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# Polyisobutylcyanoacrylate nanocapsules containing an aqueous core for the delivery of oligonucleotides

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**Abstract**

Antisense oligonucleotides (ODNs) with base sequences complementary to a specific RNA can, after binding to intracellular mRNA, selectively modulate the expression of a gene. However, these molecules are poorly stable in biological fluids and are characterized by a low intracellular penetration. In view of using ODNs as active molecules, the development of nanocapsules containing ODNs in their aqueous core was considered. Nanocapsules were prepared by interfacial polymerization of isobutylcyanoacrylate (IBCA) in a W/O emulsion. After ultracentrifugation and re-suspension in water, the nanocapsules displayed a size of $350 \pm 100$ nm. Oligonucleotide loading did not significantly influence the zeta potential, suggesting that they were located within the core of the nanocapsules. Fluorescence quenching assays confirmed this localization. When encapsulated in the nanocapsules and incubated in the presence of serum, the ODNs were efficiently protected from degradation by nucleases, whereas ODNs adsorbed onto nanospheres were less efficiently protected. This paper describes, for the first time, a nanotechnology able to encapsulate ODNs, rather than adsorbing them at the surface of a solid support. Such a formulation has great potential for oligonucleotide delivery. 

*Keywords:* Antisense; Nanocapsules; Nanoparticles; Nanospheres; Oligonucleotides; Polyisobutylcyanoacrylate

Colloidal carriers, such as polymeric nanospheres, have been proposed to improve the administration of phosphodiester oligonucleotides (ODNs) (Fattal et al., 1998). The ideal case for optimal activity would be to entrap the ODNs within the internal core of polymeric nanocapsules in order to mask them and to prevent them from any interaction with proteins. This is the reason why we have developed a new process of preparation of aqueous nanocapsules containing ODNs, which were successfully suspended in a water medium. The capsule structure has been

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confirmed by quenching of fluorescence studies (Lambert et al., 2000).

To prepare the nanocapsules, 200 μl of ethanol were added to 800 μl of demineralized water containing the ODNs and adjusted to pH 7.4. This solution was added under homogenization (1 min, 24 000 rpm) to an organic phase containing 8 g of Miglyol 812 and 1.5 g Span 80. A100 μg of the monomer, isobutylcyanoacrylate (IBCA) were, then, added to the emulsion kept under stirring at 500 rpm. After 4 h, complete polymerization was achieved and the water droplets became surrounded by the polymer, thus, leading to the formation of nanocapsules with an aqueous core containing the ODNs. Resuspension of nanocapsules in an aqueous medium was achieved after ultracentrifugation in a centrifuge tube, which was filled with both demineralized water and the suspension. The measured size of the particles was $350 \pm 100$ nm. Unloaded nanocapsules displayed a zeta potential of $-38$ mV. When ODNs were encapsulated, zeta potential remained unchanged, suggesting that the ODNs are located into the nanocapsules, rather than at their surface.

The total amount of encapsulated ODN increased while increasing ODN concentration until 5 μM, for which a plateau was reached corresponding to a value of 2.5 nmol ODN per mg of polymer.

Freeze fracture electron microscopy experiments confirmed the large size polydispersity. Most of the observed particles revealed a fracture plane, which propagated at their periphery, revealing concave and convex fractures (Fig. 1). The obtention of these liposome type images may be explained by the fact that the surfactant, the Span 80 could form a double layer surrounding the nanocapsules during the centrifugation process (Fig. 2).

To localize ODNs in nanocapsules aqueous core, collisional quenching of fluorescence studies were carried out using fluorescein labeled ODN and potassium iodide (KI) as an aqueous quencher. Quenching of the fluorescent ODN in the nanocapsule suspension (ODNinNC) was compared with free fluorescein labeled ODN in solution (Free ODN). Data were analyzed according to the Stern–Volmer equation for collisional quenching.

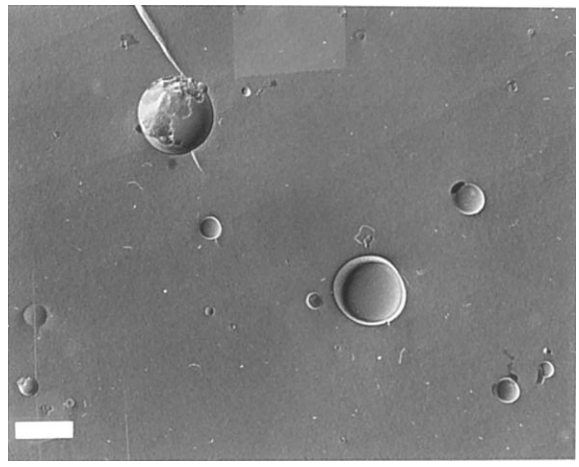

Fig. 1. Freeze fracture electron microscopy. Bar = 500 nm.

$$\frac{F_0}{F} - 1 = K_{sv} \cdot Q$$

$F_0$ and $F$ are the fluorescence intensities in the absence and in the presence of quencher, respectively; $Q$, the molecular concentration of the quencher and $K_{sv}$, the Stern–Volmer quenching constant. $K_{sv}$ is a reliable indication of the bimolecular rate constant for collisional quenching of fluorescein through the aqueous phase. The Stern–Volmer constant was low, when the ODN

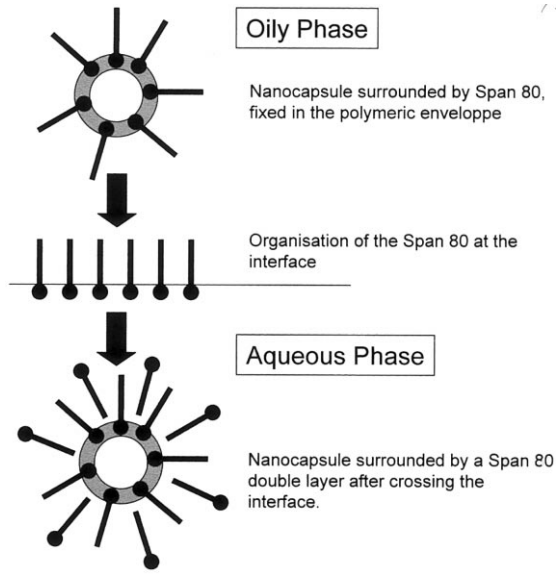


Fig. 2. Formation of a Span 80 double layer.

Table 1
Amount of non-degraded 20 mer ODN in serum from 30 s to 1 h

| | 30 s | 1 min | 5 min | 10 min | 30 min | 60 min |
|---|---|---|---|---|---|---|
| Free ODN | 53% ± 2 | 40% ± 25 | 9% ± 2 | 5% ± 3 | 2% ± 1 | 1% ± 0 |
| ODN nanoparticles | 71% ± 1 | 42% ± 2 | 22% ± 1 | 18% ± 18 | 13% ± 5 | 7% ± 4 |
| ODN nanocapsules | 83% ± 3 | 76% ± 18 | 63% ± 5 | 54% ± 20 | 36% ± 5 | 31% ± 9 |

were inside the nanocapsules 'ODNinNC' ($K_{sv}$ = 0.64 $M^{-1}$), whereas the value of $K_{sv}$ was of 4.03 $M^{-1}$ for 'Free ODN'. These data show that the fluorescein labeled ODNs were almost inaccessible to the quencher, when they were located in the aqueous core of nanocapsules, whereas they were highly accessible to the quencher when free in solution.

To evaluate the stability of ODNs in the presence of serum, nanocapsules were prepared as described using $^{33}P$ radio labeled ODN. Free ODNs and ODNs adsorbed to nanoparticles by ionic interactions (Chavany et al., 1992) were used as controls. The preparations were mixed with fetal calf serum at 37°C for various time intervals. After incubation, the mixture was placed at 70°C for 15 min to achieve inactivation of the serum enzymes. ODN release from nanocapsules was obtained by incubation with NaOH. ODN integrity was assayed by electrophoresis on a 20% polyacrylamide-7M urea sequencing gel (PAGE) followed by an analysis using a multi-channel radioactivity counter (Berthold, Germany) (Aynie et al., 1996). The free 20 mer ODN migrated at 3.7 cm in our experimental conditions and γ33ATP at approximately 12.5 cm (data not shown). The amount of intact ODN for each sample was evaluated by integration of the region corresponding to the first half of the 20 mer oligothymidylate peak (0–3.7 cm) to avoid interference from peaks corresponding to partially degraded ODNs. Free ODNs as well as ODNs adsorbed to nanoparticles were rapidly degraded in the presence of serum (Table 1). The ODN nanocapsules showed a radically different degradation profile. The main peak obtained for each experimental time point remained at a distance of 3.7 cm, which means that intact ODN was still detected during the course of the incubation period. After 1 h of contact, 31% of the 20 mer ODN remained undegraded (Table 1).

This study shows, for the first time, a successful methodology for ODN encapsulation in aqueous nanocapsules. The advantage of nanocapsules compared with other nanoparticulate strategies is that, as shown in the paper, the ODN are encapsulated and not simply adsorbed at the surface of the particles. As a consequence, a better protection from degradation by nucleases is expected as well as a reduced burst release after administration in vivo. Since anti-ras ODN-coated nanospheres have been shown to be efficient against tumor growth after intratumoral administration (Schwab et al., 1994), we could expect to obtain still greater efficiency with ODN encapsulated within these newly designed nanocapsules. Additionally, since after intravenous administration, ODN-coated nanospheres were not effective, probably, because of rapid desorption/degradation in the blood stream (Nakada et al., 1996), encapsulation technology would appear very interesting. This new technology may also offer interesting perspectives for DNA and peptide delivery and transport.

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