Резюмета на научните трудове на Крум Кафеджийски

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Свойствата на различните тиолатни полимери, които са синтезирани и характеризирани в тази дисертация, имат много предимства от гледна точка на контролираното освобождаване на лекарствата.

В рамките на тази дисертация, четири нови тиомери на базата на добре установените мукоадхезивни ексципиенти бяха синтезирани: Ch-TEA, Ch-GSH, PAA-GSH и HA-Cys. Предимството на тиоалкиламидинната субституция на Ch-TEA е, че се изключва формирането на циклични нетиолни странични продукти по време на синтеза и съхранение за разлика при конюгата Ch-TBA, където 2-iminothiolane се използва като модифициращ агент. Пълен факториален дизайн се използва за оценка на влиянието на различните фактори по време на присъединителната реакция. По-стриктен контрол върху образуването на дисулфидни връзки може да се осъществи чрез въвеждането на допълнителен етап в синтезата на конюгата като третиране на получения продукт с подходящ редуциращ агент като tris(2-carboxyethyl)phosphine hydrochloride (TCEP). Една нова стратегия, която също е обект на тази дисертация, е директната имобилизация на свободен GSH върху chitosan или poly(acrylic acid), което довежда до формирането на един нов клас тиомери, които предлагат сравнително по-високи увеличаващи пърмишъна свойства. Целта на тиолната модификация на хиалуронова киселина е да се синтезира един нов конюгат с подобрени мукоадхезивни свойства и значително по-ниска скорост на биоразграждане. Ние описваме тук успешната химическа модификация на chitosan и хиалуронова киселина посредством двойната каталитична система- carbodiimide/ N-hydroxysuccinimide. Изследванията проведени с конюгата Ch-GSH разкриват забележимите му качества, които са добра основа за разработването на оптимизирани мукоадхезивни системи за контролирано освобождаване. Конюгатът проявява удължено време на адхезия върху мукоза, което е в следствие на балансирани кохезивни и мукоадхезивни свойства. Гореспоменатото подобрение в свойствата на конюгата може да се обясни чрез комбинирането на следните ефекти. Избирането на лигандата глутатион се основава на pK_a стойността на тиолната й група и на скоростта на разтваряне. Стабилността на конюгата Ch-GSH спрямо автоокисление при физиологически условия също е важно свойство за неговите потенциални клинични приложения. Вследствие на установените оптимални реакционни условия, получената степен на модификация беше по-висока от 250

μmol/g, което разбира се със сигурност осигурява достатъчна концентрация на тиолни групи върху полимера. Резултатите показват директна корелация между съдържанието на тиолни групи и времето на адхезия. Кохезивността се увеличава незабавно във водна среда поради протичащ процес на омрежване в самия полимер. По този начин той осигурява целостта и стабилността на полимерната матрица по време на адхезия.

Резултатите потвърждават ясното предимство на новата стратегия за разработването на тиомер/GSH увеличаваща пърмишъна система като се базира от една страна на имобилизирането на GSH върху полимерния скелет, за да се избегне разреждането на несвързания GSH в гастроинтестиналния тракт и от друга, разработването на един нов тип тиомери притежаващи по-силни редукционни свойства. Редукционните свойства на конюгатите Ch-GSH и PAA-GSH се определят от естеството на лигандата. Вероятният механизъм, който е отговорен за увеличения пермеабилитет в присъствие на конюгата.

Ch-GSH, се основава на инхибирането на ензима протеин тирозин фосфатаза (PTP) от редуцираната форма на GSH. Този ензим дефосфорилира тирозиновите остатъци на оклудина, за който се предполага, че влияе върху отварянето на здравите връзки между клетките. Следователно, инхибирането на PTP от редуцирания глутатион следователно ще доведе до фосфорилиране и отваряне на здравите връзки между клетките. Поради автоокисление на GSH, количеството на активния GSH намалява, като по този начин води до намаляване на концентрацията му, от което следва и намаление на увеличението на пърмишъна. Следователно, присъствието на тиолатния полимер е от съществено значение, тъй като предпазва окислението на глутатиона на повърхността на мукозата.

Въпреки химическата модификация, тиомерите са все още биоразграждащи се и проявяват по-слабо разграждане в сравнение с немодифицираните полимери. Беше установено, че скоростта на разграждане силно зависи от количеството тиолни групи, стойността на pH на реакционната среда, структурата на конюгатите (естеството на лигандата и вида на химическата връзка), омрежването на тиомерите и молекулната маса на конюгатите.

Тиолната модификация на полимерите модулира скоростта на освобождаване от матричните таблетки, което води до кинетика, отклоняваща се от закона на Фик, и до удължено освобождаване на веществото. Скоростта на освобождаване зависи също и от молекулната маса на моделното вещество. Като се има предвид факта, че в системата протича процес на окисление на конюгатната матрица *in situ,* което води до формирането на дисулфидни връзки и омрежване на полимера, освобождаването на моделното вещество може би се контролира от този допълнителен механизъм. Процесът на омрежване намалява скоростта на дифузия на макромолекулите и допринася до зависимост на скоростта на освобождаване от молекулната маса, съответно от размера на молекулата.

Освен силно изразеният ефект на увеличаване пърмишъна на главно парацелуларно транспортирани хидрофилни съединения, тиомерите значително увеличават аборбцията на липофилни субстрати на P-gp и multidrug resistance protein (MRP) както сакуинавир. Поради прибавянето на 0.5% (w/v) Ch-TBA и 0.5% (w/v) GSH, пърмишъна на сакуинавир се подобрява 1,6 пъти като се използва Caco-2 monolayer и 2,1 пъти през интестинална мукоза от плъх. Тези резултати предполагат, че тиолатните полимери могат да бъдат потенциално ценни средства за подобряване оралната бионаличност на субстрати на P-gp и multidrug resistance protein (MRP). Както беше демонстрирано в това изследване, ко-администрирането на системата Ch-TBA/ GSH води до трикратно увеличение на пърмишъна на микропротеина McoEeTI. Прибавянето на инхибитора BBI към системата Ch-TBA/ GSH, не оказва никакво влияние върху пърмишъна на McoEeTI in vitro. Въпреки, че не се получава никакво подобрение in vitro, смята се, че ко-администрирането на BBI има съществено влияние върху оралната бионаличност на микропротеина McoEeTI. Като се инхибират протеолитичните ензими разграждащи McoEeTI, като трипсин и химотрипсин, би могло да се очаква подобряване на бионаличността им in vivo.
Като се основава на всички тези опити, новите тиомери се смятат, че са многообещаващи нови ексципиенти за разработването на различни системи за контролирано освобождаване.

I. Списък на публикации в периодични научни издания, които са свързани с докторската дисертация

1. **Krum Kafedjiiski**, Alexander H. Krauland, Martin H. Hoffer, Andreas Bernkop-Schnürch. Synthesis and in vitro evaluation of a novel thiolated chitosan. Biomaterials 2005; 26: 819-862. [IF = 8.312]

In order to achieve the same properties as chitosan-TBA and to overcome at the same time its insufficient stability, the aim of this study was to evaluate the imidoester reaction of isopropyl-S-acetylthioacetimidate for the chemical modification of chitosan and to study the properties of the resulting chitosan-thioethylamidine (TEA) derivative. The thioalkylamidine substitute was introduced without the formation of N-substituted non-thiol products**.** The resulting conjugates exhibited 1.05 ± 0.17% or 139.68 ± 17.13 µmol immobilized free thiol groups per gram polymer and a total amount of reduced and oxidized thiol groups of 1.81 ± 0.65% or 179.46 ± 67.95 µmol/g polymer. By the immobilization of thiol groups, mucoadhesion was strongly improved due to the formation of disulfide bonds with mucus glycoproteins. Chitosan-TEA was investigated regarding to its mucoadhesive properties via tensile studies and the rotating cylinder method. In tensile studies the total work of adhesion (TWA) of chitosan-

TEA was increased 3.3-fold in comparison to unmodified chitosan. Results from the rotating cylinder method showed an improvement ratio of 8.9 for chitosan-TEA compared with unmodified chitosan. In spite of the immobilization of thiol groups onto chitosan, its swelling behavior in aqueous solutions was not significantly altered. Cumulative release studies out of matrix tablets comprising the chitosan-TEA and the model compound fluorescence labeled dextrane (FD_4) demonstrated a controlled release over three hours with a trend towards a pseudo zero-order kinetic. Because of these features the new chitosan thioamidine conjugate might represent a promising new polymeric excipient for various drug delivery systems.

2. **Krum Kafedjiiski,** Martin H. Hoffer, Martin Werle, Andreas Bernkop-Schnürch. Improved synthesis and in vitro characterization of chitosan- thioethylamidine conjugate. Biomaterials 2006; 27: 127-135. [IF = 8.312]

The aim of this study was to establish improved reaction conditions for the synthesis of chitosan-thioethylamidine (Ch-TEA) conjugate and to evaluate the properties of the obtained Ch-TEA conjugate. The influence of different factors on the coupling reaction, such as concentration of chitosan solution, employment of reducing agent and deprotection of S-acetyl groups, was evaluated. The cohesive properties and stability of the obtained conjugate were evaluated by disintegration test and by oxidation experiments, respectively. The adhesive properties of Ch-TEA conjugate were evaluated in vitro on freshly excised porcine mucosa via tensile studies and the rotating cylinder method. The permeation-enhancing effect of Ch-TEA conjugate was evaluated in Ussing-type chambers by using rhodamine 123 as model compound.

The resulting conjugate displayed 225 µmol immobilized free thiol groups and 102 µmol disulfide bonds per gram polymer. The degree of modification depends mostly on the chitosan concentration employed and the deprotection of S-acetyl groups with hydroxylamine. During oxidation studies the amount of thiol groups decreased by 61%. Disintegration studies of tablets comprising Ch-TEA demonstrated stability for 48 h. In tensile studies, the total work of adhesion of the conjugate was determined to be 5.1-fold increased in comparison to unmodified chitosan. Results from the rotating cylinder method showed more than a 13-fold increase in the adhesion time of thiolated chitosan versus unmodified chitosan. The apparent permeability coefficient (P_{app}) of the system 0.5% (w/v) Ch-TEA conjugate with 5% (w/v) glutathione was calculated to be 5.35×10^{-8} cm/s, while the P_{app} value of the system 0.5% (w/v) unmodified chitosan was 1.73×10^{-8} cm/s. These features should render Ch-TEA useful as an excipient for various drug delivery systems.

3. **Krum Kafedjiiski**, Florian Föger, Martin Werle, Andreas Bernkop-Schnürch. Synthesis and in Vitro Evaluation of a Novel Chitosan- Glutathione Conjugate. Pharmaceutical Research 2005; 22: 1480-1488. [IF = 3.952]

Purpose. It was the aim of this study to synthesize and characterize a novel chitosan-glutathione (GSH) conjugate providing improved mucoadhesive and permeation-enhancing properties.

Methods. Mediated by carbodiimide and N- hydroxysuccinimide, glutathione was covalently attached to chitosan via the formation of an amide bond. The adhesive properties of chitosan-GSH conjugate were evaluated *in vitro* on freshly excised porcine mucosa via tensile studies and the rotating cylinder method. The cohesive properties and stability of the resulting conjugate were evaluated by disintegration test and by oxidation experiments, respectively. The permeation-enhancing effect of the chitosan-GSH/ GSH system was evaluated in Ussing chambers by using rhodamine 123 as model compound.

Results. The obtained conjugate displayed 265.5 µmol immobilized free thiol groups and 397.9 µmol disulfide bonds per gram polymer. Because of the formation of disulfide bonds within the polymer, the stability of matrix tablets could be strongly improved. In tensile studies, the total work of adhesion of the conjugate was determined to be 9.9-fold increased in comparison to unmodified chitosan. Results from the rotating cylinder method showed more than 55-fold increase in the adhesion time of thiolated chitosan versus unmodified chitosan. In addition, the conjugate in combination with GSH displayed a 4.9-fold higher permeation-enhancing effect compared with unmodified chitosan.

Conclusions. Because of the improved mucoadhesive and cohesive properties, and the strong permeation-enhancing effect of the chitosan-GSH conjugate/ GSH system, the novel thiolated chitosan seems to represent a promising multifunctional excipient for various drug delivery systems.

4. **Krum Kafedjiiski**, Martin Werle, Florian Föger, Andreas Bernkop-Schnürch. Synthesis and in vitro characterization of a novel poly(acrylic acid)-glutathione conjugate J. Drug Del. Sci. Tech., 2005; 15 (6): 411-417. [IF = 1.088]

The purpose of the present study was to improve the multifunctional properties of poly(acrylic acid) by the covalent attachment of glutathione. The adhesive properties of poly(acrylic acid)-glutathione (PAA-GSH) conjugate were evaluated *in vitro* on freshly excised porcine mucosa via tensile studies and the rotating cylinder method. The permeation-enhancing effect of the conjugate in combination with glutathione was evaluated in Ussing chambers by using sodium fluoresceine as model compound. The resulting PAA-GSH conjugate displayed 353.7 ± 41.8 (n=3) µmol immobilized free thiol groups and 309.8 ± 27.3 (n=3) µmol disulfide bonds per gram polymer. In aqueous solutions, the modified polymer demonstrated improved cohesive properties. Due to the immobilization of glutathione, the swelling velocity of the polymer was 4-fold accelerated. Tensile studies showed that the mucoadhesive properties of poly(acrylic acid) were strongly improved by the covalent attachment of glutathione. The adhesion time of PAA-GSH was more than 14-fold higher in comparison to unmodified poly(acrylic acid).

Furthermore, the conjugate exhibited a 1.77-fold higher permeation-enhancing effect compared with the control. According to the results of the present study, PAA-GSH conjugate represents a very promising novel thiomer for the development of various mucoadhesive drug delivery systems.

5. **Krum Kafedjiiski**, Florian Föger, Martin Werle, Andreas Bernkop-Schnürch. Evaluation of in vitro enzymatic degradation of various thiomers and cross-linked thiomers. Drug Develop. Ind. Pharmacy, 2007; 33: 199- 208. [IF = 2.006]

It was the aim of this study to examine the biodegradability of thiomers and of cross-linked thiomers in comparison with unmodified polymers. Disulfide-crosslinked conjugates were prepared by air oxidation at room temperature. Thiomers were investigated by viscosity measurements and spectrophotometric assays. The influence of different factors on the hydrolysis rate, such as the degree of modification of thiomers, structure of the conjugates, pH value of the reaction medium and the impact of the process of crosslinking were evaluated. Due to the modification, thiolated chitosans degraded 12.9 - 24.7 % less than unmodified chitosan in the framework of viscosity measurements. In addition, the hydrolysis degree of thiolated alginates and modified carboxymethylcelluloses was 25.6 - 32.4 % and 18.4 - 27.0 % lower, respectively, in comparison to the corresponding unmodified polymers. Conjugates with higher coupling rate of thiol groups were degraded even more slowly. Moreover, the crosslinking process via disulfide bonds additionally reduced the rate of thiomers degradation. The range of degradation rates achieved in vitro could be modified by alterations of the contents of thiol and disulfide groups, as well as by suitable design of the polymer structure and ligands used.

These results represent helpful basic information for the development of mucoadhesive drug delivery systems, implantable delivery systems and tissue engineering constructs.

6. **Krum Kafedjiiski**, Ram Jetti, Florian Föger, Herbert Hoyer, Martin Werle, Martin Hoffer and Andreas Bernkop-Schnürch. Synthesis and in vitro evaluation of thiolated hyaluronic acid for mucoadhesive drug delivery. Int. J. Pharm., 2007; 343: 48- 58. [IF = 3.785]

It was the aim of this study to synthesize and characterize a novel hyaluronic acid- cysteine ethyl ester (HA-Cys) conjugate providing improved mucoadhesive properties and a significantly lowered biodegradation rate. Mediated by carbodiimide and N- hydroxysuccinimide, L-cysteine ethyl ester hydrochloride was covalently attached to hyaluronic acid (HA, hyaluronan) via the formation of an amide bond. The adhesive properties of HA-Cys conjugates were evaluated in vitro on a freshly excised porcine mucosa via the rotating cylinder method. The cohesive properties of the resulting conjugates were evaluated by oxidation experiments. Biodegradability studies were carried out by viscosity measurements and spectrophotometric assays. Release studies were performed with fluorescein isothiocyanate-dextrans (FD) as model compounds. The obtained conjugate displayed 201.3 ± 18.7 µmol immobilized free thiol

groups and 85.7 ± 22.3 μmol disulfide bonds per gram polymer. Results from the rotating cylinder method showed more than 6.5-fold increase in the adhesion time of HA-Cys versus unmodified HA. In aqueous solutions, the obtained conjugate demonstrated improved cohesive properties. The hydrolysis degree of HA-Cys was lower compared with the corresponding unmodified HA in the framework of viscosity experiments. In addition, the crosslinking process via disulfide bonds additionally reduced the rate of degradation of the new derivative. Cumulative release studies out of matrix tablets comprising HA-Cys and the model compound FD demonstrated a sustained drug release for more than 12 h due to in situ formation of inter- and intramolecular disulfide bonds in the thiomer matrix. According to the results of the present study, this novel thiolated polymer seems to represent a promising multifunctional excipient for the development of various drug delivery systems.

7. **Krum Kafedjiiski** (2004). Multifunctional Polymeric Excipients in Non-Invasive Delivery of Hydrophilic Macromolecular Drugs: The Thiomer-Technology. The Drug Delivery Companies Report Autumn/Winter 2004, 47-50

Due to the immobilisation of thiol groups on polymeric excipients such as chitosans and poly(acrylates) their mucoadhesive, permeation enhancing and enzyme inhibitory properties are signifi cantly improved. Compared with oral drug delivery systems comprising unthiolated polymers, the effi cacy of delivery systems comprising thiolated polymers is signifi cantly higher. Thiomers appear to represent a promising new generation of multifunctional polymers for non-invasive delivery of hydrophilic macromolecular drugs. Companies already making use of this novel technology are MucoBiomer, Leobendorf, Austria and ThioMatrix, Innsbruck, Austria.

8. Florian Föger, **Krum Kafedjiiski**, Herbert Hoyer, Brigitta Loretz, Andreas Bernkop-Schnürch. Enhanced transport of P-glycoprotein substrate saquinavir in presence of thiolated chitosan. Journal of Drug Targeting, 2006; 00 (0): 1- 8. [IF = 2.768]

It was the aim of this study to investigate the effect of chitosan-4-thiobutylamidine (Ch-TBA) and reduced glutathione (GSH) on the absorption of P-glycoprotein (P-gp) and multidrug resistance protein (MRP) substrate saquinavir in vitro and in vivo. Bidirectional transport studies were performed with Caco-2 cell monolayers and additionally with freshly excised rat small intestinal mucosa mounted in Ussing type chambers. Furthermore, a delivery system based on Ch-TBA and GSH was
evaluated in vivo in rats. The functional activity of the efflux pumps in Caco-2 cells and rat intestinal mucosa during the experiment was proven by the efflux ratio of saquinavir, which was 6.4 for Caco-2 cells and 2.1 for rat intestinal mucosa, respectively. Ch-TBA and particularly the combination of Ch-TBA with GSH enhanced apical (AP) absorption and decreased the secretory transport of saquinavir. In

presence of 0.5% Ch-TBA and 0.5% GSH, the uptake of saquinavir was 1.6-fold improved in Caco-2 monolayer and 2.1-fold improved in rat intestinal mucosa. In vivo, the area under the plasma concentration time curve (AUC) of saquinavir was 1.4-fold and Cmax 1.6-fold increased, in comparison with control.

Results of this study showed that Ch-TBA in combination with GSH can be an interesting tool for increasing the oral bioavailability of actively secreted compounds.

9. Martin Werle, **Krum Kafedjiiski**, H. Kolmar, Andreas Bernkop-Schnürch. Evaluation and improvement of the properties of the novel cystine-knot microprotein McoEETI for oral administration. Int. J. Pharm., 2007; 332: 72- 79. [IF = 3.785]

Cystine-knot microproteins exhibit several properties that make them highly interesting as scaffolds for oral peptide drug delivery. It was therefore the aim of the study to evaluate the novel clinically relevant cystine-knot microprotein McoEeTI regarding its potential for oral delivery. Additionally, based on the gained results, important features of McoEeTI were improved. Enzymatic degradation was caused by chymotrypsin, trypsin and porcine small intestinal juice whereas McoEeTI was stable towards elastase, membrane bound proteases, pepsin and porcine gastric juice. Only minor McoEeTI degradation was observed during a 24 h incubation period in rat plasma. In the presence of various physiological ions about 50% of McoEeTI formed di- and/or trimers. Papp value of McoEeTI was determined to be (7.4±0.4)×10−6 cm/s. Sodium caprate and polycarbophil-cysteine (PCP-Cys) had no beneficial effect on McoEeTI permeation, whereas the utilization of a chitosan-thiobutylamidine (Chito-TBA) system improved McoEeTI permeation 3-fold. Enzymatic stability could be strongly improved by the utilization of Bowman-Birk-Inhibitor (BBI) as well as PCP-Cys. In conclusion, this study indicates that McoEeTI represents a promising candidate as a novel scaffold for oral peptide drug delivery.

10. Andreas Bernkop-Schnürch, Martin H Hoffer, **Krum Kafedjiiski** (2004). Thiomers for oral delivery of hydrophilic macromolecular drugs. Expert Opinion On Drug Delivery, 1(1), 87-98. [IF = 4.116]

Within recent years thiolated polymers— designated thiomers— appeared as promising new tool in oral drug delivery. Thiomers are obtained by the immobilisation of thiol bearing ligands to mucoadhesive polymeric excipients. By formation of disulfide bonds with mucus glycoproteins, the mucoadhesive properties of thiomers are up to 130-fold improved compared to the corresponding unmodified polymers. Due to the formation of inter- and intramolecular disulfide bonds within the thiomer itself, matrix tablets and particulate delivery systems show strong cohesive properties resulting in comparatively higher

stability, prolonged disintegration times and a more controlled drug release. The permeation of hydrophilic macromolecular drugs through the gastrointestinal mucosa can be improved by the use of thiomers. Furthermore some thiomers exhibit improved inhibitory properties towards gastrointestinal peptidases.

The efficacy of thiomers in oral drug delivery could be demonstrated by various in vivo studies. A pharmacological efficacy of 1%, for instance, was ahieved in rats by oral administration of calcitonin tablets comprising a thiomer. Furthermore, tablets comprising a thiomer and pegylated insulin resulted in a pharmacological efficacy of 7% after oral application to diabetic mice. Low molecular weight heparin embedded in thiolated polycarbophil led to an absolute bioavailability of at least 20% after oral administration to rats. In all these studies, formulations comprising the corresponding unmodified polymer had only a marginal or no effect. According to these results drug carrier systems based on thiomers seem to be a promising tool for oral delivery of hydrophilic macromolecular drugs.

II. Списък на публикациите в периодични научни издания, които не са свързани с докторската дисертация

II.1. Публикации в чужди научни списания

11. Florian Föger, Herbert Hoyer, **Krum Kafedjiiski**, Michael Thaurer, Andreas Bernkop-Schnürch. In vivo comparison of various polymeric and low molecular mass inhibitors of intestinal P-glycoprotein. Biomaterials 2006; 27: 5855-5860. [IF = 8.312]

Several polymers have been reported to modulate drug absorption by inhibition of intestinal P-glycoprotein (P-gp). The aim of the present study was to provide a direct in vivo comparison of delivery systems based on Pluronic P85, Myrj 52 and chitosan-4-thiobutylamidine (Ch-TBA) in vivo in rats, using rhodamine-123 (Rho-123) as representative P-gp substrate. Furthermore, the postulated low molecular mass P-gp inhibitors 6-mercaptopurine and reduced glutathione (GSH) were evaluated in vitro and in vivo. In vitro, the permeation enhancing effect of 6-mercaptopurine, GSH, Pluronic P85, Myrj 52, and the combination of Ch-TBA with GSH was evaluated by using freshly excised rat intestinal mucosa mounted in Ussing-type diffusion chambers. In comparison to buffer only,

Rho-123 transport in presence of 100 mM 6-mercaptopurine, 0.5% (w/v) GSH, 0.5% (w/v) Pluronic P85, 0.5% (w/v) Myrj 52 and the combination of 0.5% (w/v) Ch-TBA/ 0.5% (w/v) GSH, was 2.1, 1.6, 1.9, 1.8, 3.0-fold improved, respectively. In vivo in rat, entericcoated tablets based on Pluronic P85, Myrj 52 or Ch-TBA/GSH increased the area under the plasma concentration time curve (AUC0–12) of Rho-123 1.6-fold, 2.4-fold, 4.3-fold, respectively, in comparison to control only. Contrariwise, the low molecular mass excipients 6-mercaptopurine and GSH showed no significant effect in vivo at all.

This in vivo study showed that polymeric P-gp inhibitors and especially the delivery system based on thiolated chitosan significantly increased the oral bioavailability of P-gp substrate Rho-123.

12. Sayeh Majzoob, Fatemeh Atyabi, Farid Dorkoosh, **Krum Kafedjiiski**, Brigitta Loretz, Andreas Bernkop-Schnürch. Pectin-cysteine conjugate: synthesis and in-vitro evaluation of its potential for drug delivery. Journal of Pharmacy and Pharmacology, 2006: 58 (12): 1601-1610. [IF = 2.161]

This study was aimed at improving certain properties of pectin by introduction of thiol moieties on the polymer. Thiolated pectin was synthesized by covalent attachment of cysteine. Pectin–cysteine conjugate was evaluated for its ability to be degraded by pectinolytic enzyme. The toxicity profile of the thiolated polymer in Caco-2-cells, its permeation enhancing effect and its mucoadhesive and swelling properties were studied. Moreover insulin-loaded hydrogel beads of the new polymer were examined for their stability in simulated gastrointestinal conditions and their drug release profile. The new polymer displayed 892.27 ± 68.68 mmol thiol groups immobilized per g polymer, and proved to have retained its biodegradability, upon addition of Pectinex Ultra SPL in-vitro, determined by viscosity measurements and titration method. Pectin–cysteine showed no severe toxicity in Caco-2 cells, as tested by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. Moreover, the synthesized polymer exhibited a relative permeation enhancement ratio of 1.61 for sodium fluorescein, compared to unmodified pectin. Pectin–cysteine conjugate exhibited approximately 5-fold increased in in-vitro adhesion duration and significantly improved cohesive properties. Zinc pectin–cysteine beads showed improved stability in simulated gastrointestinal media; however, insulin release from these beads followed the same profile as unmodified zinc pectinate beads. Due to favourable safety and biodegradability profile, and improved cohesive and permeation-enhancing properties, pectin–cysteine might be a promising excipient in various transmucosal drug delivery systems.

13. Herbert Hoyer, **Krum Kafedjiiski**, Florian Föger, Andreas Bernkop-Schnürch. Design and evaluation of a new gastrointestinal mucoadhesive patch system containing Chitosan-Glutathione. Drug Develop. Ind. Pharmacy, 2007; 33 (12): 1289- 96. [IF = 2.006]

Within this study, a novel gastrointestinal patch system was developed and investigated regarding water-absorbing capacity, adhesive properties, in vitro release, unidirectional release and permeation enhancing effect. Water uptake studies revealed that the weight of patch systems with Ch-GSH increased about 44.5 ± 2.3 mg (127%) after 90 min. This patch system remained even after 180 h on the mucosa and released 49.7 ± 0.7% of FD4 within 8 h. A 2.5- fold higher transport of FD4 can be obtained in contrast to control. In conclusion this patch system could be an interesting possibility

for the transport through the intestinal mucosa of macromolecules which will normally be degraded in the intestinal tract.

14. Herbert Hoyer, Wolfgang Schlocker, **Krum Kafedjiiski** and Andreas Bernkop-Schnürch. Preparation and Evaluation of Microparticles from Thiolated Polymers via Air Jet Milling. Eur. J. Pham., Biopharm., 2008: 69 (2): 476- 485. [IF = 4.245]

Microparticles were formulated by incorporation of the model protein horseradish peroxidase in (thiolated) chitosan and (thiolated) poly(acrylic acid) via co-precipitation. Dried protein/polymer complexes were ground with an air jet mill and resulting particles were evaluated regarding size distribution, shape, zeta potential, drug load, protein activity, release pattern, swelling behaviour and cytotoxicity. The mean particle size distribution was 0.5–12 µm. Non-porous microparticles with a smooth surface were prepared. Microparticles from (thiolated) chitosan had a positive charge whereas microparticles from (thiolated) poly(acrylic acid) were negatively charged. The maximum protein load for microparticles based on chitosan, chitosan–glutathione (Ch–GSH), poly(acrylic acid) (PAA) and for poly(-acrylic acid)–glutathione (PAA–GSH) was 7 ± 1%, 11 ± 2%, 4 ± 0.2% and 7 ± 2%, respectively. The release profile of all microparticles followed a first order release kinetic. Chitosan (0.5 mg), Ch–GSH, PAA and PAA–GSH particles showed a 31.4-, 13.8-, 54.2- and a 42.2-fold increase in weight, respectively. No significant cytotoxicity could be found. Thiolated microparticles prepared by jet milling technique were shown to be stable and to have controlled drug release characteristics. After further optimizations the preparation method described here might be a useful tool for the production of protein loaded drug delivery systems.

II. 2. Публикации в научни списания в България

15. **Крум Кафеджийски**, Стефан Кафеджийски. Разработване на стабилен фармацевтичен състав на разтвор за перорални капки, съдържащ Metamizole sodium monohydrate, МЕДИЦИНСКИ ПРЕГЛЕД, № 2/2015г.

Дефиниран е основния проблем при разработване на състав, основан на метамизол натрий - стабилност по отношение на разпадни продукти на активното вещество.

Изследвани са ефекта на вида на буфера, капацитета на буфера и pH на разтвора върху стабилността на метамизол натрий във воден разтвор.

В резултат са определени оптималните условия и състав на разтвора за перорални капки: цитратен буфер, капацитет 125 mM и pH 7.7.

16. **Крум Кафеджийски,** Евгени Григоров, Тони Веков. Ин витро изследване на нова Alginate Raft – forming oral suspension, МЕДИЦИНСКИ ПРЕГЛЕД, № 3/2015г.

Проведени са ин витро сравнителни изпитвания на нова Alginate Raft – forming перорална суспензия спрямо търговския продукт Gaviscon Cool Mint Liquid oral suspension (Reckitt Benckiser Healthcate, UK).

Резултатите показват, че при всяка стойност на pH, Alginate Raft susp. и Gaviscon susp. създават алгинатни гелове със сравними характеристики.

Представените данни потвърждават предлагания механизъм на действие на новата Alginate Raft суспензия.

17. **Крум Кафеджийски,** Евгени Григоров, Тони Веков. Разработване на фармацевтичен състав с приятни органолептични характеристики, съдържащ Acetylcysteine, МЕДИЦИНСКИ МЕНИДЖМЪНТ И ЗДРАВНА ПОЛИТИКА, № 2/2015г.

Разработен е фармацевтичен състав на прах за перорален разтвор, съдържащ Acetylcysteine чрез прилагане на подходяща и ефективна комплексна система за маскиране вкуса и мириса на acetylcysteine.

Установен е фармацевтично приемлив стабилизатор на acetylcysteine – citric acid и оптималната концентрация в условия на перорален воден разтвор – топла напитка.

Успешно е приложен нов метод за маскиране неприятния вкус и мирис на acetylcysteine и негови примеси чрез механично смесване на активното вещество с Kleptose Linecaps 17 в определено тегловно съотношение.

Използвано е походящо разреждащо вещество в продукта – полиол xylitol, който може да се използва и от диабетици.

18. **Крум Кафеджийски**, Евгени Григоров, Тони Веков. Ин витро сравнително освобождаване на ацетилсалицилова киселина от нови стомашно-устойчиви 100 mg таблетки, МЕДИЦИНСКИ ПРЕГЛЕД, № 4/2015г.

Изследван е профила на освобождаване на acetylsalicylic acid от продукта ASA 100 mg gastro-resistant tablets в различни среди съгласно ръководството „Quality of oral modified release product”, EMA/CHMP/QWP/428693/2013. Доказано е подобие на профила на разтваряне спрямо референтен продукт Aspirin Protect 100 mg gastro-resistant tablets чрез статистически фактор на подобие f2.
В изследването за стабилност е оценено поведението на продукта относно характеристиката на разтваряне и съдържание на разпаден продукт свободна салицилова киселина.

19. Валентина Белчева, **Крум Кафеджийски**, Евгени Григоров. История и развитие на концепцията за Исхемична болест на сърцето (ИБС), Списание Сърдечно-съдови заболявания, № 1/2015г.

Статията проследява някои от най-важните моменти от историята на Исхемична болест на сърцето. Тя разкрива и същността на утвърждаването на концепцията за рисковите фактори като предпоставка за развитието на ИБС.

III. Списък на резюмета от международни конгреси, симпозиуми, конференции (без пълен текст на доклада), публикувани в научни списания или сборници с резюмета на научната проява

20. **Krum Kafedjiiski** (12- 17 June, 2005). Synthesis and in Vitro Characterisation of a Novel Chitosan-Glutathione Conjugate. Pharmaceutical Sciences Fair § Exhibition, Nice, France; ДОКЛАД, Abstract SC-54

It was the aim of this study to synthesize and evaluate a novel chitosan-glutathione (GSH) conjugate providing improved mucoadhesive and permeation enhancing properties. Mediated by carbodiimide and N- hydroxysuccinimide, glutathione was covalently attached to chitosan via amide bond. The adhesive properties of chitosan-GSH conjugate were evaluated in vitro via tensile studies and the rotating cylinder method. The permeation enhancing effect of chitosan-GSH/ GSH system was evaluated in Ussing chambers by using rhodamine 123. The obtained conjugate exhibited 265 micromol immobilised free thiol groups and 398 micromol disulfide bonds per gram polymer. The total work of adhesion of the conjugate was determined to be 9.9-fold increased compared to unmodified chitosan. Results from the rotating cylinder method showed more than 55-fold increase in the adhesion time of the thiolated chitosan. In addition, the conjugate displayed a 5-fold higher permeation enhancing effect compared with unmodified chitosan. Because of the improved mucoadhesive and cohesive properties, and the strong permeation enhancing effect of the chitosan-GSH conjugate/ GSH system, the novel thiolated chitosan seems to represent a promising tool for various drug delivery systems.

21. **Krum Kafedjiiski** (22- 26 July 2006). Synthesis and in vitro evaluation of hyaluronic acid-cysteine conjugate. 33 rd Annual Meeting and Exposition of the Controlled Release Society, Vienna, Austria

Hyaluronic acid (HA) has been investigated as a drug delivery agent for various routes of administration including ophthalmic, nasal, pulmonary, parenteral and topical. It was the aim of this study to synthesize and characterize a novel hyaluronic acid-cysteine (HA-Cys) conjugate providing improved mucoadhesive properties and a significantly lowered biodegradation rate. Another purpose of that thiol modification was to combine the mucoadhesive potential of hyaluronic acid with the new thiomer technology for the improvement of mucoadhesion.

22. **Krum Kafedjiiski** (15 March 2004). Synthesis and in vitro evaluation of a novel thiolated chitosan. APV International Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Nuremberg, Germany

Different chitosan derivatives have been developed based on the known concept of the immobilization of thiol groups to the primary amino groups of chitosan. In such a way chitosan-cysteine conjugates, chitosan-thioglycolic acid conjugates and chitosan-4-thio-butyl-amidine conjugates have been obtained. The aim of this study was to evaluate the imidoester reaction of isopropyl-S-acetylthioacetimidate for the chemical modification of chitosan and to study the properties of the resulting chitosan-thioethylamidine (TEA) derivative. It was achieved by the modification of chitosan with the new reagent: isopropyl-S-acetylthioacetimidate (i-PATAI.HC1). Apart from the chemical modification, it was the purpose of this study to evaluate the mucoadhesive properties and swelling behavior of the new thiolated chitosan and to prove whether a controlled release can be provided out of such polymer conjugates when used as drug carrier matrix.

23. Hoyer H, Schlocker W, **Kafedjiiski K**, Bernkop-Schnürch A, Preparation and Evaluation of Chitosan-Glutathione Microparticles via Air Jet Milling. INNANO, October 2006, Innsbruck, Austria

The development and investigation of a mucoadhesive microparticulate delivery system based on thiolated polymers via air jet milling was the purpose of this study.

24. Wiwat Pichayakorn, Garnpimol C. Ritthidej, Brigitta Loretz, Ronny Martien, Thierry Schmitz, **Krum Kafedjiiski**, and Andreas Bernkop - Schnürch. Oral Gene Delivery: Comparison of Different Thiolated Chitosans as pDNA Carrier Matrix,33 rd Annual Meeting & Expositions of the Controlled Release Society. Jul 22 - 26, 2006. Vienna, Austria. CD - Rom. 2 pages (Poster #619 by W. Pichayakorn)

Different thiolated chitosans-pDNA nanoparticles were prepared by complex coacervation. The polymer-pDNA ratios directly influencing the encapsulation efficiency were observed by zeta potential and gel electrophoresis. Polymer types and ratios to pDNA affected the transfection rates, which were higher than pDNA solution used as control, due to higher stability and low cytotoxicity.

25. **Krum Kafedjiiski** (05 June 2005). Thiomers: Novel Promising Excipients for Oral Delivery of hydrophilic macromolecular Drugs. 4th Congress of Pharmacy, Sofia, Bulgaria

Within recent years thiolated polymers— designated thiomers— appeared as promising new tool in oral drug delivery. Thiomers are generated by the immobilisation of thiol bearing ligands to mucoadhesive polymeric excipients. By the formation of disulfide bonds with mucus glycoproteins, the mucoadhesive properties of thiolated polymers are up to 130-fold improved. Due to the formation of inter- and intramolecular disulfide bonds within the thiomer itself, matrix tablets or particulate delivery systems exhibit strong cohesive properties, resulting in comparatively higher stability, prolonged disintegration time and a more controlled drug release of the incorporated drug. The permeation of hydrophilic macromolecular drugs through gastrointestinal mucosa can be improved by the use of thiomers. In addition, some thiomers display improved inhibitory properties towards gastrointestinal peptidases. The efficacy of thiomers in oral drug delivery could be demonstrated by various in vivo studies. A pharmacological efficacy of 1% was achieved in rats by oral administration of calcitonin tablets comprising a thiomer. Tablets comprising a thiomer and pegylated insulin resulted in a pharmacological efficacy of 7% after oral application to diabetic mice. Furthermore, low molecular weight heparin incorporated in thiolated polycarbophil led to an absolute bioavailability of at least 20% after oral administration to rats. In these studies, formulations consisting of the corresponding unmodified polymer had only a marginal or no effect. According to these results, drug carrier systems based on thiomers appear to be a promising novel tool for oral delivery of hydrophilic macromolecular drugs.

26. **Krum Kafedjiiski** (03-07 November 2000): Technological and biopharmaceutical studies on Loratadine antihistamine drug substance, Abstract book. p. 27.

Loratadine is a long-acting tricyclic antihistamine with selective peripheral histamine H1- receptor antagonistic activity. The pronounced hydrophobic character of Loratadine drug substance requires compulsory, preliminary technological and biopharmaceutical studies with the purpose of creating of a drug form with an optimal therapeutic effect. Essential physical-chemical technological and in vitro biopharmaceutical properties of Loratadine were determined such as solubility in different solvents, pKa-value, distribution coefficient in octanol-water and hexan-water system, diffusion through artificial gastric and intestinal mucosa, dissolution in 0,1n HCl and 0,1n NaOH at temperature 25C and 37C. High sensitive physical-chemical methods were used such as Roentgen structural analysis, densitometrical analysis, resorption pattern, etc. Interaction between Loratadine and basic types of excipients used in tablet production as microcrystalline cellulose, lactose monohydrate, maize starch, mannitol, povidone K25 and magnesium stearate was studied by the method of differential scanning calorimetry.

27. BG66379 (B1) — 2013-11-29; PHARMACEUTICAL COMPOUND CONTAINING L-ALPHA-GLYCERYL-PHOSPHORYL-CHOLINE; **KAFEDZHIYSKI KROUM** [BG];DRAGANOV GEORGI [BG];

(57) Изобретението се отнася до фармацевтичен състав, съдържащ L-алфа-глицерилфосфорилхолин, предназначен за изготвяне на лекарствени форми с ноотропна терапевтична активност. Фармацевтичният състав, обект на изобретението, е стабилен, притежава добри реологични характеристики и се получава по опростена технологична схема на производство. Съставът е под формата на капсули, които съдържат активното вещество L-алфа-глицерилфосфорилхолин и помощни вещества: стабилизатор, синтетичната аморфна форма на магнезиев алуминометасиликат и магнезиев стеарат. Съставът може да включва допълнително и вещества като ацетил-1-карнитин хидрохлорид, алфа липоена киселина, витамин **B6,** витамин B9 и фолиева киселина или техни смеси.

28. BG66380 (B1) — 2013-11-29; POWDER MIXTURE FOR PERORAL SOLUTION CONTAINING L-ALPHA-GLYCERYL-PHOSPHORYL-CHOLINE; **KAFEDZHIYSKI KROUM** [BG]; DRAGANOV GEORGI [BG];

(57) Изобретението се отнася до прахообразна смес за перорален разтвор, съдържаща L-алфа-глицерилфосфорилхолин, която намира приложение във фармацевтичната промишленост за изготвяне на лекарствени форми с ноотропна терапевтична активност. Прахообразната смес съгласно изобретението съдържа активното вещество L-алфа-глицерилфосфорилхолин и стабилизатор, представляващ смес от синтетична аморфна форма на магнезиев алуминометасиликат и P-манитол, както и хлъзгащи вещества, подсладител и аромати. Сместа

може да включва допълнително и други активни вещества като ацетил-1-карнитин хидрохлорид, алфалипоена киселина, витамин B6, витамин B12, фолиева киселина или смеси от тях. Сместа за перорален разтвор съгласно изобретението притежава добри реологични характеристики, стабилна е при съхранение, има добра разтворимост във вода и добри органолептични свойства, получава се по опростена технологична схема на производство и е предназначена за дозиране в сашети.

29. 8358.000-EP (2012) ORAL DOSAGE SELF-EMULSIFYING COMPOSITIONS CONTAINING COMPLEXES OF DERIVATIZED INSULIN PEPTIDES WITH ALKYL SULFATE PERMEATION ENHANCERS; **Krum Kafedzhiyski**, Franklin Okumu, Ulrik Lytt Rahbek;

The submitted invention relates to self-nanoemulsifying compositions and nanoemulsion compositions for the oral administration of derivatized insulin peptide comprising a hydrophobic ion-pair complex of insulin derivative with an alkyl sulfate permeation enhancer. Compositions according to the invention have improved oral bioavailability compared to conventional pharmaceutical compositions such as solutions.

30. 8391.000-EP (2012) ORAL DOSAGE SELF-EMULSIFYING COMPOSITIONS CONTAINING COMPLEXES OF DERIVATIZED INSULIN PEPTIDES WITH MEDIUM CHAIN FATTY ACIDS PERMEATION ENHANCERS; **Krum Kafedzhiyski**, Franklin Okumu, Ulrik Lytt Rahbek.

The submitted invention relates to self-nanoemulsifying compositions and nanoemulsion compositions for the oral administration of derivatized insulin peptide comprising a hydrophobic ion-pair complex of insulin derivative with medium chain fatty acids permeation enhancer. Compositions according to the invention have improved oral bioavailability compared to conventional pharmaceutical compositions such as solutions.