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Surfactant effects on alginate microspheres

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Summary

In the production of calcium alginate microspheres involving an emulsification process, the surfactants incorporated can affect the formation of the microspheres. In this study, the influence of the blend of surfactants, hydrophile-lipophile balance (HLB) and the sequence of addition of surfactant on the properties of the microspheres was determined. The findings showed that maintaining the same HLB, surfactants with more fatty acid chains produced larger microspheres which also have higher drug content, whereas for surfactants with longer polyoxyethylene chains, the microspheres were smaller. Varying the HLB, whilst keeping the same blend of surfactants, resulted in changes in the size and shape of the microspheres. The incorporation of the hydrophilic surfactant into the aqueous phase containing the drug gave rise to larger microspheres. These had a lower drug encapsulation efficiency. The drug release rate was faster, and this is attributed to the presence of unencapsulated drug crystals in the product.

Introduction

Calcium alginate microspheres are commonly prepared by extruding sodium alginate solution as droplets into calcium chloride solution (Deasy, 1984). As the method of preparation is simple and does not require elevated temperature, these microspheres are used to encapsulate living cells (Lim and Sun, 1980; Lim and Moss, 1981; Sun and O'Shea, 1985; Cai et al., 1989). Further studies on these microspheres as drug carriers showed their potential as a controlled release drug delivery system (Salib et al., 1978; Badwan et al., 1985; Bodmeier et al., 1989; Wheatley et al., 1991 Ostberg and Graffner, 1992).

An alternative method based on an emulsification process was developed for the production of calcium alginate microspheres (Wan et al., 1990, 1992, 1993). The effects of various operational and formulation factors on the properties of the microspheres were investigated. The surfactants used played an important role in the formation of the microspheres. An adequate amount of surfactant was essential for the production of round and discrete microspheres. The hydrophile-lipophile balance (HLB) of a blend of sorbitan trioleate and polyoxyethylene (20) sorbitan trioleate was found to affect the size distribution, shape and drug release profile of the microspheres but have very little influence on the drug encapsulation efficiency.

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It is useful to establish whether the characteristics of the microspheres are solely dependent on the HLB irrespective of the type of surfactant as this will provide important information for the selection of surfactants for the preparation of the microspheres. The above parameter was investigated in the present study by using different blends of surfactants with similar HLB. The effect of the order of incorporating the hydrophilic surfactant into the aqueous phase was also determined.

Experimental

Materials

Sodium alginate (BDH Chemicals, U.K.), calcium chloride and isooctane (analytical grade, Merck, Germany) were used as supplied. The surfactants used were sorbitan trioleate, sorbitan monooleate, sorbitan tristearate, sorbitan monostearate, POE (20) sorbitan trioleate, POE (20) sorbitan monooleate, POE (5) sorbitan monooleate, POE (20) sorbitan tristearate and POE (20) sorbitan monostearate obtained from Honeywill-Atlas, U.K. The model drug, sulphaguanidine (BP grade) was passed through a 75 μm sieve before use.

Preparation of calcium alginate microspheres

50 g of aqueous solution containing 5% w/w sodium alginate and 1% w/w sulphaguanidine were dispersed in 75 g of isooctane containing the lipophilic surfactant using a mechanical stirrer (Eyela MDC-2R, Japan) at 1000 rpm for 10 min. 5 g of aqueous solution containing the hydrophilic surfactant were then added. The dispersion was stirred for another 5 min, after which 20 g of 15% w/w calcium chloride solution was added and allowed to react with the sodium alginate globules for 10 min. The microspheres were collected by filtration and washed with 20 ml of distilled water three times before drying in an oven at 70°C.

Evaluation of size and shape of microspheres

The size and shape of the microspheres were determined using a microscope (Olympus BH-2, Japan) connected to an image analyser (Dapple System, Imageplus, U.S.A.). The shape was defined by the form factor where:

$$\text{Form factor} = 4\pi(\text{area})/(\text{perimeter})^2$$

The form factor is a measure of sphericity with the value of unity corresponding to a perfect circle. Each mean value reported was obtained from a total of more than 300 particles. Photographs of the microspheres mounted in glycerin were also taken.

Determination of drug content

Known amounts of microspheres were accurately weighed and diluted to 50 ml each using distilled water. The samples were placed in an ultrasonic water bath for three consecutive periods of 20 min each with a resting period of 30 min in between. They were then left to equilibrate for 1 day at 29 ± 2°C. Aliquot samples were removed through a 0.45 μm filter, diluted appropriately with distilled water and assayed spectrophotometrically (Perkin Elmer 550, U.S.A.) at 269 nm. Controls consisting of blank microspheres were also assayed. Each determination was carried out in triplicate and the mean drug content calculated.

Determination of drug release profile

Dissolution testing was carried out in 1000 ml deaerated distilled water at 37°C using the paddle method (USP Apparatus 2, Vankel VK6010, U.S.A.). The paddle was rotated at 50 rpm. Filtered 8 ml samples were collected using an autosample collector (Vankel VK6000, U.S.A.) at specified intervals of time and assayed spectrophotometrically at 260 nm (Hewlett Packard HP8452A, U.S.A.). At least three dissolution runs were carried out for each batch of microspheres and the results averaged.

Results and Discussion

Varying proportions of the component surfactants of different blends were used to give the specified HLB values (Table 1). Blends consisting

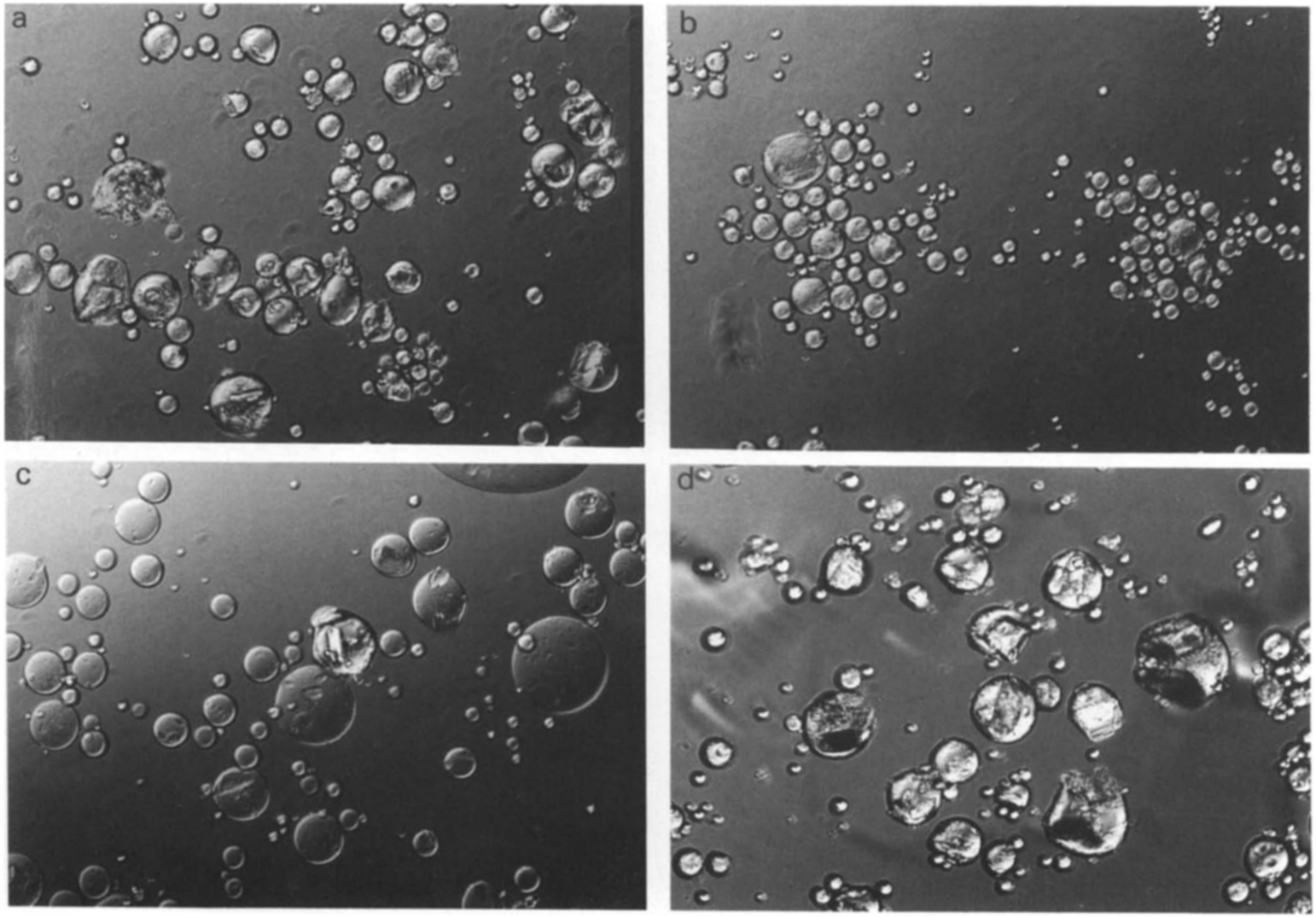

Fig. 1. Microspheres prepared from sorbitan monooleate and POE (20) sorbitan monooleate with different HLB: (a) 4.5, (b) 5.0, (c) 5.5, (d) 6.0. Scale 1 mm = 8.33 μm.

TABLE 1

Drug contents of microspheres prepared from different blends of surfactants

HLB	Amount of sorbitan trioleate (g)	Amount of POE (20) sorbitan trioleate (g)	Drug content (%)
4.5	1.837	0.763	18.07 ± 0.12
5.0	1.696	0.904	17.25 ± 0.06
5.5	1.554	1.046	17.28 ± 0.18
6.0	1.413	1.187	17.25 ± 0.16
	Amount of sorbitan monooleate (g)	Amount of POE (20) sorbitan monooleate (g)	Drug content (%)
4.5	2.551	0.049	11.90 ± 0.38
5.0	2.430	0.170	11.40 ± 0.40
5.5	2.309	0.291	11.90 ± 0.54
6.0	2.187	0.413	11.20 ± 0.48
	Amount of sorbitan monooleate (g)	Amount of POE (5) sorbitan monooleate (g)	Drug content (%)
4.5	2.509	0.091	7.90 ± 0.14
5.0	2.281	0.319	13.90 ± 0.18
5.5	2.053	0.547	12.80 ± 0.61
6.0	1.825	0.775	11.20 ± 0.42

of sorbitan tristearate with POE (20) sorbitan tristearate and sorbitan monostearate with POE (20) sorbitan monostearate were found to be unsuitable because of their poor solubility in the respective phases. The microspheres produced were highly irregular and showed marked clumping. This observation agreed with the results of earlier studies which showed that a certain amount of surfactants must be present to prevent immature microspheres from being distorted or fused together (Jalil and Nixon, 1990; Wan et al., 1993).

The HLB of a blend of sorbitan trioleate and POE (20) sorbitan trioleate was found to affect the shape and size distribution of the microspheres (Wan et al., 1993). The general appearance of the microspheres prepared from a blend of sorbitan monooleate and POE (20) sorbitan monooleate with different HLB values is shown in Fig. 1. It is clearly seen that HLB produced marked variation in the shape and size of the microspheres. The same applied to a blend of sorbitan monooleate with POE (5) sorbitan monooleate. When the microspheres prepared from the three blends of surfactants with the same HLB were compared, a significant difference in the size of the microspheres was noted (Fig. 2). Sorbitan monooleate with POE (20) sorbitan monooleate produced smaller microspheres than sorbitan trioleate with POE (20) sorbitan trioleate. Other workers who investigated microencapsulation using poly(L-lactic acid) also found that emulsifiers consisting of straight chain saturated fatty acid produced smaller microcapsules than those consisting of three fatty acid chains (Jalil and Nixon, 1990). This was explained by the closer and more uniform packing by the first type of emulsifier at the interface of the dispersed globules. In the present study, it was also found that sorbitan monooleate with POE (5) sorbitan monooleate produced much larger microspheres, suggesting that surfactants with shorter polyoxyethylene chains produced larger microspheres.

An effective method of microencapsulation should have a high efficiency of entrapping drug. The effect of HLB of different blends of surfactants on the drug encapsulation efficiency was studied by comparing the drug contents of the respective microspheres (Table 1). The drug content was found to be significantly affected by the blend of surfactants. For example, at the same HLB of 5.0, the drug content of sorbitan trioleate with POE (20) sorbitan trioleate was 17.25%, sorbitan monooleate with POE (20) sorbitan monooleate 11.40% and sorbitan monooleate with POE (5) sorbitan monooleate 13.90%. A comparison of the drug content and size of the microspheres showed no definite relationship between the two parameters. The higher drug content of sorbitan trioleate with POE (20) sorbitan trioleate relative to that of sorbitan monooleate with POE (20) sorbitan monooleate could be attributed to a looser packing of the surfactants at the interface of the sodium alginate globules

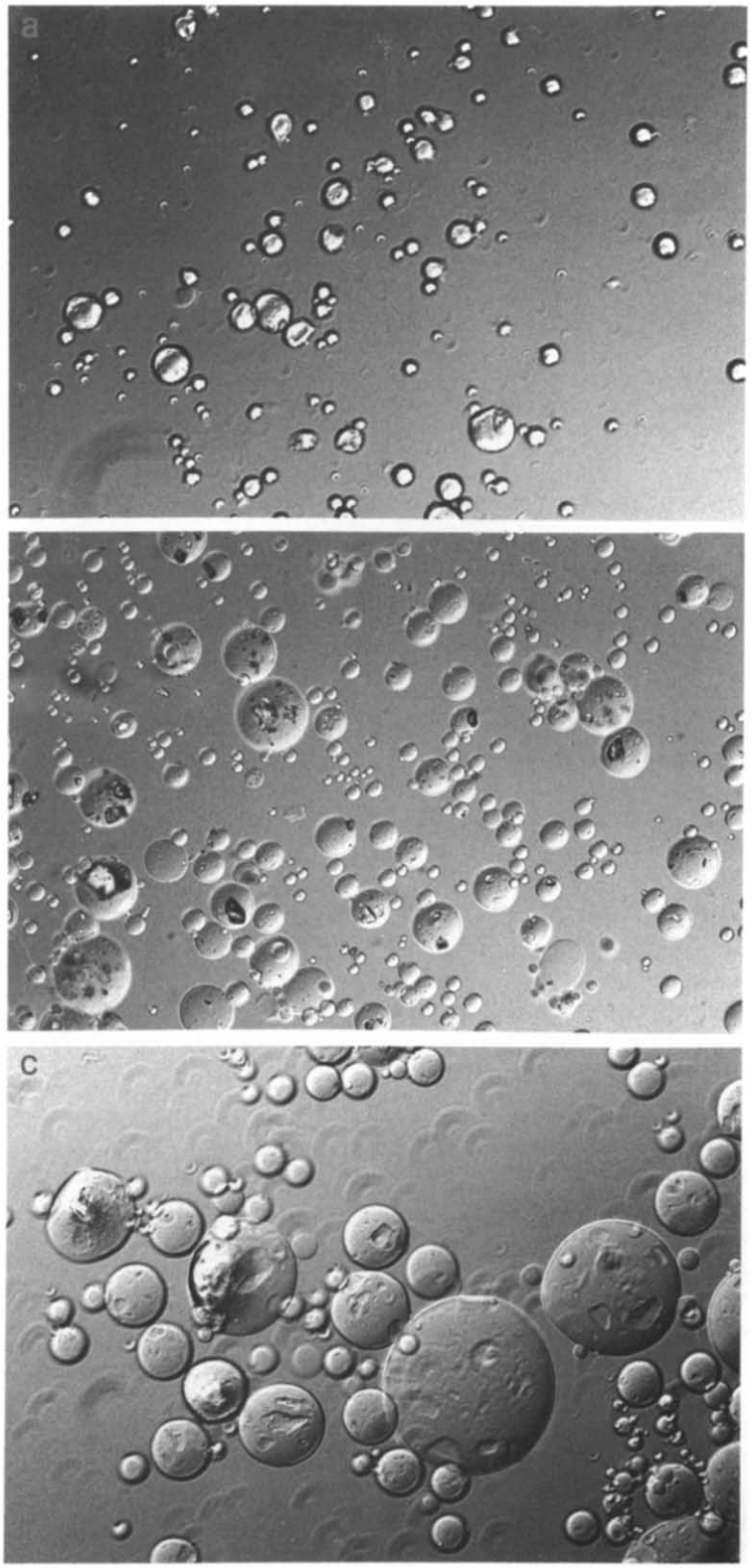

Fig. 2. Microspheres prepared from different blends of surfactants with similar HLB: (a) sorbitan monooleate with POE (20) sorbitan monooleate, (b) sorbitan trioleate with POE (20) sorbitan trioleate, (c) sorbitan monooleate with POE (5) sorbitan monooleate. Scale 1 mm = 8.33 μm.

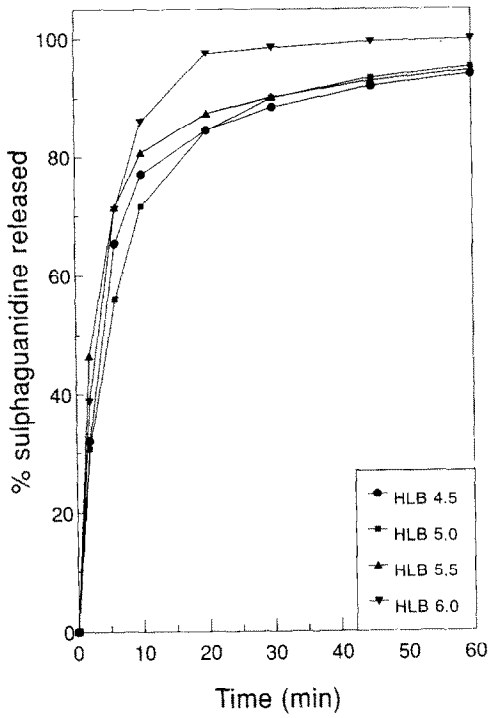

Fig. 3. Drug release profiles of microspheres prepared from a blend of sorbitan monooleate and POE (20) sorbitan monooleate with varying HLB: (●) 4.5, (■) 5.0, (▲) 5.5, (▼) 6.0.

which allowed easier penetration of the calcium ions. This would result in the formation of a denser matrix and consequently less drug loss during the washing process. Besides the number of fatty acid chains, it appeared that the drug encapsulation efficiency was also affected by the number of ethylene oxide units. It was very interesting to note that the drug contents of microspheres prepared from surfactants with 20 ethylene oxide units were not significantly affected by HLB whereas marked variation in drug contents was observed at varying HLB of surfactants with five ethylene oxide units.

Dissolution study of microspheres prepared from a blend of sorbitan monooleate and POE (20) sorbitan monooleate with varying HLB values showed that the rate of drug release was generally lower at lower HLB (Fig. 3). The microspheres prepared from a blend of sorbitan monooleate and POE (5) sorbitan monooleate

TABLE 2

Properties of microspheres prepared by method A (H4.5A, H5.5A) and method B (H4.5B, H5.5B)

Sample code	Means size (μm)	Mean form factor	Drug content (%)
H4.5A	16.92 ± 7.10	0.89 ± 0.16	18.07 ± 0.12
H4.5B	21.72 ± 7.80	0.93 ± 0.07	14.86 ± 0.12
H5.5A	18.87 ± 7.25	0.93 ± 0.07	17.28 ± 0.18
H5.5B	20.42 ± 6.95	0.92 ± 0.08	14.01 ± 0.14

exhibited a similar characteristic. As reported in a previous study, this phenomenon was most likely due to the presence of the lipophilic surfactant on the microspheres, thereby retarding the release of drug out of the microspheres (Wan et al., 1993).

Further investigations were also carried out to study the effect of different sequence of adding the hydrophilic surfactant. A blend of sorbitan trioleate with POE (20) sorbitan trioleate with HLB of 4.5 and 5.5 was used. In method A, the sodium alginate solution containing the drug was dispersed in the organic phase for 10 min before adding the hydrophilic surfactant after which stirring was continued for 5 min. In method B, the

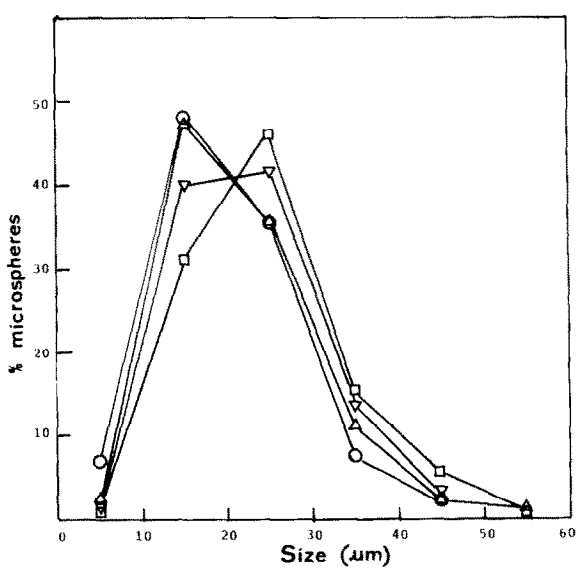

Fig. 4. Size distribution of microspheres prepared by method A (H4.5A, H5.5A) and method B (H4.5B, H5.5B): (○) H4.5A, (△) H5.5A, (□) H4.5B, (▽) H5.5B.

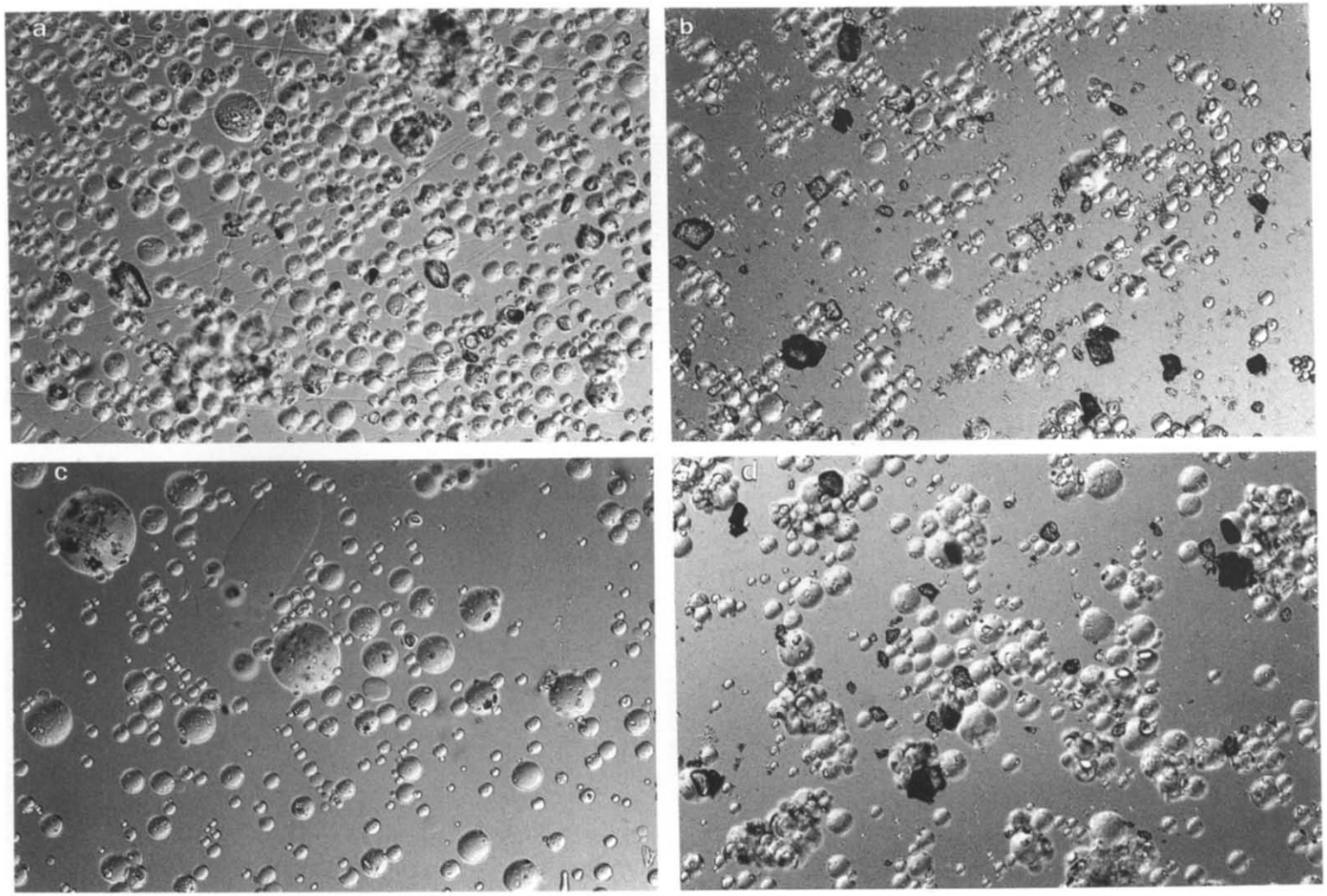

Fig. 5. Photographs of microspheres prepared by method A: (a) H4.5A and (c) H5.5A and method B (b) H4.5B and (d) H5.5B.

hydrophilic surfactant was added to the aqueous phase before dispersion in the organic phase.

The properties of the microspheres obtained at HLB 4.5 and HLB 5.5 by method A (H4.5A and H5.5A, respectively) and method B (H4.5B and H5.5B, respectively) are shown in Table 2. All the microspheres produced were fairly spherical as the mean form factors were about 0.9. Size analysis of the microspheres showed unimodal distribution, with method B producing higher proportions of larger microspheres (Fig. 4). The mean size of H4.5B and H5.5B (21.72 and 20.42 μm, respectively) was larger compared to that of H4.5A and H5.5A (16.92 and 18.87 μm, respectively). This indicated that method B was less effective in dispersing the aqueous phase or preventing the immature microspheres from fusing together.

It was also very interesting to note that H4.5B and H5.5B showed abundant free drug as this was not observed in H4.5A and H5.5A (Fig. 5). In method B, the hydrophilic surfactant was added to the aqueous phase before dispersion. The presence of the free drug could be due to adsorption of the hydrophilic surfactant on the surface of the drug particle, thereby favouring the partition of the drug into the organic phase containing the lipophilic surfactant. The solubilised drug would be lost during the collection of the microspheres by filtration. Hence, the drug contents of H4.5B and H5.5B (14.86 and 14.01%, respectively) were markedly lower than those of H4.5A and H5.5A (18.07 and 17.28%, respectively). The loss of surfactant to the drug would also result in less surfactant being available for the sodium alginate globules, which explained the formation of larger microspheres. The production of larger microspheres by lower concentrations of emulsifiers was confirmed by other workers (Jalil and Nixon, 1990; Jeffery et al., 1991).

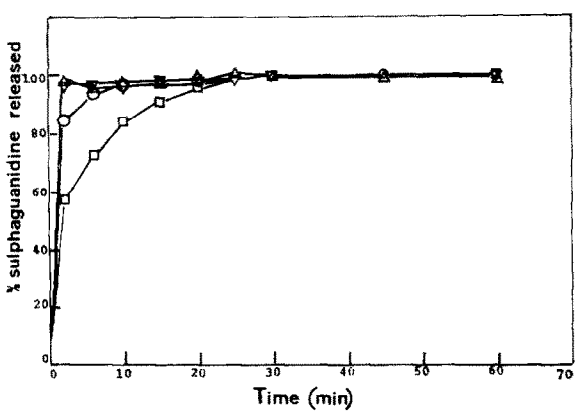

Fig. 6. Drug release profiles of microspheres prepared by method A ((□) H4.5A, (○) H5.5A)) and method B ((△) H4.5B, (▽) H5.5B)).

Dissolution studies showed that the drug release profiles of H4.5B and H5.5B were similar. The rates of drug release of these samples were significantly higher than those of H4.5A and H5.5A (Fig. 6). This could be accounted for by the free drug which dissolved faster than the encapsulated one. It is clearly seen that the order of incorporating the surfactants had a significant influence on the properties of the microspheres.

Conclusion

Surfactants play an important role in the formation of microspheres by emulsification. The properties of the microspheres are affected by both the HLB and type of surfactants. At the same HLB, surfactants consisting of more fatty acid chains produce microspheres which are larger and have higher drug contents. In contrast, surfactants with longer polyoxyethylene chains produce smaller microspheres. Within the same blend of surfactants, HLB produces marked variation in the size of the microspheres. The effect of HLB on the drug content is, however, not definite, with the effect being insignificant for some blends of surfactants but marked for others. The rate of drug release is generally retarded by surfactants with low HLB. The method of preparation is also important. Addition of hydrophilic surfactant to the aqueous phase containing the drug produces larger microspheres and lower drug encapsulation efficiency. Abundant free drug is found in the product, resulting in faster drug release.

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