Biopolymeric Microcapsules for Controlled Release of Pralidoxime Chloride

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Pralidoxime chloride (PAM) was encapsulated in biopolymeric membranes in order to evaluate the kinetics of release in aqueous solution. PAM is an efficient cholinesterase reactivator with a pronounced hydrophilic character. The biopolymeric membranes were prepared by crosslinking, directly in aqueous solution and the monodisperse spherical microcapsules made by this method had diameters between 1 and 3 mm. PAM was embedded "in situ" in microcapsules prepared from natural polymers as sodium alginate, chitosan and gelatin. The release data were fitted by first order kinetic equation, Weibull, Peppas and Higuchi semiempirical equations to obtain the kinetic parameters of controlled PAM release. The embedding of PAM in biopolymeric membranes was achieved in order to prepare a sustained release pharmaceutical formulation for oral administration.

Keywords: biopolymeric microcapsules, Pralidoxime chloride, drug release

Microcapsules mean spherical particles with diameters ranging from a few micrometers to several milimeters and whose core made up of a certain substance is separated from the external environment by a polymeric semipermeable membrane. During the micro-encapsulation process, the semipermeable membrane which separates two aqueous liquid media is formed around the microcapsule core containing the active substance. The process of encapsulation with polymeric membranes is used in order to ensure a slow and controlled release of the active substance [1]. In pharmaceutical formulations, this encapsulation is made by means of membranes prepared from natural macromolecules.

The biopolymers like alginate, chitosan, collagen, gelatin, starch, cellulose are biodegradable, biocompatible and nontoxic compounds. These properties make biopolymers to be a good choice in pharmaceutical, cosmetical, and food formulations and in tissue engineering [2-5].

Sodium alginate is a copolymer of sodium salts of D-mannuronic (M) and L -guluronic (G) acids and its structure is shown in figure 1.

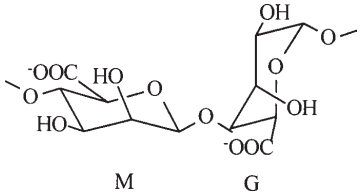

Fig. 1. Molecular structure of alginate

Alginate is a naturally occurring polysaccharide extracted from brown marine algae which can form hydrophilic gels by an interaction with bivalent metallic ions. It has been demonstrated by circular dichroism that calcium ions react preferentially with the polyguluronic segments of alginate [6] leading to the formation of an „egg-box" type structure (fig. 2).

Sodium alginate is used in pharmaceutics as an excipient in pills with controlled release of the drugs [3].

Fig. 2. "Egg-box" type structure of calcium alginate

Due to the intrinsic properties (biocompatibility, mucoadhesiveness, porosity and easy handling), calcium alginate gels can be used for cell encapsulation and tissue regeneration [4].

Calcium alginate capsules are employed as drug vectors with controlled release [5, 7, 8] Encapsulation in calcium alginate gels is a procedure widely used due to its low cost and simple preparation and administration.

The microcapsule characteristics such as thickness of the gel membranes and permeability of different substances through these membranes are easy controlled by changing the gelling conditions. One of the most important natural polymers is chitin, which is the second widestly spread natural polymer after cellulose. Chemical structure of chitin is: 2-amino-2-deoxy-(1→4)-β-D-glucopyranan. Chitosan (CH) is deacetylate chitin, that is a natural cationic polymer which is extracted from crab and shrimp shells. The basic unit of chitosan is 2-deoxy-2-acetyl amino glucose. These units are bound in positions 1-4 and form a linear chain polymer (fig. 3).

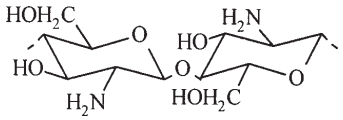

Fig. 3. Molecular structure of chitosan

The most practical applications are based on chitosan capacity to form gels and increase system viscosity. Complexes with anionic surfactants have gelling properties [9]. Chitosan improves the release rate of the encapsulated

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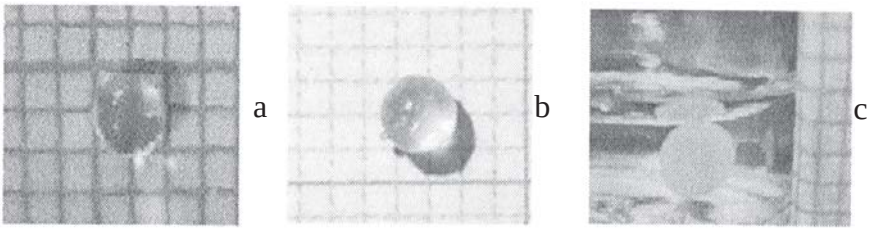

Fig. 4. Images of microcapsules of a) calcium alginate b) CH/NaOH and c) CH/SDS obtained with a video camera

hydrophilic active substances and therefore can be used for enhancing the bioavailability of these substances [10].

Chitosan is admitted in many countries as medicine (allias „magnet" of fats) and food aditive, thus having many practical applications. In pharmaceutical formulations it is used as excipient, sustained release vector for hydrophilic drugs and for enhancing the bioavailability of hydrophobic active substances.

Pralidoxime chloride (PAM) belongs to the class of cholinesteraze reactivators of a pyridine oxime type and it is employed in the treatment of intoxications with organofosforic products. Administration of cholinesteraze reactivators has to be sustained or repeated also in high doses as they are hydrophilic compounds and only a small amount passes through the membrane barriers [11, 12]. The therapeutic doses of PAM are limited by its own toxicity (~1,5 g daily). PAM encapsulation in colloidal vectors of microcapsule type could solve the issue of a sustainable release of a higher amount of drug to be administrated orally.

Experimental part

Materials and methods

Sodium alginate obtained from brown algae, high molecular weight chitosan (M_r ~ 600000) and type B gelatin were all Fluka products. The surfactants were sodium dodecyl sulfate (SDS) from Merck and sodium bis(2-etyl hexyl) sulfosuccinate (AOT) from Sigma. Calcium chloride, sodium hydroxide, acetic acid and ethyl alcohol were Merck reagents. All the reagents were of analytical grade. Bidistilled water was used throughout the study.

Preparation and characterization of biopolymeric microcapsules with PAM embedded

The chitosan solution (1wt%) was prepared in 10wt% PAM solution in 1wt% acetic acid. Sodium alginate solutions 1 and 2wt% were obtained by dispersing the biopolymer in 10wt% PAM aqueous solution. The biopolymeric solutions were stirred at a rate of 500 rpm for 24 h at 40°C by means of a stirrer provided with a heating device (IKA Labortechnik). Gelatin powder (1 and respectively 0.2g) was dissolved in 10 mL of 2wt% sodium alginate/PAM solution under stirring for 3 h at 50°C.

The microcapsules are formed instantaneously when the drops of biopolymeric solutions come into contact with a coagulant solution. The solution which contains chitosan and PAM was dripping by means of a microsyringe into the coagulant aqueous solutions of SDS (0.03 mol/L), AOT (0.03 mol/L) and NaOH (0.1 mol/L) in order to obtain CH/NaOH, CH/SDS and CH/AOT microcapsules. The 5wt% calcium chloride solution was used as coagulant for 1 and 2wt% alginate/PAM and 2wt% alginate/gelatin (1:1 and 1:5 mass ratio)/PAM solutions.

„In vitro" release of PAM from microcapsules.

The study on PAM release from a fixed quantity of microcapsules freshly prepared and washed with distilled water was performed by a spectrophotometric monitoring of the PAM amount released in 500 ml distilled water. PAM concentration in the aqueous phase was determined spectrophotometrically at λ = 294 nm (spectrophotometer UNICAM).

Experiments were carried out at temperatures between 20 and 25°C.

Results and discussions

The microcapsules of CH/NaOH, CH/SDS and CH/AOT have external diameters between 1 and 3 mm, while those of alginate and alginate/gelatin between 2 and 3 mm. In figure 4, selected images of alginate, chitosan and CH/surfactant microcapsules are depicted.

The membranes of CH/surfactant gel are very thin and have a high flexibility (they got deformed in air) but, in the same time, exhibit a good enough mechanical resistance. The alginate gel membranes are rigid and preserve their spherical form in air.

The diffusion rate of PAM through polymeric membranes was modified by varying polymer concentration, porosity and diffusion coefficient. PAM transport through macroporous membranes is facilitated by the presence of the aqueous medium for the release in the porous network. Cumulated amounts, expressed as mass % of PAM released from alginate microcapsules are presented in figure 5.

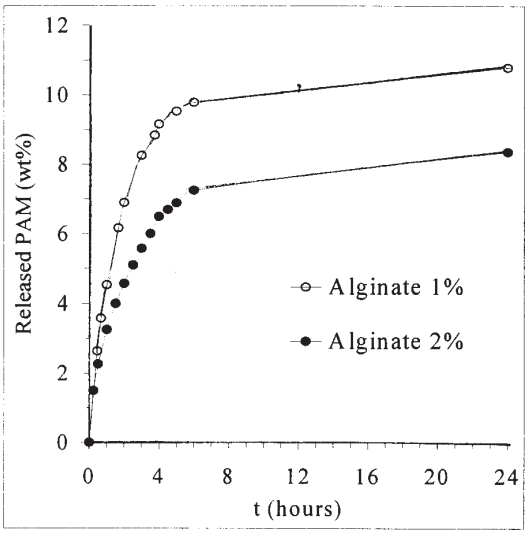

Fig. 5. Cumulative curve of PAM released from calcium alginate microcapsules prepared from sodium alginate solutions of different concentrations

The total quantity of PAM released from alginate microcapsules in 48 h does not exceed 10wt%. It is to be expected that a small encapsulated molecule such PAM to be totally released in a short time due to the high porosity (11.2 - 20nm) of the calcium alginate gel membrane [13]. Because of the ionic interaction between pyridine nitrogen of PAM and alginate polyanion, PAM remains electrostatically bound within the microcapsule. Calcium ions interact preferentially with carboxylic groups of the polyguluronic segments of the alginate [2] and thus favour PAM binding to the alginate mannuronic segments which connect together the "egg-box" type structures. An

increased sodium alginate concentration implies an increase of the number of guluronic groups with which calcium ions interact. This interaction leads to the formation of a gel having a much more dense structure. The number of mannuronic groups where PAM is bound increases in the same time and therefore the amount of PAM released by diffusion from microcapsules decreases correspondingly.

The presence of type B gelatin with positive electrical charge determines its binding to the alginate and blocks the negative centers of the polyanion carboxylic groups. The amount of PAM released from 2wt% alginate/gelatin/PAM microcapsules increases with the gelatin amount in the polymeric mixture (fig. 6). After 6 h, PAM molecules are released from alginate/gelatin microcapsules in proportion of 74% for a 1:5 alginate/gelatin ratio and 66% for 1:1 alginate/gelatin ratio (fig. 6).

PAM is released from CH/NaOH microcapsules in 6 h (fig.7).

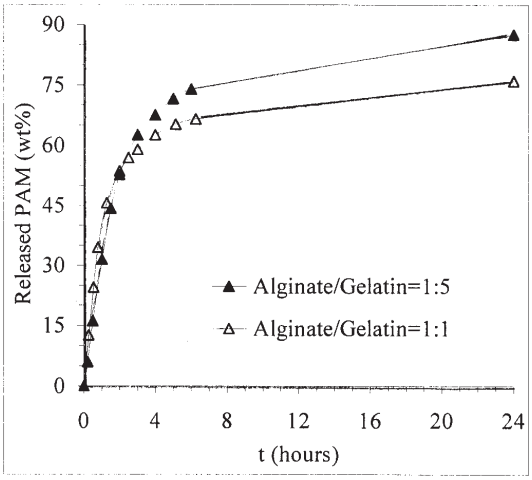

Fig. 6. Curves of PAM release from microcapsules prepared from alginate and gelatin B

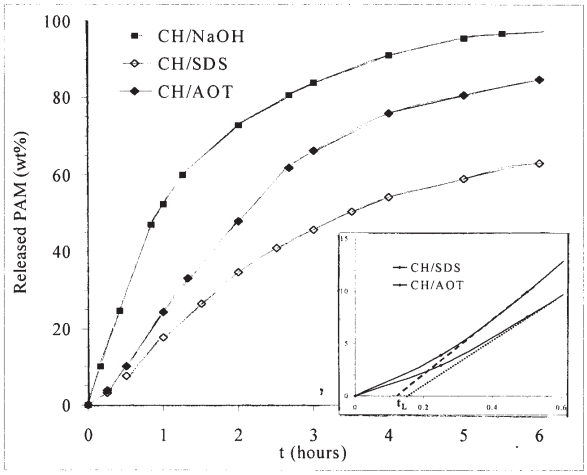

Fig. 7. Profiles of curves of PAM release from chitosan microcapsules

Chitosan builds a network with a uniform pore structure in an aqueous medium [14]. The chitosan microspheres prepared by coagulation in an alkaline medium have porous structures with average pore dimensions of 0.157μm [15]. The porosity of CH/NaOH microcapsules can be held responsible for the rapid release of the micromolecules. CH/NaOH microcapsules can be employed as colloidal transport systems but still, the presence of toxic sodium hydroxide in the preparation recipe of the pharmaceutical form is a disadvantage. Surfactant utilization as coagulant eliminates the problem of toxicity and the diffusion of PAM through CH/SDS and CH/AOT membranes is retarded.

The experimental concentration values of the active substance released as a function of time were processed for establishing the release kinetics. The following first order equation was used [16]:

$$M_t / M_\infty = 1 - exp(-k_1 t) \quad (1)$$

where M_t is the amount of drug released at time t, M_∞ the amount of drug release after infinite time and k_1 the release rate constant.

A characteristic drug release profile of the drug delivery systems is represented by the Weibull's equation [16]:

$$M_t / M_\infty = 1 - exp(-k_W \times (t - t_L)^n / n) \quad (2)$$

where t_L is lag time, k_w the release rate constant and n a formal parameter whose value depends on the evolution of the release kinetics. Weibull equation describes well the release curves, mainly those exponential and sigmoid ones.

In order to understand the mechanism drug release from polymeric-controlled delivery systems of different geometries, the first 60% of drug release was fitted to the Korsmeyer-Peppas model by an empirical power equation [16]:

$$M_t / M_\infty = k_P t^m \quad (3)$$

where k_P is a release rate constant incorporating structural and geometric characteristics of the delivery system and m is the diffusional exponent indicative of the mechanism of drug release. Only the two extreme values 0.5 and 1.0 for thin films have a physical meaning. At the lower extreme values, pure Fickian diffusion operates and results in diffusion-controlled drug release. When m has the value equal with 1, zero-order kinetics (Case II transport) are justified. Finally, the intermediate values of m indicate a combination of Fickian diffusion and Case II transport, which is usually called anomalous transport.

The dissolution data was also fitted to the Higuchi's equation [16]:

$$M_t / M_\infty = k_H \sqrt{t} \quad (4)$$

where k_H is the diffusion rate constant. The linear plot of the cumulative amount of drug released (utilizing data up to 60% of the release curve) versus the square root of time is routinely used as an indicator for diffusion-controlled drug release from delivery systems.

Kinetic parameters for the first 6 h of release are listed in tables 1-4.

The value of n parameter obtained by fitting experimental data with Weibull equation, offers information concerning the existence of free active substance in the polymeric membrane. PAM is present in the most polymeric membranes ($n \leq 1$), respectively in the liquid that soakes membrane pores, with the exception of CH/surfactant gel membranes ($n>1$) where the release curves exhibit a lag time (fig. 7). The lag time characterizes the sigmoid release curves and represents the period of time needed by the release substance to pass through the membrane and establish an uniform concentration gradient.

PAM release from the studied microcapsules follows a first order kinetics for the first 6 hours of release. The rate constants are given in table 2.

Table 1
KINETIC PARAMETERS CORRESPONDING TO WEIBULL EQUATION (EQ.2) FOR PAM RELEASE FROM POLYMERIC MICROCAPSULES

System	k_W (hour^{-1})	n	R^2
Alginate 1%	0.6271	0.98	0.9976
Alginate 2%	0.5243	0.87	0.9890
Alginate/gelatin 1:1	0.8001	0.94	0.9970
Alginate/gelatin 1:5	0.6299	1.05	0.9993
CH/NaOH	0.7085	0.96	0.9974
CH/SDS	0.4223	1.29	0.9982
CH/AOT	0.4355	1.36	0.9995

Table 2
KINETIC PARAMETERS CORRESPONDING TO FIRST ORDER EQUATION (EQ. 1) FOR PAM RELEASE FROM POLYMERIC MICROCAPSULES

System	k_I (hour^{-1})	R^2
Alginate 1%	0.6322	0.9975
Alginate 2%	0.5417	0.9837
Alginate/gelatin 1:1	0.8473	0.9959
Alginate/gelatin 1:5	0.5956	0.9962
CH/NaOH	0.7275	0.9971
CH/AOT	0.4327	0.9793
CH/SDS	0.4242	0.9828

Table 3
KINETIC PARAMETERS CORRESPONDING TO KORSMEYER-PEPPAS EQUATION (EQ. 3) FOR PAM RELEASE FROM POLYMERIC MICROCAPSULES

System	k_P (hour^{-1})	m	R^2
Alginate 1%	0.4910	0.45	0.9812
Alginate 2%	0.4515	0.46	0.9959
Alginate/gelatin 1:1	0.5400	0.51	0.9864
Alginate/gelatin 1:5	0.4131	0.70	0.9916
CH/NaOH	0.5296	0.83	0.9971
CH/AOT	0.2700	1.00	0.9976
CH/SDS	0.3283	0.66	0.9903

The lower values of correlation coefficients and the rate constants were obtained for the release of active substance from CH/surfactant microcapsules according with n values. The much more compact structure of CH/surfactant membranes explains the lower value of k_I.

Table 4
KINETIC PARAMETERS CORRESPONDING TO HIGUCHI EQUATION (EQ. 4) FOR PAM RELEASE FROM POLYMERIC MICROCAPSULES

System	k_H (hour$^{-1/2}$)	R^2
Alginate 1%	0.4529	0.9763
Alginate 2%	0.4339	0.9941
Alginate/gelatin 1:1	0.5432	0.9863
Alginate/gelatin 1:5	0.4660	0.9714
CH/NaOH	0.5100	0.9660
CH/AOT	0.3559	0.9226
CH/SDS	0.4021	0.9748

Korsmeyer-Peppas equation allows for defining the release mechanism starting from the m parameter value.

PAM is released from both alginate and alginate/gelatin 1:1 microcapsules accordingly to a diffusion mechanism. For the other systems, the PAM diffusion coefficient is not constant as it is affected by the membrane higher density, increased viscosity of the liquid filling the membrane pores, as well as interactions with membrane components.

The release of the encapsulated substance is not always controlled only by the polymeric membrane, but also by the active substance diffusion inside the microcapsules. One can see from Table 3 that the highest influence of the diffusion inside the system is registered in the case of alginate microcapsules whose interior contains sodium alginate which slows down PAM diffusion due to the possible electrostatic interactions with the latter. At a higher concentration of alginate the rate constant, k_p, decreases.

The increasing of gelatin concentration in alginate microcapsules lead to obtain a higher value of exponent m which indicates a retarded diffusion of PAM and dissolution the excess of gelatin gel. The decreasing diffusion of PAM in membrane pores with positive gelatin filling is also evidenced by diminution of k_p.

The kinetics of PAM release from CH/surfactant microcapsules is affected by the way whereby the surfactants are bound physically to chitosan in order to form an insoluble complex [17]. The CH/SDS complex is formed by grafting the surfactant spherical micelles onto the polycation chain. Physical binding of AOT surfactant to chitosan in order to form stable capsules occurs at surfactant concentrations which correspond to the formation of lamellar micelles.

The dense lamellar structure of CH/AOT membrane can explain the zero order retarded release of PAM characterized by the value of m which is equal with 1 and the low value of k_p. The anomalous release of PAM from CH/NaOH and CH/SDS (m values between 0.5 and 1) indicates that the diffusion takes place in the same time with the water uptake which produce the errosion of biopolymeric membrane. The penetration of water is due to the porosity of CH/NaOH membrane and the spherical micelles/chitosan structure of CH/SDS membrane.

Kinetic parameters corresponding to Higuchi equation are according with those obtained from Korsmeyer-Peppas equation. The correlation coefficients with high values were obtained for the diffusion controlled release (m=0.5).

The lower values of correlation coefficients obtained for PAM release from CH/NaOH and CH/AOT microcapsules confirm the values of m parameters obtained by fitting experimental data with Korsmeyer-Peppas equation.

Conclusions

In order to obtain a pharmaceutical formulation for oral administration with controlled and sustained release, PAM was embedded in alginate, alginate/gelatin, CH/NaOH and CH/surfactant microcapsules. The present work illustrates the fact that kinetic parameters of release depend on microcapsule structure and interactions of the active substance with the components of the drug vector.

PAM is present in the most biopolymeric membranes where it was encapsulated, with the exception of CH/surfactant gel membranes whose release curves present lag time. The kinetic parameters obtained by processing the dissolution data give information on the release mechanism, as well as interactions between the release substance and membrane components.

Alginate microcapsules can be used as vectors for cationic active substances which can be controlled release by diffusion. The presence of gelatin at 1:1 weight ratio in alginate/gelatin microcapsules leads to a quick release of positive active substances which was evidenced by obtaining of a high rate constant from processing of dissolution data with first order and semiempirical kinetic equations. The gelatin with positive electrical charge from 1:5 weight ratio in alginate/gelatin microcapsules is present in excess in the pores of alginate membrane and impedes the diffusion of positive active substance by electrostatic repulsions and increasing the viscosity of diffusion medium.

CH/NaOH microcapsules are not a good vector for active substances because toxicity of NaOH and quick release of the encapsulated substance. The retarded release of positive active substances like PAM from CH/SDS and CH/AOT microcapsules is influenced by the structure of biopolymeric membranes.

The information gathered in this study allow for modelling the release kinetics of the active substances with a structure similar to that of PAM in alginate, alginate/gelatin and CH/surfactant microcapsules.

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