Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 338 (2007) 284–290
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

In vitro evaluation of calcium binding capacity of chitosan and thiolated chitosan poly(isobutyl cyanoacrylate) core–shell nanoparticles

I. Bravo-Osuna, G. Millotti, C. Vauthier, G. Ponchel *

CNRS UMR 8612, Université de Paris Sud, Laboratoire de Physicochimie, Pharmacotechnie et Biopharmacie 5, Rue J.B. Clément, 92296 Chatenay-Malabry, France

Received 5 December 2006; received in revised form 23 January 2007; accepted 27 January 2007
Available online 3 February 2007

Abstract

The ability of chitosan and its derivatives to bind cations is well known. Chitosan and thiolated chitosan were recently associated with poly(isobutyl cyanoacrylate) (PIBCA) nanoparticles leading to very promising results in terms of bioadhesion and permeation enhancement properties. Taking into account the influence that cations concentration have in the maintenance of both the permeation and the enzymatic barrier of the oral route, the possible cation binding capacity of these colloidal systems might be interesting in the use of these nanocarriers for the oral administration of pharmacologically active peptides. The aim of the present work was to in vitro evaluate the capacity of these colloidal systems to bind calcium, a model cation of physiological interest in the intestinal tract. The presence of chitosan on the nanoparticle surface importantly increased the calcium binding ability, in comparison to non-coated PIBCA nanoparticles. In addition, its presentation in the gel layer surrounding the nanoparticles, also beneficiated its binding capacity, obtaining 2–3 folds higher values when the polymer coated the nanoparticles than when it was in solution. The cross-linked structure observed for thiolated chitosan, due to the formation of inter- and intra-chain disulphide bonds, diminished the accessibility of cation to active sites of the polymer, decreasing the binding capacity of the calcium ion. However, when the amount of free thiol groups on the nanoparticle surface was high enough, the binding behaviour observed was higher than for nanoparticles elaborated with non-modified polymer.

Keywords: Chitosan; Thiolated chitosan; Poly(isobutyl cyanoacrylate); Core–shell nanoparticles; Calcium binding capacity; Cross-linking

1. Introduction

Chitosan has been extensively studied for its cation binding capacity in neutral or weakly acid media (Guibal et al., 2002). The free electronic doublet of nitrogen, which is present in the polymer in more than 7% (w/w) (Guibal et al., 2002; Chassary et al., 2004), is responsible for the sorption of many cations, especially divalent cations (Lima and Airoldi, 2003; Monteiro and Airoldi, 1999), According to Lima and Airoldi (2003), the high hydrophilicity of chitosan owing to the large number of hydroxyl groups, and the flexible polymeric chain structure which favours the adjustment of the cations dispersed in solution for complex formation, play also a role in the cation binding capacity of this polymer. In addition, chemically grafting of active groups, typically occurring on $–CH_2OH$ and $–NH_2$ groups (Guibal et al., 2002), can improve the cation binding capacity of the polymer (Becker et al., 2000; Jeon and Holl, 2003; Lima and Airoldi, 2003).

In the biopharmaceutical field, the cation binding ability of chitosan can be very useful. For example, the intactness of the drug absorption barrier in the intestine is linked to the presence of Ca^{2+} and Mg^{2+} cations. It is well-known that the reduction of extracellular Ca^{2+} concentration, for example by chelant agents such as EDTA or EGTA, can result in the opening of the tight junctions that interconnect the epithelial cells (Thanou et al., 2001; Schulzke et al., 2005), promoting the paracellular transport of active molecules (Ameye et al., 2001; Roumi et al., 2001). Thus, polymers able to bind extracellular Ca^{2+} might influence the absorption by paracellular route (Borchard et al., 1996; Kriwet and Kissel, 1996). In addition, most proteolytic enzymes present in the gastrointestinal tract are metallopeptidases that require cations such as Mg^{2+} or Zn^{2+} as cofactors at their active sites (Carboxypeptidase A, Leucine aminopeptidases, etc.). Furthermore, other peptidases, such as trypsin, need cations (Ca^{2+}) to maintain their active structure (Woodley, 1994; Ameye et al., 2001; Madsen and Peppas, 1999). The depletion of

* Corresponding author. Tel.: +33 146835919; fax: +33 146619334.
E-mail address: gilles.ponchel@u-psud.fr (G. Ponchel).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.01.039

such as cations provokes the inhibition of proteolysis (Ameye et al., 2001), which might be also very beneficial for the efficient administration of peptidic active molecules by the oral route (Bernkop-Schnürch et al., 2004).

Chitosan and thiolated chitosan core–shell poly(isobutyl cyanoacrylates) nanoparticles were recently developed and characterised by our group (Bravo-Osuna et al., 2006, 2007a). The elaboration of those nanoparticles was based on emulsion radical polymerisation of alkyl cyanoacrylate monomers from carbohydrate chains. The block copolymer created is able to spontaneously self-associate forming particles with a hydrophobic core coated by hydrophilic polysaccharide (Chauvierre et al., 2003a,b,c). The presence of chitosan and thiolated chitosan onto the surface improved the mucoadhesive characteristics to the system (Bravo-Osuna et al., 2007b). Those colloidal systems constitute a novel approach in oral peptide delivery.

Following with the biopharmaceutical evaluation of these colloidal systems as nanocarriers for the oral delivery of peptides, the aim of this work was to evaluate in vitro the capacity of these core–shell nanoparticles to bind divalent cations of physiological interest. Among them, calcium was selected because its modulation can play an important role in the maintenance of the intestinal physiological barriers hence, in the absorption of peptidic drugs by the oral route. The effect of chemical modification of chitosan (changes in molecular weight and inclusion of thiol groups) on the cation binding capacity was evaluated in this work.

2. Materials and methods

2.1. Materials

Isobutyl cyanoacrylate (IBCA) was kindly provided as a gift by Loctite (Dublin, Ireland). Chitosan Mw 400,000 g/mol, and L-cysteine HCl were purchased from Fluka (Saint-Quentin Fallavier, France). Eriochrome® Black T was obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France). 2-Iminothiolane HCl (Traut's reagent) was synthesised in the Department of Organic Chemistry (Biocis UMR CNRS 8076), Faculty of Pharmacy, University Paris-XI (Chatenay-Malabry) France.

All other chemicals were reagent grade and used as received.

2.2. Methods

2.2.1. Chitosan modifications and characterisation

Chitosan was selectively depolymerised following the method developed by Huang et al. (2004). By reaction with sodium nitrite at different concentrations (7.0 and 2.7 g/l), two final molecular weight were obtained: 20,000 g/mol (Chito20) and 50,000 g/mol (Chito50) respectively. ^{1}H NMR analysis (Bruker MSL-400 spectrometer, Bruker Instrument Inc. Wissembourg, France) showed no changes in the percentage of deacetylation after the depolymerisation process, with values around 86–88% in all cases. The capillary viscosity (viscometer AVS400, Schott Gerate) measurements showed molecular weight values according to the predicted ones in agreement with previous works (Bravo-Osuna et al., 2007a).

The inclusion of thiol groups in the different hydrolysed chitosan was carried out by reaction with 2-iminothiolane following the method developed by Bernkop-Schnürch et al. (2003). The resulting polymers were chitosan-4-thiol-butylamidine (chitosan-TBA), named Chito20-TBA and Chito50-TBA according to the original molecular weight of the corresponding non-modified polymers.

Sulphur elemental analysis (Analyzer LECO SC144, Service central d'analyse du CNRS, Vernaison, France) was performed in order to determine the total sulphur content: 4.9%, and 4.7% for Chito20-TBA and Chito50-TBA respectively. These values give the total amount of sulphur present in the sample, but they do not provide any information about the chemical state of the sulphur, whether it exists as a reactive thiol or whether it has been oxidised (unreactive disulphide). The determinations of the reactive thiol groups was performed using the iodine titration method that allows the exclusive determination of the amount of reduced sulphur groups in the polymer, which resulted to be the 35% and 36% of the total amount of sulphur for Chito20-TBA and Chito50-TBA, respectively.

2.2.2. Nanoparticles preparation

PIBCA core–shell nanoparticles were prepared by the redox radical emulsion polymerisation method developed by Chauvierre et al. (2003a,c) and optimised by Bravo-Osuna et al. (2006, 2007a). Mixtures of modified and non-modified hydrolysed chitosan (%chitosan/%chitosan-TBA: 0/100; 25/75; 50/50; 75/25; 100/0) were used as shell component.

Control non-coated PIBCA nanoparticles were elaborated by anionic emulsion polymerisation according to Monza da Silveira et al. (1998) using Poloxamer as stabiliser agent.

2.2.3. Characterisation of the nanoparticles

2.2.3.1. Size measurement. The hydrodynamic mean diameter and the size distribution of the nanoparticles were determined at 20 °C by quasi-elastic light scattering using a Nanosizer® N4 PLUS (Beckman-Couter, Villepinte, France). The scattering angle was fixed at 90°. Samples were diluted in acetic acid solution (16 μmol/l) in MilliQ® water in order to achieve a signal level ranging from 5×10^4 to 5×10^6 counts per second. The results were expressed as the mean hydrodynamic diameter, the standard deviation and the polydisperisity index of the size distribution. This lately parameter is linked with the width of the curve of hydrodynamic radius distributions, small values indicating narrower distributions. Results corresponded to the average of three determinations.

2.2.3.2. Determination of the ζ potential. The ζ potential of the polymer particles was deduced from the electrophoretic mobility of the particles measured by Laser Doppler Electrophoresis (Zetasizer Nano serie. Malvern Instruments Ltd., Worcestershire, UK) in a NaCl 1 mmol/l solution (pH 6.8) after suitable dilutions (1/200, v/v) of the different nanoparticles suspensions.

2.2.3.3. Determination of thiol content. The quantification of reduced thiol groups on the nanoparticle surface was determined using a modified iodine titration method. In brief, 0.25 ml

of nanoparticle suspension were mixed with 0.25 ml of acetate buffered solution (pH 2.7). Then, 1 ml of starch solution (1%, w/v) and 0.1–0.5 ml of iodine (1 mmol/l) were added at each preparation. The reaction was allowed to proceed for 24 h at room temperature and protected from light. Then, samples were centrifuged (10 min, 3500 rpm) and the supernatant measured at 560 nm (Spectrophotometer UV/VIS lambda 11 Perkin Elmer. Norwalk, USA). Control samples were prepared from nanoparticles elaborated with non-modified chitosan. The amount of thiol moieties was calculated from the corresponding standard curve elaborated in the same conditions with L-cysteine HCl solutions (0.04–0.124 mmol/l). Iodine promotes the oxidation of the free thiol groups. To avoid interferences with oxygen, all samples were degassed by argon bubbling after the addition of iodine. The excess of iodine was able to react with starch giving a quantitative blue complex easily measured by spectrometry. This indirect dosage method allowed the determination of the amount of reduced thiol groups present in the sample.

2.2.4. Evaluation of the calcium binding capacity

The method used for the evaluation of the calcium binding capacity was performed after modifications of the method described by Bernkop-Schnürch and Krajicek (1998). One millilitre of $CaCl_2$ solution 0.01 mol/l (pH 6.5) was incubated with 0.2 ml of nanoparticles suspension (0.1; 0.4; 1.0 and 4.0 mg np). After incubation for 30 min under gentle stirring, samples were ultracentrifugated for 30 min at 40,000 rpm. 0.25 ml of NH_4Cl/NH_4OH buffer were added to 0.75 ml of supernatant to raise the pH to 10. The so-prepared sample was mixed with 0.05 ml of Eriochrome® Black T indicator (EBT) solution (1 g/l; pH 10). At this condition, samples became coloured in red. Different volumes of EDTA 0.01 mol/l were added until a change of colour from red to blue was observed, considering this point the end of the titration. The presence of magnesium improves the sharpness of the end-point. With this objective, a small amount of Mg–EDTA complex (1:1) was added along with the titration mixture. This addition does not affect the final determination.

The amount of calcium-bound polymer was calculated from the difference between total amount of calcium added and the amount of free calcium measured in the supernatant.

2.2.5. Statistical analysis

Results obtained were statistically analysed by using Mann–Whitney's *t*-test with a 95% confidence level ($P < 0.05$).

3. Results and discussion

3.1. Nanoparticle preparation and characterisation

Size and zeta potential of nanoparticles prepared with different proportions of chitosan/chitosan-TBA are presented in Table 1. Results were in agreement with our previous works (Bravo-Osuna et al., 2007a, 2007b). The partial insolubilisation of Chito50-TBA in the polymerisation medium made impossible the elaboration of nanoparticles with percentage higher than 75%. For each series of nanoparticles elaborated with chitosan of the same molecular weight, the hydrodynamic mean diameter of particles increased with the percentage of chitosan-TBA, which was explained by the presence of intra- and inter-molecular disulphide bonds formation as was suggested for other thiolated products. The mean hydrodynamic diameter of these types of nanoparticles showed a clear increase with the molecular weight of chitosan. Finally, the carbohydrate free amino groups were responsible for the measured positive ζ potential values obtained for all formulations. (Bravo-Osuna et al., 2006, 2007a).

3.2. Evaluation of the calcium binding capacity

In Fig. 1 the amount of bound calcium was plotted against amount of chitosan present in the incubation medium. For nanoparticles, the amount of chitosan was calculated considering the total amount of chitosan present in the polymerisation medium. The same amount of chitosan and thiolated chitosan was evaluated in solution. Binding studies were also performed with non-coated PIBCA nanoparticles (white circle).

As can be observed in Fig. 1, while non-coated PIBCA nanoparticles, with anionic surface (Table 1), showed a negligible sorption pattern, cationic coated PIBCA nanoparticles were able to bind calcium ions, demonstrating that the cation binding phenomenon was not based on electrostatic interaction, but it was intimately related with the presence of chitosan on the nanoparticle surface.

Table 1
Nanoparticles characterisation: mean hydrodynamic diameter (H_D), polydispersity index (PI), surface charge (ζ) and free thiol content on the surface of nanoparticles elaborated

Chitosan (g/mol)	%Chitosan/chitosan-TBA	H_D (nm) ±S.D.	PI	ζ potential (mV) ± S.D.	Thiol groups (μmol SH/cm^2)
20,000	100/0	215 ± 39	0.039	+38.0 ± 2.3	–
	75/25	223 ± 61	0.132	+26.7 ± 1.5	2.014×10^{-4}
	50/50	245 ± 98	0.365	+29.3 ± 2.5	2.040×10^{-4}
	25/75	318 ± 91	0.374	+30.4 ± 1.7	3.036×10^{-4}
	0/100	338 ± 102	0.310	+29.2 ± 1.1	5.040×10^{-4}
50,000	100/0	301 ± 69	−0.161	+41.2 ± 1.2	–
	75/25	313 ± 96	0.161	+34.2 ± 1.6	2.140×10^{-4}
	50/50	317 ± 97	0.177	+30.9 ± 0.4	2.408×10^{-4}
	25/75	357 ± 125	0.284	+31.0 ± 0.9	4.046×10^{-4}
PIBCA		318 ± 54	−0.131	−17.7 ± 0.3	–

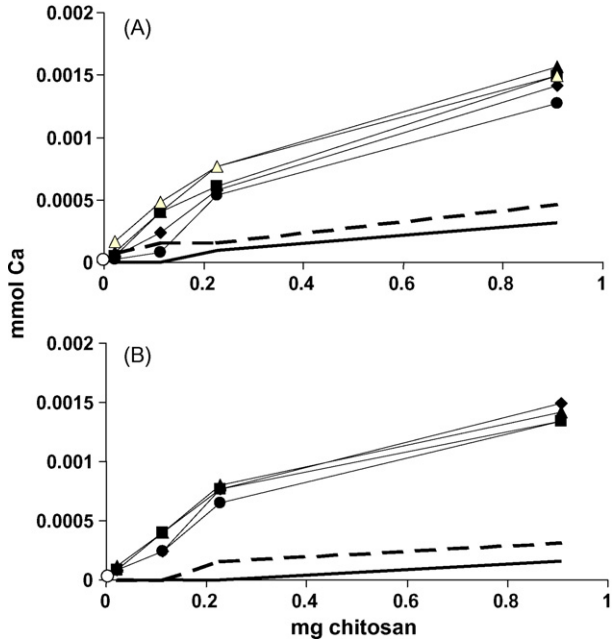

Fig. 1. Amount of calcium bound (mmol) as function of the chitosan (mg) present in solution (chitosan: continuous line; thiolated chitosan: dotted line) or onto the nanoparticle surface: %chitsosan/%chitosan-TBA: 100/0 (▲); 75/25 (■); 50/50 (●); 25/75 (♦); 0/100 (△). Non-coated PIBCA nanoparticles were also present (◯). (A) Chito20 (20,000 g/mol); (B) Chito50 (50,000 g/mol).

The reactive groups of chitosan (amine sites and hydroxyl groups, especially in the C-3 position) may interact with metal ions through different mechanisms including chemical (chelation) or electrostatic interactions (ion exchange, formation of ion pairs, Dzul Erosa et al., 2001; Guibal, 2004). In the case of the chitosan–calcium system, the competitive sorption of proton and metal ions on chitosan seems to be the mechanism more probable (Jeon and Holl, 2003).

The pH of the solution presents one of the most important parameters affecting the chitosan cation binding ability (Guibal, 2004). All experiments were carried out at pH 6.5. The selection of this pH was made taking into account the pH of the jejunum where occurs the higher part of intestinal absorption (Balimane et al., 2000). The solubility limitations of the polymer at higher pH was also considered. At pH 6.5 most of the amino groups of chitosan are protonated, this weakly acidic medium might be optimal for the calcium binding behaviour of the polymer.

Surprisingly, 2–3 folds higher tendency to bind calcium was observed for chitosan and modified chitosan present in the surface of nanoparticles than in solution (Fig. 1). In agreement with the results presented in this work, other authors have also demonstrated that physical changes in chitosan (beads or films manufacturing) can improve the cation binding capacity (Chassary et al., 2004; Guibal, 2004). According to Rhazi et al. (2002), the sorption performance of chitosan is strictly controlled by structural parameters of the polymer, which govern not only the fraction or number of the free amine groups available for metal uptake (degree of deacetylation) but also the number of accessible-free amine groups. Indeed, the total number of free amine groups is not necessarily accessible to metal uptake. Some of these amine sites are involved in hydrogen bonds (intra- or inter-molecular bonds) (Jeon and Holl, 2003; Guibal, 2004). It seems evident that these changes in the presentation of the polymer from solution to gel layer onto the PIBCA core, with micro-domains with high chitosan concentrations, might be favourable for calcium binding, improving the sorption performance of chitosan.

As can be observed in Fig. 1, the inclusion of thiol groups in the polymer structure seems to moderately increase the cation adsorption behaviour of chitosan in solution. The free active primary amino group of chitosan backbone presents high reactivity enabling reactions to give interesting chemical modifications of this polymer (Lima and Airoldi, 2003; Guibal, 2004; Sashiwa and Aiba, 2004). Among the different chemical modifications, the grafting of sulphur compounds (thioures, rubeanic acid, thiosemicarbazide) on chitosan has been the subject of many studies for the design of chelating chitosan-based resins. Several possible mechanism have been suggested: (i) ion pair formation by exchange mechanism (in the case of protonated forms); (ii) coordination by ligand exchange mechanism, via nitrogen and/or sulphur donor atoms (in the case of unprotonated forms); (iii) ion pair binding mechanism followed by slow ligand exchange (Guibal et al., 2000, 2002; Guibal, 2004).

According to Bernkop-Schnürch et al. (2003), the grafting of 2-iminothiolane on chitosan involves consumption of amine groups. The consumption of amino groups diminishing the availability of these chelating sites (Becker et al., 2000; Dzul Erosa et al., 2001; Guibal, 2004). However, the presence of thiol groups, able to improve the chelating of metal cations, seems to overcome it, leading to a global enhancement of calcium sorption.

It is interesting to remark that, while the increment of chitosan in the incubation medium raised the calcium binding capacity, this increment was not linear with the amount of incubated nanoparticles. A saturation tendency of the calcium binding was observed at higher chitosan concentration (0.9 mg chitosan, that is nanoparticles concentration of 4 mg/ml) with 15–20% of initial calcium bound. This saturation tendency was confirmed when the number of incubated nanoparticles was plotted against the amount of bound calcium (Fig. 2). Logically, a increase in the millimoles of Ca bound with the increment of nanoparticles incubated, however, this increase was no linear, reaching a plateau at high nanoparticle concentration. The analysis of the results has been then focused on the data obtained at low nanoparticles content (0.1–1 mg of nanoparticles).

To analyse the potential differences in calcium sorption among the nanoparticles elaborated, the binding capacity of the different coated nanoparticles was calculated and presented in Fig. 3. The proportional less binding tendency observed for higher nanoparticle concentration was also visible in the Fig. 3D.

The three main observations that can be drawn from Fig. 3 are: (i) Statistical analysis ($P < 0.05$) demonstrated a higher binding capacity (mmol Ca^{2+}/g polymer) for Chito50 formulations than for Chito20 ones in most of the cases (diamond marks). (ii) From a qualitatively point of view, a similar behaviour was observed for Chito20 and Chito50 formulations when thiolated chitosan was included, with a significant decrease of the binding capacity of nanoparticles elaborated with intermediate percentages of chitosan-TBA (25–75%) in comparison to formulations

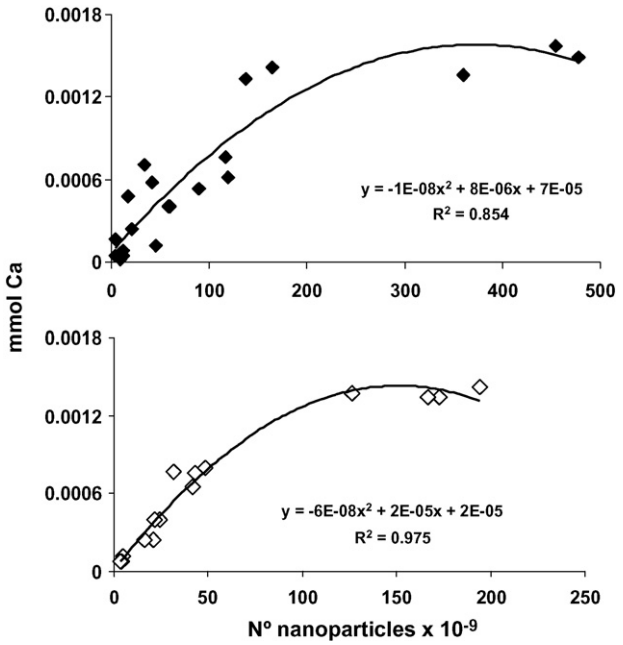

Fig. 2. Amount of calcium bound (mmol) as function of the total number of nanoparticles incubated. Chito20 (black symbols) and Chito50 (white symbols).

elaborated with non-modified biopolymer, and finally (iii), an increase in the binding capacity was observed for the formulation elaborated with 100% Chito20-TBA.

Several parameters can influence the nanoparticles binding capacity that could explain the results obtained.

According to Jeon and Holl (2003), the absorption capacity of chitosan increases with percent deacetylation and the molecular weight of the chains. Although both Chito20 and Chito50 have the same degree of deacetylation (86–88%), differences can be expected according to their different molecular weight (Chito20: 20,700 g/mol; and Chito50: 50,000 g/mol).

In previous works, it was shown by electron microscopy that the thickness of the chitosan gel layer surrounding the PIBCA core increased with the molecular weight of chitosan. Values of 8 ± 3 nm and 22 ± 10 nm ($n = 20$) for Chito20 and Chito50 nanoparticles were obtained, respectively (Bravo-Osuna et al., 2007a). The different thickness of the chitosan layer could explain the higher binding capacity observed for Chito50 formulations.

One of the parameters that can influence the binding capacity of nanoparticles is their large specific surface area that gives these pharmaceutical dosage forms a high interactive potential with biological fluids. The hydrodynamic diameter of particles increased with the increment of chitosan-TBA in the nanoparticles gel layer (Table 1). Taking into consideration that the binding capacity was analysed at four fixed points of nanoparticle mass and assuming the same density for all of them, at each point, the total surface of contact decreased with the hydrodynamic diameter of the particles. It means that the incubated total surface area decreased with the increment of chitosan-TBA in the formulation. To establish if this fact can explain the decrease of binding capacity observed in presence of moderate percentages of chitosan-TBA, the amount of calcium bound was plotted against the total contact surface at each point of the analysis (Fig. 4). However, as can be observed, no direct relation was observed, demonstrating that the calcium caption by the systems analysed is not a simply surface phenomenon. Other hypotheses were raised to explain the obtained results.

Several author have pointed out the extreme importance of the degree of cross-linking in the binding capacity of polymers (Becker et al., 2000; Dzul Erosa et al., 2001; Jeon and Holl, 2003; Guibal, 2004). All of them concluded that cross-linking can limit the accessibility of cations to active centres of the polymer by reducing the diffusion of the ion across the polymer chains and their binding capacity.

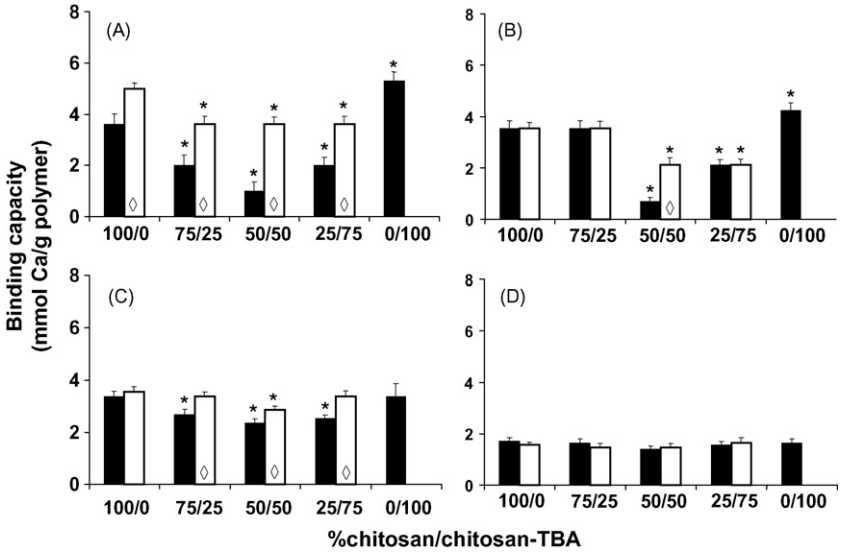

Fig. 3. Binding capacity (mmol Ca/g polymer) of chitosan onto nanoparticles surface after incubation of 0.1 mg (A), 0.4 mg (B), 1.0 mg (C) or 4.0 mg (D) of nanoparticles elaborated with Chito20 (black bars) or Chito50 (white bars) with different proportions of unmodified and thiolated chitosan. ◊Statistical differences ($P < 0.05$) between Chito20 and Chito50 formulations. *Statistical differences ($P < 0.05$) with the formulation elaborated with non-modified chitosan.

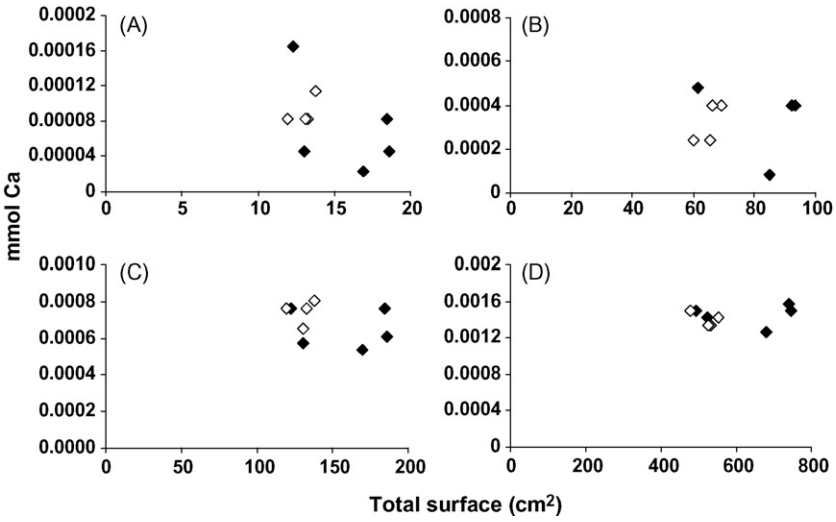

Fig. 4. Calcium bound (mmol) after incubation of 0.1 (A), 0.4 (B), 1.0 (C) or 4.0 (D) mg of nanoparticles as function of the total contact surface (cm^2). Chito20 (black symbols) and Chito50 (white symbols).

According to Table 1, an increase of "active" or "reactive" (reduced) thiol groups in the nanoparticle surface was observed with the increase of the percentage of chitosan-TBA in the formulation. A deeper analysis of sulphur distribution in the thiolated chitosan coated PIBCA nanoparticles was made in a previous work. It was observed that the increment in chitosan-TBA contain promoted an increase of the formation disulphide bonds, leading to a higher cross-linked structure. Values of percentage of reduced thiol groups from 50 to 7% and from 14 to 7% of the total sulphur content were obtained for Chito20-TBA and Chito50-TBA formulations respectively. The increasing presence of disulphide intra- and inter-chain bonds in the gel layer of core–shell nanoparticles could explain the partial lost of binding capacity observed in formulations elaborated with intermediate percentages of chitosan-TBA. The remaining question is why the formulation elaborated with 100% of Chito20-TBA, which might have the higher cross-linked structure of the whole family of nanoparticles, performed the best binding ability. The explanation for this behaviour must be found in Table 1. The high density of free thiol groups present in the nanoparticle surface elaborated with 100% of Chito20-TBA might be enough to overcome the negative effect of the cross-linking structure assumed for the gel layer of these nanoparticles.

4. Conclusions

The presence of chitosan and thiolated chitosan onto the PIBCA nanoparticles clearly conferred calcium binding ability to the colloidal system. In addition, in the present work, we have demonstrated that these chitosan-coated nanoparticles are able to bind much higher concentration in calcium than the corresponding free polymer, which might be explained by an improvement in the accessibility of binding sites of the polymer on the nanoparticle surface. Formulations elaborated with intermediate amount of thiolated biopolymer, showed a cross-linked structure in the gel layer by the formation of disulphide bonds significantly decreased the accessibility of cations to the active sites of the polymer. The positive effect of the thiol groups in the binding capacity of the biopolymer was only denoted for nanoparticles elaborated exclusively with thiolated polymers, when the amount of reduced (reactive) thiol groups was high enough to overcome the cross-linked structure.

Acknowledgements

Authors want to thank Dr. K. Broadley from Loctite (Dublin, Ireland) for his kindness in providing the isobutylcyanoacrylate monomer. Authors want also to thank the Department of Organic Chemistry (Biocis UMR CNRS 8076), Faculty of Pharmacy, University Paris-XI (Chatenay-Malabry, France) for their help in the synthesis of 2-iminothiolane and the "Service central d'analyse du CNRS" (Vernaison, France) for the elemental analysis of thiolated polymers.

References

Ameye, D., Voorspoels, J., Foreman, P., Tsai, J., Richardson, P., Geresh, S., Remon, J.P., 2001. Trypsin inhibition, calcium and zinc ion binding of starch-g-poly(acrylic acid) copolymers and starch/poly(acrylic acid) mixtures for peroral peptide drug delivery. J. Control. Release 75, 357–364.

Balimane, P.V., Chong, S., Morison, A., 2000. Current methodology used for evaluation of intestinal permeability and absorption. J. Pharmacol. Toxicol. Methods 44, 301–312.

Becker, T., Schlaak, M., Strasdeit, H., 2000. Adsorption of nickel(II), zinc(II) and cadmium(II) by new chitosan derivatives. React. Funct. Polym. 44, 289–298.

Bernkop-Schnürch, A., Kast, C.E., Guggi, D., 2003. Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: thiomer/GSH systems. J. Control. Release 93, 95–103.

Bernkop-Schnürch, A., Krajicek, M.E., 1998. Mucoadhesive polymers as platforms for peroral peptide delivery and absorption: synthesis and evaluation of different chitosan-EDTA conjugates. J. Control. Release 50, 215–223.

Bernkop-Schnürch, A., Krauland, A.H., Leitner, V.M., Palmberger, T., 2004. Thiomer: potential excipients for non-invasive peptide delivery systems. Eur. J. Pharm. Biopharm. 58, 253–263.

Borchard, G., Lueßen, H.L., de Boer, A.G., Verhoef, J.C., Lehr, C.M., Junginger, H.E., 1996. The potential of mucoadhesive polymers in enhancing intestinal

peptide drug absorption III. Effect of chitosan-glutamate and carbomer on epithelial tight junction in vitro. J. Control. Release 39, 131–138.

Bravo-Osuna, I., Schmitz, T., Bernkop-Schnürch, A., Vauthier, C., Ponchel, G., 2006. Elaboration and characterization of thiolated chitosan-coated acrylic nanoparticles. Int. J. Pharm. 316 (1–2), 170–175.

Bravo-Osuna, I., Ponchel, G., Vauthier, C., 2007a. Tuning of shell and core characteristics of chitosan-decorated acrylic nanoparticles. Eur. J. Pharm. Sci. 30 (2), 143–154.

Bravo-Osuna, I., Vauthier, C., Farabollini, A., Palmieri, G.F., Ponchel, G., 2007b. Mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core–shell nanoparticles. Biomaterials 28 (13), 2233–2243.

Chassary, P., Vincent, T., Guibal, E., 2004. Metal anion sorption of chitosan and derivative materials: a strategy for polymer modification and optimum use. React Funct. Polymers 60, 137–149.

Chauvierre, C., Labarre, D., Couvreur, P., Vauthier, C., 2003a. Radical polymerization of alkylcyanoacrylates initiated by the redox system dextran-cerium (IV) under acidic aqueous conditions. Macromolecules 36, 6018–6027.

Chauvierre, C., Labarre, D., Couvreur, P., Vauthier, C., 2003b. Plug-in spectrometry with optical fibres as a novel analytical tool for nanoparticle technology: Application to the investigation of the emulsion polymerisation of the alkylcyanoacrylate. J. Nano. Res. 5, 365–371.

Chauvierre, C., Labarre, D., Couvreur, P., Vauthier, C., 2003c. Novel polysaccharide-decorated poly(isobutyl cianoacrylate) nanoparticles. Pharm. Res. 20 (11), 1786–1793.

Dzul Erosa, M.S., Saucedo Medina, T.I., Navarro Mendoza, R., Avila Rodríguez, M., Guibal, E., 2001. Cadmium sorption on chitosan sorbents: kinetic and equilibrium studies. Hydrometal 61, 157–167.

Guibal, E., Vincent, T., Navarro-Mendoza, R., 2000. Synthesis and characterization of a thiourea derivative of chitosan for platinum recovery. J. Appl. Polym. Sci. 75, 134–199.

Guibal, E., Von Offenberg Sweeney, N., Vincent, T., Tobin, J.M., 2002. Sulfur derivatives of chitosan for palladium sorption. React. Funct. Polym. 50, 149–163.

Guibal, E., 2004. Interactions of metal ions with chitosan-based sorbents: a review. Sep. Purif. Technol. 38, 43–74.

Huang, M., Khor, E., Lim, L.Y., 2004. Uptake and cytotoxicity of chitosan molecules and nanoparticles: effects of molecular weight and degree of deacetylation. Pharm. Res. 21 (2), 344–353.

Jeon, C., Holl, W.H., 2003. Chemical modification of chitosan and equilibrium study for mercury ion removal. Water Res. 37, 4770–4780.

Kriwet, B., Kissel, T., 1996. Poly (acrylic acid) microparticles widen the intercellular spaces of Caco-2 cell monolayers: an examination by confocal laser scanning microscopy. Eur. J. Pharm. Biopharm. 42, 233–240.

Lima, I.S., Airoldi, C., 2003. Interaction of cooper with chitosan and succinic anhydride derivative—a factorial design evaluation of the chemiosorption process. Col. Surf. A: Physicochem. Eng. Aspects 229, 129–136.

Madsen, F., Peppas, N.A., 1999. Complexation graft copolymer networks: swelling properties, calcium binding and proteolytic enzyme inhibition. Biomaterials 20, 1701–1708.

Monteiro, O.A.C., Airoldi, C., 1999. Some thermodynamic data on copper-chitin and copper-chitosan biopolymer interactions. J. Colloidal Interface Sci. 212, 212–219.

Monza da Silveira, A., Ponchel, G., Puisieux, F., Duchene, D., 1998. Combined poly(isobutylcyanoacrylate) and cyclodextrins nanoparticles for enhancing the encapsulation of lipophilic drugs. Pharm. Res. 15 (7), 1051–1055.

Rhazi, M., Desbrières, J., Tolaimate, A., Rinaudo, M., Vottero, P., Alagui, A., El Meray, M., 2002. Influence of the nature of metal ions on the complexation with chitosan. Application to the treatment of liquid waste. Eur. Polym. J. 38, 1523–1530.

Roumi, M., Kwong, E., Deghenghi, R., Locatelli, V., Marleau, S., Souich, P.D., Béliveau, R., Ong, H., 2001. Permeability of peptidic GH secretagogues hexarelin and EP51389 across rat jejunum. Peptides 22, 1138–1229.

Sashiwa, H., Aiba, S.I., 2004. Chemically modified chitin and chitosan as biomaterials. Prog. Polym. Sci. 29, 887–908.

Schulzke, J.D., Gillet, A.H., Mankertz, J., Spiegel, S., Seidler, U., Amasheh, S., Saitou, M., Rsukita, S., Fromm, M., 2005. Epithelial transport and barrier function in occluding-deficient mice. Biochim. Biophys. Acta 1669, 34–42.

Thanou, M., Verhoef, J.C., Junginger, H.E., 2001. Chitosan and its derivatives as intestinal absorption enhancers. Adv. Drug Del. Rev. 50, S91–S101.

Woodley, J.F., 1994. Enzymatic barriers for GI peptide and protein delivery. Crit. Rev. Ther. Drug 11, 61–95.