MONTMORILLONITE-ALGINATE NANOCOMPOSITE BEADS AS DRUG CARRIER FOR ORAL ADMINISTRATION OF CARBOPLATIN – PREPARATION AND CHARACTERIZATION

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Scopul acestei lucrări este prepararea şi caracterizarea unui material nanocompozit pe bază de montmorillonit (MMT) şi alginat de sodiu în care a fost încorporat medicamentul antitumoral carboplatin (CP). Acest material compozit poate funcționa ca sistem cu eliberare de medicament (DDS) şi poate reprezenta alternativa administrării pe cale orală a CP, citostatic care în prezent este administrat numai pe cale intravenoasă. Hibridul rezultat în urma încorporării CP în MMT este combinat cu alginatul de sodiu. În continuare, prin tehnica gelifierii ionotropice se obțin sfere de material compozit CP-MMT-alginat. Materialul hibrid şi cel compozit au fost caracterizate prin difracție de raze X (XRD), spectroscopie în infraroşu (FT-IR), analiză termică (ATD-TG) şi microscopie electronică de baleiaj (SEM). Eficiența încorporării medicamentului în MMT şi în alginat a fost determinată cu ajutorul spectroscopiei UV-Vis.

The scope of the present study was the preparation and characterization of carboplatin nanocomposite beads based on montmorillonite (MMT) and sodium alginate as drug carriers. This composite material is a potential drug delivery system (DDS) for oral administration of the antitumoral drug carboplatin (CP), being an alternative of the present drug intravenous administration. After carboplatin incorporation into MMT, the resulting hybrid was compounded with alginate. Then, CP-MMT-alginate nanocomposite beads were obtained by ionotropic gelation technique. The hybrid and composite materials were characterized by means of X-ray diffraction (XRD), IR spectroscopy (FT-IR), thermal analysis (TG-DTA) and scanning electron microscopy (SEM). Carboplatin incorporation efficiency in MMT and alginate beads was determined by UV-Vis spectroscopy.

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1. Introduction

In drug therapy, it is important to provide therapeutic levels of drug to the site of action (or at least in the blood) and maintain them during the treatment [1]. Furthermore, it is desirable to minimize temporal variations in drug concentration by using modified drug delivery systems (MDDS) to avoid periods of over-dosing or under-dosing. The modifications involve changes in the rate and/or time and/or site of drug release in comparison with conventional formulations by delaying drug release (extended release systems), targeting drug release (site-specific release systems), prevention or reduction of side effects, taste masking or improving drug solubility. New strategies are focused on increasing drug stability and on simultaneously modifying drug delivery patterns (particulate delivery systems) [2]. These changes are achievable by several possible mechanisms, including modifications in pharmaceutical formulations and/or method of preparation.

Clay minerals (layered silicates) have been proposed as fundamental constituents of several MDDS, with different purposes and acting through various mechanisms [3]. The intercalation of organic species into layered inorganic materials provides a useful and convenient route to prepare organic-inorganic hybrids that combine the properties of both inorganic host and organic guest [4, 5]. A very interesting possibility is to use clay mineral polymer composites to modify drug release. Although clay minerals and polymers are frequently used as single drug carriers, this type of drug delivery system (DDS) often does not meet all requirements. Preparation of polymer layered silicate composite offers the possibility of improving the single components properties: those of the clay mineral particles (stability of the clay mineral dispersions and changes in its ion exchange behavior) and, more frequently, those of the polymer (mechanical properties, swelling capacity, film forming abilities, rheological properties, bioadhesion or cellular uptake) [6].

Montmorillonite clay (MMT) belongs to the smectite group, composed of silica tetrahedral sheets layered between alumina octahedral sheets at a ratio of 2:1, respectively [7]. It has large specific surface area, exhibits good adsorbance ability, high cation exchange capacity, standout adhesiveness, and drug-carrying capability. Thus, MMT is a common ingredient in pharmaceutical products, both as excipient and as active support. It was reported [8] that drug incorporation into clays takes place by adsorption, both by intercalation into the clay structure within the interlayer spacing (by replacing the water molecules), and also on the surface. The most important interactions taking place between the two components of the hybrid system are ionic [9]. The ionic exchange process may take place by mixing

ion exchangers with ionic drugs in solution. In biological fluids, "counterions" can displace the drug from the substrate and deliver it into the body, while the exchanger is eliminated. The positively charged edges on the layers of MMT can interact with anionic polymer like alginate (sodium salt of alginic acid) to form unique polymer silicate materials, having superior capability to incorporate drug molecules. Involvement of MMT to alginate composites decreases the drug release rate by increasing drug/matrix adsorbtion capacity/entrapment efficiency [10].

Alginate is widely used as delivery vehicle for controlled release of therapeutic agents [11]. The capacity of alginate to form gel in the presence of multivalent cations (ionotropic gellation technique) has been exploited to prepare multi-particulate systems, incorporating numerous drugs, proteins, cells or enzymes. Alginate is a linear, naturally occurring polysaccharide extracted from brown seaweed of species Laminaria. Alginic acid contains D-mannuronic (M) and L-glucuronic (G) acids which are arranged in homopolymeric MM or GG blocks with an alternating structure [12]. Sodium alginate is the water soluble form, that upon quenching with Ca^{2+} crossliks to the water insoluble form of calcium alginate. The divalent cation bridges the gap between two polimer chains, stabilizing the network in turn. The crosslinking source is calcium chloride. Dropwise addition of aqueous alginate solution to the aqueous solution containing di or polyvalent cations causes spherical gel formation, denoted as "alginate bead".

Alginate shrinks at low pH (gastric environment) and the encapsulated drug cannot be released in the stomach [13]. As the hydrogel passes down the intestinal tract, along with pH increase, the degree of swelling increases, which facilitate its disintegration and thus a controlled release of the drug [14]. This way, acid sensitive drugs are protected from gastric juice, being consequently released from the beads in the intestine [15].

A low entrapment efficiency of water-soluble drugs in the alginate beads is a problem for developing a drug delivery system. This is due to the leakage of drug molecules from the wet beads during the cross-linking process. Other major disadvantages of alginate beads are their fast disintegration in intestinal fluid and their high porosity, which result in rapid drug release. An alternative approach to improve drug entrapment efficiency and modulate drug release involves the incorporation of water insoluble materials, like clays [16].

Biopharmaceutical characteristics of alginate beads prepared by ionotropic gelation depend on their size and shape, as well as their morphology. It was demonstrated that optimization of bead shape, size and morphology can be achieved by altering the processing parameters like hardening time, temperature and concentration of calcium chloride solution and drying conditions [17]. Drying technique can influence, besides the size and shape of the beads, their mechanical

properties, swelling properties, drug release kinetics and drug migration to the periphery of the beads along with water during drying. Concerning the latter, the accumulation of drug on the surface of the beads could be accountable for several difficulties, i.e. burst effect in drug release, ineffective taste masking of drugs with unpleasant taste or drug loss due to crumbling of the drug from the bead surface. Drug migration during bead drying can be avoided by freeze-drying which involves sublimation, but not evaporation of water molecules.

Oral chemotherapy offers great challenges in drug delivery in order to maintain an appropriate drug concentration in the circulation and to achieve prolonged exposure of the drug to the cancerous cells. This will increase citostatic drug efficiency and decrease its side effects. Unfortunately, most anticancer drugs are not orally bioavailable, i.e. not absorbable in the gastrointestinal tract.

Carboplatin (Figure 1) [Pt(CBDCA-*O,O'*)$(NH_3)_2$] (CBDCA=cyclobutane-1,1-dicarboxylate) is a platinum-based antineoplasic drug used to treat many types of cancer. It was introduced in the late 1980s and has since gained popularity in clinical treatment due to its vastly reduced side effects compared to its parent compound cisplatin.

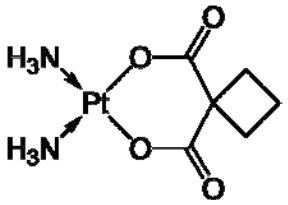


Fig. 1. Carboplatin structure

The scope of the present study was the preparation and characterization of carboplatin nanocomposite beads based on montmorillonite (MMT) and sodium alginate as drug carriers. After carboplatin incorporation into MMT, the resulting hybrid was compounded with alginate in order to obtain CP-MMT-alginate nanocomposite beads. The beads are expected to bypass the gastric environment and deliver the drug in the intestine, due to the presence of alginate. Considering that nowadays carboplatin (CP) is administered only by i.v. route, this new composite material has the potential of becoming a DDS in oral chemotherapy. This capacity will be further confirmed by in vitro and in vivo experiments.

The hybrid and composite materials were characterized by means of X-ray diffraction (XRD), IR spectroscopy (FT-IR), thermal analysis (DTA-TG) and scanning electron microscopy (SEM). Carboplatin incorporation efficiency (IE) in MMT and in alginate beads was determined by UV-Vis spectroscopy (λ_{max}=233 nm).

2. Experimental

Materials. Sodium montmorillonite (Cloisite Na^+) was obtained from Sowthern Clay Products Inc. Carboplatin (purity min. 98%) was purchased from Sigma Aldrich, and alginic acid sodium salt (low viscosity), from Alfa Aesar. Calcium chloride dihydrate (99.99%) was from Sigma Aldrich. All chemicals were used as received, without further purification.

CP-MMT hybrid material preparation. CP-MMT hybrid material (initial drug:clay mass ratio of 40:60) was obtained by mixing the active substance aqueous solution with the swelled clay, as described in our former article [21]. In order to establish carboplatin incorporation efficiency (IE) in MMT, the resulting hybrid stable suspension was centrifuged, and the drug concentration in the supernatant was determined by UV-Vis spectroscopy at λ_{max}=233 nm (the amount of the drug incorporated in the clay was calculated as the difference between the initial amount of CP and the amount of CP in the supernatant). The hybrid material was dried in air.

CP-MMT-alginate composite beads preparation. The preparation of CP-MMT-alginate composite beads (initial hybrid:alginate mass ratio of 15:85), presented in figure 2, implied the following steps: (*i*) preparation of 2% (w/v) sodium alginate aqueous solution; (*ii*) preparation of the composite suspension (0.175 g of CP-MMT hybrid powder was slowly added under magnetic stirring to the alginate solution; the temperature was kept at 60ºC and the stirring speed was fixed at 600 rpm throughout the 4 hour interval in order to obtain an homogeneous solution); (*iii*) preparation of the CP-MMT-alginate composite beads by means of the ionotropic gelation technique [10, 16, 18].

For the preparation of CP-MMT-alginate composite beads, a volume of 39 cm^3 of bubble free, homogeneous CP-MMT suspension was dropped from the tip of a 22-guage hypodermic needle fitted with a rubber tubing to a peristaltic pump (2 cm falling distance, 2.5 ml/min pumping rate) into 100 ml of 0.27M (3.1% w/v) calcium chloride aqueous solution, which was magnetically stirred (200 rpm). The formed beads were allowed to cure in the gelling medium for 30 minutes and then they were three times washed with water and separated by decantation.

Carboplatin encapsulation efficiency in alginate beads (IE) was determined by measuring the drug UV absorbance (λ_{max}=233 nm) in the resulting calcium chloride solution (the amount of drug encapsulated in alginate beads was calculated as the difference between the initial amount of CP added as CP-MMT hybrid and the amount of CP in the gelling medium).

The beads were dried using two different methods, air-drying and freeze-drying. Besides the size and the shape of the beads or their swelling properties, the drying technique can influence the drug release kinetics and the drug migration to the periphery of the beads along with water during drying. The accumulation of

the drug on the surface of the beads could be accountable for several difficulties, such as burst effect in drug release or drug loss due to crumbling of the drug from the bead surface. Drug migration during bead drying can be avoided by freeze-drying which involves sublimation, but not evaporation of water molecules.

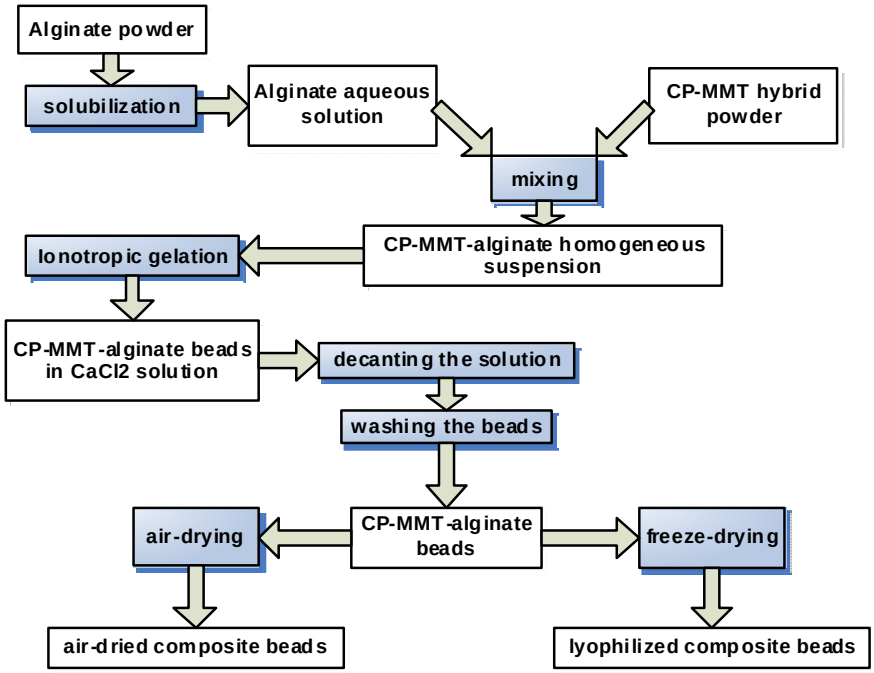


Fig. 2. Flow chart of CP-MMT-alginate composite beads preparation

Characterization. For identifying the crystalline phases of the hybrid and composite powders, X-ray diffraction analysis was performed on a Shimadzu diffractometer XRD 6000. Additional information regarding the structure and the chemical bonds between chemical species was carried out by infrared spectroscopy in 4000-200 cm^{-1} wavenumber range using a Bruker Tensor 27 spectrophotometer (CsI pellet technique). Thermal analysis (TG-DTA) was performed using a differential thermal analyzer Shimadzu DTG-TA 51H (30 - 1000ºC temperature range and 10ºC/min heating rate). Morphological study of the synthesized materials was carried out using a scanning electron microscope Quanta Inspect F (1.2 nm resolution). Carboplatin incorporation efficiency was determined by Jasco UV-Vis V560 spectrophotometer at λ_{max}=233 nm.

3. Results and discussion

Carboplatin incorporation efficiency. Table 1 comprises the calculated values of CP incorporation efficiency (IE) and incorporation yield (η) in the hybrid and composite materials, using the equations (1) and (2), where: Q_{pCP} = practical drug quantity in the material (hybrid or composite), determined spectrometrically; Q_{tCP} = theoretical drug quantity in the material (amount of the initial load); $Q_{t\ material}$ = theoretical quantity of the material.

$$IE = (Q_{pCP}/Q_{t\ material}) \cdot 100 \quad (1)$$

$$\eta = (Q_{pCP}/Q_{tCP}) \cdot 100 \quad (2)$$

Table 1

Carboplatin incorporation efficiency (IE) and incorporation yield (η) determined using spectrometrical data

Material	MMT (% w)	CP (% w)	Alginate (% w)	IE (% w)	η (%)
CP-MMT hybrid	60	40	-	7	17.6
CP-MMT-alginate composite	11.5	3.5	85	0.17	16.4

X-ray diffraction

Fig. 3 shows XRD images for MMT, carboplatin and for CP-hybrid and composite powders.

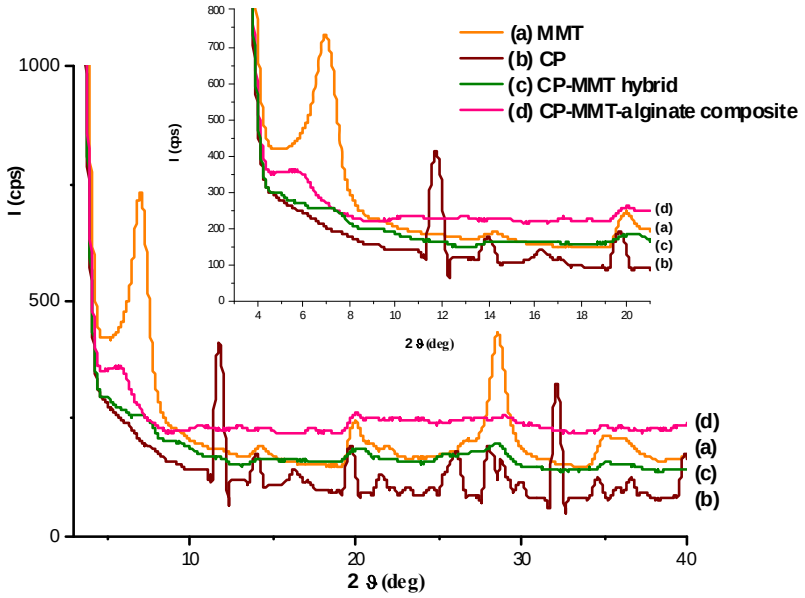


Fig. 3. XRD patterns for: (a) - montmorillonite clay- MMT; (b) - carboplatin-CP; (c) - CP-MMT hybrid; (d) - CP-MMT-alginate composite

For CP-hybrid material, MMT characteristic peaks decreased in intensity or even disappeared. Instead, drug characteristic peaks are present (some of them appear at diffraction angle values of: 11.93, 14.31, 15.11, 16.13, 16.86, 32.67, 39.91, and 42.44 to 62.96 deg).

Characteristic diffraction peak of the [001] plane for MMT appears in the pure clay diffractogram at 2θ=7.23º (basal spacing d=12.22 Å), while for CP-MMT it shifts to 2θ=6.17º (d=14.31 Å). According to Bragg's law, the peak shifting to a lower diffraction angle is due to an increase in the clay basal spacing. This fact indicates that the clay-drug ionic exchange took place and carboplatin entered the montmorillonite interlayer spacing.

Interaction between CP-MMT and alginate results in a tactoid formation [22], as it can be observed in fig. 3 from the broadening of MMT [001] peak and from the slight increase of the corresponding basal spacing (d=14.44 Å at 2θ=6.11º).

From the XRD analysis it can be concluded that alginate is not intercalated into MMT structure, but it interacted with the clay surface hydroxyl groups. Alginate and MMT formed electrostatic and intermolecular hydrogen bonds, which brought about numerous contact points and created a three-dimensional network. Alginate chains could also serve as a bridge between neighboring silicate layers, if a higher content of MMT was incorporated.

FT-IR spectroscopy

The absorption bands from FT-IR spectrum of MMT are assigned as follows: 3450 and 3631 cm^{-1} correspond to OH stretching mode in molecular water and in Si-OH, Al-OH bonds, respectively; 1640 cm^{-1} corresponds to the bending vibration of water; 1044, 623 and 523 cm^{-1} are attributed to Si-O stretching in $[SiO_4]^{4-}$ tetrahedra; 916 cm^{-1} is for Al-Al-OH bending vibration; 798 cm^{-1} corresponds to Si-O vibration in SiO_2 and 467 cm^{-1} to Si-O-Si and Na-Al-OH vibrations.

The most intense peaks in carboplatin spectrum (fig. 4.a.) are attributed to esther groups (C=O at 1642 cm^{-1} and C-O at 1348 cm^{-1}) and to associated NH group (at 3270 cm^{-1}). The bands at 2958 and 2862 cm^{-1} correspond to asymmetric and symmetric CH stretching vibrations, respectively. Characteristic IR peaks for Pt-NH_2 bond appear at 1380 and 1289 cm^{-1}. The peaks at 1610 and 1464 cm^{-1} are attributed to the bending vibrations of NH and NH_2, respectively.

Sodium alginate showed asymmetric and symmetric stretching vibrations at 1627 and 1415 cm^{-1} due to carboxyl anions and at 1034 cm^{-1} for oxygen stretching in cyclic ether bridge. The band at 3439 cm^{-1} corresponds to OH stretching vibration.

FT-IR spectrum of carboplatin-loaded MMT hybrid system (fig. 4.b.) resembles to the superimposition of the spectra corresponding to the drug and to

the clay. This similarity between the spectra indicates that the interaction between the drug and the clay is rather weak. The band due to the deformation mode of molecular water, originally recorded at 1640 cm^{-1} in MMT spectrum, is much weaker and might be overlapped by the carboplatin peaks (1642 cm^{-1} and 1610 cm^{-1} – due to C=O and N-H bending vibration, respectively), suggesting that drug intercalation has displaced (probably not completely) the water molecules from the clay interlayer space. The fact that the relative intensity of the broad peak due to OH vibration in hydrogen-bonded hydroxyl has decreased (although a contribution from structural hydroxyl groups to this band cannot be ruled out) shows that carboplatin is adsorbed also onto the surface.

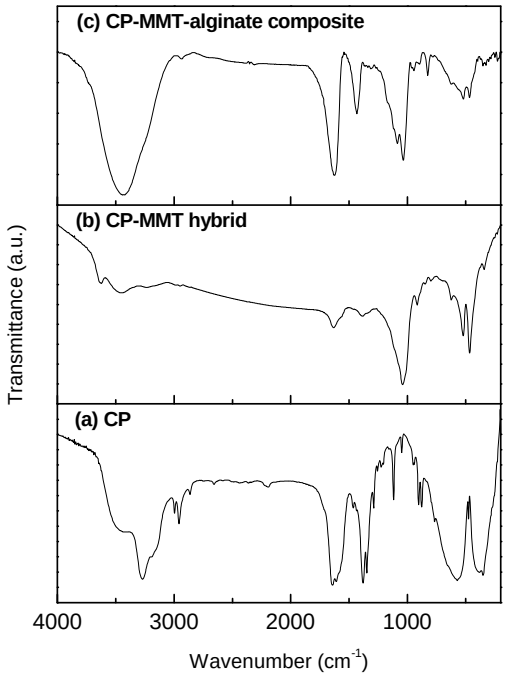


Fig. 4. FT-IR spectra for carboplatin (CP), CP-MMT hybrid and CP-MMT-alginate composite

From FT-IR spectrum of the composite material (fig. 4.c.) it can be observed that their chemical structure is similar to alginate, which is the major fraction in their composition. Carboxyl stretching peaks of alginate decrease in intensity and change as wave number. The negative charge of the carboxyl groups may have an electrostatic interaction with the positively charged sites situated at the edges of MMT (in accordance with [19]). OH stretching peak of silanol group

(3626 cm^{-1}) disappeared in the CP-MMT-alginate composite and OH stretching peaks of alginate are shifted. These observations put in evidence the existence of intermolecular hydrogen bonding and electrostatic forces between alginate and montmorillonite, as confirmed also by XRD analysis (fig.3).

Thermal analysis (DTA-TG)

Thermal analysis (DTA-TG) (fig. 5) brings additional information regarding chemico-mineralogic composition of the hybrid and composite materials. The obtained data are presented in table 2.

For MMT-type mineral clay, the total weight loss of 17.03% is attributed to the loss of adsorbed water (11.47%), with an exothermic effect on DTA curve having a maximum at approx. 80ºC and to the loss of structural water (2.76%), presenting an exothermic effect on DTA curve with a maximum at approx. 658ºC.

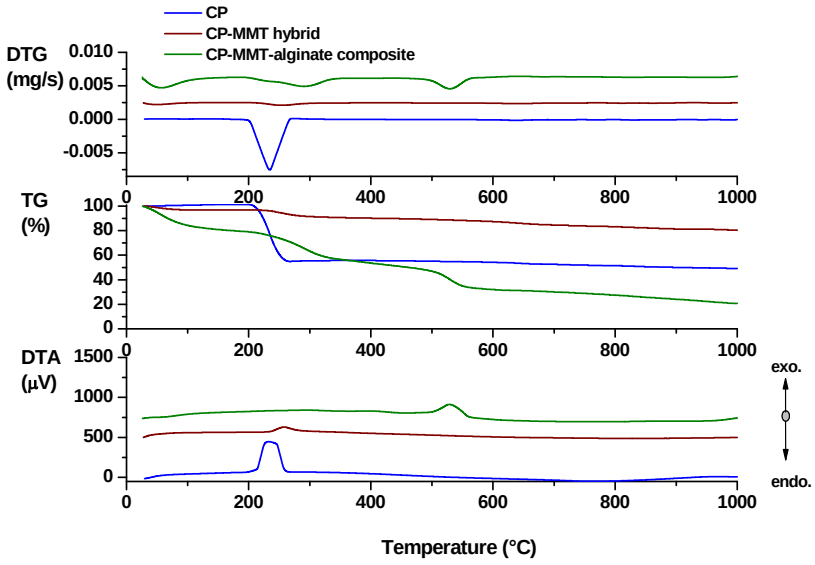


Fig 5. Derivatograms for carboplatin (CP), CP-MMT hybrid and CP-MMT-alginate composite

Carboplatin shows a strong weight loss in the 200-270ºC interval, with an exothermic effect on DTA curve having a maximum at 235ºC and another one, very weak, in the temperature region of 700-1000ºC. The strong exothermic effect on DTA curve with a maximum rate at 235ºC is coupled with an effect on DTA curve and with weight loss on TG curve, suggesting that it corresponds to drug decomposition. The weight loss in the 700-1000ºC interval may be due to the burning of the carbon (probably with a low crystallinity degree) which results from drug decomposition.

The CP-MMT hybrid loses weight in four steps: the first weight loss takes place at approx. 47ºC, due to adsorbed water evaporation (i.e. physisorbed drug, weakly bonded on the surface); the second weight loss, in the 200-350ºC interval (exothermic effect on DTA curve with a maximum at 255ºC, associated with an effect on DTG curve and with a weight loss on TG curve) corresponds to drug decomposition; the third weight loss in the 550-700ºC temperature region is due to structural water removal (i.e. strongly bonded drug, intercalated drug molecules); the fourth weight loss in the 700-1000ºC interval may be due to the burning of the carbon (probably with a low crystallinity degree) which results from drug decomposition. Being cationic in nature, carboplatin was intercalated into the MMT interlayer by ionic exchange, replacing the sodium ions. In the temperature range of 550-700ºC, the MMT weight loss due to structural OH group release is 2.76%, whereas for CP-MMT hybrid, the weight loss is higher (3.69%). This fact pertains to the total replacement of the clay structural hydroxyl groups by carboplatin during intercalation (according to [20]).

Alginate powder derivatogram presents three exothermic effects on DTA curve at 241ºC, 341ºC and 586ºC, coupled with weight loss on TG curve, which are associated to alginate decomposition. The weight loss in the 700-1000ºC interval, with a maximum at 967ºC, may be due to the burning of the carbon (probably with a low crystallinity degree) resulting from alginate decomposition.

In CP-MMT-alginate composite derivatogram, the main effects correspond to that of alginate, which is the major fraction in its composition.

Table 2

Material weight loss (Δm) determined using thermal analysis data.

Material symbol	Temperature, (ºC)						Δm, (%) 30-1000ºC
	30-150	150-200	200-270	270-550	550-700	700-1000	
CP	0.25	0.2	44.61	0.83	1.98	3.12	50.99
MMT	11,47	1,7			2.76	1.09	17.03
CP-MMT	3.14	0.1	4.08	4.07	3.69	4.33	19.41
CP-MMT-alginate	18.65	1.96	10.7	31.8	5.05	8.7	76.86
Alginate	13.6	2.24	29.1	10	13.82	13.87	82.63

SEM analysis

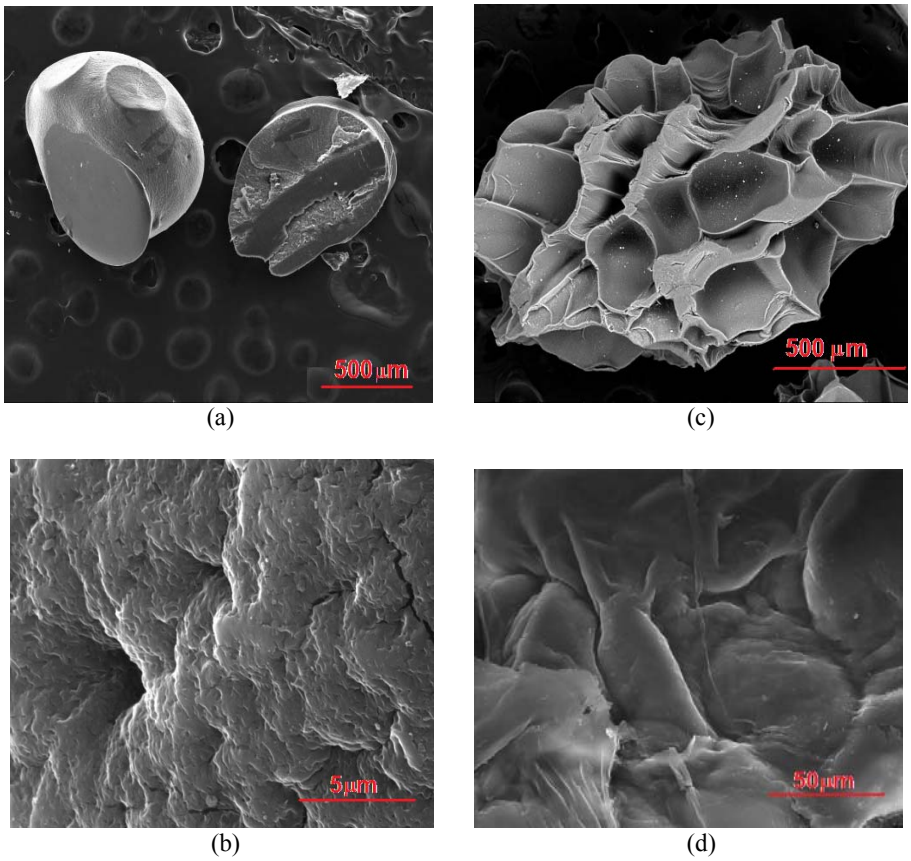
As it can be observed in fig. 6, which presents the morphological characteristics of CP-MMT-alginate composite beads, the drying technique has influenced the size and the shape of the beads.

The freeze-dried beads are larger than the air-dried ones, as they remained almost the same size as before drying. Furthermore, they are the most spherical and have a cellular structure. Such characteristics of the lyophilized beads are due to the fast sublimation of frozen water from the matrix, thus the beads having no time to shrink, resulting in cell formation in the areas of former ice crystals. The

air-dried composite beads have a smooth surface, while the walls of the freeze-dried beads have reduced porosity and relatively smooth surface.

By freeze-drying process, drug migration to the periphery of the beads along with water and its accumulation on the bead surface can be avoided.

It is expected that, as compared to the air-dried beads, the lyophilized ones – which have a cellular structure, and thus a better swelling capacity – will ensure a near to "zero order" drug release kinetics, without any burst effect during drug release.



(a) (c)

(b) (d)

Fig. 6. SEM images of carboplatin-based alginate beads: (a, b) air dried and (c, d) lyophilized

4. Conclusions

The incorporation of carboplatin into MMT by ion exchange mechanism has been performed, both within the interlayer spacing and on the surface. The resulting hybrid was further compounded with alginate. The nanocomposite beads were successfully prepared by ionotropic gelation technique. Their XRD and FT-IR analysis reveal the formation of an intercalated composite. SEM analysis shows that the drying technique has influenced the morphology of the beads. As compared to the air-dried beads, the lyophilized beads are larger, more spherical in shape, and have a cellular structure. Drug incorporation efficiency, determined by UV-Vis spectroscopy, was found low for both the hybrid and the composite material. This behaviour has been explained by the high water solubility of carboplatin. The quantity of drug incorporated in alginate composite was much lower as compared to the hybrid.

Consequently, the nanocomposite beads represent a potential DDS for oral administration of carboplatin. The results should be further confirmed by in vitro and in vivo release experiments.

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