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Note

Alginate microparticles for enzyme peroral administration

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Abstract

In order to protect protein and peptide drugs against inactivation by different barriers in the gastro-intestinal tract and to improve their absorption, alginate microparticles as a carrier of L-lactate dehydrogenase, were developed by spray-drying technique. However, alginate complexation and spray-drying conditions led to enzyme activity loss. Such a drawback was overcome by using protectant additives (carboxymethylcellulose sodium salt, polyacrylic acid sodium salt, lactose) preventing the enzyme inactivation by both interaction with alginate and experimental conditions, lactose having the most protective effect. Nevertheless, only polyacrylic acid sodium salt provided a microparticulate structure required for the target of the Peyer's patches.

Keywords: Alginate; Spray-drying; Oral delivery; Protein; Activity preservation

Microparticles as potential oral delivery systems for proteins have been described (Couvreur and Puisieux, 1993), since they represent a possible strategy to overcome some problems in the oral protein administration, such as pH and proteolytic degradation and poor diffusivity through biological barriers. Furthermore, it was shown that the microparticles are able to be taken up by the Peyer's patches (Jani et al., 1992).

The spray-drying technique was reported as a method of preparing microcapsules (Tabata and Ikada, 1998). In carrier selection, natural polymer hydrogels may be preferable, because they are biosafe and do not need organic solvents. In this regard, the use of alginate gelled by the addition of calcium ions has been reported as protein release matrix (Gombotz and Wee, 1998).

Alginate microparticles, produced by spray-drying and crosslinked by calcium ions and chitosan for the transport of bovine serum albumin, as a model protein, was proposed (Coppi et al., 2001).

Complexation between alginate and cationic drugs led to increased drug loadings into the microsystem, however a decreased biological activity is possible (Mumper et al., 1994). In addition, during the spray-drying process, the lost of protein hydration and the shear forces in the spray nozzle may alter the secondary structure of the protein (Millqvist-Fureby et al., 1999).

The use of stabilizing additives could be an approach to protect the protein molecule from inactivation (Mumper et al., 1994).

In this regard, the applicability of the spray-drying technique in producing an alginate mi-

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Table 1
Relative enzymatic activity of samples at different LDH/Na-A ratios

LDH/Na-A (w/w)	Relative activity (%)
1:50	9 ± 2
2:50	22 ± 3
3:50	36 ± 4
4:50	38 ± 3
5:50	41 ± 4

Table 2
Enzymatic activity of LDH in physical mixtures and spray-dried microparticles

Sample	Physical mixtures activity (%)	Spray-dried microparticles activity (%)
LDH	69 ± 7	2 ± 2
LDH/Na-A 2:50 (w/w)	22 ± 3	4 ± 3
LDH/Na-A/Na-CMC 2:50:50 (w/w/w)	34 ± 5	4 ± 2
LDH/Na-A/Na-PAA 2:50:50 (w/w/w)	63 ± 5	40 ± 5
LDH/Na-A/lattosio 2:50:50 (w/w/w)	58 ± 4	56 ± 5

crosystem as a carrier of enzymes has been evaluated. In particular, the enzyme activity in the absence and presence of protectant molecules in alginate complex and in spray-dried products has been considered.

The L-lactate dehydrogenase (LDH, from rabbit muscle with 165 U/mg, from Fluka Chemie, Buchs, Switzerland), as an enzyme model, was considered. An 'optimized standard method' conforming to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (Roche Diagnostics GmbH, Mannheim, Germany) as assay for enzyme activity was used.

A complete loss of native LDH activity (corresponding to 100%) was found in water, whereas residual relative activity of 69 and 90% were observed in physiological solution and in phosphate buffer pH 7.5, respectively. Since all the samples were prepared in physiological solution that did not affect the subsequent procedure, the residual activity of the enzyme in such a medium was considered as the referring value.

The addition of sodium alginate (Na-A, MW $\approx$ 147,000, containing 62% mannuronic acid and 38% guluronic acid, from Kelco International, Bagnolex, Cedex, France) into LDH solutions resulted in a loss of activity according to the LDH/Na-A ratio (Table 1). The enzymatic activity decreased as the decreasing of LDH/Na-A

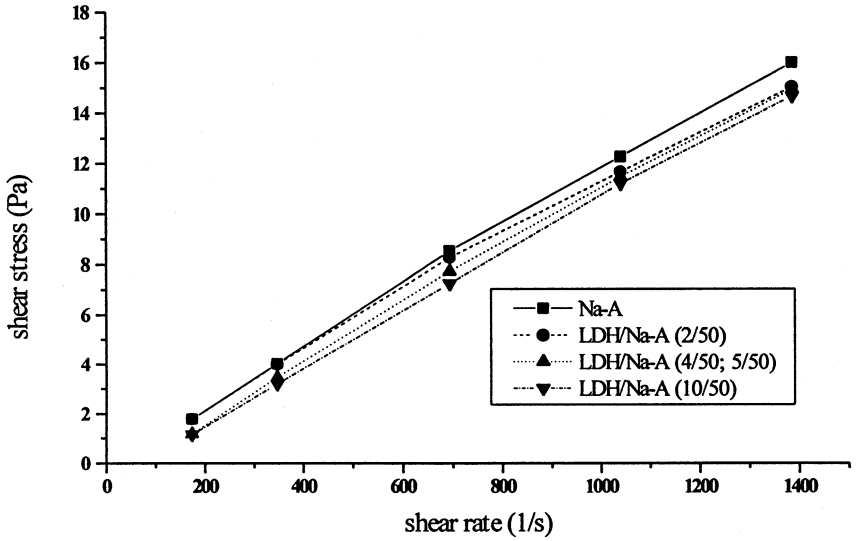

Fig. 1. Flow behaviour of solutions at different LDH/Na-A ratios.

ratio, suggesting the formation of a complex between the enzyme and alginate as previously reported for other protein molecules (Coppi et al., 2001). Presumably, such an interaction would lead to an inactive compound, so the decreasing in LDH/Na-A ratio resulted in diminished free bioactive LDH.

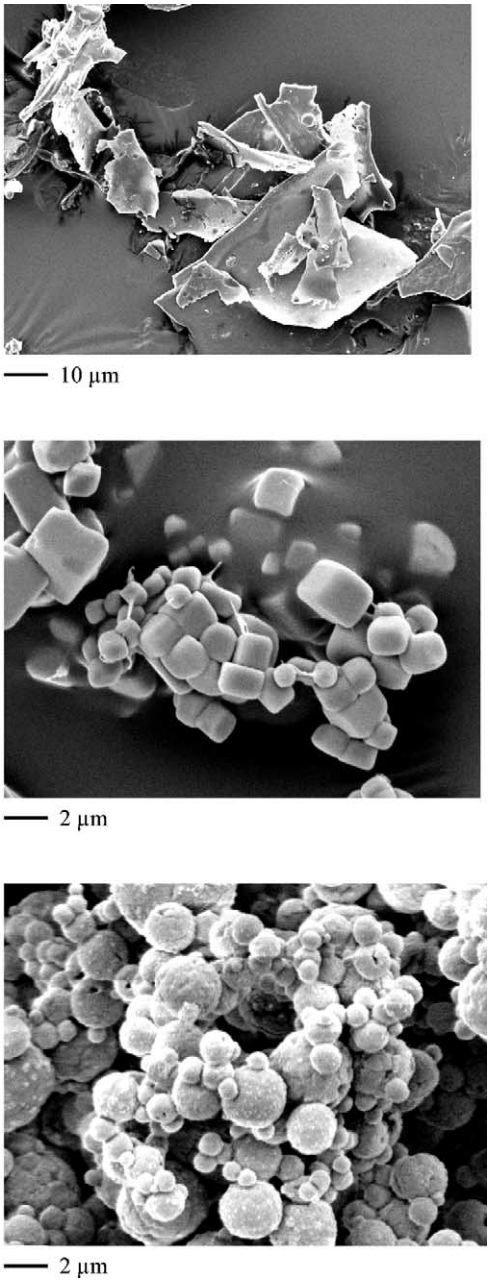

Fig. 2. SEM micrographs. (A) Commercial LDH sample; (B) spray-dried LDH microparticles; and (C) spray-dried LDH/Na-A/Na-PAA (2:50:50) microparticles.

In order to verify the occurring of the interaction between the enzyme and alginate, rheological investigations were carried out at 25 °C in a coaxial cylinder (radii ratio = 1.02) rheometer (Rotovisco RV 12 Haake, Karlsruhe, Germany) measuring the shear stress as a function of the shear rate. Dilute solutions (0.5%) of the polyanionic sodium alginate in the presence of oppositely charged compounds show a decreased intramolecular repulsion leading to a tighter configuration and, consequently, to a reduced viscosity ('electroviscous effect') (Fuoss, 1948).

The flow behaviour of dilute Na-A solutions in presence of different LDH amounts showed a decreased viscosity as the increase of LDH, indicating the occurring of an electrostatic interaction between LDH and alginate (Fig. 1).

To shield LDH from interaction with alginate and then from inactivation, protectant additives could be incorporated. Therefore, sodium carboxymethylcellulose (Na-CMC, from Fluka Chemie) and polyacrylic acid sodium salt (Na-PAA, Fluka Chemie) as polyanionic compounds acting through competitive interaction with alginate (Mumper et al., 1994) and lactose (Fluka Chemie), capable of forming hydrogen bounds with the protein (Millqvist-Fureby et al., 1999) preventing protein complexation with alginate were considered.

Physiological solutions of mixtures LDH/Na-A/protectant (2:50:50) were assayed for the LDH activity (Table 2).

The retained enzymatic activity values of the mixture solutions demonstrated that the protective effect towards alginate inhibition was poor by using Na-CMC and nearly complete by using Na-PAA or lactose.

After spray-drying (Buechi Laboratoriums, Technik AG, Flawil, Switzerland) (inlet temperature 140 °C, outlet temperature 45–50 °C, pump setting 15 ml/min, spray flow 500 Nhl^{-1}, aspirator setting 10, nozzle body cooled with water and 0.5 mm nozzle cap), the bioactivity of the LDH and the complex LDH/Na-A was completely lost.

In fact, both protein dehydration and shear stress in the spray nozzle may alter the secondary structure of the protein, leading to activity loss.

The activity resulted preserved by the addition of Na-PAA and lactose, lactose being the best protectant according to the 'water replacement' hypothesis (Millqvist-Fureby et al., 1999), no protective effect was shown by Na-CMC (Table 2).

The observations by scanning electron microscope (SEM, XL-40 Philips, Eindhoven, The Netherlands) revealed that the spray-drying procedure provided microparticulate structures only in the presence of Na-PAA (Fig. 2), which is probably capable of neutralizing the effect of NaCl of the physiological solution in preventing microparticulate formation. The obtained microparticles were < 5 μm in size, therefore suitable to be taken up by the Peyer's patches (Heldridge et al., 1990).

In conclusion, the development of alginate microparticles for the oral administration of enzymatic compounds requires careful consideration of some factors involved in the protein inactivation, such as complexation reactions or denaturations by the experimental conditions.

Protectant substances may be used to reduce the activity loss. Among the substances assayed in the present study, Na-PAA can be considered as the most convenient, combining bioactivity protection and morphology preservation.

References

Coppi, G., Iannuccelli, V., Leo, E., Bernabei, M.T., Cameroni, R., 2001. Chitosan-alginate microparticles as a protein carrier. Drug Dev. Ind. Pharm. 27, 393–400.

Couvreur, P., Puisieux, F., 1993. Nano- and microparticles for the delivery of polypeptides and proteins. Adv. Drug Deliv. Rev. 10, 141–162.

Fuoss, R.M., 1948. Viscosity function for polyelectrolytes. J. Polym. Sci. 3, 603–604.

Gombotz, W.R., Wee, S.F., 1998. Protein release from alginate matrices. Adv. Drug Deliv. Rev. 31, 267–285.

Heldridge, J.H., Hammond, C.J., Meulbroek, J.A., Staas, J.K., Gilley, R.M., Tice, T.R., 1990. Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. J. Control. Rel. 11, 205–214.

Jani, P.U., McCarthy, D.E., Florence, A.T., 1992. Nanosphere and microsphere uptake via Peyer's patches: observation of the rate of uptake in the rat after a single oral dose. Int. J. Pharm. 86, 239–246.

Millqvist-Fureby, A., Malmsten, M., Bergenstahl, B., 1999. Spray-drying of trypsin-surface characterisation and activity preservation. Int. J. Pharm. 188, 243–253.

Mumper, R.J., Hoffman, A.S., Puolakkainen, P.A., Bouchard, L.S., Gombotz, W.R., 1994. Calcium-alginate beads for the oral delivery of transforming growth factor β_1 (TGF-β_1): stabilization of TGF-β_1 by the addition of polyacrilic acid within acid-treated beads. J. Control. Rel. 30, 241–251.

Tabata, Y., Ikada, Y., 1998. Protein release from gelatin matrices. Adv. Drug Deliv. Rev. 31, 287–301.