Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Physicochemical characterization of protein-loaded pectin-chitosan nanoparticles prepared by polyelectrolyte complexation

P. Ahlin Grabnar, J. Kristl

Received July 5, 2010, accepted July 26, 2010

Dr. Pegi Ahlin Grabnar, University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia
ahlinp@ffa.uni-lj.si

Pharmazie 65: 851–852 (2010)
doi: 10.1691/ph.2010.0197

Recent advances in nanotechnology applied to proteins are directed towards safer and simpler methods of preparation, using naturally occurring polymers such as alginate, pectin and chitosan. In this study, pectin-chitosan nanoparticles (NPs) were designed by the mild process of polyelectrolyte complexation, which occurs at room temperature without using sonication or organic solvents. NPs with a mean diameter between 300 and 400 nm and 45 to 86% protein association efficiency were obtained by varying the pectin:chitosan mass ratio and initial protein concentration. A prolonged release profile without burst effect of investigated ovalbumin from pectin-chitosan NPs was determined.

Nanoparticulate delivery systems have the potential to improve protein stability, prolong the therapeutic effect and permit non-parenteral administration. Polyelectrolyte complexes, which are formed when oppositely charged polymers interact via electrostatic interactions, present new possibilities in drug formulation. Polymers such as pectin, alginate and chitosan have been described as biocompatible, biodegradable and mucoadhesive, enabling numerous pharmaceutical and biomedical applications including the design of controlled release devices. Among many different methods for preparation of protein-loaded NPs, the double emulsion method is frequently used. However, although polyelectrolyte complexation has been used for preparation of hydrophilic gels for a long time, studies using polyelectrolyte complexation for preparation of NPs are scarce. Among the published studies alginate-chitosan NPs appear promising as an oral delivery system for therapeutic proteins, such as insulin (Sarmento et al. 2007a, b). It has been confirmed that polyelectrolyte complexation does not induce conformational changes to the insulin structure. The complex protects the protein, has biocompatible and biodegradable characteristics, and limits the release of entrapped materials more effectively than either alginate or chitosan alone.

In the present study we designed pectin-chitosan polyionic nanocomplexes, which form through interactions between the carboxyl groups of pectin and the amine groups of chitosan. Pectins are anionic, soluble polysaccharides extracted from the primary cell walls of plants. They form gels by controlled calcium-mediated interchain association to give an extended, uniformly regular junction zone, presumably similar to that depicted in the eggbox model proposed for calcium alginate (Liu et al. 2003). Chitosan is the cationic deacetylated form of chitin obtained from exoskeletons of marine arthropods and is widely used in NP preparation (Rinaudo 2006).

Table: The effect of initial OVA concentration on the NPs characteristics. Pectin: chitosan mass ratio was 1: 0.25

OVA (mg/ml)	d (nm)	PI	ZP (mV)	EE (%)
0.1	408.9	0.41	−26.0	73.1
0.5	302.4	0.29	−21.7	86.6
1	visible	0.91	−1.5	84.9
2	visible	1.00	5.6	51.9
4	391.2	0.56	31.6	45.3

The main scope of the current study was the design, formulation and physicochemical characterization of pectin-chitosan NPs. Such complexes are suitable for protein drug incorporation and mucosal delivery. Protein ovalbumin (OVA) was used as a model drug.

The mean particle size, polydispersity index and zeta potential of the NPs were affected by the initial ratio between the mass of pectin and chitosan used to prepare NPs. The zeta potential was close to zero for pectin:chitosan mass ratio between 1:0.36 and 1:0.38, which indicates that there is a balance between positive and negative charges. These particles had also the highest mean particle size. The most physically stable NP dispersion with pectin:chitosan ratio 1:0.25, with the mean diameter of 419 nm and zeta potential − 30.4 mV, was used in further studies for OVA incorporation.

OVA is an amphoteric molecule and it can be electrostatically attached to NPs composed of pectin and chitosan. As presented in the Table, NPs with a mean size between 300 and 400 nm and 45 to 86% OVA association efficiency were obtained by varying the pectin:chitosan mass ratio. The zeta potential of these NPs was inverted from negative to positive values when 1.2 mg/ml concentration of OVA in pectin solution (pectin: chitosan: OVA = 1:0.25:1.98) was used. Visible particles were formed as a consequence of aggregation.

Release profiles of OVA from pectin:chitosan NPs was evaluated by comparison with the diffusion of free OVA and solution OVA:pectin through the dialysis membrane. As shown in the Fig., prolonged release profile of OVA from pectin:chitosan NPs was observed, which confirms the strong interaction of OVA with the polymers.

It can be concluded, that polyelectrolyte complexation between pectin and chitosan in low concentrated solutions is a mild process, which could be used for preparation of NPs at ambient temperature without using sonication or organic solvents. Potentially it is a suitable procedure for incorporation of sensitive materials such as proteins. The automation of the process could be achieved by using a vibrating nozzle device, which has been previously successfully applied for preparation of poly(lactide-co-glycolide) NPs (Zvonar et al. 2009).

Experimental

1. Materials

Components of the carrier systems were chitosan (low viscosity, 96% degree of deacetylation; Fluka, Swiss), pectin (low methoxy pectin with 36% degree of esterification; GENU LH101ASZ, Kelco BioPolymers, Denmark), ovalbumin (Sigma, USA) and $CaCl_2$ (Merck, Germany).

2. Preparation of nanoparticles

Pectin solution was prepared in deionized water and chitosan was dissolved in 1% acetic acid solution. The pH of pectin and chitosan solutions was initially adjusted with HCl to 4.2 and 3.9, respectively. Pectin:chitosan NPs were prepared in a two-step procedure based on the ionotropic pre-gelation

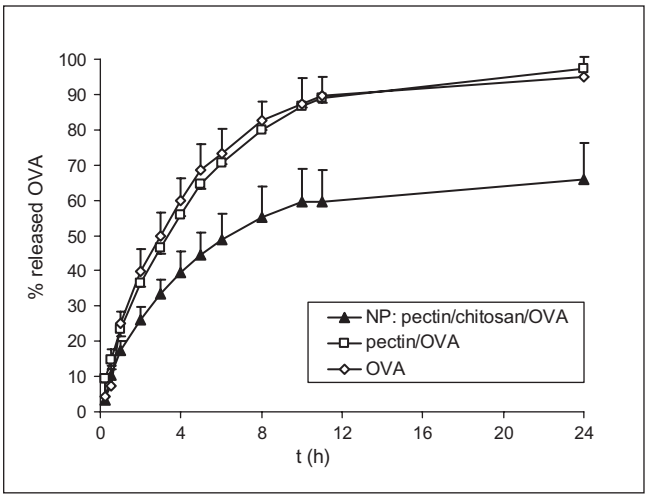

Fig.: Release profiles of OVA from pectin-chitosan NPs in comparison with the diffusion of free OVA and solution OVA/pectin through the dialysis membrane (mean, SD, n = 3)

of pectin with $CaCl_2$ followed by chitosan crosslinking. 1.05 ml of $CaCl_2$ solution (0.0943 mg/ml) was injected under gentle stirring into a beaker containing 16.5 ml of pectin solution (0.6 mg/ml) and stirred for 10 min to provide a pre-gel (Ca^{2+}/pectin = 0.01). Then, 3.55 ml of chitosan solution was injected into the pre-gel under stirring. For OVA-loaded NPs, OVA was mixed with the pectin solution before addition of the $CaCl_2$ solution.

3. Size and zeta potential measurements

The mean particle size was estimated by a photon correlation spectroscopy and zeta potential by laser Doppler anemometry using a Zetasizer Nano ZS (Malvern, UK).

4. Entrapment efficiency

Entrapment efficiency was calculated as the difference between the total amount of OVA used to prepare NPs and the amount present in the supernatant after centrifugation at 28000 rpm for 1 h. Samples were analysed for OVA content using HPLC (gel filtration column: Zorbax GF-250, Agilent).

5. In vitro release studies

The release of OVA from pectin/chitosan NPs in phosphate buffer pH 7.4 was assessed using a dialysis technique (Spectrapor, Thomas Scientific) under sink conditions over a 24 h period. Samples were analysed for OVA content using HPLC (gel filtration column: Zorbax GF-250, Agilent).

References

Liu LS, Fishman ML, Kost J, Hicks KB (2003) Pectin-based systems for colon-specific drug delivery via oral route. Biomaterials 24: 3333–3343.

Rinaudo M (2006) Chitin and chitosan: Properties and applications. Prog Polym Sci 31: 603–632.

Sarmento B, Ribeiro AJ, Veiga F, Ferreira DC, Neufeld RJ (2007a) Insulin-loaded nanopartilces are prepared by alginate ionotropic pre-gelation followed by chitosan polyelectrolyte complexation. J Nanosci Nanotechnol 7: 2833–2841.

Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D (2007b) Alginate/chitosan nanoparticles are effective for oral insulin delivery. Pharm Res 24: 2198–2206.

Zvonar A, Kristl J, Kerč J, Ahlin Grabnar P (2009) High celecoxib-loaded nanoparticles prepared by a vibrating nozzle device. J Microencapsul 26: 748–759.