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European Journal of Pharmaceutics and Biopharmaceutics 53 (2002) 335–342

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Factors influencing the entrapment of hydrophilic compounds in nanocapsules prepared by interfacial polymerisation of water-in-oil microemulsions

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Received 24 July 2001; accepted in revised form 27 November 2001

Abstract

This study demonstrates the effect of drug properties and method of loading (sorption and encapsulation) on entrapment within poly(alkyl cyanoacrylate) nanocapsules prepared by interfacial polymerisation of biocompatible water-in-oil microemulsions. For small molecular weight compounds (<1000 Da), entrapment efficiency is more dependent on charge of the compound than on the method used for entrapment. Entrapment efficiency within the negatively charged nanocapsules (zeta potential approximately −30 mV) was in the order cationic compound > neutral compound > anionic compound. Only minimal differences for entrapment efficiency were noted between sorption (addition of the compound 4 h after initiation of the polymerisation) and encapsulation (addition of the compound to microemulsion prior to polymerisation). For high molecular weight compounds, the method used for entrapment however, is very important. For hydrophilic macromolecules such as proteins, high entrapment efficiencies can only be achieved by encapsulation. Entrapment of such compounds seems to be independent of the net charge of the compound being encapsulated but depended on the molecular weight. For nanocapsules prepared by interfacial polymerisation of water-in-oil microemulsions, these findings are useful as a foundation in the development of nanocapsules with desired properties.

Keywords: Entrapment efficiency; Encapsulation; Sorption; Interfacial polymerisation; Microemulsion; Nanocapsules

1. Introduction

Poly (alkyl cyanoacrylate) (PACA) nanoparticles have gained extensive interest as drug carriers because of the biocompatibility and biodegradability of the polymer and the simplicity of the polymerisation process [1]. They can be prepared by based–catalysed interfacial polymerisation using micelles, submicron emulsions or microemulsions as polymerisation templates.

Micellar polymerisation is carried out by adding alkylcyanoacrylate monomer to an acidic aqueous solution containing surfactant. This method leads to the formation of nanospheres [2]. Entrapment efficiency of hydrophilic bioactives such as proteins and peptides by this method, however, can be low because of the high water solubility of these bioactives [3].

PACA nanocapsules having an aqueous core prepared by interfacial polymerisation of water-in-oil sub-micron emulsions have higher entrapment efficiency of hydrophilic bioactives [4–6]. However, in this technique, high energy input is required to reduce the particle size of the liquid-in-liquid dispersion that act as a template for the interfacial polymerisation [4–7]. Furthermore, the particle size distribution of the nanocapsules obtained by this method is rather wide [4–6,8]. These problems can be overcome by using a water-in-oil microemulsion as a template, a technique first proposed by Gasco and Trotta [9].

As microemulsions are spontaneously forming and thermodynamically stable [10–12], water-in-oil microemulsions require only minimal input of energy to obtain small and uniform aqueous dispersed droplets. Thus, high energy input to create and maintain a sufficiently dispersed aqueous compartment is avoided. In the system proposed by Gasco and Trotta [9,13], an organic solution containing isopropyl myristate, aerosol AOT and butanol was used for microemulsion formulation. Separation of the nanocapsules from the medium following polymerisation was therefore necessary for in vivo use, which can lead to problems of aggregation of the dispersed nanocapsules [14].

Recently, Watnasirichaikul et al. [15] proposed a simple, single-step method for the preparation of biodegradable

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0939-6411/02/$ - see front matter
PII: S0939-6411(01)00245-4

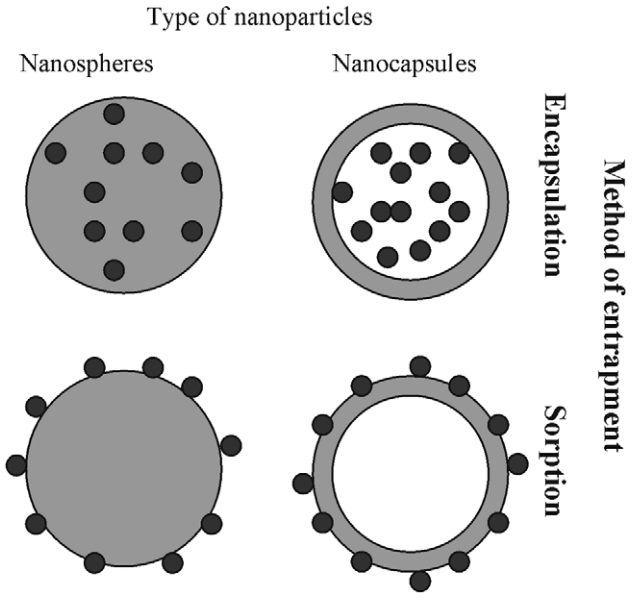

Fig. 1. Types of nanoparticles and entrapment methods of bioactives.

PACA nanocapsules by interfacial polymerisation of biocompatible microemulsions. The use of biocompatible oils and surfactants in the formulation process may overcome the need for isolation of nanocapsules from the preparation medium following polymerisation.

Association (*entrapment*) of a hydrophilic drug with the nanoparticles can, in principle, occur in two ways, as shown in Fig. 1 [1,3,16]. On the one hand, the hydrophilic drug can be added prior to polymerisation, resulting in the *encapsulation* of the drug within the nanoparticles. A high entrapment of peptides and proteins by this method has been achieved for nanocapsules prepared from water-in-oil sub-micron dispersions [5,6]. On the other hand, the drug can be added after the polymerisation, resulting in *sorption* of the drug onto the surface of the nanoparticles. The extent of sorption depends on the affinity between the polymer and the drug and the nature of the dispersion medium. The mechanism of sorption of hydrophilic compounds on nanospheres is believed to be due mainly to an ionic interaction [17–24].

Both encapsulation and sorption methods have advantages and disadvantages. The encapsulation technique renders better protection of the bioactive from the external milieu (e.g. pH, luminal and brush border enzymes, P-glycoprotein and Cytochrome P-450) which often are the cause of low bioavailability of orally administered bioactives [25], reduces burst release in vivo [5] and may prolong association of the bioactive with the nanocapsules thus allowing for a controlled release of the bioactives. However, chemical interactions between drug and polymer during polymerisation may occur [1,3,16,20,26,27]. The addition of drug following polymerisation can overcome the possibility of such interactions at the expense of reducing entrapment efficiency and protection of the drug.

Besides the method used for entrapment, it can be anticipated that the physico-chemical characteristics of the compounds to be entrapped will have a significant effect on the entrapment efficiency. The aim of this study, therefore, was to determine the effect of charge and molecular weight of hydrophilic model compounds on the entrapment efficiency in nanocapsules prepared by interfacial polymerisation of a water-in-oil microemulsion when the compounds were added before and after interfacial polymerisation (encapsulation versus sorption). The in vitro release profiles of the model compounds from the nanocapsules were also investigated to further understand the association between the compounds and the nanocapsules.

2. Materials and methods

2.1. Materials

Caprylic/capric triglycerides (Crodamol GTCC™), polysorbate 80 (Crillet 4™) and sorbitan mono-oleate (Crill 4™) were supplied by Croda Surfactants NZ (Auckland, NZ). Caprylic/capric mono-/diglycerides (Capmul MCM™) was a gift from Abitec Corp. (Columbus, OH, USA). Methylene blue and erythrosine were obtained from Koch-Light Laboratories (Buckinghamshire, UK). ^{14}C-sucrose (50 μCi/250 μl) and biodegradable counting scintillant were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Ethyl 2-cyanoacrylate, sucrose, fluorescein isothiocyanate-dextran (FITC-dextran) 10 and 70 kDa, ovalbumin (OVA; grade V) and fluorescein 5-isothiocyanate (isomer I) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Preparation of FITC-OVA

FITC (20 mg) was dissolved in 10 ml of carbonate buffer (pH 9.5, 0.005 mol/l) and 100 mg of OVA was added to the solution. The solution was gently stirred in the dark at 4°C for 18 h. Unbound FITC was removed by ultrafiltration with repeated washing using a regenerated cellulose membrane (molecular weight cut-off 10,000, Millipore, MA). The resulting FITC-OVA solution was then freeze-dried and stored at 4°C. The isoelectric point (IEP) of FITC-OVA was determined by measurement of the zeta potential of the protein as a function of the pH of the solution using a Zeta-sizer 3000 (Malvern Instruments Ltd, UK) and determined to be 3.75.

The zeta potential (ξ) can be calculated from the electrophoretic mobility using the Henry equation;

$$\mu_E = \varepsilon\xi f(\kappa a)/6\pi\eta$$

where μ_E is the electrophoretic mobility, ε is dielectric constant of the medium, η is viscosity of the medium, κ is Debye–Hückel parameter and $f(\kappa\, a)$ is a correction factor which takes into account the thickness of the double layer and particle diameter (a). The unit of κ is a reciprocal

length. $1/\kappa$ is frequently described as the thickness of the electrical double layer.

In practice an approximation is made for $f(\kappa a)$, this is $f(\kappa a) = 1.0$ for non-polar media (Hückel approximation), $f(\kappa a) = 1.5$ for particle dispersions in polar media (Smoluchowski approximation).

2.2.2. Preparation of nanocapsules

To investigate the effect of charge on entrapment efficiency and release from the nanocapsules, three low molecular weight compounds, methyleneblue (MW:374), sucrose (MW:342) and erythrosine (MW:880) werechosenascationic, neutralandanionic model compounds, respectively. FITC-dextrans with a molecular weight of 10 and 70 kDa were used to examine the effect of molecular weight on entrapment. The effect of charge ofahighmolecularweightbioactiveonentrapmentwasassessed using FITC-OVA and microemulsions in which the pH of the aqueous fraction was adjusted to pH 2.5, 3.75 and 7.4 (below, at andaboveIEP)usingHCl, acetate-orphosphate-buffer, respectively.

Poly (ethyl 2-cyanoacrylate) nanocapsules were prepared according to the method of Watnasirichaikul et al. [15]. Briefly, a microemulsion containing 7.6 g of oil mixture (Crodamol GTCC: Capmul MCM 3:1 w/w), 1.4 g of surfactant mixture (Crillet4: Crill4 3:2 w/w) and 1.0 g of 0.001 M phosphate buffer (pH 7.4) was prepared. Ethyl 2-cyanoacrylate monomer (600 mg) dissolved in 1800 mg chloroform was slowly added into the microemulsion. Polymerisation under stirring at 4°C proceeded overnight.

For encapsulation studies, the model compound was dissolved in the aqueous fraction prior to addition to the oil/surfactant blend. For sorption studies, 200 μl of the aqueous fraction containing the same amount of model compound as in the encapsulation studies (Table 1) was added after 4 h to the nanocapsule dispersion prepared as above and was stirred overnight.

2.2.3. Characterisation of empty nanocapsules

The particle size and zeta potential of empty nanocapsules were measured by photon correlation spectroscopy (Zetasizer 3000, Malvern Instruments Ltd, UK) following isolation of the nanocapsules from the microemulsion by repeated washing in ethanol and centrifugation at $18{,}500 \times g$ for 10 min at 25°C. Nanocapsules were dispersed in 1 mM NaCl for zeta potential measurement. The zeta potential of empty nanocapsules was also measured in McILvaine buffer solutions at various pHs. The structure of nanocapsules was visualised by freeze fracture transmission electron microscopy (TEM; Philips 410LS, The Netherlands). Water content of microemulsions and nanocapsule dispersions was determined by Karl Fischer titration (736 GP Titrino, Metrohm Ltd, Switzerland).

2.2.4. Determination of entrapment efficiency

Percentage entrapment within nanocapsules was calculated according to the difference between the total amount of the model compound added to the formulation and the unentrapped amount detected in the supernatant of the resulting nanocapsule dispersion.

For methylene blue and erythrosine, free drug was measured in the supernatant following separation of nanocapsules from the polymerisation medium by centrifugation of the nanocapsule dispersion at $51{,}500 \times g$ for 1 h at 25°C (Beckman J2/MC Centrifuge, JA 20.1 rotor). The supernatant (1.6 g) was diluted to 10 ml with water adjusted to pH 2.5 by addition of hydrochloric acid. Three hundred microlitres of the dispersion was mixed with 300 μl 80% (v/v) methanol and then centrifuged (Biofuge15, Heraeus Sepatech GmbH, Germany) at $12{,}000 \times g$ for 12 min at room temperature. The amount of non-associated methylene blue and erythrosine was detected from the aqueous supernatant, which was diluted with pure methanol, by absorbance measurements at 663 and 530 nm, respectively (8452 A, Hewlett Packard, UK).

To determine the entrapment efficiency of ^{14}C-sucrose, 0.4 g of the supernatant of the nanocapsule dispersion was mixed with 4 ml of biodegradable counting scintillant following centrifugation at $51{,}500 \times g$ for 1 h at 25°C and associated activity measured by liquid scintillation counting (LS 3801, Beckman, USA).

Entrapment of two FITC-dextrans was determined by mixing 200 mg of the nanocapsule dispersion with 1 ml of 50% (v/v) aqueous methanol. The nanocapsules and the oil phase were separated from the resulting aqueous phase by centrifugation at $12{,}000 \times g$ for 12 min at room temperature. The aqueous supernatant was diluted as required with pure

Table 1
Overview of the model compounds used in this study

Model compound	Molecular weight	Final concentration of model compound (mg/ml)	pH of water compartment of microemulsions	Net charge of model drug
Methylene blue	374	1	7.4	Positive
Erythrosine	880	5	7.4	Negative
Sucrose	342	1	7.4	Neutral
FITC-dextran 10	9500	1	7.4	Neutral
FITC-dextran 70	77,000	1	7.4	Neutral
FITC-OVA	45,000	0.1	7.4	Negative
		0.05	3.75	Neutral
		0.1	2.5	Positive

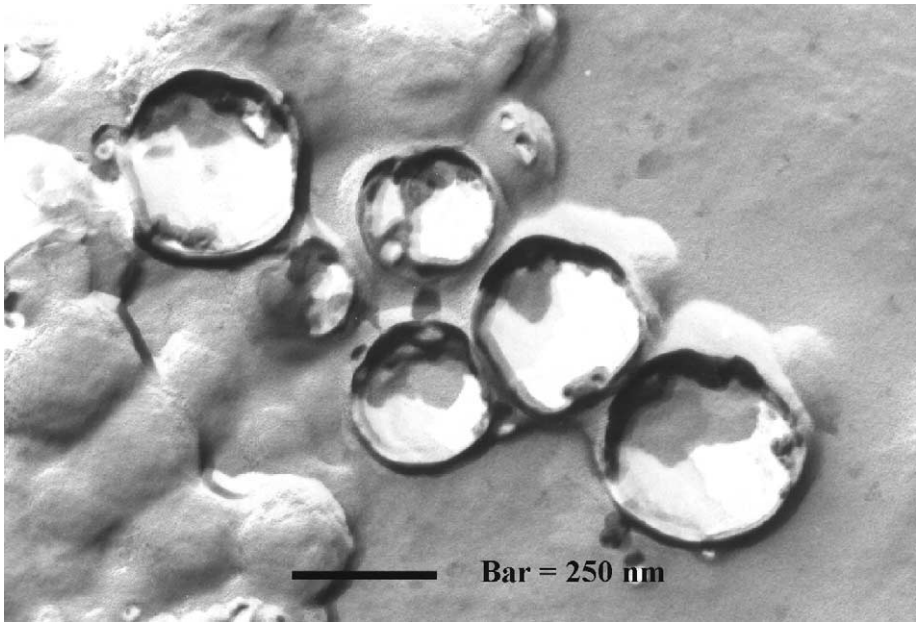

Fig. 2. Freeze fracture transmission electron micrograph of poly (ethyl 2-cyanoacrylate) nanocapsules.

methanol and dextran was then quantified by spectrofluorometry (RF540, Shimadzu, Japan) using an excitation and emission wavelength of 495 and 515 nm, respectively.

To determine the entrapment efficiency of FITC-OVA, the clear supernatant of the nanocapsule dispersion was subjected to fluorescence intensity measurements (excitation and emission wavelength of 498 and 519 nm, respectively) directly after centrifugation at 51,500 × *g* for 1 h at 25°C.

Microemulsions containing the different model compounds were used as controls for all experiments.

2.2.5. Release studies

Four grams of the nanocapsule dispersion or microemulsion were diluted to 25 ml with release medium and then transferred to a double-walled thermostated beaker (37°C). The sample was continuously stirred using a magnetic flea at a rate of 200 rpm. At selected times, the release medium was withdrawn and mixed with 80% (v/v) methanol. The mixture was subjected to centrifugation at 12,000 × *g* for 12 min at room temperature. The clear aqueous supernatant was diluted with pure methanol as required. The amount of methylene blue was quantified by UV spectrophotometry at 663 nm and that of FITC-dextran was determined by spectrofluorometry (excitation 500 nm; emission 520 nm). Microemulsions were used as controls for all experiments.

3. Results and discussion

3.1. Characterisation of empty nanocapsules

A transmission electron micrograph of nanoparticles obtained by the interfacial polymerisation of water-in-oil biocompatible microemulsions is shown in Fig. 2. From the micrograph the size of the nanoparticles can be estimated to be between 200 and 300 nm. The size of the nanoparticles was also measured by photon correlation spectroscopy and was found to be about 250 nm. The polydispersity index was less than 0.1 in all cases, indicating a narrow particle size distribution.

TEM and PCS investigations indicate that the size of the nanoparticles is significantly larger than the size of microemulsion droplets. The particle size of microemulsion was estimated by photon correlation spectroscopy to be around 15–20 nm which is in agreement with that reported in the literature [28]. The discrepancy in size between that measured for the microemulsion and that measured for the nanocapsules proposes some kind of structural collapse of the microemulsion occurred during polymerisation. This would suggest that the rate of polymerisation is slower than the droplet dynamics in the microemulsion [29,30], where a dynamic equilibrium occurring in the microsecond time scale maintains an equilibrium droplet size [29].

The zeta potential of empty nanocapsules was approximately −30 mV when dispersed in 1 mM NaCl solution and was dependent on pH (Fig. 3). The water content of the dispersion medium was determined after polymerisation and separation of the nanocapsules. It was found to be almost 100% of the water content of the microemulsion template suggesting that water can readily diffuse through the forming polymeric wall.

3.2. Entrapment of model compounds in nanocapsules

Entrapment efficiency of low molecular weight compounds following encapsulation or sorption methods was in the order: cationic compound > neutral compound > negative compound (Fig. 4).

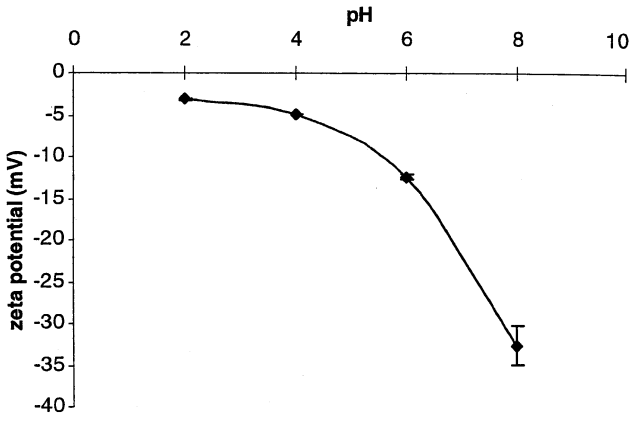

Fig. 3. Zeta potential-pH profile of empty poly (ethyl 2-cyanoacrylate) nanocapsules (values represent mean ± SD, $n = 3$).

These results suggest that an ionic interaction between the model compound and the polymeric nanoparticles, that have a slightly negative charge, play an important role in the entrapment of low molecular weight compounds. This finding is in agreement with the results of Losa et al. [18] who reported that amikacin sulphate was adsorbed onto poly (butyl 2-cyanoacrylate) nanoparticles by a mechanism of electrostatic interaction between drug and polymeric nanospheres. Douglas et al. [19] found that rose bengal, an anionic compound, adsorbed more onto poly (butyl 2-cyanoacrylate) nanoparticles produced using diethylaminoethyl dextran as a stabiliser (particles bear a net positive charge) than onto those produced using dextran 70 as a stabiliser (particles bear a net negative charge). Fattal et al. [24] found that the association of oligonucleotides with polyalkylcyanoacrylate nanoparticles increased by adding the oligonucleotides to the polymeric dispersion in the presence of cetyltrimethylammonium bromide as this reduced the negative charge of the nanoparticles.

Only a small difference was found in the entrapment efficiency of low molecular weight compounds between the two methods investigated (Fig. 4). The release profiles of methylene blue from nanocapsule dispersions loaded by both methods in pH 7.4 were found to be similar (Fig. 5). An immediate release of 55–65% was measured. This immediate release was much higher than the amount of methylene blue not entrapped in the nanocapsules, i.e. the methylene blue remaining in the dispersion medium (approximately 15–25%). Surprisingly, the immediate release was followed by a slight decrease in the amount of compound detected in the release medium. In comparison, the 100% release of methylene blue from microemulsions occurred immediately upon dilution in the release medium (data not shown). The similarity of the profiles obtained for both encapsulation and sorption suggests that the distribution of the drug in the nanocapsules is similar following entrapment by these two methods. The high burst release further supports the hypothesis that the association of the model compound in both methods is by sorption at the surface of the nanocapsules or close to the surface of the polymeric nanocapsule wall. As stated above, a high percentage of the water in the aqueous microemulsion was recovered in the dispersion medium after polymerisation. It would therefore appear that low molecular weight compounds, together with water, may diffuse through the forming polymeric wall at the initial stage of the polymerisation and are solubilised in the water droplets of the microemulsion outside the nanocapsules. The compounds may then bind to the surface of the forming nanocapsules to different degrees, depending on the charge of the model compound. Thus for low molecular weight bioactives it would appear that association to nanocapsules will to

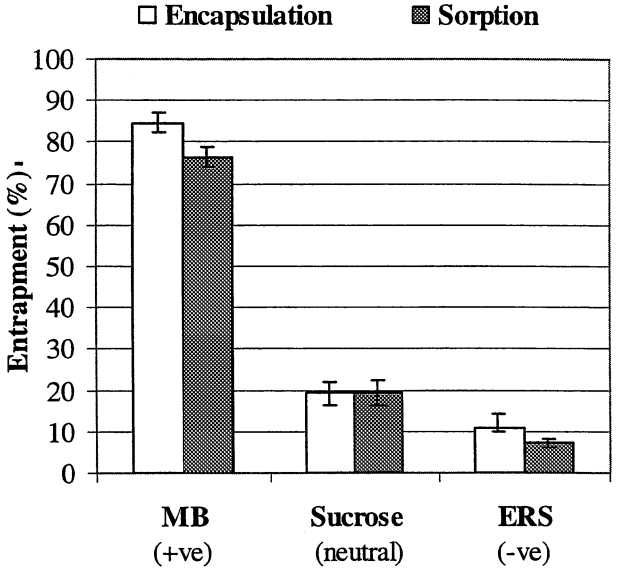

Fig. 4. Effect of charge and preparation method on entrapment of the small molecular weight compounds: methylene blue (MB), sucrose and erythrosine (ERS) (values represent mean ± SD, $n = 4$).

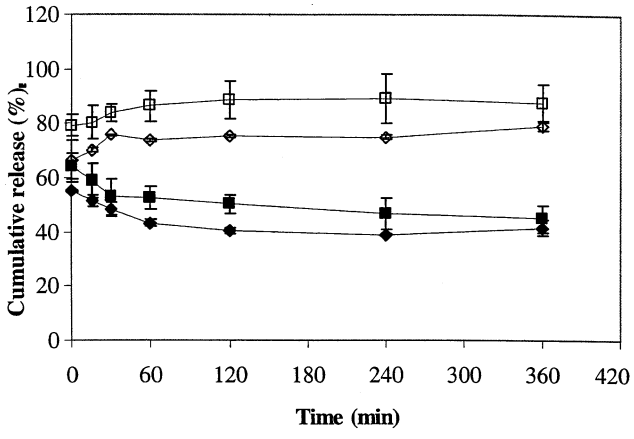

Fig. 5. Release profiles of methylene blue from nanocapsule dispersions obtained by encapsulation (◆, ◇) and sorption (■, □) methods into phosphate buffer pH 7.4 (closed symbols) and HCl solution pH 2.5 (open symbols), respectively (values represent mean ± SD, $n = 4$).

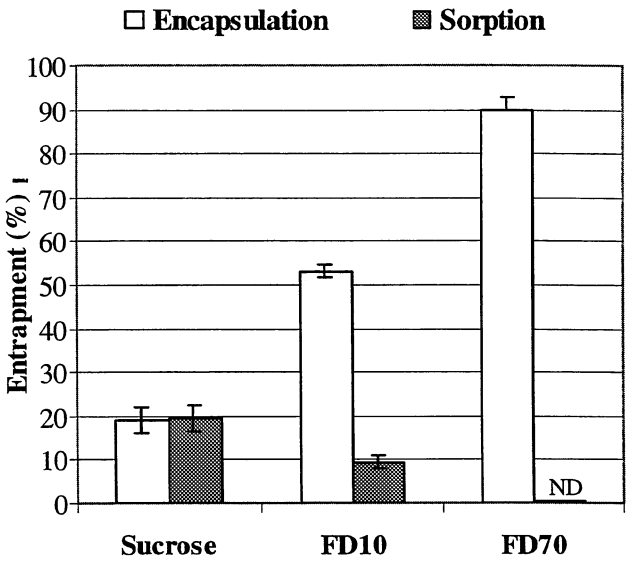

Fig. 6. Effect of molecular weight and preparation method on entrapment of the model compounds: sucrose, FITC-dextran 10 (FD10) and FITC-dextran 70 (FD70) (values represent mean ± SD, $n = 4$). ND, not detectable.

some degree be similar following loading by either encapsulation and sorption methods.

The slight decrease in the amount of methylene blue released at pH 7.4 in the first 60 min may be explained by an increase in ionic interaction between the drug and the nanoparticles due to the change of the dispersion medium of nanocapsules from a microemulsion to pH 7.4 aqueous release medium. As a consequence, the surrounding free drug in the release medium is bound back to some extent to the surface of the particles (approximate zeta potential, −25 mV). This assumption is supported by the release studies of methylene blue in pH 2.5 in which the ionisation of the polymer is suppressed (approximate zeta potential, −5 mV). Release of methylene blue from the nanocapsule dispersions prepared by both methods in release medium pH 2.5 was again found to be similar (albeit faster than in release medium pH 7.4), again confirming the similar distribution of drug within the nanocapsules following loading by the two different methods.

In both release media (pH 7.4 and 2.5), the release of methylene blue from nanocapsule dispersions loaded by sorption was slightly higher than from those loaded by encapsulation. This difference is in agreement with the slight difference in entrapment efficiency noted between the two methods (Fig. 4). This may be due to intervention of the model compound in polymerisation when it is added prior to polymerisation process [31]. These results are in line with the findings of Fawaz et al. [23] who reported a slightly more rapid release of ciprofloxacin from poly (isobutylcyanoacrylate) nanoparticles prepared by adsorption compared to those prepared by encapsulation.

The dramatic effect of molecular weight on the entrapment of model compounds in nanocapsules is clearly illustrated in Fig. 6. The encapsulation efficiency of the model compounds increased with increasing molecular weight and for a high molecular weight compound was not influenced by charge (Fig. 7). The encapsulation efficiency of nanocapsules containing FITC-OVA was about 97% in all cases. On the other hand, the sorption efficiency of the model compound decreased with increasing molecular weight and no sorption was detectable for FITC-dextran 70 or FITC-OVA. Again, charge of the high molecular weight model compound did not affect the sorption efficiency.

The difference between the encapsulation of low and high molecular weight compounds may be explained by the difference in the ability of these compounds to diffuse through the forming polymeric wall at the initial stage of the interfacial polymerisation. The higher the molecular weight of the model compounds, the more difficult it will be for these compounds to escape through the forming polymeric wall, i.e. the model compounds are retained in the nanocapsules.

The decrease in sorption efficiency with increasing molecular weight of the model compound may be explained by the possibility that low molecular weight compounds may not only adsorb on the surface of the nanocapsules but may also diffuse into the network of the polymeric wall to interact with binding sites inside the polymer wall. In the case of a high molecular weight compound, accessibility of binding sites other than at the surface of the nanocapsules may be less and hence association reduces with increasing molecular weight. It must also be borne in mind that the nanocapsules are dispersed in a water-in-oil microemulsion. A hydrophilic compartment is thus available for the hydrophilic compounds in which they can be solubilised.

The hypothesis that high molecular weight compounds

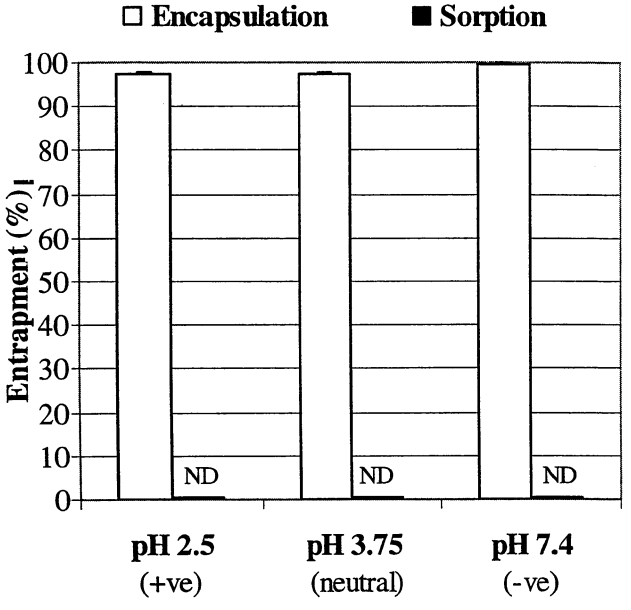

Fig. 7. Effect of charge and preparation method on entrapment of the high molecular weight compound, FITC-OVA (values represent mean ± SD, $n = 4$). pH represents pH of the water fraction of the microemulsion in which the protein was solubilised. ND, non-detectable.

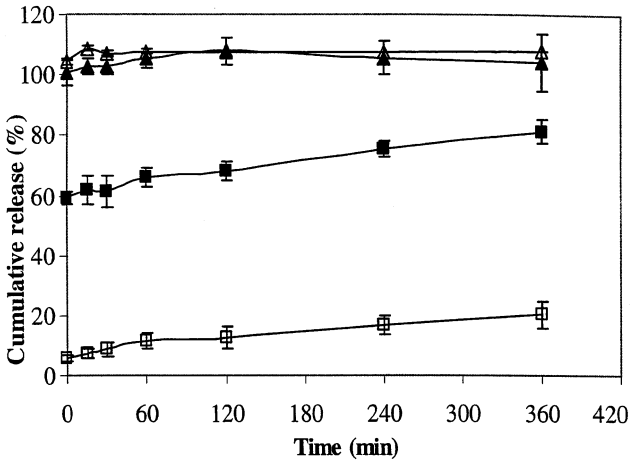

Fig. 8. Release profiles of FITC-dextran 10 kDa (closed symbols) and 70 kDa (open symbols) from nanocapsule dispersions obtained by encapsulation (■ and □) and sorption (▲ and △) methods into phosphate buffer pH 6.5 (values represent mean ± SD, $n = 4$).

are in fact encapsulated in the nanocapsules following loading by the encapsulation method is confirmed by the differences found between the release profiles of the two FITC-dextrans from nanocapsule dispersions loaded by both methods (Fig. 8). As could be expected, for FITC-dextran 70 prepared by the sorption method, an immediate and complete release of the model compound was found because the entrapment was negligible and hence the model compound was solubilised in the microemulsion (Fig. 6). For FITC-dextran 10 (sorption method), the same release profile as for FITC-dextran 70 was obtained. The small amount of model compound adsorbed to the nanocapsules (approximately 10%, Fig. 6) desorbed quickly suggesting that FITC-dextran 10 is bound superficially on the nanocapsules rather than being internalised.

For FITC-dextran 10 (encapsulation method), an immediate release from nanocapsules dispersed in the polymerisation medium, i.e. microemulsion, of about 60% of the model compound was detected (Fig. 8). The small difference between this value and the amount of model compound which was not encapsulated in the nanocapsules, but solubilised in the dispersion medium (approximately 50%), is likely to be due to a fast desorption of the fraction that was adsorbed onto the surface of the nanocapsules. This difference of approximately 10% corresponds to the amount of drug entrapped following loading by sorption, which was also released immediately and hence supports this hypothesis. After the immediate release, however, a slow release phase was noted which might be due to the release of the model compound being encapsulated. For FITC-dextran 70, a similar release profile was obtained, but due to the fact that a high amount of the model compound was encapsulated, only a very limited immediate release (~5%) was observed. This is also in agreement with the release following sorption which showed no detectable association of the FITC-dextran 70 to the nanocapsule surface.

4. Conclusions

This study has demonstrated that the interfacial polymerisation of these water-in-oil microemulsions using ethyl 2-cyanoacrylate leads to a dispersion of nanocapsules in a dispersion medium that has retained its water component, i.e. a microemulsion. Model compound associates with these nanocapsules by encapsulation or sorption but entrapment efficiency resulting from these loading methods is dependent on the physico-chemical properties of the compound. For low molecular weight compounds, entrapment efficiency is more dependent on charge of the compounds than on the method used for entrapment (positive > neutral > negative). For high molecular weight compounds, the method used for entrapment is very important. For hydrophilic macromolecules, high entrapment efficiencies can only be achieved by encapsulation. Entrapment of such compounds seems to be independent of the net charge of the compound being encapsulated but dependent on the molecular weight. For nanocapsules prepared by interfacial polymerisation of water-in-oil microemulsions, these findings are useful as a foundation for the development of nanocapsules with desired properties.

Acknowledgements

The authors are grateful to the University of Otago for providing a PhD scholarship to T.P.

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