Design, In Vitro and In Vivo Characterization of Chitosan-Dextran Sulfate
Microparticles for Oral Delivery of Insulin

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Abstract

Microparticles were fabricated by layer-by-layer deposition of chitosan (Ch) and dextran sulfate (DS) on microaggregates formed by human insulin and DS. Consecutive treatment of the negatively charged microaggregates with Ch, DS, and Ch yielded small (ca. 10 μm) positively charged microparticles with high insulin encapsulation efficiency (65% of initial amount of insulin) and loading (50% w/w). Virtually all immobilized protein remained insoluble in the pH range 1.0–6.0 corresponding to the aggressive media of stomach and upper small intestine, while at pH 7.4, about 90% of the insulin was released during one-hour incubation. Encapsulated insulin was more resistant to the protease action than native insulin in solution: after 1-h incubation in simulated pancreatic juice only 60% of encapsulated insulin degraded, while insulin in solution degraded almost completely. Experiments in vivo demonstrated that insulin encapsulated in microparticles preserved biological activity and exerted a prolonged hypoglycemic effect after peroral administration in rabbits and diabetic rats. Bioavailability of the encapsulated insulin administered per os was 11%. The produced microparticles are biocompatible, biodegradable, and mucoadhesive and may be used for the development of oral insulin delivery systems in humans.

Introduction

Insulin is used for treatment of type 1 diabetes mellitus, which is characterized by lack of the internally produced hormone. Traditional injection dosage forms of insulin have a number of serious shortcomings, e.g., permanent violation of skin, variable absorption, and significant intra- and interindividual differences of the pharmacological effect. However, the main problem is the pharmacological profile of traditional insulin preparations that are not able to maintain the plasma concentration of insulin at the physiological level.

Various alternative ways of insulin delivery are intensively developed [1]. Nevertheless, there is only one noninvasive form of insulin (transbuccal Oral-Lyn™, Generex, USA), which is available in Ecuador and India [2]. Peroral formulation is still considered as the most safe, consistent with natural way of insulin delivery, and of high patient compliance. Insulin adsorbed in intestine transverses liver through portal vein, similar to the normally secreted insulin. Liver, in turn, controls the peripheral insulin concentration [3].

However, bioavailability of orally administered insulin does not exceed 1–2% [4] due to the protein denaturation in gastric juice with extremely low pH values, protein cleavage by gastric and intestinal proteolytic enzymes, and poor permeability of intestinal epithelium for the protein molecules [5].

In the last decade, chitosan-polyelectrolyte micro- and nanoparticles with encapsulated insulin were extensively studied as perspective carriers improving bioavailability of the peroral insulin [1]. Cationic polysaccharide chitosan, a deacetylated derivative of chitin, is structurally similar to mucopolysaccharides of human mucosa, e.g., hyaluronic acid, chondroitin, or heparin [6]. Chitosan is a biocompatible, biodegradable, and mucoadhesive polymer with antibacterial activity [7]. Mucoadhesive properties of chitosan can provide intimate and prolonged contact of perorally delivered drug with intestinal epithelium, thus inducing a high drug concentration gradient near the site of adsorption [8]. Chitosan is also able to increase paracellular drug adsorption owing to its ability to open epithelial tight junctions [9]. Chitosan ability to inhibit pepsin can reduce the protein degradation in stomach [10].

Methods of ionotropic gelation [11,12] and consecutive adsorption of polyelectrolytes onto hormone-containing aggregates [13,14] are perspective for preparation of insulin-containing polyelectrolyte micro- and nanoparticles. Both methods are simple and performed in aqueous solutions in the absence of organic solvents. However, low protein content in the particles prepared by ionotropic gelation (no more than 15%) and their insufficient stability in acidic media make the method of consecutive adsorption more attractive. As it was reported earlier [15], the use of chitosan and dextran sulfate (modified natural polysaccharide carrying on the average 2.3 sulfate groups per one monosaccharide unit) for alternating polyelectrolyte sorption allows fabricating nanostructured microparticles with various proteins. Appropriate pH-sensitivity of the microparticles protects encapsulated proteins from the aggressive stomach medium [13]. Microparticle composition and some release properties were previously published for chitosan-DS microparticles with different proteins, including, porcine insulin and rapid-acting analogues of human insulin [13,16,17]. In
in this work we present a further study on selection of microparticle preparation condition, more detailed release & degradation studies, and in vivo evaluation of biological activity after subcutaneous injection and oral administration.

Materials and Methods

Materials

Human recombinant insulin zinc salt (Ins) was provided by the Institute of Bioorganic Chemistry, Russia. Dextran sulfate sodium salt, Mₙ 500 kDa (DS); N-benzoyl-L-arginine ethyl ester (BAEE), and N-benzoyl-L-tyrosine ethyl ester (BTEE) were purchased from Sigma, USA. Chitosan with degree of deacetylation 75–85%, Mₙ 400 kDa (Ch); α-chymotrypsin, and trypsin were purchased from Fluka, Germany.

Preliminary characterization of microparticles

Microparticles were prepared by mixing equal volumes of the protein (20 mg/ml) and dextran sulfate (5.00–8.75 mg/ml) solution at pH 3.0, as described in [17]. Further Ch and DS deposition was performed in 0.15 M NaCl, 1 mM HCl at 20°C as described in [15]. The prepared microparticles were stored at 4°C as suspension; otherwise, they were washed three times with 1 mM HCl and freeze-dried.

Analysis of microparticle composition

Lyophilized microparticles were preliminary resuspended in 0.1 M NaOH in order to destroy Ch–DS polyelectrolyte complexes. Ins concentration was measured by the Lowry method [18] and DS concentration by the Dubois method [19]. Ch concentration was determined by the colorimetric assay developed earlier [20], based on the derivatization reaction of the chitosan primary amino groups with o-phthalaldehyde and N-acetyl-l-cysteine. Percentage of the microparticle components was determined as the ratio of the component mass to the mass of the lyophilized microparticles. Encapsulation efficiency was calculated as the ratio of the Ins or DS content determined in the microparticles to the respective amounts of Ins or DS used for the microparticle preparation.

Release of Ins and DS in vitro

Microparticle suspensions with final protein concentration 0.20–0.25 mg/mL were incubated in a shaker-bath with stirring (100 rpm) in universal buffer (0.02 M H₃PO₄, 0.02 M CH₃COOH, 0.02 M H₃BO₃ + 0.1 M NaOH, pH 2–8) at room temperature. After 1-h incubation the samples were centrifuged (10000 g, 5 min) and concentrations of Ins and DS in the supernatant were measured. The component release was characterized by the ratio of protein concentration in the supernatant to the initial protein concentration in the microparticle suspension.

Evaluation of proteolytic degradation of Ins

Aliquots of Ins-containing microparticles or Ins solution with protein content 0.5 mg/mL were incubated at 37°C under gentle stirring (100 rpm) either in 0.05 M Tris-buffer (pH 7.1) in the presence of trypsin (700 BAEE U) and chymotrypsin (4 BTEE U) for 1 h [21] or in 0.05 M Tris-buffer (pH 7.8) in the presence of trypsin (140 BAEE U) for 6 h [22]. Trypsin and chymotrypsin activities of the commercial preparations (9000 BAEE U/mg and 39 BTEE U/mg respectively) were measured as described in [23]. Proteolysis was stopped by the addition of trifluoroacetic acid to final concentration of 0.1%, samples were centrifuged (10000 g, 5 min), and the content of the remaining undegraded Ins in supernatants was measured by high-performance liquid chromatography (Agilent 1100 Series, Hewlett Packard, USA). A reversed-phase C18 column and gradient elution with a mobile phase composed of 0.1% acetonitrile and 0.1% aqueous trifluoroacetic acid solutions at a flow rate of 1 ml/min was used. Protein was detected by UV spectroscopy at 215 nm. The gradient changed from 20:80 (acetonitrile solution: water solution, v/v) to 50:50 (v/v) within 5 min; for further 10 min isocratic elution at 50:50 (v/v) was performed. Ins degradation was calculated as a relative difference (%) of the Ins peak areas before and after proteolysis.

In vivo studies of biological activity of microparticles in rabbits

Chinchilla male rabbits (2.5–3.5 kg) were kept on standard diet in the vivarium of the university in an air-conditioned animal room (22±2°C) under a 12 h light/dark cycle. The animals were fasted 3 h before and during experiments and had free access to water. Ins preparations were injected subcutaneously in a volume of 1 ml containing a dose of 4 IU/kg. During peroral administration the same dose of 4 IU/kg was introduced in 3 ml of microparticle suspension by the base of tongue and then washed by additional 5 ml of water. Control group did not get any Ins-containing preparations. Each group numbered 5–6 animals. Blood sampling was performed from the right ear before and three h during experiments and had free access to water. Ins concentration was measured by the Lowry method [18] and DS concentration by the Dubois method [19]. Ch concentration was determined by the colorimetric assay developed earlier [20], based on the derivatization reaction of the chitosan primary amino groups with o-phthalaldehyde and N-acetyl-l-cysteine. Percentage of the microparticle components was determined as the ratio of the component mass to the mass of the lyophilized microparticles. Encapsulation efficiency was calculated as the ratio of the Ins or DS content determined in the microparticles to the respective amounts of Ins or DS used for the microparticle preparation.

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Kinetics of the protein release was studied as follows. HCl solution (pH 1.1) was added to the microparticle suspension up to protein concentration of 0.20–0.25 mg/mL and the mixture was gently stirred (100 rpm) at room temperature for 2 h. Then the sample was centrifuged (200 g, 2 min), supernatant was collected and the equal volume of the universal buffer (pH 6.0) was added to the sediment. The resulting suspension of microparticles was incubated with gentle stirring for 2 h and centrifuged, then the supernatant was replaced with the universal buffer (pH 7.4), and the microparticle suspension was incubated with gentle stirring for 4 h. Every hour after the HCl solution was first added, an aliquot of the suspension was collected, centrifuged (10000 g, 5 min) and the protein content in the supernatant was determined. Protein release from the microparticles was characterized by the ratio of protein concentration in the supernatant to the initial protein concentration in the microparticle suspension.

In vivo studies of biological activity of microparticles in diabetic rats

Male Wistar rats (250–350 g) were housed in cages in the vivarium of the university in an air-conditioned animal room (22±2°C) under a 12 h light/dark cycle fed laboratory animal standard diet and provided with tap water. Rats were rendered diabetic by a single intraperitoneal injection of 50 mg/kg streptozotocin (26.6 mg/mL in citrate solution,
pH 4.5). Two weeks later, rats with fasting blood glucose level above 13 mM were randomly grouped into four: control, treated with 10 IU/kg or 25 IU/kg insulin microparticles and reference groups, each numbering 5-7 animals. The rats were fasted 12 h before and 24 h after insulin administration and had free access to water.

In the treated groups and in the control group the samples (0.5 mL), containing microparticle suspension or equivalent volume of 0.15 M NaCl, 1 mM HCl, respectively, were administered by oral gavage. In the reference group insulin was administered by subcutaneous injection (2.5 IU/kg). In each group blood samples (0.05 mL) were collected from the tail vein. Blood glucose level was determined using the Glukoza-agat Kit, (Agat-med, Russia; range 2-20 mM) and expressed as a percent of the baseline plasma glucose level. Plasma glucose levels were plotted over time to evaluate the cumulative hypoglycemic effect over time after insulin administration. Results and Discussion

Preparation of polyelectrolyte microparticles

Insulin-containing microparticles were prepared by consecutive adsorption of oppositely charged DS and Ch onto microaggregates of insoluble (Ins–DS) polyelectrolyte complex in 0.15 M NaCl, pH 3.0 (Figure 1). The choice of polyelectrolytes (Ch, 400 kDa and DS, 500 kDa) for the microparticle preparation has been substantiated in previous studies [13,16]. The aim of the first step was to obtain Ins–DS aggregates (particle size of 1–10 µm) with high protein content and a negative surface charge that would provide further electrostatic interaction of the insulin with oppositely charged chitosan (Figure 1). Micron size of the aggregates is required for effective centrifugation and resuspension during microparticle preparation. The presence of 2.3 sulfate groups per one glucosyl residue in DS provides a high density of negative charge (mass/charge ratio is 183 m/z), which is largely unchanged at all pH values. The human recombinant insulin zinc salt mainly persists in solution as a hexamer [24]. Theoretical calculations using the modified Tanford–Kirkwood model [25,26] revealed that insulin hexamer (pI = 5.35) at pH 3.0 is a sphere with a radius of 25 Å and a charge of +26, which corresponds to a mass/charge ratio of 1340 m/z. These calculations were confirmed by the results of turbidimetric titration of insulin solution by DS solution with Ins:DS weight ratios from 14:1 to 4:1. A maximum on the titration curves at the component ratio 7:1 indicated a complete neutralization of the protein. This ratio corresponds to a charge of hexameric Ins of +24 (+4 per monomer), which is close to the theoretically calculated value of +26. Assuming that not all Ins molecule charges are exposed, ζ-potential measurements should show neutralization at smaller ratio, that is generally consistent with the data shown in (Table 1). A zero ζ-potential corresponded to weight ratio ca. 5:1 and during further titration acquired a negative charge. In the following experiments, negatively charged Ins–DS aggregates prepared at the ratio of 4:1 were used. Under these conditions the efficiency of the protein and DS incorporation was the highest with mean size of microaggregates about 10 µm (Table 1). On the average, one molecule of DS bound to 23 sulfate groups per one glucosyl residue in DS provides a high density of negative charge (mass/charge ratio is

![Image](https://via.placeholder.com/150)

Figure 1: Scheme of the microparticles preparation by consecutive adsorption of the oppositely charged polyelectrolytes onto Ins–DS microaggregates.

<table>
<thead>
<tr>
<th>Ins:DS ratio during microaggregate preparation, (w/w)</th>
<th>Ins encapsulation efficiency, %</th>
<th>ζ-potential, mV</th>
<th>Mean size, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:1</td>
<td>38±1</td>
<td>19±2</td>
<td>3±1</td>
</tr>
<tr>
<td>5:1</td>
<td>94±2</td>
<td>7±1</td>
<td>3±2</td>
</tr>
<tr>
<td>4:1</td>
<td>98±3</td>
<td>-29±3</td>
<td>9±3</td>
</tr>
</tbody>
</table>

Table 1: Characterization of microaggregates of Ins–DS complexes.

**Results and Discussion**

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*Ratio of the component to microparticle weight

<table>
<thead>
<tr>
<th>Microparticles</th>
<th>No of adsorption steps (s)</th>
<th>Ins encapsulation efficiency, %</th>
<th>Content, w/w %*</th>
<th>Mean size, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ins–DS)</td>
<td>1</td>
<td>97±3</td>
<td>81±4</td>
<td>17±5</td>
</tr>
<tr>
<td>(Ins–DS)+Ch</td>
<td>2</td>
<td>67±2</td>
<td>63±3</td>
<td>21±3</td>
</tr>
<tr>
<td>(Ins–DS)+Ch-DS</td>
<td>3</td>
<td>65±3</td>
<td>57±2</td>
<td>28±5</td>
</tr>
<tr>
<td>(Ins–DS)+Ch-DS-Ch</td>
<td>4</td>
<td>65±2</td>
<td>57±4</td>
<td>26±3</td>
</tr>
</tbody>
</table>

Table 2: Characterization of insulin-containing polyelectrolyte microparticles.
polysaccharide competitively replaces Ins from the microaggregates forming more stable polyelectrolyte complex with anionic DS. The following alternating treatment had no significant effect on the protein encapsulation efficiency; microparticle size was about 10 µm (Table 2). The more sorption stages were carried out the higher was the DS and Ch percentage. Microparticles with number of stages of the polyelectrolyte adsorption (s) from 1 to 4 were prepared. Ins:DS:Ch ratio in final particles (s=4) was about 2.5:1:1 [11].

The sign of ζ-potential depended on the polyelectrolyte of the outmost layer (Figure 2). The microparticles possessed positive or negative surface charge after the adsorption of Ch or DS, respectively. The value of ζ-potential not only allowed estimation of the fullness of the polyelectrolytes sorption but also made it possible to predict mucoadhesive properties of the microparticles. It is well-documented that mucus layer of intestine is negatively charged due to terminal sialic acid residues of mucopolysaccharides [27]. That is why positive charge of the chitosan-coated microparticles could improve their mucoadhesive properties.

Microscopic studies indicated that microparticles retained the shape of the initial microaggregates, i.e., irregular-shaped particles with size range from 3 to 15 µm (Figure 3).

**pH-Sensitivity of the micro particles**

The main goal of the research was to develop an Ins preparation that would retain its activity and efficiency when administered orally. In other words, the protein should be protected from pepsin in strongly acidic stomach medium and be released and absorbed in the small intestine. pH-Sensitivity of the microparticles (Figure 4) seems to be appropriate. According to the results of analysis of the protein content in supernatants (Figure 4A), microparticles (s = 2) prepared by sorption of chitosan onto Ins–DS aggregates released Ins in strongly acidic media. This can be explained by positive ζ-potential of microparticles after chitosan sorption and competitive replacement of DS-bound Ins by the added Ch. In acidic media, the charge of Ins is increased forming the excess of positive charge on microparticles that results in displacement of a part of Ins molecules from the complex. As far as the polysaccharides in Ch–DS complex are more tightly bound than the protein in Ins–DS complex, some of the Ins molecules are released. Further polyelectrolyte coating of the microparticles prevents insulin from leakage in acidic medium. It is important that in the pH range of 6–7, a cooperative destruction of the microparticles with almost complete release of Ins occurred. This is clearly caused by Ins molecule recharging (pI = 5.35) and competitive displacement of the negatively charged protein from the insoluble particles. In the same pH range, deprotonation of Ch (pKa=6.4) takes place along with the Ins recharging. This should facilitate the formation of soluble polyelectrolyte complexes of Ch–DS [28], since solubilizing ability of highly charged DS increases as the charge of the cationic polysaccharide decreases. This assumption is supported by the analysis of anionic polysaccharide concentration in supernatants (Figure 4B). All DS chains remained in microparticles at pH up to 6.0 (s = 2–4) and then released into solution almost linearly. Note that DS molecules released much less effectively than the protein did (Figure 4A). Thus, only a quarter of the original content of the polysaccharide in the microparticles was found in the supernatant at pH 8.0. This value remained constant for all the microparticles (s = 2–4). A complete release of DS was likely prevented by formation of hydrogen bonds with deprotonated amino groups of Ch that stabilized the insoluble complex.

It should be noted that Ins was almost completely removed from the microparticles at pH 7.0 (Figure 4A), whereas only 10% of DS was released (Figure 4B). These results are in agreement with a previous report confirming that Ins is released from the microparticles as hexamer, which does not associate with the polyelectrolytes (according to gel chromatography) [29].

**Kinetics of Ins release from microparticles**

The kinetics of Ins release was studied under the conditions simulating the change of luminal pH of the gastrointestinal tract (s=4, Figure 5). For the first 2 h microparticles were incubated at pH 1.1 imitating acidic stomach medium; then for 2 h, at pH 6.0 corresponding to the medium acidity of duodenum, and after that, for 4 h, at pH 7.4 corresponding to the lower sections of small intestine. Release curves confirmed that Ins was retained in the microparticles at pH 1.1. At pH
6.0 a slight release of the protein (no more than 2%) was observed. This differs from the data shown in (Figure 4A), when almost 30% of Ins was released after 1-h incubation at pH=6.0. This discrepancy is likely associated with stabilizing effect of the incubation of microparticles at low pH prior to the application of pH=6.0: in the acidic medium Ch and Ins attain more positive charges stabilizing their complex with DS. This effect weakens at pH 7.4, and about 85% of Ins releases within the first hour of incubation at pH 7.4. The rest of Ins was released from the microparticles within the next 3 h of incubation.

Thus, polyelectrolyte microparticles prepared by four cycles of polyelectrolyte sorption exhibit a high efficiency of protein encapsulation (about 65%), small size (about 10 μm), stability at low pH (from 2 to 5) corresponding to the acidity of stomach medium, and release of Ins at pH over 6 corresponding to pH of intestine medium. The use of cationic polysaccharide Ch at the last cycles of sorption provides positive ζ-potential of the microparticles and should enhance their adhesion to intestinal epithelium. All these features make the particle a perspective vehicle for peroral Ins delivery.

**Protease resistance of microencapsulated insulin**

Conditions recommended by A. Bernkop-Schnürch were chosen to study Ins proteolysis in small intestine. These conditions imitate two different divisions of human intestine: pancreatic juice (pH 7.1) [21] and luminal juice of the lower parts of the small intestine (pH 7.8) [22] (Table 3). Ins encapsulation into polyelectrolyte microparticles reduced but did not completely prevent proteolytic cleavage of Ins. In pancreatic juice the protective effect was the most significant: 40% of Ins avoided proteolysis in microparticles, whereas almost all native Ins was hydrolyzed after 1 h incubation. In simulated lower parts of the small intestine the amount of undegraded Ins in microparticles was 2 times more than that of native Ins in solution. The protective properties of the microparticles can derive from formation of DS–protease complex, Ca²⁺-binding ability of DS decreasing enzyme activity [17], and from the steric effect of polyelectrolyte network hindering the access of proteases to the encapsulated Ins. Another possibility is sorption of proteases on the microparticles remaining after Ins release; such microparticles acquire negative charge (−39 mV) and may bind proteases thus reducing their concentration in the medium.

**Hypoglycemic activity of microencapsulated Ins in vivo**

*Healthy rabbits:* Biological activity of Ins-containing microparticles (s=4) was evaluated in healthy rabbits. After a subcutaneous injection of soluble or encapsulated Ins at a dose of 4 IU/kg blood glucose level decreased by 40% within one hour (Figure 6).

For *in vivo* testing of peroral insulin preparations, diabetic rats [12,30], mice [31] and, less frequently, rabbits [32] are usually used. However, rabbits seem to be an appropriate object for studies of peroral preparations as gastrointestinal tract of rabbits bares similarity to the human’s one by such parameters as relative intestine surface area, pH values of intestine divisions, microflora, etc. [33]. Because of low bioavailability of oral preparations of Ins, high doses of Ins – from 20 [30] to 100 IU/kg [12] – are usually used. We studied a hypoglycemic activity of Ins-containing microparticles after peroral administration of a low Ins dose of 4 IU/kg, similar to subcutaneous injections. Animals were divided into two groups depending on their initial blood glucose level (Figure 6). In the first group with normal initial glucose concentration, no hypoglycemic activity was observed. In the other group with high initial blood glucose level (>5.5 mM), hypoglycemic effect (by up to 17%) was manifested for 150 min. These findings correspond well to the previously reported observations [34] that revealed a hypoglycemic effect of peroral insulin preparation only in diabetic animals or in animals with elevated initial blood glucose level.

**Diabetic rats:** The next step was to study hypoglycemic activity and to evaluate pharmacological availability of peroral microencapsulated insulin using streptozotocin-induced rat model of diabetes. In this study the doses were lowered down to 10 and 25 IU/kg since an efficiency at
against cleavage by intestinal proteases has been established. All these
released within one hour. The ability of the microparticles to protect Ins
started to be released, and at pH 8.0 practically all Ins molecules were
acidic media. At neutral pH values imitating small intestine lumen, Ins
pH range, from strongly acidic media imitating stomach to weakly
sorption, featured a relatively high Ins loading and stability in a wide
Positively charged microparticles obtained after four-step (s=4)
biocompatible polysaccharides DS and Ch onto Ins–DS microaggregates.
prepared by layer-by-layer adsorption of oppositely charged

Conclusions

properties, together with microparticle mucoadhesivity due to the Ch
coating, notably improve Ins bioavailability upon oral administration
of microparticles.

Preservation of Ins biological activity during encapsulation into
polyelectrolyte microparticles was confirmed. Hypoglycemic activity of
Ins-loaded microparticles was revealed at a dose of 4 IU/kg after peroral
administration to healthy rabbits with elevated blood glucose level. A
prolonged (up to 10 h) and dose-dependent hypoglycemic effect was
ascertained after peroral administration of polyelectrolyte insulin-
containing microparticles to diabetic rats at doses of 10 and 25 IU/kg.
Bioavailability of perorally administered microencapsulated insulin
was estimated to be 10.7% as compared to subcutaneous injection.

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References


insulin control of liver glycogen synthase b into a conversion. Biochem Biophys 

249-257.

barriers and developments. BioDrugs 1: 165-177.

of mucoadhesive properties of chitosan and some other natural polymers. Int J 
Pharm 78: 43-48.


and their encapsulation with biodegradable polyelectrolytes via the layer-by-

Polyelectrolyte assembling for protein microencapsulation. J Drug Del Sci Tech 
16: 315-319.


