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Short communication

Physico-chemical characterization of insulin-loaded poly(isobutylcyanoacrylate) nanocapsules obtained by interfacial polymerization

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Abstract

Insulin could be encapsulated very efficiently in oily containing poly(isobutylcyanoacrylate) nanocapsules obtained by interfacial polymerization. In addition, these nanocapsules showed unexpected biological activity after intragastric administration. The hypoglycemic effect was characterized by a lag time period of 2 days and a prolonged effect over a period of 20 days. To explain, the high encapsulation rate of insulin achieved in these nanocapsules and the biological effect, this work was focused on the characterization of the nanocapsules and on the study of the mechanism of nanocapsule formation. Results showed that insulin was found unmodified during the nanoencapsulation process. This was due to the large amount of ethanol used in the preparation of the nanocapsules that initiated the polymerization of isobutylcyanoacrylate preserving the peptide from a reaction with the monomer. Results also showed that insulin was located inside the core of the nanocapsules and not simply adsorbed onto their surface.

Keywords: Insulin; Nanocapsules; Poly(isobutylcyanoacrylate); Isobutylcyanoacrylate; Polymerization initiation

Intragastric administration of insulin-loaded poly(isobutylcyanoacrylate) nanocapsules induced a reduction of the glycemia to normal level in streptozotocin diabetic rats (Aprahamian et al., 1987; Damgé et al., 1988, 1990) and in alloxan induced diabetic dogs (Damgé et al., 1995). The hypoglycemic effect was characterized by surprising events including a lag time period of 2 days and a prolonged effect over a period of 20 days. Insulin is a very hydrosoluble peptide and should

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be inactivated by the enzymes of the gastrointestinal tract. Thus, the reason why insulin could be encapsulated with high efficiency in nanocapsules containing an oily core and why these nanocapsules showed so unexpected biological effect remained unexplained. Nanocapsules were prepared by interfacial polymerization of isobutylcyanoacrylate (Al Khouri Fallouh et al., 1986). The polymerization of such a monomer could be initiated by any nucleophilic group including those of some of the aminoacids of insulin (Leonard et al., 1966). In this case insulin could be found covalently attached to the polymer forming the nanocapsule wall as it was recently demonstrated with insulin-loaded nanospheres (Damgé et al., 1997). The aim of the work was to elucidate the mechanism which allowed a high rate of insulin encapsulation within the nanocapsules and to investigate if insulin was covalently attached to the polymer forming the nanocapsules.

Insulin-loaded nanocapsules were obtained by dissolving 5 mg of insulin in 25 ml of absolute ethanol (Carlo Erba, Italy) containing 1 ml Miglyol® 812N (Hüls, France), and 125 μl of isobutylcyanoacrylate (IBCA) (Loctite, France) and adding this ethanolic solution to an aqueous solution of Lutrol® F68 (poloxamer 188, BASF, Germany) at the concentration of 0.25%. The nanocapsules formed immediatly and ethanol was removed by rotoevaporation under vacuum. The diameter of the nanocapsules were evaluated by quasi elastic light scattering (Nanosizer N4MD, Coultertronic, France) and were found to be 250 ± 35 nm. Unloaded nanocapsules were obtained by the same method excepted that insulin was not used in the preparation. Unloaded nanocapsules showed the same size as insulin-loaded nanocapsules.

As discussed above, aminoacids of insulin could theoretically initiate the polymerization of IBCA leading to the covalent linkage of the peptide to the polymer forming the nanocapsules. In order to evaluate whether or not insulin interacted with the monomer during the preparation of the nanocapsules, the total amount of insulin still present within the nanocapsule dispersion has been directly determined after dissolution of the polymer with acetonitrile. Insulin determination has been performed using a high-performance liquid chromatography (HPLC) method consisting of a reverse phase column C18 (μBondapack Waters, Millipore, France ref.P/N 27324), a linear gradient at a flow rate of 1 ml min^{-1} over 50 min performed with 90–0% solvent A (0.1% TFA in water) and 10–100% solvent B (0.1% TFA/49.9% water/50% acetonitrile), and a detection at 220 nm. With this method, insulin showed up at a retention time of 29.86 min (Fig. 1(A)). The chromatogram given by the nanocapsule dispersion highlighted a single peak identical to the peak given by a reference solution of insulin (Fig. 1). The quantitative analysis revealed that 96% of the amount of insulin used for the preparation of the nanocapsules was recovered unchanged after the nanocapsules were formed. The rate of insulin association within the nanocapsules has been determined after separation of the nanocapsules from the dispersing medium by ultracentrifugation. The amount of non-encapsulated insulin was measured by HPLC within the dispersing phase. It represented 10% of the initial amount of insulin used in the preparation of the nanocapsules. This means that 90% of the insulin introduced in the medium for nanocapsule preparation has been associated with the nanocapsules.

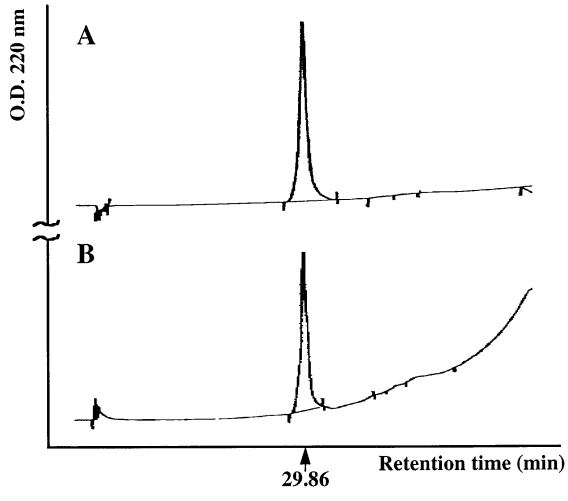

Fig. 1. Chromatograms of a reference solution of insulin (A) and the insulin obtained from the nanocapsule dispersion after dissolution of the nanocapsules (B).

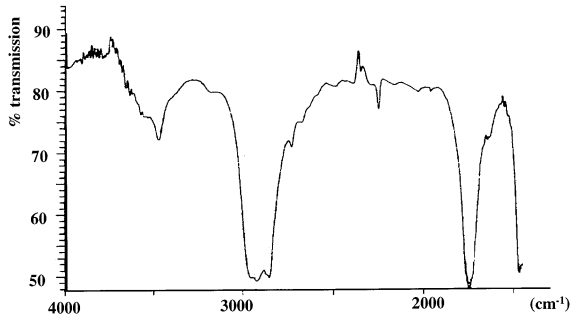

Fig. 2. Infrared (IR) spectrum of the polymer purified from the insulin-loaded nanocapsules.

The polymer forming the nanocapsules has been purified from nanocapsule dispersion and analyzed by size exclusion chromatography (SEC) and infrared (IR) spectroscopy. Results obtained with polymers purified from insulin-loaded nanocapsules has been compared to the results produced by the analysis performed in same conditions but with purified polymers arising from unloaded nanocapsules. PIBCA exctracted from insulin-loaded and unloaded nanocapsules showed the same SEC chromatograms with a peak corresponding to the polymer at a retention time of 12.02 min. The molecular weight was 149 000 as referred to a calibration performed with polystyrene. Since the molecular weight of PIBCA was unchanged whether the polymer was formed in the presence or in the absence of insulin, it was concluded that insulin did not interfere with the mechanism of IBCA polymerization involved during the formation of the nanocapsules. This is contrary to recent results published by Damgé et al. (1997) who showed that insulin induced a significant modification of the molecular weight of PIBCA in case of nanospheres prepared in the presence of insulin. The main difference between the two methods of preparation (nanospheres or nanocapsules) is the use of a large amount of ethanol in the preparation of the nanocapsules. On the other hand, the IR spectrum given by the insulin-loaded nanocapsules (Fig. 2) was identical to the spectrum given by the polymer purified from unloaded nanocapsules (data not shown). Peaks corresponding to the OH and the CN group showed up at 3600 and 2250 cm^{-1}, respectively. According to Leonard et al. (1966), the fact that these signals showed up indicated that the polymerization of IBCA was not initiated by the aminoacids of insulin. The presence of the signal for the OH group suggested that the polymerization was initiated by a component containing an OH group. As mentioned above, ethanol was used in large amount for the preparation of nanocapsules and could therefore be responsible for the initiation of the polymerization.

From these data it appeared that the high rate of insulin association with the nanocapsules as measured in the first part of this study could not be explained by an interaction of the peptide with the polymer. Zeta potential measurements were performed to determine whether insulin was truly encapsulated within the core of the nanocapsules or simply adsorbed onto the nanocapsule surface. These determinations performed on insulin-loaded nanocapsules and unloaded nanocapsules have been performed in NaCl 1 mM using a Zetasizer® IIc (Malvern). The results obtained showed identical zeta potential (-19 mV) for both types of nanocapsules. This suggested that the surface of insulin-loaded nanocapsules was of the same composition as the surface of the unloaded nanocapsules which in turn led to hypothesize that insulin was truly encapsulated within the core of the nanocapsules rather than simply adsorbed at the nanocapsule surface. This was further confirmed by the fact that the zeta potential of unloaded nanocapsules was increased from -19 to -10 mV when 500 μg/ml insulin were added to the dispersion. In this later case, insulin could only adsorbed onto the nanocapsule surface.

All the results obtained in this study strongly suggested that insulin was not involved within the polymerization process of IBCA during nanocapsule preparation and that insulin was truly encapsulated within the oily core of the nanocapsules. The mechanism involved for insulin encapsulation appeared very interesting since it allowed the encapsulation of intact peptide in an oily core preserving its biological activity.

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