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Thermogravimetric characterization of chitosan/alginate microparticles loaded with different drugs

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Summary

The thermal behavior of several chitosan/alginate /drug microparticle formulations containing ciprofloxacin, ibuprofen, ketoprofen or tannic acid was investigated by thermogravimetry in inert atmosphere in the temperature range of 25 – 900°C. The chemical composition and the method used for drug incorporation influenced the thermal stability of the products. It was found that the entrapment of the drug in chitosan, alginate or chitosan/alginate complex modifies the degradation mechanism introducing new degradation steps by comparison with raw polymers. The activation energy for the main degradation step is also changed.

Keywords

chitosan, alginate, microsphere, thermogravimetry, chitosan/alginate/drug, ciprofloxacin, ketoprofen, ibuprofen, tannic acid

Introduction

The efficiency of many drugs is often limited by their potential to reach the site requiring therapeutic action. In most conventional dosage forms only a small fraction of the administered quantity reaches the target site, while the majority of the drug distributes throughout the rest of the body. It is therefore a challenging task to develop a drug delivery system that optimizes the pharmaceutical action a drug while keeping a low dose. The potential use of micro- and nanoparticles as drug carriers has led to the development of many different methods and preparation techniques. A basic requirement for these polymers that are going to be used in humans or animals is that they are able to degrade into molecules with no toxicity for biological environments. The natural polymers, chitosan and alginate correspond to these requirements. Chitosan, poly-β(1-4)-D-glucosamine is a modified natural carbohydrate polymer (figure 1) prepared by partial N-deacetylation of chitin. By comparison to other natural polymers, chitosan is a hydrophilic polymer with positive charge due to the presence of weak basic groups along the polymeric chain, which confer it special technologically interesting characteristics. The D-glucosamine unit has a pKa value of 7.5 [1]. Chitosan molecule possesses many ideal properties as polymeric carrier for

micro- and nanoparticles because it is biocompatible, biodegradable, nontoxic, and inexpensive.

Alginic acid is a natural polysaccharide that is found in the cell walls of a large number of species of brown seaweed. It is a polyuronide type polysaccharide composed of different proportions of β-D-mannuronic acid (M) and α-L guluronic acid (G) units, linked by β-1-4 and α- 1−4 bonds (see Figure 1). The proportions 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 these proportions and sequences. The alginates have a very interesting ion-bonding feature that has attracted considerable attention and many studies focused on this property have been carried out. The pK_a values of M and G residues are 3.38 and 3.65, respectively.

The formation of an alginate-chitosan complex is driven by an electrostatic mechanism, where charge neutralization and possible local compensation or different bridging induces attraction between topologically separated segments of the two polyelectrolytes. Detailed studies about the alginate–chitosan interactions and of the polyelectrolyte complex stability were earlier investigated [2-4].

The thermogravimetric analysis has been widely used to investigate the thermal degradation behavior of polysaccharides or its derivates [5-8]. The objective of the study was to investigate the effect of the loaded drug and of the preparation method on the thermogravimetric stability of complexes with chitosan/alginate.

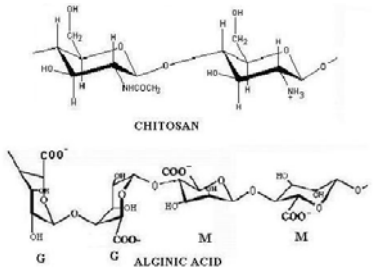

Figure 1. Structures of Chitosan and Alginic acid

Materials and methods

Materials

Chitosan with M_w = 310,000g/mol and degree of deacetylation of 79.7 was provided by Vanson Chemicals Redmond WA, S.U.A. Low viscosity sodium alginate (viscosity 30.2 cP, 1% aqueous solution) extracted from brown algae was purchased from Fluka Co. Ltd., Switzerland. The pharmacological agents used for complex preparation are presented in Table 1. Before usage, the drugs were dissolved either in water or in 0.1M acetic acid solution. All compounds were used without further purification.

Microparticle preparation

Alginate and chitosan solutions were prepared by dissolving a known amount of powder in 0.1 M acetic acid solution. The concentrations of these solutions were 0.5 % w/v and pH 3.6 and 3.0 respectively. In a few cases these solutions were diluted till

Table 1. Pharmacological agents used in the study

Pharmacological name	Chemical name	Molecular weight, g mol^{-1}	Producer
Ketoprofen	2-(benzoylphenyl)propanoic) acid $C_{16}H_{14}O_3$	254.28	BIDACDHEM s.p.a, Fornovo S. Giovanni, Italia
Ibuprofen	2-[4-(2methylpropyl)phenyl] - propanoic acid $C_{13}H_{18}O_2$	206.28	Shasun Chemicals and Drug Ltd India
Tannic acid	$C_{76}H_{52}O_{46}$	1701.20	Sigma-Aldrich, USA
Ciprofloxacin	1-cyclopropyl-6-fluoro-4-oxo-7-piperazine-1-yl-quinoline-3-carboxylic acid $C_{17}H_{18}FN_3O_3$	331.34	S&D Chemicals Ltd, Cunningham House, Harrow, Middlesex, Anglia

0.2 or 0.3 %. . The drugs were dissolved either in water or in 0.1 M acetic acid solution, depending on their solubility requirements. The microparticles were prepared by dropwise addition of the solution of one component (drug or polymer) into a vigorously stirred solution of the other component (such as the 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 liquid solution was pumped with a 1 - 4 mL/min flow rate using a peristaltic pump. Before mixing, all polymer solutions were filtered through a Millipore membrane (50 MILLEX AP).

Two methods were used for preparing the polymer/polymer/drug complexes. In the first method the drug solution was added into the Na-Alginate solution (either by direct mixing or by dropwise addition), and then the chitosan solution was slowly added to this mixture. These microparticles were designated as (alginate/drug)/ chitosan. The second method of preparation involved adding the drug solution dropwise into the chitosan solution, followed by the Na-Alginate solution addition to the already formed suspension under vigorous stirring. The final hardening of the gellified particles was achieved by modifying the pH in suspension to an alkaline value of 7.5, by adding 0.1M NaOH or 0.2 M phosphates buffer solution. These microparticles were designated as (chitosan/drug)/alginate.

The microparticle were subsequently washed with deionized water, freeze-dried using a Freeze Dry System Freezone 6 (Labconco Corporation), and characterized regarding size distribution and mean diameter. Particle size analyses were performed with a Laser Diffraction Particle Size Analyzer of SALD-7001 type using particle samples suspended in a 1% Tween 80 aqueous solution.

The amount of drug entrapped in the microparticle was determined by simulated digestion of the samples in concentrated HCl aqueous solution followed by spectrophotometric analysis of the drug. The synthesis conditions and particle characteristics are presented in Table 2.

In this table the gravimetric ratios alginate/chitosan/drug correspond to the total starting quantities of the components involved in each synthesis. The particle size is expressed as mean diameter (D_m) both from number distribution curve and volume distribution curve.

Table 2. Microparticle formulation and characteristics

No	Microparticle designation	Gravimetric ratios Alg:Chit:Drug	Mean Diameter D_m (μm)		Drug content (%)
			Number	Volume	
1	(Alginate/Ciprofloxacin)/Chitosan	1 : 1.2 : 1	4.5	51	28.1
2	(Alginate/Tanic acid)/Chitosan	1 : 1.3 : 5	4.1	108	8.9
3	(Alginate/Ibuprofen)/Chitosan	1 : 0.8 : 1.3	13.5	320	18.8
4	(Alginate/Ketoprofen)/Chitosan	1 : 0.8 : 1.3	2.24	2.45	11.8
5	(Chitosan/Ciprofloxacin)/Alginate	1 : 1.2 : 1	4.1	44	9.6
6	Alginate/Ciprofloxacin	1 : 0 : 2	0.12	158	13.4
7	Chitosan/Ciprofloxacin	0 : 1 : 2	0.023	0.22	13.3

The most particle samples have dimensions in the micrometric range on the number distribution and volume distribution. There is a relatively large difference between the number and volume mean values, which is probably due to the presence of a bimodal population.

Thermal characterization

Thermal stability was studied with the Mettler Toledo TGA-SDTA 851e apparatus. All measurements were carried out in inert atmosphere under constant purging of nitrogen at a 20 mL/min flow rate. The weight of the sample used was situated between 3.3 and 4.3 mg excepting tannic acid where the sample's weight was of only 1.55 mg – due to the fact that higher quantities cause a spontaneous combustion at temperatures over 198°C. Thermogravimetric data were recorded in the temperature range of 25°C to 900°C, with 10 K/min heating rate. Thermal analysis in dynamic conditions (TG, DTG and DTA) was also performed and STAR software was used for data investigation.

Results and discussions

Thermogravimetric technique can yield information about humidity and ash content in a simple and rapid manner. Since the polymers employed in this study contain hydrophilic groups they usually interact strongly with water molecules, the adsorbed humidity may influence their properties and it is an important to determine this characteristic [9].

The heating rate has a strong influence upon the resolution of the TG curve [10] and the definition of this parameter is fundamental for a thermogravimetric investigation. In this study a constant heating rate of 10°C/min was used, as in many other studies, permitting clear evidence of all degradation processes taking place during the sample heating. The thermogravimetric parameters for precursors and complexes are presented in Table 3, considering all the degradation steps. In this table, $\mathbf{T_{onset}}$ is the temperature at which the degradation begins in each step, $\mathbf{T_{endset}}$ – the temperature at which degradation is finished in each given step, $\mathbf{T_{peak}}$ – 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.

Table 3. Thermogravimetric parameters

Sample	Step	T_{onset}	T_{peak}	T_{endset}	W%	Residue
Na-Alginate	I	50	91	205	7.81	39.84
	II	205	226	268	40.00	
	III	268	440	488	12.35	
Chitosan	I	50	88	118	5.12	32.34
	II	272	283	472	62.54	
1. (Alginate/Ciprofloxcin)/Chitosan	I	50	124	150	7.20	25.48
	II	231	245	301	40.08	
	III	301	420	465	12.35	
	IV	465	774	900	14.89	
2. (Alginate/ Tannic Acid)/Chitosan	I	50	81	112	15.68	24.54
	II	193	218	356	44.07	
	III	356	465	900	15.71	
3. (Alginate/Ibuprofen)/Chitosan	I	50	70	109	8.01	16.73
	II	229	263	290	17.73	
	III	360	382	398	38.98	
	IV	442	463	508	9.68	
	V	697	737	900	8.79	
4. Alginate/Ketoprofen)/Chitosan	I	50	78	115	6.22	62.75
	II	116	151	175	5.14	
	III	175	187	205	16.48	
	IV	205	221	321		
	V	355	423	535	9.41	
5. (Chitosan/Ciprofloxacin)/Alginate	I	50	105	130	6.44	37.65
	II	224	294	339	29.15	
	III	339	392	532	26.76	
6. (Alginate/Ciprofloxacin)	I	77	95	112	8.98	20.10
	II	208	226	245	22.32	
	III	317	337	539	48.60	
7. (Chitosan/Ciprofloxacin)	I	76	103	112	12.67	27.67
	II	307	334	346	18.30	
	III	346	422	486	41.36	
Tannic acid	I	50	58	99	5.65	21.12
	II	240	259	328	52.21	
	III	328	456	900	21.01	
Ibuprofen Na	I	<50	53	98	2.02	63.46
	II	100	134	175	20.54	
	III	305	316	323	6.00	
	IV	439	454	511	7.98	
Ibuprofen	I	201	276	288	94.95	5.04
Ketoprofen	I	265	372	427	92.53	7.47

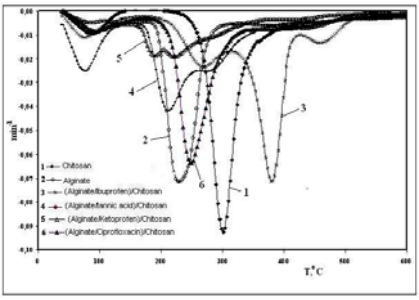

Figure 2. DTG curves for chitosan, alginate and various (alginate/drug)/ chitosan complexes

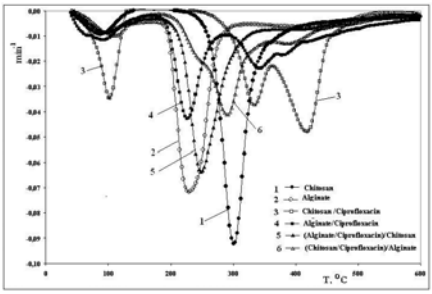

Figure 3. DTG curves for chitosan, alginate, polymer/ciprofloxacin systems and (polymer 1/ciprofloxacin)/polymer 2 systems

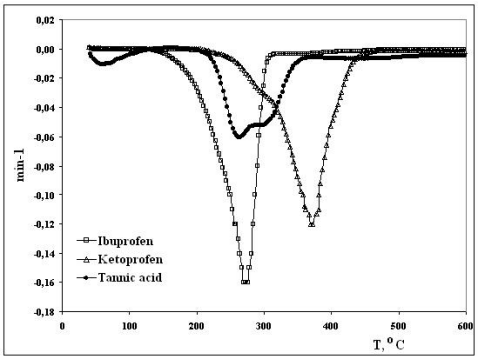

Figure 4. DTG curves for ibuprofen, ketoprofen and tannic acid

A clearer distinction between the different stages of the degradation process is evidenced in the derivative of the thermogravimetric curve (DTG curve). DTG curves for precursors and drug-loaded microparticles are presented, comparatively, in figures 2 - 4, considering only the initial and main stages of the degradation process (for the temperature range of 25 to 600°C).

It was not possible to obtain an experimental thermogram for ciprofloxacin due to sublimation during thermal decomposition.

Analyzing the data presented in Table 3 and in Figures 2, 3 and 4, one can ascertain that chitosan decomposed in two successive steps, with a percent mass loss of approximately 68 %, while the Na-alginate decomposed in three steps, with a percent mass loss of 60 %, in good agreement with data reported by Zohuriaan and Shokrolahi [11]. In the case of Na-Alginate the first step (a minor dehydration) is followed by degradation into Na_2CO_3 and a carbonized material that decomposes slowly in the three steps. The formation of sodium carbonate was also observed by Soares et al. [7] who reported a similar behavior for Na-Alginate decomposition in nitrogen atmosphere and pointed out that the alginic acid decomposes in two steps without residue.

The chitosan/alginate/ciprofloxacin complex samples 1 and 5 respectively, which were obtained by two different methods, show a different degradation mechanism, depending on the preparation method. Sample 1, prepared in sequence succession chitosan→(ciprofloxacin→alginate), decomposes in four steps, showing the highest

weight loss (40 %) between 220 and 300°C – this being the main degradation step, closer in behavior to that of the raw Na-alginate than the chitosan.
Sample 5, obtained by sequence succession Na-alginate→(ciprofloxacin→chitosan), on the other hand, decomposes in three steps, but the third stage is an extension of the second one. More important is the fact that the total weight loss in these two combined steps is equal to that of the main step in chitosan degradation.
One can notice that the ciprofloxacin entrapped into complex by ionic interaction on chitosan or alginate does not decomposes with explosion as it is the case of pure ciprofloxacin (t_e = 372°C); therefore incorporation into polymer complex has a stabilizing effect.
The thermograms presented in Fig. 3 allow qualitative differentiation of the modifications produced in thermal behavior by the complexation process [12]. The main degradation peak in the case of chitosan/alginate/ciprofloxacin microparticles is positioned between those of the raw precursors. Moreover, for sample 1, which was prepared by dropwise addition of the drug in Na-alginate with subsequent chitosan addition, the main peak is close to that of the pure alginate, while the main peak for sample 5, prepared by first adding the dug in chitosan, is shifted towards the peak characteristic for chitosan. This behavior is consistent with the weight losses and it seems to indicate that the microparticle core mainly consists in a drug/polymer complex, the polymer being the one initially mixed with the drug, while the other polymer is distributed in a thin layer on the outside.
The thermal decomposition of sample 1 to 4, obtained by process sequence chitosan→(drug→alginate), shows three, four or five steps, depending on the nature of the drug loaded in microparticle. The degradation mechanism is quite complex, the peak positions indicating polymer-drug and polymer-polymer interactions; the T_{peak} values are different from those of the corresponding precursors. As a criterion of stability one was considered the temperature T_{onset} for the main degradation step. A thermostability series which suggest the drug influence upon the complex thermal stability can be established from the thermogravimetric data, overlooking the dehydration step, as following:

Ketoprofen<Tannic acid<Ibuprofen<Ciprofloxacin

The bi-component complexes alginate/ciprofloxacin (sample 6) and chitosan/ ciprofloxacin (sample 7) show three decomposition stages with different weight loss amounts, the steepest being the third stage. For the second step in the degradation of sample 7 (chitosan/ciprofloxacin) the corresponding peak is very close to the peak obtained in the case of raw chitosan, while for sample 6 (alginate/ciprofloxacin), T_{peak} is close to that of the alginate as in the case of the corresponding tri-component complexes.
In the case of (alginate/tannic acid)/chitosan sample the second step corresponds to the alginate thermal decomposition and the third to the chitosan/tannic acid complex degradation, which is more stable. From quantitative point of view, the percentage of chitosan in microparticles is probably less than that of the alginate.
The study was extended with the kinetic processing of thermogravimetric data. Freemann-Caroll [13] method application, based on the equation:

$$\frac{\Delta \ln \frac{d\alpha}{dT}}{\Delta \ln(1-\alpha)} = n - \frac{Ea}{R} \times \frac{\Delta\left(\frac{1}{T}\right)}{\Delta \ln(1-\alpha)} \qquad (1)$$

has lead to the kinetic characteristics shown in Table 4.

Table 4. Kinetic characteristics

Sample	Step	lnA	Ea (KJ/mol)	n
Na-Alginate	II	11.83±1.01	69.63±4.07	1.05±0.058
Chitosan	II	63.07±0.93	318.86±4.29	2.96±0.040
1. (Alginate/Ciprofloxcin)/Chitosan	II	61.12±0.88	281.15±3.68	4.66±0.057
2. (Alginate/ Tannic Acid)/Chitosan	II	4.08±0.1	39.39±1.12	1.24±0.083
3. (Alginate/Ibuprofen)/Chitosan	II	16.11±0.37	96.37±1.60	1.75±0.030
	III	23.03±0.43	153.27±2.23	0.7±0.025
	IV	27.69±0.71	199.34±4.22	1.82±0.031
4. (Alginate/Ketoprofen)/Chitosan	II	81.13±1.49	324.52±5.57	1.17±0.038
	III+IV	2.35±0.96	34.51±3.73	1.57±0.012
5. (Chitosan/Ciprofloxacin)/Alginate	II	14.75±0.79	92.76±3.44	1.70±0.070
6. (Alginate/Ciprofloxacin)	II	73.85±2.34	319.83±9.41	5.30±0.14
	III	22.44±1.59	140.70±7.82	1.61±0.090
7. (Chitosan/Ciprofloxacin)	II	41.4±0.82	260.08±3.99	2.91±0.047
	III	25.1±0.47	172.90±2.62	1.41±0.022
Tannic acid	II	26.68±1.04	140.72±4.48	1.69±0.056
Ibuprofen	I	4.41±0.11	47.04±1.08	0.12±0.001
Ketoprofen	I	9.88±0.30	80.89±1.45	0.48±0.029

(A – pre-exponential factor; E_a - apparent activation energy; n - reaction order)

By plotting the graph of $\Delta\ln(d\alpha/dT)/\Delta\ln(1-\alpha)$ versus $\Delta(1/T)/\Delta\ln(1-\alpha)$ the slope of the straight line yields the activation energy E_a and the intercept gives the reaction order n. The pre-exponential factor is computed using the equation:

$$\frac{d\alpha}{dT} = \frac{1}{a} A \exp(\frac{-Ea}{RT}) f(\alpha) \qquad (2)$$

The calculated values for the activation energies and reaction orders, presented in table 4, confirm the complexity of the products degradation process, suggesting a free radical mechanism. If we compare the values of the activation energies in about the same temperature range (200-300ºC), the thermal stability series is confirmed for the samples chitosan/alginate/drug, previously determined from thermogravimetric characteristics.

For ibuprofen and the samples 2 and 4 we obtained lower levels of the activation energies in stages I, II, respectively III and IV. The very low value of the activation energy found in the main degradation step of the (alginate/tannic acid)/chitosan complex can probably be attributed to the fact that in this case the process is initiated by decomposition of tannic acid to gallic acid and this step is favored by the presence of a chitosan film on the particle surfaces. This supposition is supported by the fact that in absence of chitosan the decomposition of tannic acid is characterized by activation energy 3.5 times higher.

The presence of about 10 % ketoprofen in the tri-molecular complex leads also to a reduction of the activation energy in the main decomposition step. On the other hand,

when ibuprofen is present in a proportion of 19 % the diminution of the activation energy is less important, although the activation energy of ibuprofen is very small (47 kJ/mol) and a zero order reaction takes place. The inclusion of ibuprofen into alginate brings an increased stability both for alginate and the drug.
The presence of ciprofloxacin in the complexes has a completely different influence upon the stability of the products. The complexes containing alginate and ciprofloxacin decompose by a new mechanism reflected in the high and unusual reaction order (almost 5). The same amount of ciprofloxacin does not show this effect when chitosan/ciprofloxacin complexes are prepared. In this case the reaction order is similar to that of chitosan (n=3) and the activation energy is only 18% lower than that of raw chitosan.

Conclusions

The following conclusion can be drawn from the thermogravimetric study of some interpolymeric complexes chitosan/alginate loaded with various drugs (quantity loaded in the range of 10 to 30 %):

- The microparticle preparation method has a significant influence upon the resulting chitosan/alginate ratio, and the structure of the microparticle although the initial precursor ratios in the preparation process were unchanged. The location of the main peaks on the DTG curves and the weight loss amounts in these steps indicate that the microparticle core is principally constituted from a drug/polymer complex, the polymer being the one which was initially mixed with the drug, while the other polymer distributed in a thin layer around the core.
- The entrapment of a drug in chitosan, alginate or chitosan/alginate complex modifies the degradation mechanism introducing new steps compared with the raw polymer degradation.
- Using the parameter T_{onset} for the main degradation step as a thermal stability criterion, the drug influence on thermal stability, for chitosan→(drug→alginate) samples the following series of thermostability can be established:

Ketoprofen<Tannic acid<Ibuprofen<Ciprofloxacin

- From the kinetical point of view it was determined that the presence of the drug in the interpolymeric complex leads to modified thermal behavior and degradation mechanism:

- In the case of (alginate/drug)/chitosan microparticle, the presence of alginic acid and ibuprofen lowers significantly the activation energy for the main degradation step, while ibuprofen and ciprofloxacin lead to activation energy values positioned between those of alginate and chitosan
- The presence of ciprofloxacin in the complexes has a completely different influence upon the stability of the products. The reaction order and activation energy are changed when alginate is part of the complex. This effect is less significant in the complexes containing chitosan and ciprofloxacin.

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