyglutamic acid (PGA)/alginate PEC hydrogel was formulated to achieve better control of piroxicam release (9) (Tab. I). Mucoadhesiveness of chitosan/alginate-based PECs has been proven in various *ex vivo* models, such as isolated rat jejunum (64) and the proximal part of the porcine colon (65).

2.2. Chitosan/hyaluronic acid-based PECs

Hyaluronic acid (HA) is a natural non-toxic, biocompatible, and biodegradable polysaccharide, i.e., glycosaminoglycan with recurrent β-1,4-D-glucuronic acid-1,3-N-acetyl-D-glucosamine disaccharide units. HA is present in all tissues, particularly in connective, epithelial, and nervous tissue, and has a high molecular weight, from 100 kDa in serum to 8000 kDa in the vitreous body. Because of the presence of carboxylate groups in its structure, HA is a natural anionic polyelectrolyte with the ability to form PECs with chitosan. Chitosan/HA-based PECs have a potential for application in biomedicine owing to their colloidal stability, low cytotoxicity, and ability to protect drugs from enzymatic degradation. By combining highly mucoadhesive properties of HA and chitosan permeability enhancement effect, chitosan/HA PEC-based carriers may be more suitable than many other carriers to deliver drugs *via* mucous membranes and enhance their intestinal absorption (5, 7, 66).

Nanoparticle systems based on chitosan/HA PECs are considered suitable carriers for drug delivery *via* different administration routes. For example, chitosan/HA nanoparticles are used for the ocular delivery of drugs without exhibiting eye discomfort or irritation (67).

Furthermore, enhanced mucoadhesion with improved pharmacodynamics of loaded drugs and reduced systemic absorption in an animal model was reported (68). Dexamethasone-sodium phosphate (DEX)-loaded chitosan-sodium tripolyphosphate nanoparticles (CS-NPs) were prepared using the ionotropic gelation method and coated with HA to obtain discrete, free-flowing NPs which would improve their mucoadhesion onto corneal and conjunctival epithelial surfaces and extend the drug retention in the eyes. Physicochemical characteristics of DEX-CS-NPs suspensions were appropriate for ocular use, with good stability for 3-months storage, while the size of NPs was increased from 305 to 400 nm after coating with HA. *In vitro* drug release testing in simulated tear fluid demonstrated that 75.84% of DEX was released during 12 h from HA-coated CS-NPs (69) (Tab. I). *In vitro* transcorneal permeation on the excised rabbit cornea and *in vivo* ocular irritation tests indicated the ocular safety of DEX-loaded HA-CS-NPs (70) (Tab. I).

The preparation of nasal inserts based on chitosan/HA PECs with mucoadhesive properties for nasal drug delivery was also reported (71) (Tab. I). *In vitro* swelling, mucoadhesion, and drug release investigations were done to evaluate the potential of vancomycin and insulin delivery in the nasal cavity. The obtained results revealed that the selection of suitable preparation conditions enabled the modification of insert swelling and prolonged drug release during 6 h. Sponges or films based on chitosan/HA PECs for wound treatment were also reported (72–74).

Lalevée *et al.* (75) obtained chitosan/HA-based PECs in a homogeneous mixture of polymers at high salt concentrations. Then, the mixture was dialyzed, which led to the controlled self-assembly of the polymers. The assembly of chitosan and HA during the PEC formation depended mostly on chitosan acetylation degree and molar mass, the residual salt concentration, and the molar ratio of the charge of both polymers. These parameters determined if the obtained PECs will be in the form of colloidal suspensions or gel coacervates.

Nath *et al.* (76) loaded bone morphogenetic protein-2 (BMP-2) in chitosan/HA-based PEC. Free amino groups of chitosan were cross-linked with different amounts of genipin. Immobilization of BMP-2 (immobilization efficacies varied from 61% to 76%, depending on the amount of BMP-2) in chitosan/HA PEC-based scaffolds sustained the protein release for more than 30 days. Moreover, BMP-2 facilitated osteogenesis. Thus, genipin cross-linked chitosan/HA PEC showed the potential in drug and protein delivery.

2.3. Chitosan/pectin-based PECs

Pectin is among the main components of the cell walls of plants. The chemical structure of pectin is highly miscellaneous and complex but is essentially rich in 1,4-galactosyluronic acid residues, and the linear chain is composed of acetyl and methyl esters of carboxylic acids. The degree of methyl esterification and the degree of acetylation determine pectin's functional properties, especially the ability to form a gel (5, 7, 77). Pectin has been tested for the encapsulation and delivery of drugs, especially for colon-specific drug delivery (78, 79). Pectin molecules remain chemically unchanged under physiological conditions in the stomach and small intestine but can be degraded due to the action of enzymes of microbiota present in the colon. Due to its high solubility, pectin itself cannot be an effective drug carrier. Therefore, the coating of pectin and low-soluble pectin derivatives (e.g., calcium-pectinate, pectin-amide) have been used to formulate pectin-based carriers (77, 80). Chitosan is the most prominent biopolymer in the development of pectin-based PEC carriers for the controlled drug release. Strong electrostatic coupling prevents gastric dissolution and provides high resistance to enzymatic hydrolysis, enabling the controlled release of drugs when the carrier reaches the colon (5, 7, 77).

The potential of chitosan coating in the modification of drug release from pectin-amide-based hydrogels was investigated (81) (Tab. I). The release rate of indomethacin (highly lipophilic drug) and sulfamethoxazole (less lipophilic drug) from PECs after oral administration was significantly reduced compared to the pectin hydrogel without chitosan coating. Thus, encapsulation into chitosan-coated pectin-based hydrogel beads reduced the premature release of drugs in the gastrointestinal tract (GIT) and ensured their release in the colon. The pH of chitosan dispersion influenced the electrostatic interaction strength between pectin and chitosan, and pH 5.0 was considered optimal. Additionally, it was demonstrated that the encapsulation efficiency of drugs, the swelling ability, and release profiles of drugs from chitosan/pectin based PECs, depended on various preparation factors, the acetylation degree, molecular weight, and methyl esterification degree of pectin (82, 83).

Chitosan/pectin/zinc-based particles with a diameter of approximately 1000 µm were formulated for colon-specific delivery of resveratrol (84). Formulations prepared at a lower pH and a higher chitosan concentration significantly retarded resveratrol release in the simulated GIT *in vitro*. Further investigations of the drug pharmacokinetics in rats were performed to evaluate the effect of chitosan coating on the alteration in drug release. Even though the area under the curve and the maximum drug concentration were similar for the pectin/zinc and chitosan/pectin/zinc particles, the two formulations showed a significantly different drug retention profile in plasma. The stable period of plasma drug concentration growth was 2 – 4 h for pectin/zinc particles and 4 – 9 h for chitosan/pectin/zinc particles. Considering that it takes approximately 5 – 6 h for the drug to reach the colon, chitosan/pectin/zinc particles were considered more suitable than pectin/zinc particles for colon-specific resveratrol delivery.

In another study, the formulation of chitosan/zinc/pectinate/polyethylene glycol (PEG) nanoparticles for colon-specific delivery of resveratrol was investigated (23) (Tab. I). Size and zeta potential of nanoparticles obtained at pectin/zinc/chitosan ratio 10:1:3% *w/v* were 399 ± 18 nm and +25 ± 1 mV, respectively. The addition of PEG (a solvent for resveratrol) significantly lowered the size of nanoparticles to 83 ± 4 nm. The encapsulation efficiency of resveratrol into nanoparticles with PEG was approximately 63%, while for physically-loaded resveratrol (without PEG) was 26%. A low amount of drug (approximately 40%) was released from the nanoparticles without PEG during one month period at pH 4, and the addition of PEG increased the released amount of drug to approximately 51%. The remaining resveratrol in both types of nanoparticles was released in simulated colon fluid in the presence of pectinase, so they could be considered suitable for successful colon-specific delivery of resveratrol.

Neufeld *et al.* (22) investigated the suitability of chitosan/pectin hydrogels as drug carriers (Tab. I). Extended release of mesalamine, curcumin, and progesterone during 24 h under physiological conditions was demonstrated. FTIR and DSC experiments, as well as swelling analysis, showed that the interaction extent significantly affected drug release rates. Thus, it could be expected that the obtained chitosan/pectin hydrogels may reduce the frequency of drug intake.

2.4. Chitosan/carrageenan-based PECs

Carrageenan is a biopolymer obtained from red seaweed. It consists of galactose and anhydrogalactose linked by glycosidic bonds (Fig. 1). Depending on the method and source of extraction, there are three types of carrageenan, kappa (κ), iota (ι) and lambda (λ), which generally differ in the sulphate group substitution degree, and only κ- and ι-carrageenan form gels. The first forms gels that are firm and rigid, and the second forms soft and elastic gels. Owing to the presence of sulphate groups, carrageenan is negatively charged and has the potential to interact with chitosan (5, 7, 85).

Different chitosan/carrageenan PEC-based carriers, such as hydrogels (28), films (29), nanoparticles (39, 86), for different routes of administration and controlled release of drugs have been developed. Many factors can affect the physicochemical properties of these PECs, and the type of carrageenan, the mass ratio of the polymers, the solvent type used in the formulation, and ionic strength of the medium are the most important (7, 85).

At lower concentrations of chitosan and carrageenan, nanoparticles can be formed using the ionic gelation method and their surface charge depends on the mass ratio and the mixing order of polymers. Highly positively charged nanoparticles (+60 mV) were obtained by the addition of the low concentration carrageenan dispersion into the chitosan dispersion dropwise. They showed good potential as drug carriers with no cytotoxicity at the concentrations up to 3 mg/ml, which was confirmed on L929 fibroblast culture (86) (Tab. I). Rodrigues *et al.* (39) showed that the addition of TPP into carrageenan dispersion before its addition into chitosan dispersion significantly reduced the particle size from 500 nm to 200 nm and increased the yield from 20% to 35%. Maciel *et al.* (28) prepared chitosan/ι-carrageenan superabsorbent hydrogels at different pH values (from 4 to 9) and different ratios of polysaccharides (1:1, 1:2 and 2:1), with swelling degrees greater than 1000%. The hydrogel surface was irregular, rough, and porous. The mechanical tests demonstrated that the hydrogels prepared at pH 4 had greater compression strength compared to those prepared at pH 9. Chitosan/hydroxyapatite/κ-carrageenan hydrogel nanocomposites for sustained release of ciprofloxacin with antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria were also developed recently (30) (Tab. I). The chitosan/κ-carrageenan complex released 98% of ciprofloxacin throughout 120 h, and only 52% and 66% of ciprofloxacin were released from nanocomposites containing high and low content of hydroxyapatite, respectively. For that reason, such nanocomposites could be considered as drug delivery systems with extended-release ability.

2.5. Chitosan/xanthan gum-based PECs

Xanthan gum (XG) is a natural anionic polyelectrolyte produced by *Xanthomonas campestris,* which contains five repeating sugar units: the backbone consists of two β-D-glucose units, and the sidechains include two mannose units and one glucuronic acid (Fig. 1). The sidechains have pyruvic acid residues attached to approximately one half of the terminal D-mannose units and acetic acid residues attached to D-mannose units in the backbone. XG is known for its non-toxicity and suitability for the development of drug carriers (5, 7, 87).

Chitosan/XG hydrogel beads with high swelling ability are usually prepared by adding the concentrated XG dispersion dropwise into the chitosan dispersion. Network density and swelling capacity have been shown to depend more on the XG concentration and the pH of the medium than on the chitosan concentration. The high molecular weight of chitosan also led to a higher swelling degree of beads (11, 88).

Kulkarni *et al.* (24) prepared the floating mucoadhesive chitosan/XG beads by polyionic complexation technique for controlled release of glipizide with pH-dependent swelling kinetics (Tab. I). Sustained *in vitro* release of 87.50 – 100.67% of glipizide after 24 h, in phosphate buffer pH 7.4, was observed. Encapsulation efficiency was between 79.48 and 94.48%. *In vitro* bioadhesion studies showed that the beads had satisfactory bioadhesive strength.

Chitosan/XG based microspheres could be also prepared by the coacervation technique. The prepared microspheres were mixed with chlorohexidine (local antiseptic) to form injectable antibacterial hydrogels. The study of Kim *et al.* confirmed that prepared microspheres were biocompatible and had the potential in the treatment of acute or chronic periodontitis (26) (Tab. I).

Very recently, Ćirić *et al.* (12) studied the combined effect of pH adjusting agent type (hydrochloric, acetic or lactic acid) and pH value (3.6, 4.6 and 5.6) on the formation of chitosan/XG PECs, their characteristics and influence on *in vitro* ibuprofen release kinetics (Tab. I). Conductivity measurements and rheological characterization demonstrated the greatest interaction extent in PECs prepared with acetic acid at pH 3.6. Acid type and pH strongly influenced the yield and particle size of air-dried PECs. DSC, FTIR, and PXRD analysis confirmed exclusively physical interactions between the polymers. PECs comprising acetic acid prepared at pH 4.6 and 5.6 improved rehydration capacity in phosphate buffer pH 7.2. At PEC-to-drug mass ratio up to 1:2, they prolonged ibuprofen release up to 10 h.

De Morais Lima *et al.* (89) prepared six formulations of chitosan/XG based films using the casting technique and varying the chitosan-to-XG ratio at 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (% *w/w*). The films with a higher content of XG showed the highest tensile strength, while the addition of XG did not affect the water permeability, solubility, and residual moisture content of films. These results confirmed the formation of chitosan/XG PECs in the films.

A relatively new approach was proposed for the preparation of macroporous chitosan/XG PECs, cryogels, starting with the freezing of polymers' dispersion, maintaining it frozen during an appropriate period to enable the gel to freeze and form pores and then followed by lyophilization. First, XG powder is added to the concentrated dispersion of chitosan in dilute acetic acid solution, followed by freezing and then lyophilization. The electrostatic interactions between chitosan and XG enhanced during freezing, and therefore the gel was formed at a temperature below freezing temperature. The freezing method and its rate are considered the main parameters in defining the physicochemical characteristics of cryogels. A higher freezing rate (-2 °C/min) led to the formation of smaller pores (40 µm), while a lower freezing rate (-0.25 °C/min) resulted in the formation of larger pores (68 µm) (90).

2.6. Chitosan/gellan gum-based PECs

Gellan gum (GG) is a biocompatible natural polymer produced by *Pseudomonas elodeac* which contains the residues of glucose, glucuronic acid, and rhamnose in 2:1:1 ratio. Due to the high content of glucuronic acid (20%), GG is negatively charged when dissolved in water and its gelation is temperature-dependent (7).

Kumar *et al.* (91) prepared ketoconazole loaded chitosan/GG nanoparticles by electrostatic complexation technique (Tab. I). It has been shown that the influence of GG on the particle size was greater in comparison with chitosan. An increase in GG concentration significantly increased the particle size but decreased the zeta potential. On the contrary, an increase in chitosan concentration increased the zeta potential. The optimal formulation was obtained at a chitosan concentration of 0.02% *w/v* and GG of 0.01% *w/v* with particle size and zeta potential of 155.7 ± 26.1 nm and 32.1 ± 2.8 mV, respectively. Ketoconazole-loaded nanoparticles had significantly higher antifungal activity against *Aspergillus niger* compared to drug-free nanoparticles and pure drug.

3. PECs of chitosan and carboxymethylcellulose

Among cellulose ethers, carboxymethylcellulose (CMC) has been the most investigated one for the utilization in biomedicine. CMC is a water-soluble polymer with a long linear cellulose chain and carboxymethyl groups attached to some hydroxyl groups of glucopyranose units. CMC is often in the form of sodium salt, so it carries a negative charge upon dissociation of the carboxylic groups when dissolved in water (7).

Temperature-sensitive and pH-sensitive chitosan/CMC PEC-based hydrogel was evaluated as a carrier for parenteral delivery of drugs/cells. The hydrogel was prepared by mixing chitosan with a CMC/sodium hydrogen carbonate mixture at room temperature. The resulting PEC had liquid consistency at room temperature and the ability to encapsulate live chondrocytes. PEC gel implants were formed *in situ*, at body temperature and physiological pH after injection. This temperature-sensitive and pH-sensitive hydrogel had a great potential in bone and cartilage regeneration, due to the ability of *in situ* gel formation and controlled release of drugs/cells at a specific site after injection (92).

Bigucci *et al.* (31) prepared vaginal inserts based on chitosan/CMC PECs for local delivery of chlorhexidine digluconate at different chitosan/CMC molar ratios and pH around pKa interval of the polymers. PECs had high values of drug loading and increasing water uptake capacity with an increase in the CMC amount. The selection of adequate chitosan/CMC ratio (10:90) allowed the formulation of cone-like shaped solid inserts, that were easy to handle and able to hydrate, in addition to prolonged drug release during 6 h. Moreover, the obtained inserts showed antimicrobial activity against *Candida albicans* and *Escherichia coli*.

On the other hand, Cerchiara *et al.* (32) prepared chitosan/CMC-based PECs for colon-specific delivery of vancomycin (Tab. I). Different formulations of PECs, with different chitosan/CMC mass ratios (3:1, 1:1 and 1:3), were prepared and collected in the form of microparticles by spray-drying. The best formulation prepared at chitosan/CMC mass ratio 1:3 was selected considering the encapsulation efficiency, water uptake, and drug release rate. Also, microparticles could prevent vancomycin degradation and had good antibacterial activity against *Staphylococcus aureus*.

4. PECs of chitosan and synthetic polymers

4.1. Chitosan/polyacrylic acid-based PECs

Polycarbophils and carbomers are high molecular weight polymers containing acrylic acid monomers and are formed by cross-linking the polyacrylic acid chains with divinyl glycol and polyhydroxy alcohols (e.g. allyl ethers of pentaerythritol, and allyl ethers of sucrose), respectively (5).

The main complexation mechanism between chitosan and carbomer is the formation of ionic interactions between amino groups of chitosan and carboxylate groups of carbomer, as confirmed by FTIR analysis. The chitosan-to-carbomer mass ratio and the preparation medium pH strongly influenced the characteristics of PECs. The release of drugs from matrix tablets based on chitosan/carbomer PECs at different ratios showed no significant differences at pH 1.2, while they were strikingly observed at pH 6.8 (93) (Tab. I). The chitosan/polycarbophil PEC-based matrix tablets showed zero-order release kinetics for two model drugs with different water solubility (diltiazem and ibuprofen). Their release rate depended on the concentration of PEC in the formulation. In general, chitosan/polycarbophil PECs have great potential as excipients for the formulation of modified-release dosage forms (94).

Recently, He *et al.* (95) formulated novel PEC hydrogels based on chitosan and sodium polyacrylate by cross-linking the polymers with epichlorohydrin, by inhibiting the protonation effect of chitosan in alkali/urea aqueous solution. The equilibrium swelling ratio of chitosan hydrogel in water increased significantly with the introduction of sodium polyacrylate. PEC hydrogel had different swelling ratios at different pH and ionic strength, leading to smart responsive properties. Also, PEC hydrogels had relatively high compressive strength, biocompatibility, and *in vitro* biodegradability.

4.2. Chitosan/polymethacrylate copolymers-based PECs

Polymethacrylate copolymers (Eudragit®) are commonly used as coating agents for capsules and tablets. There are different types of Eudragit®, consisting of dimethylamino ethyl methacrylates, methacrylic acid esters, and methacrylic acid in different ratios. Some of them are positively charged (Eudragit® E, RL, RS, and NE) owing to dimethylamino or quaternary amino groups, and the others are negatively charged (Eudragit® L and S) owing to carboxylate groups in their structure (5) (Fig. 1). PECs of different molecular weight chitosan and Eudragit® L100 or Eudragit® L100-55 were used to formulate matrix tablets with diclofenac-sodium. PECs of these two polymers had high potential in controlled drug delivery applications. The mass ratio of polymers and chitosan molecular weight influenced the drug release rate from matrix tablets (96). Sareen *et al.* (97) prepared Eudragit® S-100-coated chitosan microspheres with curcumin for the treatment of ulcerative colitis using the emulsion cross-linking method (Tab. I). Microspheres had a uniform spherical shape and high entrapment efficiency. Uncoated curcumin/chitosan microspheres exhibited *in vitro* burst release within the initial 4 h. Eudragit® S-100 coating prevented the premature release of curcumin and enabled its controlled release during 12 h. *In vivo* study in mice demonstrated their effectiveness and revealed a significant reduction of colonic damage with curcumin loaded into microspheres in comparison with pure curcumin. The obtained microspheres are considered the promising system for colon-specific curcumin delivery.

5. Biocompatible chitosan/PVA complexes

Biocompatible chitosan/PVA complexes are similar to PECs in terms of structure, characteristics, and use. However, they are formed mainly by the formation of hydrogen bonds between hydroxyl or amino groups of chitosan and hydroxyl groups of PVA (1). They can be readily prepared using different methods, such as the autoclaving method (98) and the freeze/thaw method (99–101). The structure of complexes could be different depending on the preparation method. PVA is a non-toxic, biodegradable, and biocompatible synthetic polymer that consists mainly of 1,3-glycol units and a small percentage of 1,2-glycol units (1). The complexes prepared by the autoclaving method are formed by mixing and autoclaving the PVA and chitosan dispersions to form highly elastic hydrogels (98). Hydrogels obtained by the freeze/thaw method are formed by repeating the freezing and thawing cycles of chitosan and PVA dispersions (99–101). Complexes prepared by the autoclaving method can be dissolved under acidic conditions and are not suitable as drug carriers. Complexes prepared by the freeze/thaw method are less soluble. The swelling of these complexes is not pH-dependent, drug release is controlled by one of the already known types of diffusion and depends on chitosan mass content. Therefore, these chitosan/PVA hydrogels are promising drug carriers. Characteristics of chitosan favour the carrier adhesion to the application site and increase the bioavailability of low-permeable drugs after oral administration of chitosan/PVA gel (1, 98–101). Wu *et al.* (36) prepared chitosan/PVA based films by simple mixing and casting method. It was shown that chitosan and PVA in films interacted by intermolecular hydrogen bonds and complex based films had higher thermal stability than those made only of PVA. Chitosan addition decreased the tensile strength and light transmittance of films. The film with chitosan-to-PVA mass ratio 60:40 (% *w/w*) showed notable activity against the adhesion and inhibited the growth of *Pseudomonas aeruginosa*.

6. PECs of grafted chitosan and different polymers

Chitosan derivatives are formed by substitution of some amino and/or hydroxyl groups in their structure (43). The preparation of derivatives often requires the use of auxiliary molecules to initiate the reaction. Grafting is usually performed with molecules that have functional groups that form covalent bonds with chitosan (1).

Chitosan copolymerization with hypromellose *via* coupling reagent-mediated approach to form a non-toxic chitosan derivative soluble in water, which was then complexed with CMC to form a PEC hydrogel, was successfully performed. When compared with conventional chitosan, the grafted chitosan had a high solubility at a wide pH range and higher buffering capacity, which provided a pH-stable environment for drug delivery. Also, grafted chitosan-based PEC had the drug encapsulation efficiency of more than 90% (almost twice the encapsulation efficiency of chitosan-based PECs), with 2-3 times extended drug release (42).

PEC hydrogels based on carboxymethyl chitosan (CMCS) (Fig. 1) and alginate with high hygroscopicity were prepared. The hydrogels' swelling ratio could be increased 450 times by changing the CMCS-to-alginate mass ratio. Superabsorbent hydrogels were prepared at CMCS-to-alginate mass ratio below 1. The *in vitro* cytotoxicity test showed that hydrogels had very high cytocompatibility. Their swelling ratio and bovine serum albumin (BSA) release were pH-dependent. The hydrogels' swelling ratio (CMCS-to-alginate mass ratio 1:2) at pH 7.4 was about 34 times higher than at pH 1.2, and the BSA released amount at pH 7.4 was also significantly higher. The obtained CMCS/alginate PEC hydrogels had a high potential in oral delivery of proteins (43).

6. Conclusion

With the growing need for drug carriers of natural origin, PECs based on chitosan and natural polysaccharides are of increasing interest. The functional characteristics of these PECs can be adapted for various administration routes using different preparation methods and appropriate combinations with other polysaccharides. Recent research in this area highlights the following opportunities in the development of PECs based on chitosan and other polysaccharides: (1) design of various types of PECs such as nanoparticles, microparticles, hydrogel beads, gels, films and membranes, and optimization of drug release kinetics; (2) encapsulation of various drugs, including small molecules, macromolecular drugs, growth factors, antimicrobial agents, and achieving their controlled delivery; (3) improved mechanical properties, controlled swelling, and site-specific drug delivery. Most of the available literature deals with ionic interactions of chitosan, which are easily formed. Hydrogen bonds and hydrophobic interactions have been less studied and rarely used to achieve controlled drug release. This opened the space for more detailed investigations.

To our best knowledge, there are still no commercially available drug delivery systems based on complexes of chitosan with different polymers. On the other hand, there are many chitosan-based medical devices for wound healing, nutraceuticals, and cosmetic products on the market. Also, a drug for the treatment of onychomycosis, a nail lacquer with ciclopirox that contains a chitosan derivative (hydroxypropyl chitosan), is available on the market. Possible explanations for the absence of drug delivery systems based on complexes of chitosan with different polymers are strict regulatory aspects related to the pharmaceutical industry and a clear need to demonstrate their safety as pharmaceutical excipients. Fulfilment of these requirements can take a long time, but in the close future, such preparations may be launched onto the market, taking into account patients´ needs unsatisfied with conventional drugs.

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Biokompatibilni nekovalentni kompleksi hitozana sa različitim polimerima: svojstva i primena kao nosača lekovitih supstanci

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Kratak sadržaj

Formulacija biokompatibilnih nosača lekovitih supstanci na bazi katjonskog biopolimera hitozana i prirodnih ili sintetskih polimera predstavlja značajan istraživački interes. Stoga je cilj ovog rada sagledati njihovu potencijalnu primenu kao nosača lekovitih supstanci. Najistraženije blende polimera na bazi hitozana su polielektrolitni kompleksi (PEK) dobijeni uspostavljanjem jonskih interakcija sa biokompatibilnim polianjonima, npr. alginatom, pektinom, ksantan gumom, karagenanom, karboksimetilcelulozom i kolagenom. U zavisnosti od uslova pripreme, mogu se formulisati PEK u vidu membrana/filmova, hidrogelnih perli, nanočestica, mikročestica ili drugih tipova nosača, sa ciljem postizanja kontrolisanog (npr. produženog, odloženog, kolon-specifičnog i pH-zavisnog) oslobađanja lekovitih supstanci. PEK su pogodni za inkapsulaciju hidrofilnih ili lipofilnih lekovitih supstanci različitih molekulskih masa. Inkapsulacija obezbeđuje očuvanje njihove strukture, aktivnosti, poboljšanje apsorpcije, smanjenje štetnih efekata i dugoročnu stabilnost *in vitro* i *in vivo*. Biokompatibilne strukture nalik kompleksima na bazi hitozana mogu se formirati i uspostavljanjem vodoničnih veza, kao što je slučaj sa polivinil alkoholom. Njihovo bubrenje ne zavisi od pH. Inkapsulirane lekovite supstance se najčešće oslobađaju prema nekom od već poznatih tipova difuzije. Dodatno, različiti derivati hitozana (npr. karboksimetilhitozan, trimetilhitozan, akril derivati hitozana) sintetisani su radi poboljšanja rastvorljivosti polimera u vodi u širokom opsegu pH i povećanja kapaciteta za inkapsulaciju lekovitih supstanci tako dobijenih PEK, koji takođe predstavljaju obećavajuće nosače.

Ključne reči: hitozan, polielektrolitni kompleks, biokompatibilnost, nosači lekovitih supstanci, kontrolisano oslobađanje lekovitih supstanci

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UNIVERZITET U BEOGRADU
FARMACEUTSKI FAKULTET
03 br. 3/76
13.09.2023. godine

Na osnovu Statuta Univerziteta u Beogradu, prodekan Fakulteta je dana 13.09.2023. godine, donela

ODLUKU

ODOBRAVA se Ćirić Ani, studentu Farmaceutskog fakulteta, indeks br. 29/17, produžetak roka za završetak studija za školsku 2023/24.

Obrazloženje

Ćirić Ana, student na studijskom programu DAS: F. tehnologija, podneo je zahtev 03 br. 3/76 od 13.09.2023. godine prodekanu Fakulteta da mu se odobri produžetak roka za završetak studija.

Prodekan Fakulteta je razmatrala zahtev 13.09.2023. godine i ocenila da je zahtev osnovan, te je doneto rešenje kao u dispozitivu.

PRAVNA POUKA: Protiv ove odluke imenovani ima pravo prigovora dekanu Fakulteta u roku od 8 (osam) dana od dana prijema istog.

Odluku dostaviti: Imenovanoj-om, dekanu i Odseku za nastavu i studentska pitanja.

PRODEKAN ZA POSLEDIPLOMSKU NASTAVU
I KONTINUIRANU EDUKACIJU

Prof. dr Sandra Vezmar Kovačević