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Tannic acid incorporation in chitosan-based microparticles and in vitro controlled release

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Abstract Chitosan, a natural polycationic polysaccharide, was coupled with two polyanionic polymers: Na-alginate and carboxymethylcellulose (CMC) and with tannic acid (TA) obtaining three species of self-assembled complexes: chitosan/alginate/TA (sample 1), chitosan/TA (sample 2) and chitosan/CMC/TA (sample 3). The microparticle formation was achieved by dropwise addition of one solution into other by using a coaxial airflow sprayer. These systems were characterized with regard to particle size distribution, thermal stability, tannic acid entrapment efficiency. Sample 2 showed quite a different behavior compared to the other two samples; the particle diameter is located in the nanometric region, the quantity of incorporated tannic acid is higher than in the other two samples and the material shows better thermal stability. The release of tannic acid from these complexes was studied in water (pH = 5.89), phosphates buffer (pH = 7.04) and acetate buffer (pH = 4.11). These studies revealed two distinct periods in tannic acid delivery process: an initial period, varying between 4 and 10 h, characterized by a high release rate with a delivered tannic acid amount of approximately 80% of the incorporated polyphenol and a second period, which starts after 20 to 30 h of delivery and it ends after approximately 120 h, when the release process takes place with low and constant rate and the kinetic curve is linear—characteristic for a zero order kinetic.

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1 Introduction

Tannic acid ($C_{76}H_{52}O_{46}$) is a gallic ester of D-glucose in which the hydroxyl groups of the carbohydrate are totally esterified with gallic acid dimers. This is tannin, which can hydrolyze under mild acidic or alkaline conditions to yield carbohydrate (glucose) and phenolic acids (mainly gallic acid). Hot water or enzymes (such as tannase) also produce tannic acid hydrolysis.

Tannic acid is an important ingredient in the process of leather tanning and it is also used as a component in some wood adhesive formulations which replace phenol-formaldehyde adhesives. Its ability to strongly interact with carbohydrates and proteins is used to clear wines, by forming insoluble complexes that sediment with proteins. Tannic acid has anti-bacterial, anti-enzymatic and astringent properties and may be used as medication against diarrhea, in hemostatic and antihemorrhoidal formulations. However, this compound should not be used in high quantities because it inhibits the absorption of iron in the body. Tannic acid reduces the effectiveness of digestive enzymes [1]. Externally, tannic acid is used for treating skin ulcers, wounds and toothache. Recent animal studies have shown that tannic acid exerts cancer chemo-preventative activity [2]. Thus this compound was able to suppress the skin tumor development induced by UV-B radiation in hairless mice by approximately 70% [3]. Dietary intake in low doses can exert a strong dose-dependent chemo preventative activity against spontaneous liver tumor development in C3H male mice by 87% [4], or inhibition of the tumor cell proteasome activity [5].

Administered orally to rabbits, tannin was absorbed in the gastrointestinal tract and it was metabolized and excreted within 24 h. It has very low acute, sub chronic and chronic oral toxicity and it is non mutagenic [1] and non

carcinogenic. Thus, tannic acid is considered to be moderately toxic to practically nontoxic [6].

Entrapment of tannic acid in biodegradable polyelectrolyte complexes may increase its chances of being used in medical and pharmaceutical applications by improving the control over released quantity and delivery rate.

The aim of the present study is to incorporate tannic acid in polymeric microparticles, using biocompatible and biodegradable natural polymers, such as chitosan, alginate and carboxymethyl cellulose. We propose a technique involving the polyelectrolyte self-assembly by using oppositely charged aqueous soluble polymers thus forming chitosan-alginic acid, and chitosan-carboxymethylcellulose complexes which immobilize tannic acid. Also, a complex chitosan/tannic acid was prepared. This paper also deals with in vitro delivery of tannic acid in elution media of various pH values.

Chitosan shows ideal properties as a polymeric support for pharmaceutical micro- and nano-particles because it is biocompatible, biodegradable, nontoxic, and inexpensive. Chitosan is a modified natural carbohydrate polymer prepared by partial *N*-deacetylation of chitin. The primary unit in chitin polymeric chain is 2-deoxy-2-(acetylamino) glucose. These units are combined through (l, 4)glycosidic linkages, forming a long linear polymer chain. Chitosan dissolves in most weakly acidic solutions, including formic, acetic, tartaric, and citric acid, at pH < 6.5. The D-glucosamine unit has a pKa value of 7.5.

Alginic acid is a natural polysaccharide found in the cell walls of a large number of species of brown seaweed. It is a polyuronide type polysaccharide containing β-D-mannuronic acid (M) in various proportions and α-L guluronic acid (G) units, linked by β-1–4 and α-1–4 bonds. The proportion and sequence distribution of these units along the polysaccharide chain vary according to the natural source; in turn, the physical properties of alginic acid depend on its composition. The alginates have the very interesting ion-bonding properties that have attracted considerable attention and many studies have been carried out based on this property. These studies conclude that the affinity of alginates for divalent ions grows stronger with increasing the proportion of L-guluronic residues in the polysaccharide chain, thus promoting the formation of more GG-blocks and less of MM and MG blocks.

The pK_a values of M and G residues are 3.38 and 3.65, respectively. It has been shown that the two types of units are joined together in blocks. Three types of blocks may be distinguished: homopolymeric (GG) or (MM) and heteropolymeric alternating blocks (MG). Alginate is chemically very stable at pH values between 5 and 10, while the highly acidic media cause decarboxylation. Sodium alginate is water-soluble. Alginate was used as an entrapment matrix for cells, enzymes and drugs.

The formation of a polyanion-polycation complex is driven by an electrostatic mechanism, where charge neutralization and possible local compensation and bridging induce attraction between topologically separated segments of the two polyelectrolytes [7]. Detailed studies about the alginate–chitosan interactions and interpolymeric complex formation were earlier published [8–10].

Carboxymethylcellulose (CMC) is another anionic polysaccharide, a cellulose derivative obtained by its reaction with alkali and chloroacetic acid. The CMC structure is composed of β-(1→4)-D-glucopyranose units similar to those of cellulose. Different esterification methods may conduct to different substitution degrees; this parameter is generally in the range of 0.6–0.95 derivatives per monomer unit. This anionic polymer may also form polyelectrolyte complexes with chitosan. The self-assembly conditions for CMC-chitosan complexes, used as hydrogels for controlled release formulations [11] and for nanoparticle formation [12], were earlier analysed. In this study the chitosan/CMC microparticles with incorporated tannic acid were obtained.

2 Materials and methods

2.1 Materials

Low viscosity sodium alginate (viscosity 30.2 cP, 1% aqueous solution) extracted from brown algae was purchased from Fluka Co. Ltd, Switzerland. Medium molecular weight Chitosan ($M_w = 310{,}000$ g/mol, polydispersity index 3.26 and degree of deacetylation 79.7%) was the product CHV3 (Vanson Chemicals Redmond WA, USA). Carboxymethylcellulose sodium salt (CMC), with a number average molecular weight of $M_n = 370{,}000$ g/mol and an esterification degree of 81%, was provided by Austranal. Tannic acid was obtained from Sigma-Aldrich. All compounds were used without further purification.

2.2 Apparatus

In all cases the microparticle formation was achieved by dropwise addition of the solution of one component (drug or polymer) into a vigorously stirred solution of the other component (usually a polymer). Both drug solution and polymer solution were added dropwise through a syringe needle (0.4 mm outer diameter) connected to a coaxial airflow sprayer. The air pressure was maintained constant at 0.8 Pa, while the solution was pumped with 1–4 ml/min flow rate using a peristaltic pump. Before mixing, all polymer solutions were filtered through Millipore membrane filter (50 MILLEX AP).

2.3 Preparation of tannic acid encapsulation systems

Three types of polymer microparticles were obtained: two by polyanion-polycation complexation (alginate-chitosan and carboxymethylcellulose-chitosan, both containing tannic acid) and the other one by co-precipitation (chitosan/tannic acid microparticles).

The alginate and chitosan solutions were prepared by dissolving dry powder in 0.1 M acetic acid solution. The concentration of both solutions was 0.5% w/v while the pH was 3.6 and 3.0 respectively. The CMC solution was prepared in distilled water with a concentration of 0.3% w/v and pH = 7.58. The tannic acid solution, with a concentration of 7% in distilled water, showed a pH of 2.81 after dissolution.

2.3.1 Chitosan/alginate/tannic acid microparticles (sample 1)

One part (by volume) of the tannic acid solution was mixed, under vigorous stirring, with three parts of sodium alginate solution. After mixing, the pH of the resulting solution was 3.30. This clear solution was added dropwise with 3 ml/min flow rate in four parts chitosan solution. The gravimetric ratio of alginate/chitosan/tannic acid was 1/1.33/4.67, while taking into account the chitosan de-acetylation degree, the alginate/chitosan molar equivalent ratio was 1:1. The alginate-chitosan complex formed instantaneously, probably including many of the bulky tannic acid molecules. The final pH of the system was found to be 3.25. After mixing, the suspension was further dispersed with an Ultraturex disperser, at 15,000 rpm, and matured for 24 h. After this period of time, no sedimentation was observed. Finally, the microparticles were separated by centrifugation (4,000 rpm, 15 min) washed with acetone to remove unincorporated tannic acid and then air dried at 45°C to constant weight.

2.3.2 Chitosan/tannic acid microparticles (sample 2)

One part of tannic acid solution was added dropwise into three parts of chitosan solution. The final pH of the mixture was 3.05 and no precipitation occurred. In this experiment the gravimetric ratio chitosan/tannic acid was 1/4.67. This clear solution was added with 4 ml/min constant flow rate of into 0.2 M phosphates buffer solution, pH = 7.04. A light violet flocculent precipitate was obtained and the final pH of the suspension was 5.66. The suspension was dispersed for 10 min at 15,000 rpm and matured for 24 h. The stable suspension was then centrifuged for 15 min at 4,000 rpm. The obtained precipitate was washed with water and then with acetone to remove unincorporated tannic acid, separated by decantating and air dried at 55°C.

2.3.3 Chitosan/carboxymethylcellulose/tannic acid microparticles (sample 3)

One part (by volume) of tannic acid solution was added, at 2 ml/min flow rate, into three parts chitosan solution, stirred vigorously with the Ultraturex disperser 18,000 rpm) and left to rest for 60 min. The final pH was found to be equal to 3.08. After that, four volume parts of CMC solution were added (3 ml/min flow rate), resulting in a voluminous white suspension. The final pH was 3.47. The precipitate, separated by centrifugation at 4,000 rpm, re-dispersed in acetone and recovered by filtration was finally dried.

2.4 Equipment for physico-chemical characterization of microparticles

Microparticle size distribution and mean diameter measurements were carried out with a Laser Diffraction Particle Size Analyser of SALD-7001 type (Shimadzu, Japan).

Thermal stability was studied with the Mettler Toledo TGA-SDTA 851e apparatus. All measurements were carried out in N_2 atmosphere at a 20 ml/min flow rate, with 10 K/min heating rate. The sample weight was between 3.3 and 4.3 mg except tannic acid, for which the sample weight was only 1.55 mg due to the fact that using higher quantities causes a spontaneous combustion at temperatures over 198°C.

Infrared transmission spectra were obtained using a FTIR spectrophotometer BOMEM MB 104 (Canada). Dry samples were dispersed in potassium chloride (6% w/w), ground into fine powder using an agate mortar and then compressed into KBr disc at 10,000 psi, then scanned with a resolution of 4 cm^{-1} in the wavenumber range 400–4000 cm^{-1}.

3 Results and discussions

3.1 Particle size distribution and mean dimensions

Measurements of the particle size distribution and mean diameter of the microparticles were carried out using the suspensions obtained after solution mixing. Before measurements the suspensions were diluted and dispersed by ultrasonication.

For a real and correct interpretation of microparticle size distribution and dimensions, Fig. 1 presents both the number size distribution (frequency distribution) and volume size distribution curves.

As it can be seen from this figure there is an appreciable difference between the two types of distribution curves.

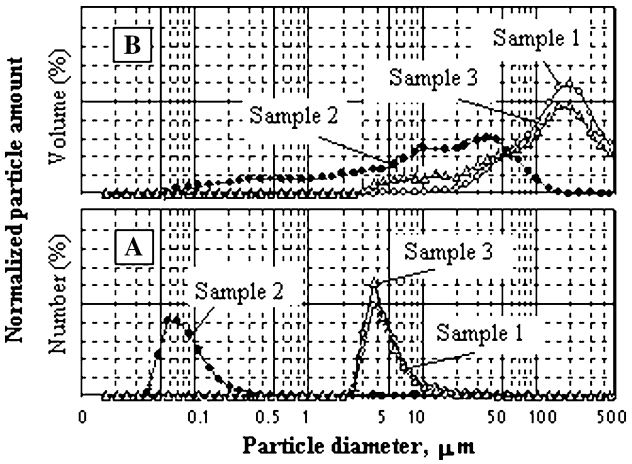

Fig. 1 Number size distribution curves (**a**) and volume size distribution curves (**b**)

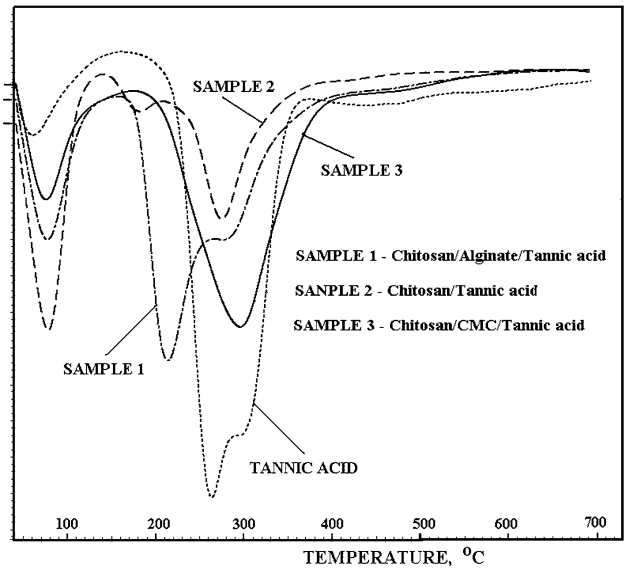

Fig. 2 DTG curves for the three-studied sample and for tannic acid

First, this difference indicates that the obtained microparticles do not have a spherical shape and present a considerable asymmetry. Samples 1 and 3, in which the microparticles are composed of polymer 1/polymer 2/tannic acid complexes, are very similar. In these samples most particles have the diameter in the range of 3–6 μm with the maximum being located around 4 μm. Sample 2 containing chitosan/tannic acid microparticles, shows the maximum of the numeric distribution curve in the nanometer range, at 80 nm. This behavior indicates the fact that, due to strong interactions between chitosan and tannin phenolic groups, the obtained complex is very compact.

The volume distribution curves also show certain similarity between samples 1 and 3, indicating the fact that the coarse microparticles contribution is more important than that of the fine microparticles, although their number is smaller, but also indicates a significant asymmetry of these two types of particles. The median value of the particle diameter in the volume distribution is 108 μm for sample 1 and 120 μm for sample 3, respectively. Sample 2 is also different from the other two in this respect. The volume size distribution curve for this sample does not show a distinct maximum in the large diameter domain, the curve extending broadly between 80 nm and 100 μm. This indicates to a certain extent a lower asymmetry of the particles. The asymmetry of microparticles and the higher values of the mean diameter found from volume distribution curves may be due to microparticle agglomeration in solution in the absence of an effective protecting agent.

3.2 Thermal stability

Thermograms (only the differential themogravimetric curves are shown) recorded in conditions described above for the three products and for tannic acid are shown in Fig. 2, while the thermogravimetric characteristics are presented in Table 1.

In this table, T_{onset} is temperature at which the degradation begins in each step, T_{endset}—the temperature at which degradation is finished in each given step, T_{peak}—the temperature at which the degradation rate is maximum, *W%*—the percentage mass loss and residue—represents the ash that is left after heating the sample to 900°C.

Examining these curves and data from Table 1, one can appreciate that the first step corresponds to dehydration processes and mass loss in this stage yields information about the sample water content.

In the case of sample 1 (chitosan/alginate/TA) the end step corresponds to the alginate thermal decomposition and the third to the chitosan/tannic acid (more stable) complex

Table 1 Thermogravimetric characteristics

Sample	Step	T_{onset}	T_{peak}	T_{endset}	*W%*	Residue
Tannic acid	I	50	58	99	5.65	21.12
	II	240	259	328	52.21	
	III	328	456	900	21.01	
Sample 1	I	50	81	112	15.68	24.54
	II	193	218	356	44.07	
	III	356	465	900	15.71	
Sample 2	I	50	78	97	18.52	46.87
	II	164	177	248	4.44	
	III	248	272	900	30.17	
Sample 3	I	50	75	97	13.13	29.55
	II	226	295	373	43.85	
	III	373	490	900	13.30	

degradation. Apparently, from a quantitative point of view, the percentage of chitosan in microparticles is less than that of the alginate.

In the case of sample 2 (chitosan/tannic acid) the main degradation period is the third step and this corresponds to the partial thermal decomposition of the chitosan/tannic acid complex. It seems that this complex is much more stable than the other two, the fact being confirmed both by the mass loss value (about 30% only) and by the large quantity of residual ash, after heating to 900°C. The second step of degradation may be attributed to partial decomposition of tannic acid into gallic acid or gallic acid dimers.

From the point of view of the thermal stability, sample 3 shows the same characteristics as the pure carboxymethylcellulose, with higher stability than that of the other two complexes. The third step is irrelevant since it is not clearly evidenced in the diagram.

3.3 FTIR spectroscopy

FTIR spectra for precursors and microparticles are prented in Fig. 3. FTIR spectra of chitosan showed a weak band at 2926 and 2879 cm^{-1} due to the C–H stretching, a band at 1655 cm^{-1}—for secondary amide carbonyl C=O stretching, a band at 1599 cm^{-1}—bonding vibration of the N–H in non-acylated 2-aminoglucose primary amines, bands 1423 and 1381 cm^{-1}—CH_3 symmetrical deformation mode [13, 14], 1321 cm^{-1} band—N–H stretching of the amide, bands 1155; 1078; 1032 cm^{-1}—oxygen bridge (C–O–C cyclic ether from saccharide cycle) (Fig. 3).

In alginate spectra one can remark the two distinct peaks at 1624 and 1419 cm^{-1} due to carboxyl anions (asymmetric and symmetric stretching vibrations). These two bands may be found in CMC spectra at 1618 and 1390 cm^{-1}. Moreover, in CMC there are noticeable bands at 3418 cm^{-1} (stretching O–H) and 2930 cm^{-1} (stretching C–H).

In chitosan/alginate/tannic acid microparticle spectra the 1599 cm^{-1} band and the peak group at 1423, 1381 and 1321 cm^{-1} (characteristic to the bending vibrations of N–H in chitosan) disappear and an intense band appears at 1412 cm^{-1} indicating that the NH_3^+ group of chitosan has interacted with the COO^-, more probable belonging to gallic acid which resulted from tannic acid decomposition, then the complex was formed. This assumption is also confirmed by the fact that the characteristic bands from alginate spectrum (2927, 1624, 1100, 1030 cm^{-1}) are found in the microparticle spectrum as well.

The tannic acid characteristic bands practically do not appear in the sample 1 spectra, probably because, on one hand, the tannic acid content represents a rather small percentage in the sample and on the other hand due to its strong interactions with chitosan. These observations

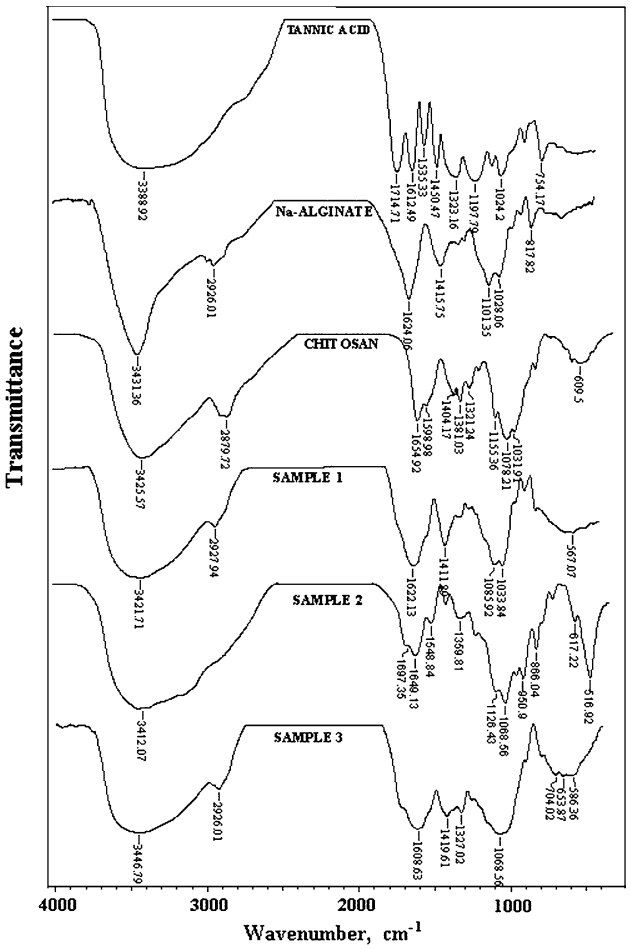

Fig. 3 The FTIR spectra of tannic acid, sodium alginate, chitosan, alginate/chitosan/TA microparticles (sample 1), Chitosan/TA microparticles (sample2) and chitosan/CMC/TA

appear to confirm the supposition that the chitosan is complexed with tannic acid (probably after decomposition in gallic acid or in gallic acid dimers) while alginate is co-precipitated at once with the chitosan/TA complex.

The band located around 3600–2800 cm^{-1} becomes broader, which indicates that hydrogen bonding is enhanced, both with alginate and chitosan.

In the chitosan/tannic acid microparticles (sample 2) the band at 1599 cm^{-1} does not appear, it is instead substituted with the band located at 1549 cm^{-1}, assigned to the symmetrical deformations of the NH_3^+ group which resulted from ionization of the primary amine group in the presence of the carboxylic group belonging to the gallic acid dimer that resulted by dissociative interactions between chitosan and tannic acid under acidic conditions (this medium and the presence of NH_3^+ cation favor tannic acid hydrolysis).

A single weak band situated at 1360 cm^{-1} replaces the band group at 1400, 1320 cm^{-1} in chitosan spectra. The 1068 cm^{-1} band (characteristic for C–O–C bonding vibrations) was attenuated and the band 1032 cm^{-1}

completely disappeared and was replaced by two distinct bands, at 951 and 866 cm^{-1}. The band located at 1714 cm^{-1} (carbonyl group) from tannic acid is attenuated and displaced to lower wavenumber and a weak band appears at 1452 cm^{-1} (electrostatically interacting carboxyl).

All these changes indicate the existence of some interactions between chitosan amino groups and the carboxyl groups located on tannic acid residues, more probable as gallic acid dimers.

In sample 3 spectrum, tannic acid characteristic bands are present, either attenuated or covered (as shoulders underneath other bands), this representing proof that the tannic acid is not included in complex at all or it is found in a very small amount in the complex. The bands at 1618 cm^{-1} (asymmetric vibration of COO^- group of CMC) and the pair 1655, 1599 cm^{-1} (characteristic for amino group of chitosan) disappear and are replaced by a broad band at 1608 cm^{-1}, thus indicating the presence of the electrostatic interactions between COO^- and NH_3^+ groups. In this case the chitosan/CMC complex formation is favored and probably leads to lower incorporation of tannic acid in these microparticles.

3.4 In vitro studies of the tannic acid release

The tannic acid release from the three prepared complexes was studied by incubating 100–150 mg microparticles in 50 ml eluent, keeping it in a shaking water bath at 25°C and monitoring, at various moments, the tannic acid concentration in elution media. The elution media were: water (pH = 5.89), acetic acid/sodium acetate buffer solution (pH = 4.11) and 0.2 M phosphates buffer (pH = 7.04).

The tannic acid concentration in elution media was determined using a NanoDrop-1000 spectrophotometer. This device is very useful for kinetic studies given its two essential features: a small quantity of solution is used (in micro liter range) and a very short period of time is required for measurements. The measurements were carried out at 277 nm, where the absorption spectra of tannic acid show a maximum; in this wavelength domain, the other solution components do not absorb.

Calibration curves were obtained for all three eluents and in phosphate buffer solution this curve was identical to that obtained in water. The equations and the mean square deviations (R^2) for these curves are:

$$A = 0.0041 \times c; \quad R^2 = 0.998 - \text{for water and phosphate buffer solution}$$

$$A = 0.0048 \times c; \quad R^2 = 0.999 - \text{for acetate buffer solution}$$

where A, is absorbance and c, is solution concentration expressed in μg ml^{-1}.

After tannic acid release, the UV absorption spectra of the resulted solution showed a maximum at a different wavelength. This is due to the fact that in acidic media the release of tannic acid takes place with partial or total transformation to gallic acid. The tannic acid presents a maximum at 277 nm wavelength in all elution media, while gallic acid shows a maximum at values between 261 and 268 nm (see Table 2). In Table 2 the values of the UV absorption maximum for pure polyphenols and the final eluents of the three studied samples are presented.

The concentrations in release media were calculated with the calibration equations described before. These equations were used for all samples indifferently of the maximum position. This is possible since, within the 2% error limits, the calibration curve equations are identical for both tannic acid and gallic acid.

The tannic acid release profiles for the three types of microparticles in specified elution media are presented in Figs. 4, 5 and 6.

In these diagrams m_t is the tannic acid quantity released from complexes at an arbitrary moment t and m_{in} is the total quantity of tannic acid entrapped in a given sample, both related to the same amount of sample. Thus, m_t/m_{in} ratio represents the fraction of encapsulated tannic acid, which is delivered at a certain moment.

The encapsulation efficiency for the three samples was determined spectrophotometrically, after complete extraction of polyphenol from the sample (30 h in 0.5 M HCl). The percentages content of tannic acid in the three samples were: 14% in sample 1; 28.9% in sample 2 and 6.3% in sample 3. The low encapsulation efficiency is probably due to steric hindrance caused by the fact that the tannic acid molecule is bulky, containing 8–10 gallic acid cycles and one glucosidic residue.

From the kinetic curves presented in Figs. 4, 5 and 6 we can remark the following:

- For all samples the kinetic curves in the all elution media show the same shape evidencing two distinct periods of polyphenol delivery. In the initial period, varying between 4 and 10 h, a rapid release takes place; the amount of delivered tannic acid is high and may reach 80% of the incorporated polyphenol. This behavior can be attributed to the fact that the main

Table 2 UV absorption maxims for polyphenols and for studied samples (λ, nm)

Sample	Eluent		
	Water pH = 5.89	Phosphates buffer pH = 7.04	Acetate buffer pH = 4.11
Tannic acid	277	277	277
Gallic acid	268	261	267
Sample 1	260…270 (broad)	260…270 (broad)	228
Sample 2	261	261	277
Sample 3	260…270 (broad)	261…277 (broad)	228

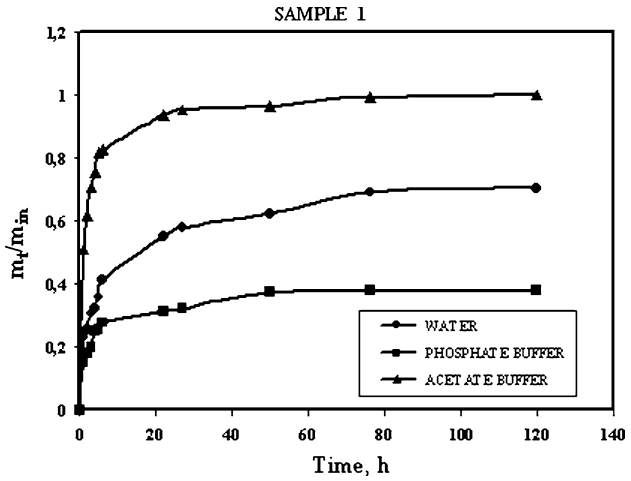

Fig. 4 In vitro release profile of tannic acid from Chitosan/alginate/tannic acid microparticles in various elution media

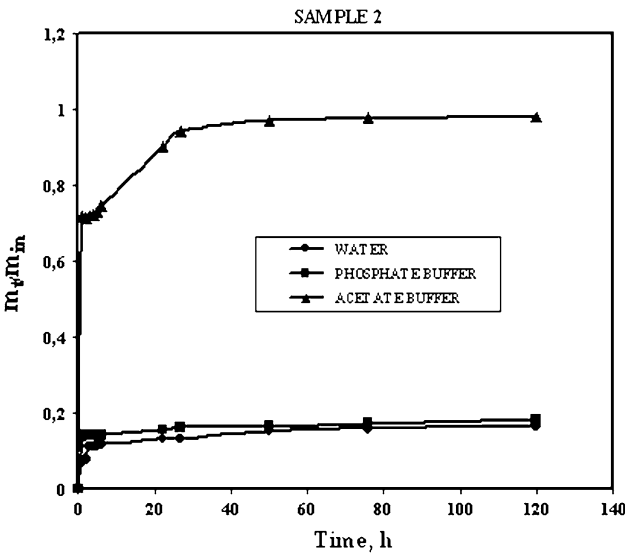

Fig. 5 In vitro release profile of tannic acid from chitosan/tannic acid microparticles

portion of encapsulated tannic acid is physically included in the complex, and only a small amount is chemically bonded. In the second period, which begins after 20–30 h and it ends after approximately 120 h, the release takes place with low and constant rate, the kinetic curve is linear—characteristic for a zero order kinetic process. The release rates calculated from the slope of these curves are presented in Table 3.

- The highest released quantities and the highest delivery rates where obtained in acidic elution medium. This behavior is perhaps due to the fact that in acidic media the non-complexed chitosan is soluble, and the swelling of the complexes is promoted due diminished electrostatic bonds.
- The chitosan/tannic acid microparticles (sample 2) show a different behavior. In this case the delivery rate is higher than that of the other two sample, but unlike these, there is a big difference between the delivery in acidic media and delivery at pH values higher than 5.5 (where the chitosan is partially insoluble); in acetate buffer, during the first 20 h, over 90% of the encapsulated tannic acid is eliminated, while at pH values of 5.97 and 7.04, <20% is released over a 120 h period. Moreover, in acidic solutions only the tannic acid was

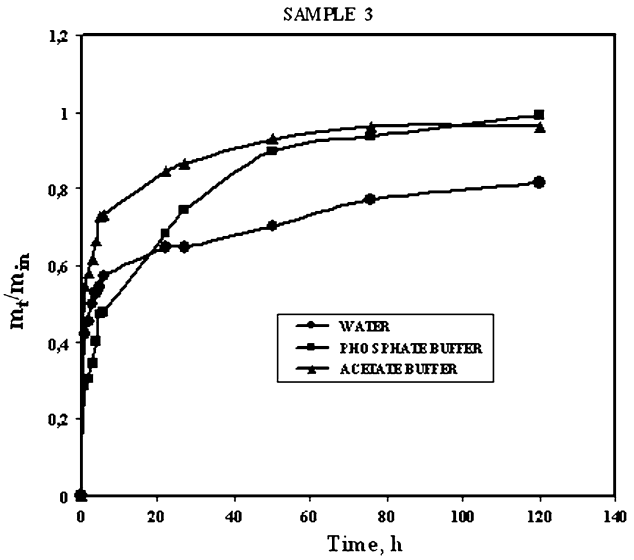

Fig. 6 In vitro release profile of tannic acid from chitosan/CMC/tannic acid microparticles

Table 3 Release rates of tannic acid for zero order kinetic domains

Eluent	Sample 1		Sample 2		Sample 3	
	v (μg/g h)	R^2	v (μg/g h)	R^2	v (μg/g h)	R^2
Water, pH = 5.89	–	–	3.00	0.976	1.50	0.982
Phosphates buffer, pH = 7.04	3.60	0.995	3.30	0.934	6.40	0.979
Acetate buffer, pH = 4.11	10.2	0.994	103.7	0.994	8.10	0.992

released from complexes (the absorption maximum is 277 nm), while in weak acid or weak alkaline media the gallic acid or its dimers are released.

- In the case of sample 1 the release rate increases linearly with decreasing pH.
- For sample 3 it is observed that higher percentages of tannic acid are released, independent of the pH value; the interaction between tannic acid and chitosan/CMC complex is weaker than in the other two types of particle. This is probable the reason for which a smaller quantity of tannic acid is incorporated in this complex.
- In the case of sample 1 and 3, eluted in water and phosphates buffer, both tannic acid and gallic acid are probably released (the absorption curve maximum is broad and covers the two characteristic maxima: 277 nm and 261 nm). In acidic medium the absorption maximum of samples 1 and 3 is situated at 228 nm, probably due to the release of gallic acid dimers from complexes.
- The encapsulation process contributes to reducing the astringent properties of raw tannic acid due to the presence of chitosan and alginate and to the fact that the concentration of the incorporated tannic acid is relatively low.

4 Conclusions

- These studies confirm the possibility of incorporating tannic acid in chitosan-based microparticles; the best method was found to be the co-precipitation of a chitosan/tannic acid solution in a neutral phosphate buffer solution. Tannic acid can be totally released from these microparticles in acidic media and only approximately 20% in weak acid or in weak alkaline media.
- The incorporated tannic acid quantity can not attain high values both due to the bulky tannic acid molecules and to the high rigidity of the polymer chains. The lowest incapsulation level was obtained in the case of sample 3 in which the active amino groups contributed to the formation of an interpolimeric complex with carboxymethylcellulose, thus excluding the voluminous tannic acid molecules.
- Some of the prepared complexes, especially sample 1 and 2 could potentially be used in toothache and superficial skin wound treatment formulations.

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