DESIGN OF NEW CHITOSAN/SILICA COMPOSITES FOR DRUG-DELIVERY SYSTEMS.

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Abstract

Due to its biocompatibility, chitosan is used in medical applications as drug delivery system. In this work, we studied the influence of the presence of a mineral part (silica) in chitosan microspheres on their drug delivery properties. Chitosan beads were soaked in a silica precursor solution, and then a condensation step led to the formation of the composite microspheres. Well-defined experimental parameters allowed achievement of homogeneous or core-shell composite microspheres with a controlled shell thickness. Kinetic releases were studied on chitosan microspheres as a reference and on the homogeneous and core-shell composite materials. *In vitro* release of a hydrophilic probe was investigated with simulated intestinal fluid using flow-through cell dissolution. Results indicated that the presence and repartition of silica modify the release kinetic profile.

Introduction

The association between inorganic compounds and biopolymers is common to many natural materials. Such a self-assembly leads to cytocompatibility and, very often, affords good mechanical properties [1]. The man-made synthesis of such materials is a rapidly-expanding field of research [2, 3]. Silica-organics hybrids have been studied both for the comprehension of the role of biomolecules on the silicic polymerization by micro-organisms [4] and for the synthesis of new supports for cells and enzymes [5]. Several polysaccharides have been used as the organic part of the hybrid. [6-9].

Among these, due to the presence of amino groups [10], chitin and especially chitosan, offer an interesting potential for the formation of hybrid materials. Airoldi and Monteiro [11], Wang *et al.*, [12] and Miao and Tan [13] have described chitosan-silica hybrid materials with a lamellar-like surface morphology for biosensor applications. In these materials, the amine groups of the silylating agents are linked to the amine groups of chitosan through linear glutaraldehyde units. Dissolved polysaccharides were used in all the cited preparations, in order to favor a close interaction between the organic moiety and the silica precursors. This approach does not allow shaping the hybrid materials by using the well-proven methods developed for the gelling of polysaccharides alone. This represents a serious drawback for the formulation of drug delivery systems, in which a fine control of morphology is needed to tailor the release properties [14]. An alternative approach can be inspired by the morphosynthetic introduction of silica in already shaped polysaccharide beads [15, 16]. Although chitosan-silica hybrids have been described as interesting materials for drug delivery systems, to the best of our knowledge, no kinetic releases have been reported in the literature.

The present work describes the influence of the presence and the repartition of the inorganic component in the release properties of some chitosan-silica composites. *In vitro* release of a

fluorescent probe, chosen as a model, was investigated with simulated intestinal fluid using flow-through cell dissolution.

Material and Methods

Preparation of chitosan microspheres

Hydrogel beads: chitosan gel beads were formed from an aqueous solution of purified chitosane [17] (Aldrich, degree of acetylation of 10 % as measured by IR spectroscopy, M_v = 700 000 g.mol^{-1} (LM_v) and M_v = 1 200 000 g.mol^{-1} (HM_v) determined by viscosimetry). Chitosan was totally dissolved using a stoechiometric amount of acetic acid with respect to the amount of NH_2 functions under stirring 2 hours at room temperature. Gelation was obtained by dropping the chitosan solution into a 4 M NaOH solution through a 0.8 mm gauge syringes needle. The chitosan beads were left in the alkaline solution for 2 hours, filtered and washed with demineralised water.

Alcogel beads: Hydrogel beads were dehydrated by immersion in a series of successive ethanol-water baths of increasing alcohol concentration (10, 30, 50, 70, 90, and 100 %) during 15 min each (these alcogel beads were directly used for the synthesis of composite chitosan-silica materials). Additional absolute ethanol was used to obtain complete exchange of water in the microspheres.

Aerogel beads: alcogel beads were dried under supercritical CO_2 (sc CO_2) (74 bars, 31.5 °C) in a Polaron 3100 apparatus.

Preparation of chitosan-silica homogeneous composite

Alcogel chitosan microspheres were put into an excess of tetraethoxysilane (TEOS) ($R_{TEOS/chitosan}$=52 w/w). Hydrolysis of TEOS proceeded for 12 hours, and NaF was added to catalyse the condensation of silica. Mixing was obtained by rotation of the flask around its horizontal axis on a Reacx 2 mechanical agitator (overhead mixer). The beads were washed thrice in absolute ethanol bath and when necessary, dried under supercritical conditions.

Preparation of chitosan-silica core-shell composite)

2.4g of alcogel chitosan microspheres were put into a 25 ml wealthon flask with 10 ml of ludox colloidal solution (∅=12 nm). The initial pH (9.84) was neutralised to 7.4 using HCl 2N. Mixing was obtained by rotation of the flask around its horizontal axis on a Reacx 2 mechanical agitator (overhead mixer). After reaction, beads were washed with absolute ethanol and when necessary dried under supercritical conditions.

Instrumentation

Scanning electron micrographs (SEM) of the dried beads were obtained on a Hitachi S-4500 apparatus after platinum metallization.

Nitrogen adsorption/desorption isotherms were recorded in a Micromeritics ASAP 2010 apparatus at 77 K after outgassing the sample at 353 K under vacuum until a stable $3 \cdot 10^{-5}$ Torr pressure was obtained without pumping. Surface area and micropore volume were evaluated by the alpha-S method by using a reference isotherm measured on non-porous fumed silica (Aerosil 200) [18]. Thermogravimetric analyses were performed with a Netzsch TG 209 C apparatus in air flow at heating rate 5 °C/min. The local composition on gel cross-sections was analysed by EDX microprobe on a Cambridge Stereoscan 260 apparatus.

Dynamic viscosity of chitosan solutions was measured using a controlled stress Haake rheometer (Rheostress RS 100). The rheometer was equipped with a double gap concentric cylinder geometry for low chitosan concentrations (below 0.3% - determination of the molecular weight) or a cone and plate geometry with a cone angle of 1° and a diameter of 60 mm for high concentrations (from 0.5% to 3 %).

In the last case, the measurements were conducted with shear stresses ranging from 0.01 to 884 Pa at a temperature of 25 (± 0.1) °C. Two distinct viscosity regions are observed on the viscosity curve: a plateau region at low shear stress corresponding to the Newtonian behaviour, and a shear thinning region at high shear stress. The viscosity was evaluated by an average of 10 points at the Newtonian plateau [19].

Loading procedure

1g of alcogel microspheres were impregnated with a solution of 5(6)-carboxyfluorescein (5(6)-CF) in PBS buffer pH 7.4 [10^{-2}M] during 1 hour. Then beads were quickly washed thrice with 10 ml of PBS buffer in order to remove the excess of 5(6)-CF molecules coated on the outer surface. The amount of 5(6)-CF remaining in the supernatant phase was determined using HPLC analysis with UV detection at 254 nm. The amount of 5(6)-CF adsorbed onto beads was determined by the depletion method. Part of the *5(6)-CF loaded hydrogel bead*s were dried at 50°C to give *5(6)-CF loaded xerogel* beads.

Release procedure

The release kinetics of 5(6)-CF were studied using a USP/EP flow-through method (dissolutest CE 1). Cells have an internal diameter of 22.6 mm. For laminar flow conditions, the conical part of the cell was filled with 1 mm diameter glass beads. A ruby bead of diameter 5 mm in the tip of the cell cone prevented solvent escaping when the cell was removed from the unit at the end of the test. For laminar operation, sample was inserted in the cell on the bed of glass beads. The dissolution medium entered the cone through a capillary bore situated on the bottom and flows upwards. The cone was separated from the cylindrical portion by a 40-mesh screen. The amount of alcogel beads used per experiment was around 1 g. The flow rate of dissolution medium across the cell was set at 4.5 ml/min and the cell was thermostated at 37°C. The dissolution fluid was a simulated intestinal medium (PBS pH 7.4). Experiments were carried out in an open loop setup and samples were collected over time during 2 hours to measure the 5(6)-CF released. The HPLC used for the dosage consisted of a LC-2010A HT system (shimadzu) equipped with a Kromasil column (C18, 5 µm, 100 Å) 250 × 4.6 mm with a guard column (BISCHOFF chromatography). The absorbance value read at 254 nm in the UV visible detector was used for quantitative analysis of 5(6)-CF on the basis of a calibration curve. The mobile phase for elution was methanol (acetic acid 0.5%) / water (acetic acid 0.5%): 55% / 45% at a flow rate of 1 ml/min. The injection volume of sample was in the range of 10-100 µl according to the 5(6)-CF concentration.

Results and Discussion

Chitosan microspheres

The chitosan beads are obtained by addition drop to drop of the polymer solution into an alkaline bath. The formation of the drop, which will control the morphology of the bead, depends on the viscosity of the solution and thus on the molecular weight and concentration of the polymer in the solution. Thus a series of solutions of different polymer concentrations were used to prepare chitosan beads. The corresponding aerogel beads were obtained by supercritical CO_2 drying. This procedure kept the porous texture quite intact by avoiding the pore collapse phenomenon. Supercritical CO_2 drying of alcogels has been suggested as the best method to obtain an image of the wet materials in the dried state. This technique is commonly processed with inorganic solids to achieve very high surface area [20], we recently successfully applied it to polysaccharide materials [21]. Information about several textural properties was obtained by nitrogen adsorption-desorption isotherms on these dried materials. The adsorption at low relative pressure allowed us to evaluate the surface area of the samples by the BET method [22] assuming a monolayer of N_2 molecule to cover 0.162 nm^2. Surface areas up to 140 $m^2.g^{-1}$ were obtained in a reproducible way.

Previous control of the textural and morphological properties of the chitosan beads was thus achieved. The results concerning the viscosities of the polymer solutions, the morphology of the particles and their textural properties are summarized in table 1. They show that the textural properties were controlled by the viscosity of the polymer solution. Both high molecular or low molecular weight chitosan solutions of iso-viscosity afforded similar surface area materials. Control of the morphology was made easier with high molecular weight polymer. Iso-viscosity solutions (71-74 mPa.s) gave lenses with the LM_v and spheres with the HM_v polymer. Indeed, above a viscosity of 678 mPa.s the LMv chitosan afforded beads with tail, when the HMv chitosan allowed the obtaining of spheres. Composites were prepared with a 2.5% LM_v chitosan solution.

Table 1: Morphology and textural properties of the materials in relation with the viscosities of the chitosan solutions.

Chitosan	Viscosity η (mPa.s)	polymer concentration % (w/V)	morphology	S (BET) $m^2.g^{-1}$
LM_v	12	0.5	no beads formation	
LM_v	29	1	no beads formation	
LM_v	74	1.5	lenses	106 ± 2
LM_v	157	2	spheres	99 ± 2
LM_v	326	2.5	spheres	143 ± 2
LM_v	678	3	Beads with tail	67 ± 2
HM_v	17	0.25	No beads formation	
HM_v	71	0.5	Weak spheres	120 ± 2
HM_v	330	0.85	spheres	144 ± 2
HM_v	426	1	spheres	nd
HM_v	2030	1.5	spheres	nd
HM_v	8350	2	Beads with tail	nd
HM_v	32550	2.5	impossible	

Composites formation

The composite beads were prepared by impregnation of chitosan beads by a silica sol issued from partial hydrolysis of TEOS in the presence of alkali fluoride (procedure 1), or by a colloidal silica sol (procedure 2). The resulting composite hydrogels were dehydrated by CO_2 supercritical drying and characterized. The amount of organics was evaluated from the loss of weight at temperature higher than 200 °C. In the evaluation of the thermogravimetric results, it was taken into account that the solid residue at 850 °C is composed only of silica. The distribution of silica inside the composites has been studied by EDX microprobe analysis of aerogel bead cross-sections.

The two procedures clearly afford two different composites in term of morphology as illustrated in figure 1.

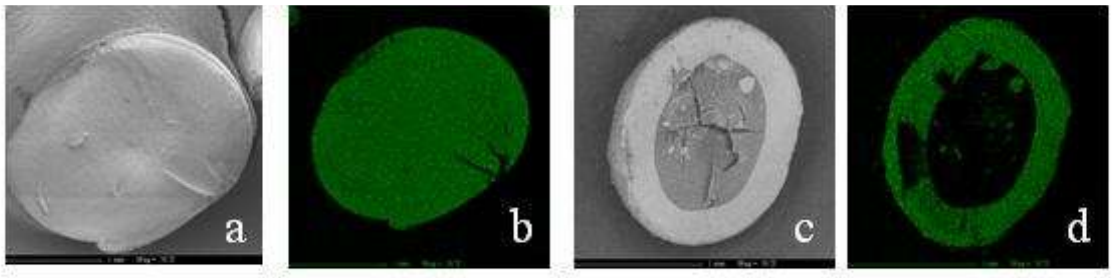

Figure 1 : SEM and Si element mapping of the chitosan-silica homogeneous composites (a, b) and core-shell composites (c, d).

The polysaccharide gel presents a very open macroporous texture with a void fraction as high as 99 %. In procedure 1, this allows an easy penetration of the silica precursor into the core of the gel beads, as witnessed by the EDX microprobe analysis results (fig 1-a,b). Whatever the reaction time, between 0.5h and 9h, the amount of silica incorporated into the chitosan beads is close to 70% wt and the samples present a silica/chitosan ratio virtually constant across the section. Composites obtained according procedure 1 are labelled “homogeneous” composites. In procedure 2, the resulting composites present a core-shell morphology (fig. 1-c, d). The silica particles don’t penetrate the chitosan beads as evidenced by the Si element EDX mapping (fig. 1-d).

The amount of silica can be varied between 65 % and 82 %, the core-shell morphology is preserved, the increase of the silica content increasing the thickness of the shell (fig. 2).

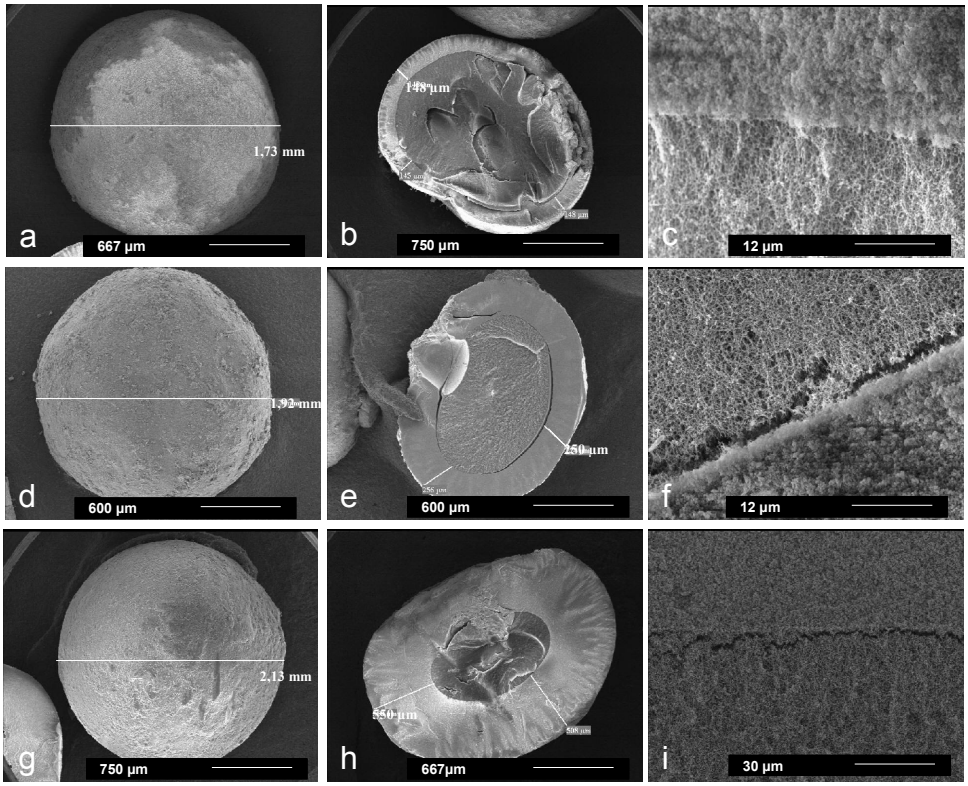

Figure 2 : SEM of different core-shell composites: whole bead (a,d,g), cross-section (b-c, e-f, h-i)

The limit between the organic and the mineral part is clearly observed in the SEM pictures (fig. 2 c, f, i) suggesting that no strong interactions exist.

Incorporation of 5(6)-carboxyfluorescein

We first determine the influence of the presence and the repartition of silica on the incorporation of a probe molecule. 5(6)-carboxyfluorescein (5(6)-CF) is a fluorescent hydrosoluble molecule commonly used as a probe molecule for the evaluation of the drug delivery properties of the materials. The incorporation was achieved by the impregnation of the alcogel beads by an PBS buffer solution of (5(6)-CF). The results of the loading of three different solids are reported in table 2.

If the amount of loaded 5(6)-CF is expressed as a percent of the total weight of the solid, it is clear that the presence of silica dramatically limits the incorporation, but the morphological distribution of the inorganic part has no effect. If the loading is reported to the mass of chitosan in the composite, the values are closed, suggesting that the incorporation is controlled by acid-base interactions between the carboxylic groups of the probe and the amino groups of chitosan.

Table 2: 5(6)-CF loading

Sample	% silica (g/g)	mg 5(6)-CF/ g solid	mg 5(6)-CF/ g chitosan
Chitosan	0	38	38
Homogeneous	72	7±1	26
Core-shell	65	9±1	26

Kinetics of release.

The release kinetics of 5(6)-CF were studied using a USP/EP flow-through method. The experiments were carried out in an open loop setup. The cell was thermostated at 37°C and the

dissolution fluid was a simulated intestinal medium (PBS pH 7.4). Samples were collected over time during 2 hours to measure the 5(6)-CF released. The solids were tested either as hydrogel materials or in their dried state after evaporation of water. The kinetics are reported in figure 3.

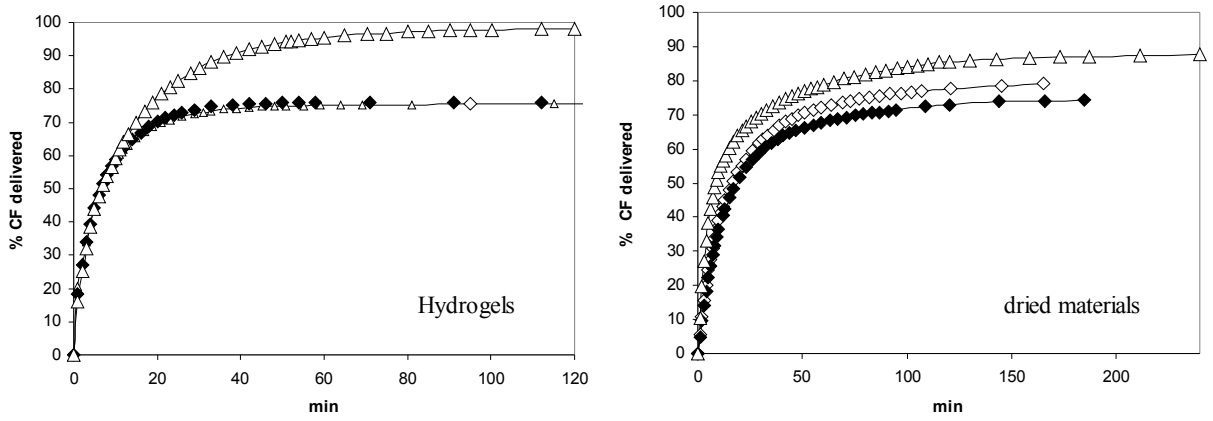

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Figure 3 : 5(6)-CF release from: (◇) chitosan, , (♦) homogeneous composite, (Δ) core-shell composite

The first observation which can be drawn from these experiments is that the releases are not total, except for core-shell hydrogel composite. This retention cannot be related to the presence of silica. Some experiments were done over 24h , the release 5(6)-CF was not increased, the beads remaining coloured by the presence of the probe molecule. This retention is favoured in the case of xerogels, in particular for the core-shell material. The presence of silica in the homogeneous composites has not effect on the release of the probe molecule, but modifies the kinetics in the core-shell materials.

Conclusion

The impregnation of the chitosan gel by a suspension of silica is an efficient method to form a composite material. Well defined experimental parameters allow achievement of homogeneous or core-shell systems with a controlled shell thickness. Such composite materials present several potential advantages for drug-delivery systems: control of the bead size through the synthesis process, ability of chitosan to complex acidic drugs and improved diffusion properties due to the stabilisation of the gel volume.

These prelimary results on the behaviour of such materials for the release of a probe molecule demonstrate that the presence of silica is not a drawback for the release of a probe molecule and that the morphology of the composite can modify the release profile.

References

[1] C. Muzzarelli, R.A.A. Muzzarelli, J. Inorg. Biochem. 92 (2002) 89.

[2] L.A. Estroff, A.D. Hamilton, Chem. Mater. 13 (2001) 3327.

[3] G.J. de A.A. Soler-Illia, C. Sanchez, B. Lebeau, J. Patarin, Chem.Rev. 102 (2002) 4093.

[4] N. Almqvist, N.H. Thomson, B.L. Smith, G.D. Stucky, D.E. Morse, P.K. Hansma, Mat. Sci. Eng. C 7 (1999) 37.

[5] Y.A. Shchipunov, T.Y, Karpenko, I.Y. Bakunina, Y.V. Burtseva, T.N. Zvyagintseva, J. Biochem. Bioph. Meth. 58 (2004) 25.

[6] T. Coradin, L. Linsnard, J. Livage, Chem. Commun. (2001) 2496.

[7] T. Coradin, J. Livage, J. Sol-Gel Sci. Tech. 26 (2003) 1165.

[8] S. Sakai, T. Ono, H. Ijima, K. Kawakami, Biomaterials 22 (2001) 2827.

[9] S. Sakai, T. Ono, H. Ijima, K. Kawakami, J. Membrane Sci. 202 (2002) 73.

[10] W. Ogasawara, W. Shenton, S.A. Davis, S. Mann, Chem. Mater. 12 (2000) 2835.

[11] C. Airoldi, O.A.C. Monteiro, J. Appl. Polym. Sci., 77 (2000) 797.

[12] G. Wang, J.J. Xu, H.Y. Chen, Z.H. Lu, Biosens. Bioelectron., 18 (2003) 335.

[13] Y. Miao, S.N. Tan, Anal. Chim. Acta, 437 (2001) 87.

[14] A. Prokop, E. Kozlov, G.W, Newman, M.J. Newman, Biotechnol. Bioeng., 78 (2002) 459.

[15] S. Mann, S.L. Burkett, S.A. Davis, C.E. Fowler, N.H. Mendelson, S.D. Sims, D. Walsh, N.T. Whilton, Chem Mater., 9 (1997) 2300.

[16] K. Molvinger, F. Quignard, D. Brunel, M. Boissière, J.M. Devoisselle, Chem. Mater., 16 (2004) 3367.

[17] A. Montembault, C. Viton, A. Domard, Biomaterials 26 (2005) 1633.

[18] K.S.W. Sing, Chem. Ind. (London), (1968) 1520.

[19] G.A.F. Roberts, J.G. Domszy, Int. J. Bio. Macromol., 4 (1982) 374.

[20] A.C. Pierre, G.M. Pajonk, Chem. Rev., 102 (2002**)** 4243.

[21] R.Valentin, K.Molvinger, C. Viton, A. Domard, F. Quignard, Biomacromol., 6 (2005) 2785.

[22]

Exemple:

[23] K. Ogama, T.Yui, K.Okuyama, Int J. Biol Mracromol, 34(2004) 1-8.