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NEW DRUGS DELIVERY SYSTEMS BASED ON POLYELECTROLYTE COMPLEXES

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Spherical microparticles based on chitosan-polybetaine complexes have been obtained by a simple and economic method. FT-IR spectroscopy, TGA analysis and water swelling measurements confirmed the formation of chitosan-polybetaine complexes. Optical microscopy also showed that the microparticles thus obtained have a good sphericity, a "core-shell" structure and an average diameter ranging between 700-900μm. Taking into account the possibility of their use in medical and pharmaceutical fields, the retention and release capacity of chloramphenicol succinate sodium salt was investigated. It was obtain that the loaded and the released amount of drug depends on the experimental conditions used for the preparation of microparticles based on such complexes.

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INTRODUCTION

The design and preparation of drug delivery systems have attracted a great deal of interest in biomedical engineering, biomaterial science and pharmaceutical research,[1, 2] because of their many advantages over conventional forms: (i) protection of the biologically active drug molecules against degradation during transport; (ii) easy administration, reduced number of dosages and maintenance of constant drug levels in the desired range for prolonged periods; (iii) improvement of bioavailability of the low soluble drugs; (iv) reduction of drugs toxicity and less adverse side-effects; (v) delivery of two or more drugs from the same formulation. The concept of a polymeric drug system, consisting of a drug and a polymer carrier capable of delivering the drug has been first introduced in the 1970s.[3]

After more than 35 years of research and development of drug delivery systems, polymer carriers have become crucial in the design and preparation of controlled release formulations.[4, 5] Both synthetic and natural polymers have applications in drug delivery. Chitosan (CH), a natural polymer, is an interesting biomaterial due to its good biocompatibility, biodegradability, low toxicity, haemostatic potential, [6] good film forming character and anti-infecting activity. The presence of amino functional groups in chitosan can lead to the formation of microparticles, by different methods: crosslinking, [7] ionic complexation/ coacervation [8] or ionic gelation. [9]

The cationic nature of chitosan and the high charge density in acidic solution are also two of the most important properties of this polymer. Based on these properties, chitosan can interact with the negatively charged polymers on contact in an aqueous environment.[10] Polybetaines, discovered in 1957, are synthetic polymers having an anion:cation pair covalently bound by an alkyl located in the same monomer unit and possessing an onium group without hydrogen atoms as a cationic site.[11] Polybetaines are polymers with growing scientific and commercial interest due to their unique and specific properties. These polymers, having a structural similarity with peptides and the living matter, can serve as a model for proteins in the vicinity of the isoelectric point, or as surface covering surfaces of biomaterials, to improve their haemocompatibility. A combination between natural and synthetic polymers can lead to new polymeric materials with specific properties. Only few studies are cited in literature on the preparation and characterization of complexes based on chitosan and

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betaines of lower molecular weight.[12] For this reason, the present work is focused on the preparation and characterization of a new type of microparticles based on thecomplexes between chitosan and two poly(carboxybetaines)(PCB), in view of their potential applications in medical fields as drug carriers.

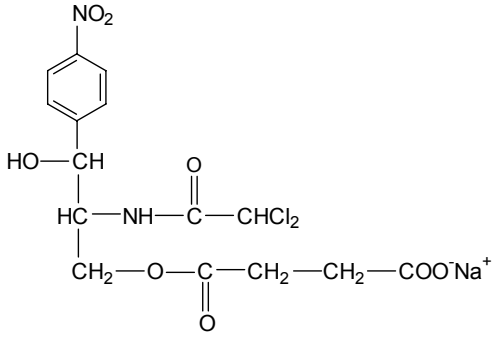
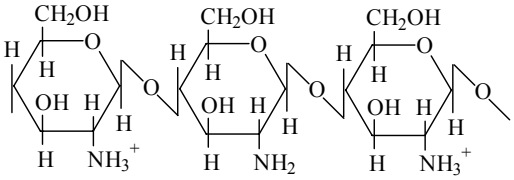
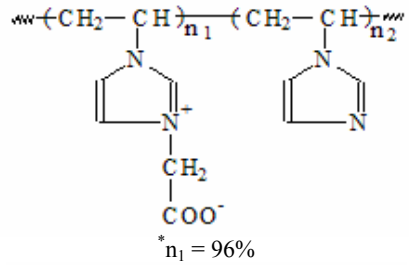
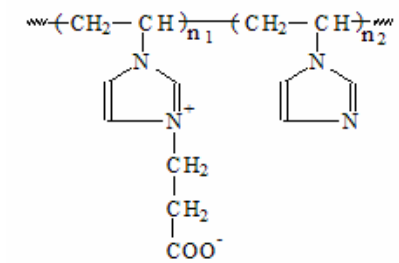
RESULTS AND DISCUSSION

To obtain a polymer-drug system, two main components are required: a polymeric support, which can be either synthetic or natural, and a drug that can be fixed on the support by chemical or physical bonds or by a combination of them, depending on the purpose of the treatment followed. In this case, the polymeric supports have been obtained by the interaction between a cationic polyelectrolyte (chitosan) and two linear poly(carboxybetaines) based on poly(N-vinylimidazole). The chemical structures of the drug and polymers used for the preparation of microparticles based on CH-PCB complexes are shown in Table 1.

The microparticles based on CH-PCB complexes were obtained by the complex coacervation method (Figure 1).

Table 1

Chemical structure of drug and starting polymers

Samples name	Chemical structure of drug and starting polymers
Chloramphenicol succinate sodium salt (CPh) Molecular formula: $C_{15}H_{15}Cl_2N_2NaO_8$	
chitosan	
Poly[1-vinyl-3-(1-carboxymethyl imidazolium betaine] PNVIB-1	 *n_1 = 96%
Poly[1-vinyl-3-(2-carboxyethyl imidazolium betaine] PNVIB-2	 *n_1 = 94%

*n_1 is the betainization degree

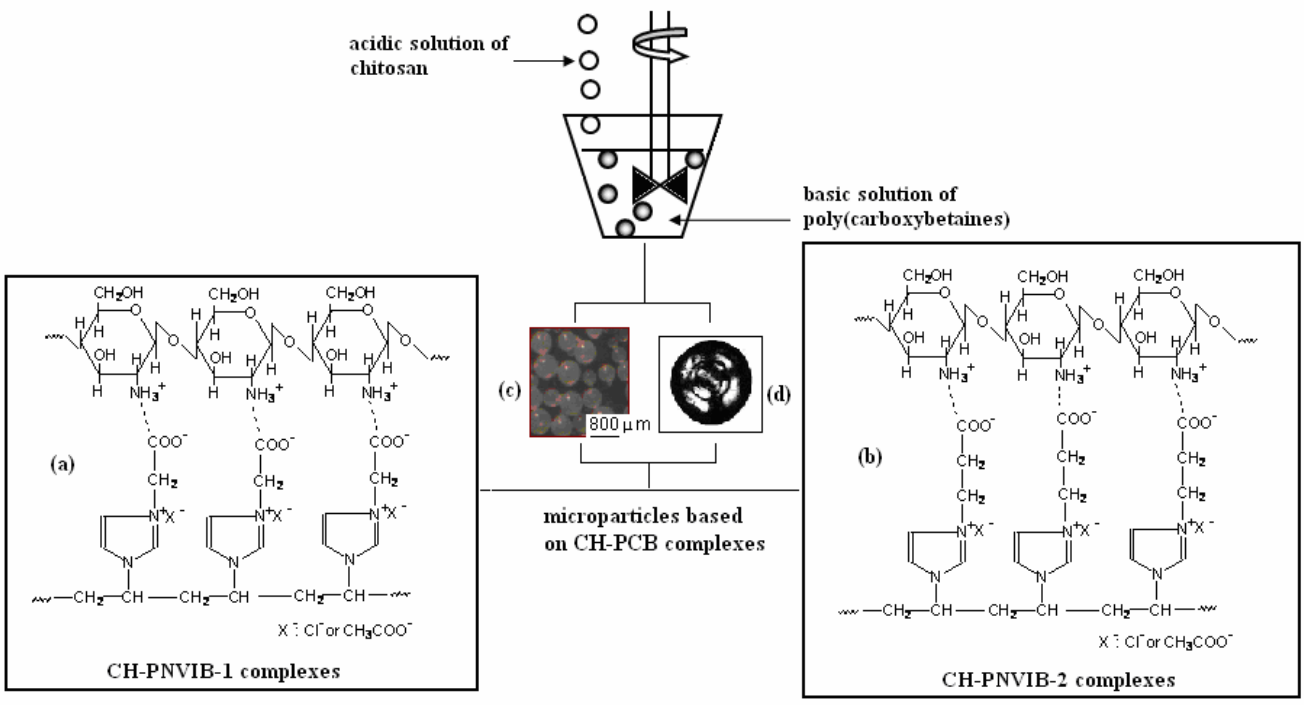


Fig. 1 – Schematic representation of complex coacervation method.

The chitosan microparticles have been prepared by the simple coacervation method similar to that used in the preparation of microparticles based on CH-PCB complexes, which a chitosan solution was added dropwise into 1N Na_2CO_3 aqueous solution.

The preparation conditions of microparticles based on chitosan and CH-PCB complexes, and the retention capacities of drug at equilibrium are shown in Table 2.

Table 2

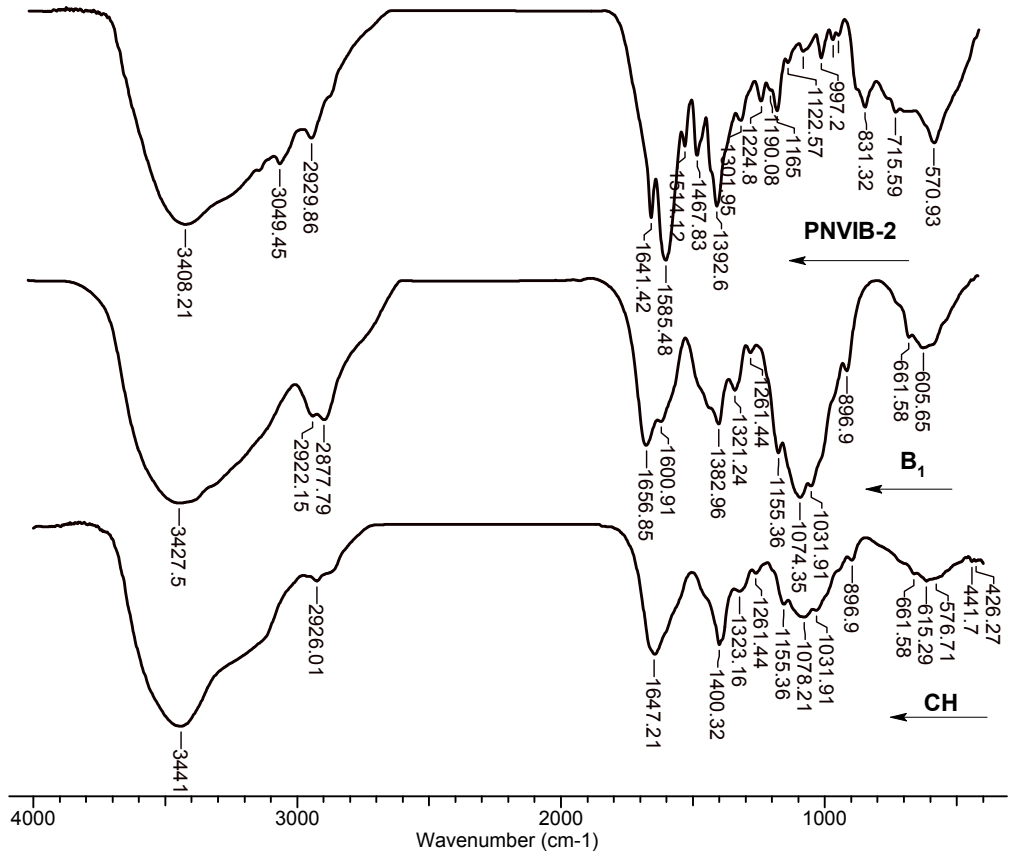
Preparation conditions and retention capacities of drug at equilibrium of C and B-type microparticles

Sample code	C_{CH} (g/l)	C_2 (g/l)	T (^{0}C)	Molar ratio CH:PCB	Chitosan solvent	C_1 (g/l)	t_C (h)	C_{Req} (mg drug/g dry microparticles)*
B_1				1:1	0.1N AcOH	-		9.26
B_2			25		0.1N HCl	-		8.70
B_3				1:2	0.1N AcOH	-	24	11.93
B_4		1.5	50		0.1N AcOH	-		10.48
B_5	15				0.1N AcOH	-	3	4.72
B_6		-		1:1	0.1N AcOH	1.5		8.11
B_7		-	25		0.1N HCl	1.5		8.62
C_1		-		-	0.1N AcOH	-	24	4.56
C_2		-		-	0.1N HCl	-		7.88
C_3		-	50	-	0.1N AcOH	-		7.33

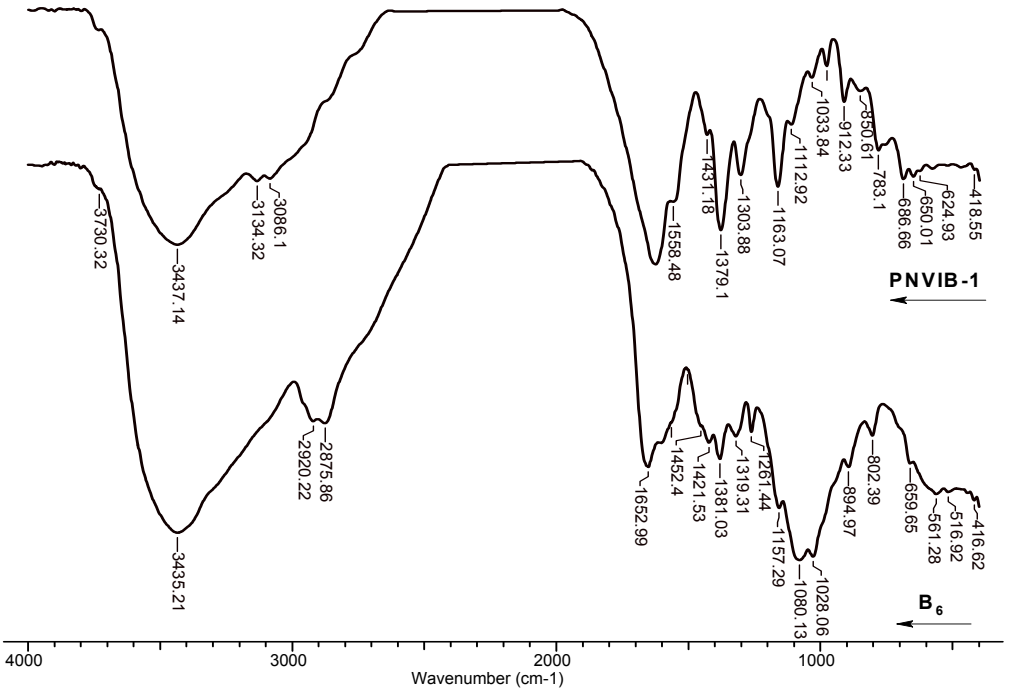
C_1 = concentration of PNVIB-1 solution; C_2 = concentration of PNVIB-2 solution; *Initial concentration of CPh solution was $7x10^{-5}$ g/L.

Formation of B-type microparticles takes place as a result of the ionic interaction between chitosan and ionic polymers (Figure 1, structures (a) and (b)).

An additional proof on the formation mechanism of microparticles based on CH-PCB complexes is illustrated by the FT-IR spectra of the starting polymers and of their complexes (Figures 2a and 2b).



(a)



(b)

Fig. 2 – FT-IR spectra of: a) chitosan, B_1 complex and PNVIB-2; b) B_6 complex and PNVIB-1.

In the FT-IR spectrum of chitosan (Figure 2a, CH spectrum), the broad band at 3441cm^{-1} is assigned to N-H stretching vibrations. The band at 2926 cm^{-1} represents the aliphatic >CH- stretching vibrations and the bands at 1647 and 1400 cm^{-1} indicate the presence of amide I and amide II groups, respectively. For polybetaines, the vibration bands at 1393 cm^{-1} (PNVIB-2) or 1379 cm^{-1} (PNVIB-1) correspond to the >C=N- groups. The absorption band at about 1600 cm^{-1} corresponds to the asymmetric vibrations of the –COO^- group. The FT-IR spectra of CH, PNVIB-1 and PNVIB-2 were compared with those of the microparticles based on CH-PCB complexes. The spectra of B microparticles exhibit many differences, such as: (1) peaks around 2920 and 2876 cm^{-1} attributed to the asymmetrical and symmetrical >CH_2 group belonging to the two starting polymers; (2) absorption bands at approximatively 1600 cm^{-1}, which correspond to the carboxylate group and which can be found only in poly(carboxybetaines); (3) the presence, in the B_6 spectrum, of absorption bands at 1503 and 802 cm^{-1}, characteristic to the –NH_3^+ and –NH_2 groups, respectively. Based on the above observations, it can be asserted that the complexes between chitosan and polybetaines are formed mainly through ionic interactions, without excluding the possibility of other type of interactions between the two polymers, *i.e.* hydrogen bonds. The microparticles based on CH-PCB complexes show a good sphericity, a core-shell structure and an average diameter size between 700 and 900μm (Figures 1d and 1c).

Thermogravimetric analysis (TG) is an analytical experimental technique performed to determine the weight changes occurring in a polymeric material as a function of temperature or time, under controlled atmosphere. The thermogravimetric characteristics of the starting polymers and of its complexes are shown in Table 3.

The temperature at which intensive degradation is initiated (T_i) was viewed as a criterion of heat stability of the complexes. Thermal analysis measurements, such as activation energy (E_a) and order of the degradation reaction (n) were calculated using the Coats-Redfren equation [13]. As shown in Table 3, the main decomposition region for chitosan starts at 185^{0}C and ends at 390^{0}C, with weight losses of 45% while, for poly(carboxybetaines), it starts at 245^{0}C PNVIB-1 and 170^{0}C PNVIB-2 and ends at 355 and 220^{0}C, with weight losses of 60% and 9%, respectively. For the B_1 complex the decomposition stage starts at 175^{0}C and ends at 410^{0}C, with weight losses of 40.5%. This behavior can be explained by the fact that PNVIB-2 starts to degrade at 170^{0}C, its use in the preparation of microparticles based on CH-PCB complexes leading to a decrease of their thermal stability, comparatively with to the thermal stability of the microparticles based on chitosan. For microparticles based on chitosan and PNVIB-1 an increase in thermal stability can be seen, compared to microparticles based on CH-PNVIB-2 complexes, which fully agrees with the thermal stability of polybetaines, the PNVIB-1 being more stable than PNVIB-2. These results confirm the formation of complexes between chitosan and poly(carboxybetaines).

The water swelling capacity, anoher important property of the microparticles based on CH-PCB complexes was also investigated in the present study. This characteristic was determined by swelling of the microparticles in bidistilled water at 25^{0}C until equilibrium was reached. The results obtained are presented in Table 4.

Table 3

Thermogravimetric characteristics of CH, PNVIB-1, PNVIB-2, B_1 and B_6 complexes

Sample code	T_i (^{0}C)	T_f (^{0}C)	w (%)	E_a (kJ/mol)	n
PNVIB-1	245	355	60	147	0
PNVIB-2	170	220	9	126	0.8
CH	185	390	45	134	2.1
B_1	175	410	40.5	130	2.9
B_6	215	415	48	141	1.6

T_i = initial degradation temperature; T_f = finally degradation temperature; E_a = activation energy; n = reaction order; w = weight loss

Table 4

Water uptake coefficients of microparticles based on CH-PNVIB-2 complex and CH-PNVIB-1 complex

Sample code	q_v
B_1	1.686
B_2	1.672
B_3	1.907
B_4	1.772
B_5	1.521
B_6	1.595
B_7	1.561

As shown in Table 4 the water uptake coefficient of the microparticles depends on their preparation conditions. Also, the water uptakes coefficients of the B_6 and B_7 are lower than for the B_1 and B_2 microparticles. This may be explained by the fact that PNVIB-1 is a less hydrophilic polymer than PNVIB-2.

For an efficient drug loading, it is important to know the time at which equilibrium is reached and how much drug will be loaded into the microparticles, which depends on the beads type and on the loading method.

Preparation of a polymer-drug system can be achieved in two ways: (a) by dissolution or dispersion of drug into the polymer solution, followed by the direct production of the polymer-drug system; (b) by physical embedding of the drug into the polymer matrix or by its adsorption onto the surface, after the formation of beads.

In the present study, the second method has been applied. As a drug, chloramphenicol succinate sodium salt, which treats or prevents infections, was loaded on the microparticles. The chemical structure of CPh is presented in Table 1 and the retained drug amount at equilibrium is shown in Table 2.

From Table 2 one can see that the microparticles based on CH-PCB complexes have a higher drug retention capacity than the microparticles based on chitosan, due to its higher swelling capacity in aqueous solution. *In vitro* release studies of CPh from microparticles based on chitosan and CH-PCB complexes were also developed. Release experiments were carried out in a phosphate buffer solution (pH = 7.4), similar to that of the intestine medium. The release profiles of CPh from the microparticles are plotted in Figure 3.

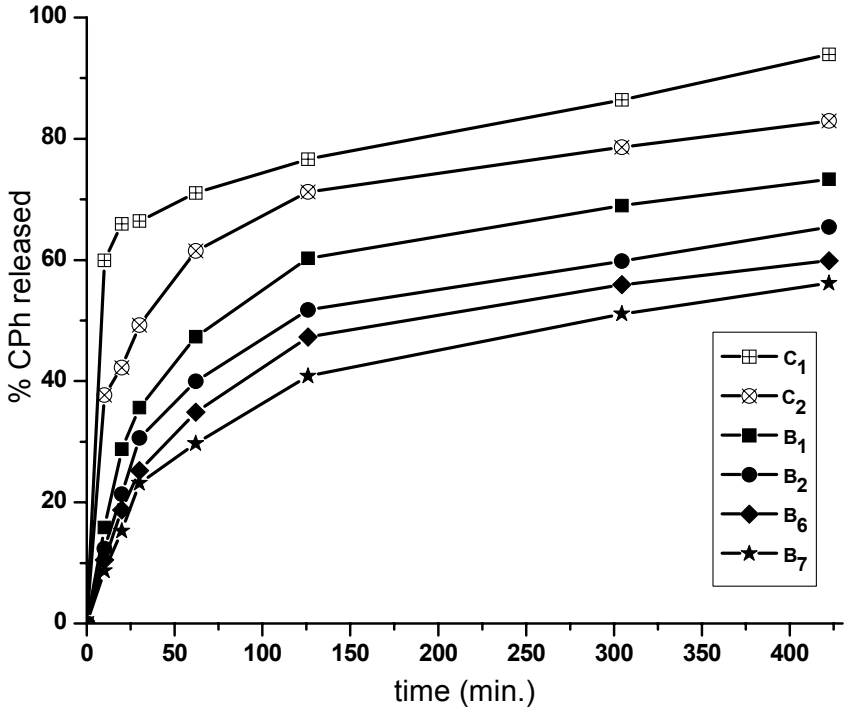


Fig. 3 – Release profiles of CPh from the microparticles based on chitosan and CH-PCB complexes.

Figure 3 shows that the microparticles based on chitosan release a higher amount of CPh than the corresponding microparticles based on CH-PCB complexes. In general, the release of CPh from microparticles exhibited an initial burst-effect, which was probably due to the release of drug from their superficial layers, followed by a much slower release rate controlled by the swelling rate of the polymer matrix and the simultaneous diffusion of CPh inside the microparticles based on chitosan. A careful analysis of the CPh release from the B microparticles shows that: (1) the burst effect was diminished compared to that of microparticles based on chitosan; (2) the CPh release from microparticles based on the CH-PNVIB-2 complex occurs faster than from the microparticles based on CH-PNVIB-1; (3) the CPh amount released at 7 hours from microparticles based on the CH-PCB complex is lower than the one from microparticles based on chitosan.

EXPERIMENTAL

1. Materials

Chitosan with high molecular weight (600,000 g/mol) and degree of acetylation 13.5% was obtained from Fluka Chemical Co. Poly(carboxybetaines) were prepared as described elsewhere.[14] Acetic acid, hydrochloric acid, and Na_2CO_3 were purchased from Fluka Chemical Co., while chloramphenicol succinate sodium salt with molar mass 445.20 g/mol from Sigma Chemical Co. All materials were used as received.

2. Methods

Preparation of microparticles based on CH-PCB complexes and chitosan. The CH solution (1.5g CH dissolved in 100 mL 0.1N AcOH or 0.1N HCl) was added dropwise in a PCB solution (0.15 g PCB dissolved in 100 mL 1N Na_2CO_3), by means of a syringe equipped with a needle of 12 mm length and 0.45 mm in diameter. In order to obtain perfect spherical particles the distance between the needle and PCB solution was of 12 cm, and the optimum stirring speed of 200 rpm. The CH solution was added to the PCB solution at two temperatures: 25^0C and 50^0C, respectively and the reaction time was of 3 and 24h, respectively. The molar ratio between CH and PCB was of 1:1 and 1:2, respectively. After the specified time (3 and 24h), the microparticles were removed from the PCB solution, washed with distilled water up to alkalinity loss and finally centrifuged for 10 min. at 1000 rpm. Such microparticles were used for drug immobilization. The microparticles based on chitosan were prepared by the same method. The solution with the same concentration was added dropwise in 1N Na_2CO_3.

FT-IR Spectroscopy. The FT-IR spectra (Bruker Vertex 70 Spectrophotometer) of the chitosan and the core shell microparticles in the 5000-400 cm^{-1} range were obtained using the KBr pellet technique.

Optical microscopy. An optical microscope (Alpha STO 5, ElectroOptika Ltd. Hungary) was used for the determination of shape and swelling measurements. The dried beads were sprinkled onto a glass plate and then examined and the image taken with a camera was attached to the microscope.

Thermogravimetric analysis. Thermogravimetric measurements were performed on a METTLER Toledo Model TGA/SDTA 851 derivatograph, using 5 mg of sample and a heating rate of 10^0C/min, under nitrogen atmosphere. The weight loss versus temperature was recorded.

Equilibrium swelling studies. The swelling behavior was determined by measuring the change in the diameter of microparticles using a microscope equipped with a micrometer. The swelling ratio for each sample determined at time t was calculated using the following equation:

$$q_v = D_t / D_0 \quad (1)$$

where D_t is the diameter of the beads at time t and D_0 is the initial diameter of the dried beads. The experiments were performed in triplicate and represented (Table 4) as a mean value.

Retention and *in vitro* release of drug. The retention process was performed by the batch method: 50 mg microparticles based on chitosan or on CH-PCB complexes were introduced in 20 mL aqueous solution of CPh and shaken at 120 rpm, using a thermostated shaker bath (Memmert, M00/M01, Germany). After the specified time (10 – 420 min), the microparticles were removed quantitatively from the aqueous solution of drug and centrifuged for 10 min, at 1000 rpm. A known

amount of CPh-loaded microparticles was suspended into a physiological saline solution (NaH_2PO_4 + Na_2HPO_4, pH = 7.4). The suspension was kept at 37^0C and stirred at 150 rpm. The retained and the released drug amounts were determined spectrophotometrically at 276 nm, on an of UV-VIS SPEKOL 1300 spectrophotometer (Analytik Jena) using a previously plotted calibration curve.

CONCLUSIONS

Some types of microparticles based on CH-PCB complexes were investigated. Characterization of microparticles by FT-IR spectroscopy, thermogravimetric analysis, optical microscopy and water uptake capacity evidences that: (1) the complexes are formed mainly by ionic interactions; (ii) the microparticles have a core-shell structure; (iii) the water uptake capacity depends on the preparation conditions of the microparticles. The drug retention capacities are larger in the case of microparticles based on CH-PCB complexes than in those based on chitosan. Based on the release profiles, it can be concluded that the microparticles based on CH-PCB complexes are suitable drug delivery systems.

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