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FORMATION OF POLY(BUTYL 2-CYANOACRYLATE) MICROCAPSULES AND THE MICROENCAPSULATION OF AQUEOUS SOLUTIONS OF [^{125}I]-LABELLED PROTEINS

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SUMMARY

Some features of the polymerization reaction of butyl 2-cyanoacrylate at different aqueous/organic solvent interfaces have been investigated. In particular, the effects of pH and the presence of protein on the formation of microcapsules by in situ interfacial polymerization of butyl 2-cyanoacrylate in w/o emulsions have been studied. [^{125}I]-labelled proteins have been used to study the procedure as a method of microencapsulating enzymes or other proteins within potentially biodegradable membranes. Preliminary in vitro degradation studies suggest that degradation of the microcapsules is inhibited by low levels of their breakdown products, thus allowing the storage of the microcapsules as aqueous suspensions for prolonged periods in sealed containers.

INTRODUCTION

The potential pharmaceutical applications of microcapsules are well known and some success has been obtained in their use in oral sustained-release formulations. However, developments for parenteral applications have been slow due to the requirement for a biodegradable and biocompatible polymer to act as a microencapsulating agent.

Several techniques can be used to encapsulate enzymes, cell contents, cells, vaccines, antigens, antiserum, co-factors, hormones and proteins (Chang, 1977). One widely studied process has been the microencapsulation of enzymes by interfacial polymerization. The resulting microcapsules, which can be regarded as "artificial cells" (Chang, 1972) have

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application in the therapy of hereditary enzyme deficiency conditions, organ failure and substrate-dependent tumours. Several different types of polymer have been used to prepare microcapsules by interfacial polycondensation reactions at the interface of w/o emulsions. Most frequently the polyamides have been the membrane-forming polymers and some of the properties of this type of microcapsule have been reviewed by Kondo (1978) but several features are still not understood adequately. In this type of system the internal core is an aqueous solution and several enzymes have been encapsulated and used successfully in animal experiments (e.g. Chang, 1972). Thus poly(piperazine terephthalamide) microcapsules containing histidase have shown promise in reducing blood levels of histidine in the histidinaemic mouse (Wood et al., 1979).

However, the question of the biodegradability of the polyamides and the ultimate fate of the degradation products has not been studied to any great extent. In this laboratory two approaches have been used to prepare potentially "biodegradable" microcapsules; the first was to form more hydrophilic polyamides and the second, discussed in this paper, involved the use of polymers which are known to be biodegradable.

The alkyl 2-cyanoacrylates are used as adhesives and polymerize rapidly, by a base-catalyzed mechanism, when in contact with traces of water or body fluids. The methyl ester is used as an instant "super glue" and the butyl monomer is available as a biodegradable tissue adhesive for surgical applications. The rate of degradation is fastest with the methyl ester, the rate decreasing as the alkyl chain length is increased (Leonard et al., 1966a; Vezin and Florence, 1980).

In a previous communication (Florence et al., 1979) we reported the preparation of potentially biodegradable microcapsules from butyl 2-cyanoacrylate, by interfacial polymerization in w/o emulsions. The present paper discusses some of the important features of the formation of poly(alkyl 2-cyanoacrylate) films at w/o interfaces and discusses the scope of this polymer for microcapsule formation.

MATERIALS AND METHODS

Butyl 2-cyanoacrylate (BCA) was prepared using the method described by Leonard et al. (1966b) and redistilled at least twice in the presence of sulphur dioxide. The level of sulphur dioxide during preparation and storage was not closely controlled and was presumably at saturation level in the monomer (at atmospheric pressure). The monomer was generally used within a few days of redistillation. Organic solvents were Analar grade from BDH. Chloroform was washed to remove the ethanol present as stabilizer and dried over anhydrous sodium sulphate. Sorbitan trioleate (Span 85) and polysorbate 20 (Tween 20) were obtained from Koch-Light. Haemoglobin was supplied by Cambrian Chemicals.

Iodinated [^{125}I]human fibrinogen injection (approximately 1 mg fibrinogen and approximately 22 mg human serum albumin with buffer salts, reconstituted to 2.2 ml with water to give a solution of 50 μCi/ml) and iodinated [^{125}I]human albumin injection (in isotonic saline with 0.9% benzyl alcohol; 50 μCi/ml; 20 mg albumin/ml) were obtained from the Radiochemical Centre, Amersham.

Microcapsule preparation

A general procedure consisted of the following: an aqueous phase (2 ml) was emulsi-

fied in an organic solvent (e.g. 10 ml chloroform–cyclohexane (1 : 4) containing 5% (v/v) sorbitan trioleate) for 1 min. A second volume (10 ml) of organic phase, containing monomeric butyl 2-cyanoacrylate (e.g. 0.25 ml), was added and the interfacial polymerization reaction allowed to proceed for 3 min. A further volume of organic solvent (20 ml) was added to dilute the reactant and prevent intercapsular cross-linking. After one minute, stirring was discontinued. The newly formed microcapsules were allowed to sediment in the organic solvent (about 15 min) and most of the solvent was drawn off. The solutions were stored in ice prior to use, and the reactions were carried out at 4°C. Butyl 2-cyanoacrylate monomer (measured in the dried plastic tips of Finn pipette) was added to dried (anhydrous sodium sulphate) solvent immediately prior to addition to the preformed w/o emulsion.

The reaction vessel was a 50 ml glass beaker (diameter 4.0 cm, height 6.0 cm) with an ice-jacket and was stirred by a magnetic follower (PTFE cylinder diameter 0.7 cm, length 4.0 cm, with a slight rib in the middle) driven by a Gallenkamp magnetic stirrer (speed setting 7).

The newly formed microcapsules, in a minimum volume of organic solvent, were washed by adding 30% (v/v) polysorbate 20 solution (20 ml), water (20 ml) and ethanol (40 ml) with continuous stirring over a period of about 2 min. The aqueous suspension was left to allow the microcapsules to sediment. The microcapsules were repeatedly washed on a centrifuge with buffer solution.

Studies with [^{125}I]-labelled protein

Each [^{125}I]-labelled protein was added as 0.1 ml volumes (5 μCi in 0.1 ml) in the aqueous phase prior to encapsulation.

Adsorption studies were carried out in isotonic buffer (pH 7.4); again 5 μCi amounts of [^{125}I]-labelled protein were used. The microcapsules were rapidly washed, on a centrifuge, 3 times with buffer solution and the activity of the microcapsules counted.

Microscopy. Droplet and microcapsule sizes were assessed from photographs taken through a light microscope provided with a graticule. Scanning electron microscopy was carried out using a Philips P SEM 500 instrument on gold-coated samples.

RESULTS AND DISCUSSION

Interfacial polymerization at unstirred w/o interfaces

Visible macroscopic films of poly(butyl 2-cyanoacrylate) were formed almost instantaneously when BCA (to produce 5% (v/v) organic solution) was carefully added to a layer of cyclohexane over an aqueous medium. The pH and ionic strength of the aqueous layer determine to some degree the rate of formation and the extent of polymerization. When water alone was used as the aqueous layer, up to 10 s were required for the appearance of a visible film and the resulting film, after a prolonged period of time, consisted of a thin layer of transparent/white polymer at the interface.

When water was replaced by a solution of sodium carbonate of increasing molarity, film formation was almost instantaneous and polymerization and precipitation of polymer proceeded from the interface to regions quite remote from it. Analogous behaviour was found when BCA monomer was injected into a layer of chloroform below an aqueous medium, in both cases the polymer precipitation occurred in the organic solvent. When

removed, the polymer had little mechanical strength, being extremely swollen with solvent and its "sponge-like" appearance can be seen in Fig. 1.

The mildly basic aqueous phase is believed to promote interfacial polymerization in two related ways. Firstly, it provides anions in the form of hydroxyl ions, which catalyze the reaction and secondly, it neutralizes the acidic polymerization inhibitor thus further promoting polymerization. The pure alkyl 2-cyanoacrylate monomers are extremely reactive and tend to polymerize "spontaneously" when inhibitors are absent.

When protein (e.g. 1% haemoglobin) was present in the aqueous phase the time for formation and the appearance of the film was similar to systems without protein. Leonard et al. (1966a) found different times for film formation and different monomer spreadability for the alkyl 2-cyanoacrylate series when drops of pure monomer were applied to simple aqueous solutions and solutions of protein and coined the term "blood effect". That study found that the methyl ester formed polymer most rapidly (<10 s) on aqueous media while the heptyl ester required more than 5 min, the reverse order being found on "blood". With the butyl ester, intermediate in the series, the differences found between the behaviour on aqueous media and "blood" were slight. In another study (Leonard et al., 1966b) it was found that nucleophiles other than hydroxyl ions (from water) preferentially initiated polymerization, and that the IR peak for OH was suppressed when compounds such as pyridine and glycine were present in the aqueous phase. Therefore it appears likely that the presence of haemoglobin or other proteins in the aqueous phase of these systems, and in the microencapsulation procedures, may affect the polymerization reaction and also become incorporated in the polymer.

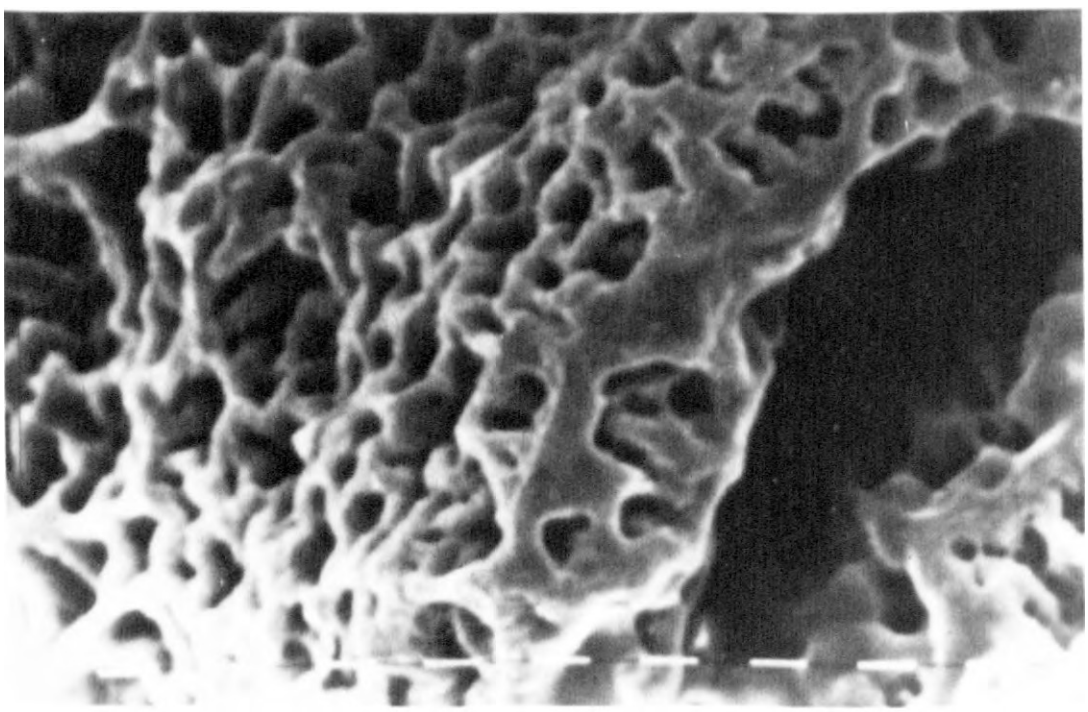

Fig. 1. Appearance of macroscopic film of poly(butyl 2-cyanoacrylate) formed at chloroform/aqueous solution (0.1 M sodium carbonate) interface (scale marker = 10 μm).

Interfacial polymerization in w/o emulsions and the formation of stable microcapsules

The simple experiments at unstirred interfaces described above indicate some of the important features of the polymerization reaction and can be used to predict conditions for the formation of stable microcapsule membranes. The isolation procedure used here required that the microcapsules be subjected to centrifugation and therefore preparation with microcapsules of poor mechanical strength were readily identified and regarded as being unsuitable.

The effects of a weakly basic molecule capable of diffusion into the organic phase and of a protein included in the aqueous phase, on the stability of the emulsion droplets are shown in Table 1. While, in the absence of haemoglobin, polymeric film was readily visible at the interface of the w/o system when examined microscopically, the droplets were seen to be able to coalesce. The introduction of a low concentration of protein (0.5% haemoglobin) resulted in the formation of membranes with sufficient mechanical strength to perform as microcapsule walls. The importance of the protein is believed to be due to its action as a cross-linking agent. It seems probable that basic groups on the protein molecule, which adsorbs at the interface, act as initiators of polymerization and become chemically incorporated in the polymer film.

The importance of the presence of mild base in addition to protein in the aqueous phase can be seen in Table 1 where a sodium carbonate concentration of 0.05 M and above was sufficient to allow rapid polymerization and the formation of microcapsules. The mild conditions in the aqueous phase in this type of microencapsulation procedure offer an advantage when pH-sensitive enzymes are encapsulated. Microencapsulating conditions with polyamides generally require a more alkaline pH of high ionic strength which may denature the enzyme.

The size of poly(butyl 2-cyanoacrylate) microcapsules can be varied over a wide range by changing stirring speed and surfactant concentration. In general, the conditions used here produced microcapsules with mean diameters which could be controlled in the range 25–250 μm.

TABLE 1

EFFECT OF HAEMOGLOBIN, SODIUM CARBONATE AND PIPERAZINE ON THE FORMATION OF POLY(BUTYL 2-CYANOACRYLATE) MICROCAPSULES

Aqueous phase concentration of:			Approximate droplet diameter (μm) [a]	Droplet stability and microcapsule quality [a]
Haemoglobin (% w/v)	Sodium carbonate (M)	Piperazine (M)		
–	0.45	0.4	60	coalescing droplets
0.5	0.225	0.2	30	stable microcapsules
0.5	0.0005	–	100–400	coalescing droplets
0.5	0.005	–	100–300	coalescing droplets
0.5	0.05	–	45	stable microcapsules
0.5	0.225	–	30– 45	stable microcapsules

[a] Microscopic assessment immediately after formation.

Fig. 2 shows the physical appearance of the microcapsules. This type of microcapsule has membranes with sufficient mechanical strength to allow the retention of their spherical shape even when the internal aqueous core was removed for SEM. Polyamide microcapsules prepared using similar techniques always collapsed to some extent when treated in this way. Fig. 3 shows the appearance of the membranes when microcapsules are crushed and indicates that the walls are a few μm thick.

[^{125}I]Albumin was included in the aqueous phase and the effect of the amount of monomer used on the percentage of [^{125}I]protein retained within the microcapsules shown in Table 2. Clearly, there is no advantage in using more than 250 μl of monomer. When [^{125}I]fibrinogen was used in similar experiments little difference was found at various levels of monomer, the average amount incorporated being about 60% in every case. The use of [^{125}I]-labelled protein in this way offers a simple assessment of the quality of microcapsules during the development of microencapsulation procedures. The concentration of [^{125}I]-protein is very small compared to the amount of haemoglobin which was used and serves as a model for the microencapsulation of enzymes.

The two different proteins are known to adsorb differently on polymeric surfaces and are believed to play an important role in the biocompatibility of polymers with blood (Brash and Lyman, 1971; Bagnall, 1978; Horbett et al., 1978).

Table 3 shows the effect of breaking the microcapsules, by ultrasonication, and measuring the amount of activity retained with the broken membrane fragments together with the proportion of these proteins which adsorbed onto microcapsules prepared using the typical procedure. Clearly most of the [^{125}I]-labelled protein retained with the micro-

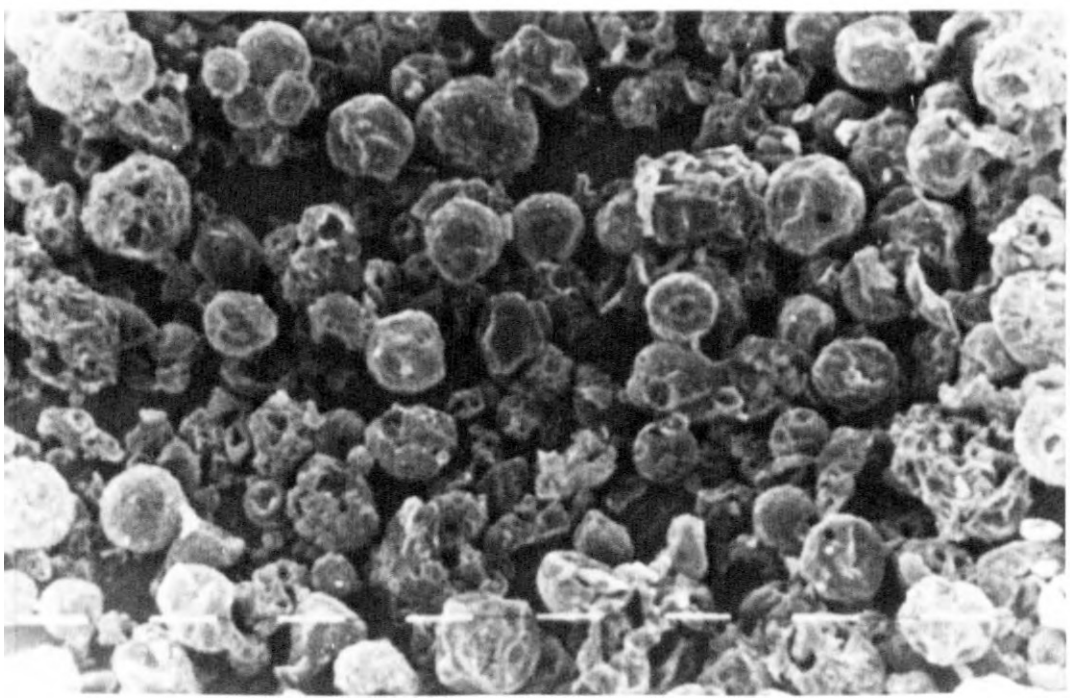

Fig. 2. Typical appearance of poly(butyl 2-cyanoacrylate) microcapsules (scale marker = 100 μm).

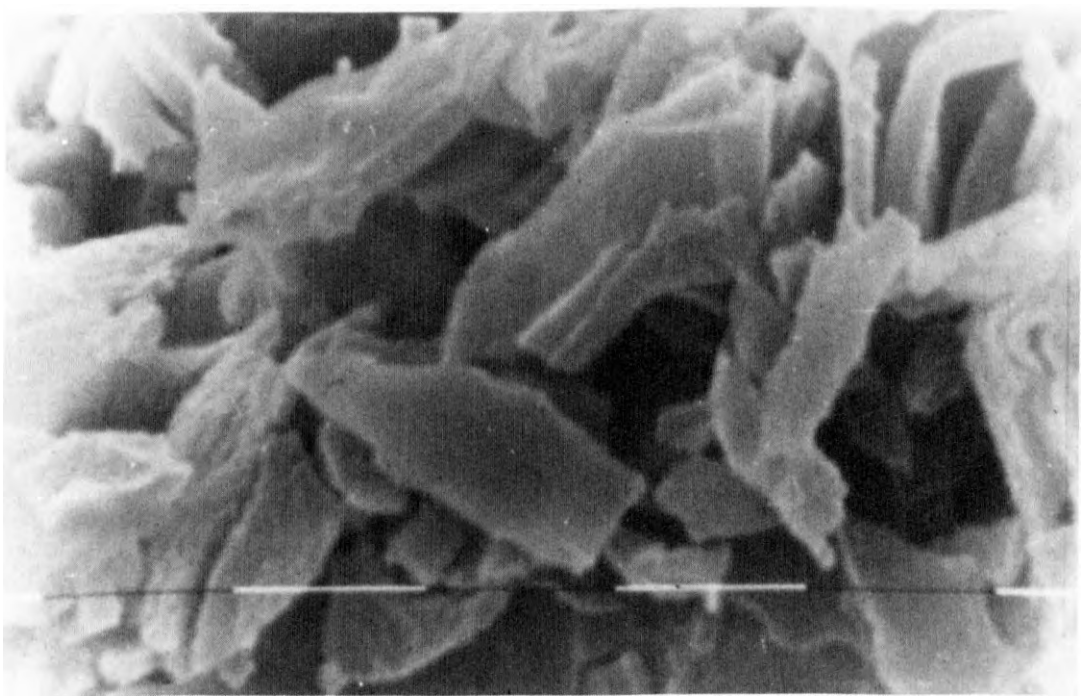

Fig. 3. Crushed poly(butyl 2-cyanoacrylate) microcapsules showing wall thickness (scale marker = 10 μm).

capsules appears to have been physically or chemically attached to the polymer and not "free" in solution in the microcapsule cores.

Similar studies with poly(piperazine terephthalamide) microcapsules (Wood et al. 1979) showed similar behaviour with [^{125}I]fibrinogen and indicated that about one-third of the protein was lost during microcapsule preparation and washing, and that the rest was attached to the polymeric membrane. In the case of [^{125}I]albumin, about 66% was

TABLE 2

EFFECT OF THE AMOUNT OF MONOMER ON THE PERCENTAGE OF [^{125}I]-LABELLED HUMAN SERUM ALBUMIN INCORPORATED IN POLY(BUTYL 2-CYANOACRYLATE) MICROCAPSULES

Volume of BCA monomer used (μl)	Percentage of initial activity in various systems			
	Organic solvent after reaction	Aqueous washings	Microcapsules	Total
100	1.2	64	16	81
200	1.0	56	35	92
250	0.4	53	39	92
300	0.9	64	38	103
400	1.1	55	40	96

TABLE 3

PERCENTAGE OF [^{125}I]-LABELLED PROTEIN ENCAPSULATED INITIALLY, RETAINED ON BROKEN MICROCAPSULE MEMBRANE FRAGMENTS AND ADSORBED ONTO POLY(BUTYL 2-CYANOACRYLATE) MICROCAPSULES

	[^{125}I]-labelled (%)	
	Albumin	Fibrinogen
Encapsulated initially	39	65
Retained with broken fragments	33	61
Adsorbed after 5 min	1	18
Adsorbed after 24 h	1	75

retained with poly(piperazine terephthalamide) microcapsules and about half of that was attached to the membranes while the rest was free to be washed away.

With poly(butyl 2-cyanoacrylate) about 40% was retained with the microcapsules and breaking by ultrasonication had little effect, implying that it was attached to the membrane. Therefore it would appear that the poly(butyl 2-cyanoacrylate) microcapsules were permeable to the relatively small albumin molecule and that any protein which was not attached to the membranes diffused out during the washing procedures.

When different organic solvents (chloroform, cyclohexane, chlorobenzene and anisole) were used to replace the usual chloroform: cyclohexane (1 : 4) mixture the amount of [^{125}I]fibrinogen retained with microcapsules was different (68, 58, 31, 34%) and there were differences in the volume of the packed-bed of microcapsules. At this time it is not understood why the differences should occur but it has previously been shown (Florence et al., 1976) that the structure of macroscopic films of poly(methyl 2-cyanoacrylate) formed at different organic solvent–water interfaces is different. Therefore the correct choice of organic solvent system and/or surfactant should allow a degree of control over the preparation of microcapsules with particular properties.

In vitro degradation of poly(butyl 2-cyanoacrylate) microcapsules

Aqueous degradation studies using mild conditions (pH 6.2, 7.4 and 9.2) have shown that an equilibrium was rapidly reached between the poly(butyl 2-cyanoacrylate) microcapsule membranes and the degradation products.

Leonard et al. (1966b) proposed a base-catalyzed degradation mechanism leading to alkyl cyanoacetate and formaldehyde and Hastings (1976) provided further confirmation. In vivo degradation is believed to occur in a similar manner (Leonard, 1970). In this study the level of formaldehyde (measured by the method of Bricker and Johnson (1949)) which was sufficient to stop the forward reaction was estimated to be 10^{-4} mole per mole of BCA. This equilibrium was reached within one or two days.

Under more severe conditions (1 M NaOH) degradation occurred rapidly and 50% of the microcapsules had degraded within 24 h and after a few days no insoluble material remained. In this case the strong base should remove formaldehyde by the Cannizzaro reaction. In the in vivo situation formaldehyde would diffuse away into body fluids and

allow degradation to proceed under essentially sink conditions.

The major advantage of this system is that poly(butyl 2-cyanoacrylate) microcapsules can be stored for prolonged periods because the degradation reaction is inhibited by a very low level of formaldehyde.

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