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Influence of hydrophile-lipophile balance on alginate microspheres

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Summary

In this study, calcium alginate microspheres were prepared by an emulsification method using surfactants. A blend of sorbitan trioleate and polyoxyethylene sorbitan trioleate of varying HLB was used to produce the microspheres. The results obtained showed that the surfactant concentration and HLB played an important role in the formation of the microspheres. The size, size distribution and to a certain extent the shape were significantly affected by the HLB. However, HLB did not alter the drug encapsulation efficiency appreciably but did affect the rate of drug release.

Introduction

Numerous techniques of microencapsulation are documented, each with its advantages and limitations (Chang et al., 1966; Luzzi, 1970; Madan et al., 1978; Deasy, 1984; Arshandy, 1988; Gupta and Hung, 1989; Jalil and Nixon, 1990). The choice of a technique depends largely on the coating material, core material and desired properties of the microspheres formed. Calcium alginate microspheres which are potential drug carriers are readily prepared by the congealable disperse phase encapsulation method using an extrusion device (Badwan et al., 1985, Matsumoto et al., 1986; and Saslawski et al., 1988).

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In earlier studies, an alternative method based on an emulsification process was developed for the production of small calcium alginate microspheres (Wan et al., 1990, 1992). In this method, the aqueous sodium alginate solution containing the drug was dispersed in an organic phase to which calcium chloride was later added to congeal the globules. The properties of the microspheres were markedly affected by the stirring speed, addition rate of calcium chloride and concentration and composition of the coating material. The drug encapsulation efficiency was enhanced by forming the microspheres by phase inversion and generally increased with the drug load.

As the present method of microencapsulation involves emulsification, the surfactants would play an important role in the production of the microspheres. In preliminary investigations, it was observed that the successful formation of micro-

spheres was to a large extent dependent on the concentration and composition of the surfactants. The objective of the present study was to investigate the effects of concentration and hydrophile-lipophile balance (HLB) of surfactants on the physical characteristics of the microspheres.

Experimental

Materials

Sodium alginate (BDH Chemicals, U.K.), calcium chloride and iso-octane (analytical grade, Merck, Germany) were used as supplied. The surfactants sorbitan trioleate and polyoxyethylene (POE) sorbitan trioleate were obtained from Honeywell-Atlas, U.K. The 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 iso-octane containing varying concentrations of sorbitan trioleate using a mechanical stirrer (Eyela MDC-2R, Japan) at 1000 rpm for 10 min, after which 5 g of aqueous solution containing varying amounts of POE sorbitan trioleate were added and the dispersion was stirred for another 5 min. This was followed by the addition of 15% w/w calcium chloride solution which was allowed to react with the dispersed sodium alginate globules for 10 min. The microspheres were collected by filtration and washed with 20 ml of distilled water thrice 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 analyzer (Dapple System, Imageplus, USA) which consists of a video camera and a microcomputer system. The mean size was expressed as the equivalent spherical (ES) diameter while the shape was defined by the form factor where:

$$\text{ES diameter} = (\text{length})^{1/3}(\text{breadth})^{2/3}$$

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

The form factor is a measure of the 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.

Determination of drug encapsulation efficiency

Known amounts of microspheres were accurately weighed, transferred to 50 ml volumetric flasks and distilled water was added to make up to volume. The samples were placed in an ultrasonic water bath for three consecutive periods of 20 min each and then left to equilibrate for 1 day. Aliquot samples were removed through a 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 encapsulation efficiency 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 5 ml samples were collected using an autosample collector (Vankel, VK6000, USA) at specified intervals of time and assayed spectrophotometrically at 260 nm (Hewlett Packard, HP8452A U.S.A.). For each batch, at least three dissolution runs were carried out and the results averaged.

Results and Discussion

Previous studies have indicated that surfactants play an important role in the production of calcium alginate microspheres by emulsification (Wan et al., 1990). In the preparation of poly(lactic acid), poly(dl-lactide) and poly(lactide-

co-glycolide) microcapsules, other workers have reported that the type and concentration of emulsifier in the aqueous phase affect the microcapsule size, shape and drug encapsulation efficiency (Wakiyama et al., 1981; Juni et al., 1985; Cavalier et al., 1986; Spenlehauer et al., 1986; Bodmeier and McGinty, 1987; Jeffery et al., 1991). In the present investigation, preliminary studies were carried out to determine the concentration of surfactants required to form discrete microspheres with and without drug. As in the earlier studies, the proportion by weight of sorbitan trioleate to POE sorbitan trioleate was kept constant at 3:2 and the total concentration was varied from 0.5, 1.0, 1.5 to 2.0% w/w.

Microscopic examination of the samples withdrawn from the dispersions before the addition of calcium chloride showed the aqueous phase dispersed as fine globules in the organic phase at all the concentrations of surfactants used. However, upon adding calcium chloride, marked clumping of round unloaded microspheres was produced at 0.5% w/w surfactants. Less clumping was observed at 1.0 and 1.5% w/w while at 2.0% w/w the extent of clumping was insignificant. The above observation showed that as little as 0.5% w/w surfactants was adequate for the production of a fine dispersion but this did not ensure the formation of discrete and spherical microspheres. It appeared that surfactants were required to prevent the immature microspheres from being distorted and/or fused together. It was also noted that a higher concentration of surfactants was needed in the presence of drug as surfactant concentrations less than 1.5% w/w produced irregular calcium alginate particles. A large number of round microspheres with little clumping was formed at 2.0% w/w, compared to 1.0% w/w for unloaded microspheres. Hence, in subsequent studies, 2.0% w/w surfactants was used.

There are many types of surfactants which may be characterised by their HLB. As the properties of microcapsules are affected by the type of surfactant, it would be useful to establish the effect of HLB on the properties of microspheres. In this study, varying proportions of sorbitan trioleate and POE sorbitan trioleate were used to give different HLB values (Table 1).

Clumps of microspheres were formed at HLB 3.5. Size analysis of the microspheres obtained from the other HLB values showed a unimodal distribution with all the microspheres less than 70 μm and the largest size fraction ranging from 10.0 to 19.9 μm (Fig. 1). Plot of percentage of microspheres in this size fraction against HLB showed that HLB 4.0 had the lowest percentage of 39.73% (Fig. 2). The percentage increased proportionally from HLB 4.0 to 5.0 and decreased to approx. 47% at HLB greater than 5.0. It was also observed that smaller microspheres were formed at HLB 4.5–5.0 as reflected by higher proportions of microspheres measuring 0–9.9 μm and the absence of microspheres larger than 50 μm. The results showed than HLB 3.5 was unsuitable as the microspheres fused together to form large aggregates. It was evident

TABLE 1

Properties of microspheres obtained at varying HLB

HLB	Amount of sorbitan trioleate (g)	Amount of POE sorbitan trioleate (g)	Mean size (μm) ($\pm$SD)	Mean form factor ($\pm$SD)	Drug content (%)
3.5	2.120	0.481	–	–	17.73
4.0	1.978	0.622	19.95 $\pm$ 8.96	0.92 $\pm$ 0.09	17.77
4.5	1.837	0.763	16.92 $\pm$ 7.10	0.89 $\pm$ 0.16	18.07
5.0	1.696	0.904	16.47 $\pm$ 6.40	0.90 $\pm$ 0.11	17.25
5.5	1.554	1.046	18.87 $\pm$ 7.25	0.93 $\pm$ 0.07	17.28
6.0	1.413	1.187	19.17 $\pm$ 7.40	0.93 $\pm$ 0.08	17.25

that the size distribution was affected by the HLB which in turn could be employed to control the proportions of varying size fractions.

The study on the effect of HLB on the mean size of the microspheres showed a curve with a minima at HLB 5.0 (Fig. 2). The mean size decreased disproportionately from 19.95 μm at HLB 4.0, 16.92 μm at HLB 4.5 to 16.47 μm at HLB 5.0. Beyond HLB 5.0, the mean size increased to 18.87 μm and 19.17 μm at HLB 5.5 and 6.0 respectively. A more efficient emulsification would produce a finer dispersion which would result in smaller microspheres. Thus, HLB had a significant effect on the efficiency of dispersing the aqueous sodium alginate solution in the organic phase and it is clearly indicated in Fig. 2 that HLB 5.0 which produced the smallest mean size and the highest percentage of microspheres in the largest size fraction was the optimal.

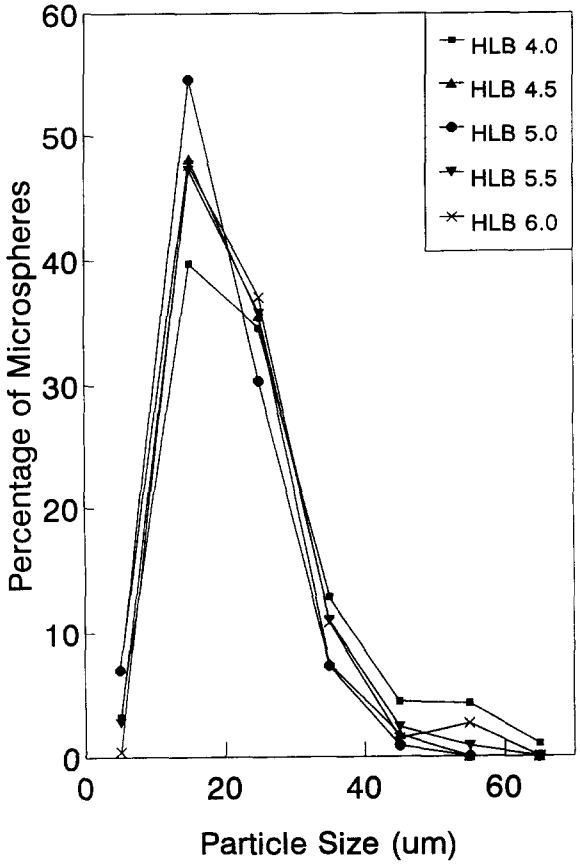

Fig. 1. Size distribution of microspheres.

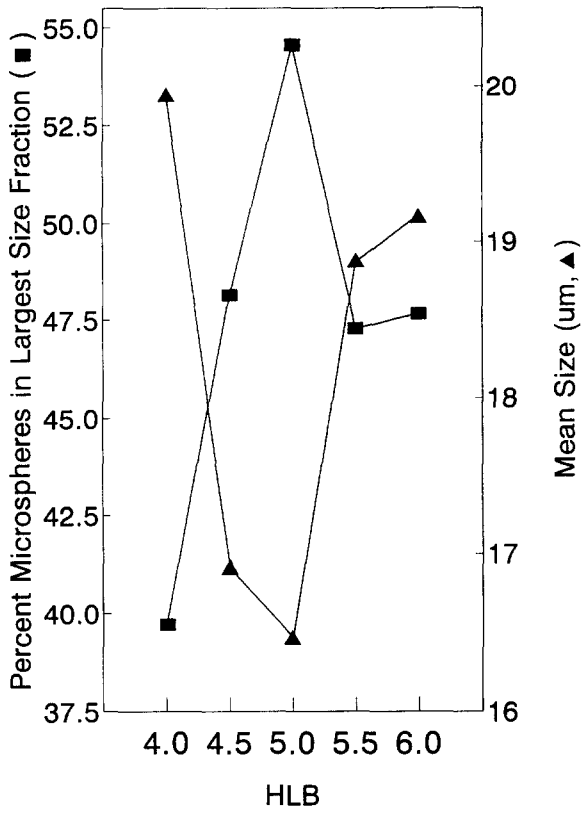

Fig. 2. Effect of HLB on percentage of largest size fraction and mean size.

Most of the microspheres obtained had a form factor greater than 0.9, indicating that they were fairly spherical (Table 2). There was no correlation between the mean form factor and size range. For example, the mean form factor of size range 40.0–49.9 μm varied from 0.92 (HLB 4.0), 0.37

TABLE 2

Mean form factor of varying size fractions

Size fraction (μm)	Mean form factor				
	HLB 4.0	HLB 4.5	HLB 5.0	HLB 5.5	HLB 6.0
0 - 9.9	0.94	0.93	0.92	0.93	0.86
10.0–19.9	0.92	0.91	0.91	0.93	0.94
20.0–29.9	0.92	0.92	0.87	0.94	0.92
30.0–39.9	0.90	0.86	0.91	0.88	0.93
40.0–49.9	0.92	0.37	0.83	0.98	1.00
50.0–59.9	0.91	–	–	0.89	0.96
60.0–69.9	0.90	–	–	–	–

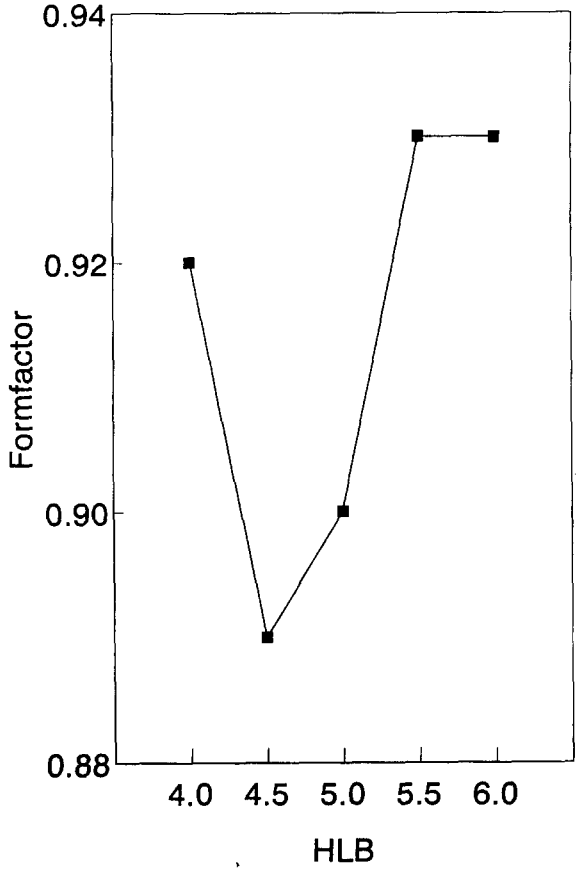

Fig. 3. Relationship between mean form factor and HLB.

(HLB 4.5), 0.83 (HLB 5.0), 0.98 (HLB 5.5) to 1.00 (HLB 6.0). Even at the same HLB, the mean form factor did not show any definite relationship with size. When the mean form factor was plotted against the corresponding HLB, the former parameter was found to decrease from HLB 4.0 to 4.5 and increase with higher HLB values (Fig. 3). This suggested that the shape of the microsphere was influenced by some other factors besides HLB. The effect of HLB was probably attributed to the packing of the surfactant molecules at the interface of the aqueous globules, forming a barrier to the calcium ions which were required to form a semi-rigid microsphere with the sodium alginate. Surfactant mixtures with a higher HLB had a higher proportion of hydrophilic surfactant and hence a greater affinity for the aqueous calcium chloride solution. Thus, the calcium alginate microspheres were completely formed before distortion of their shape occurred as a result of turbulence due to introduction of the calcium chloride solution.

Since HLB was found to affect the size distribution and shape of the microspheres significantly, further investigations were made to determine its effects on drug encapsulation efficiency. The drug contents of the microspheres are given in Table 1. Theoretically, the microspheres should contain 16.66% w/w drug. However, for all the HLB values, the drug content was found to be higher than this amount, indicating a drug encapsulation efficiency greater than 100%. This was attributed to the loss of very fine microspheres in the filtrate. These microspheres most probably did not contain any drug, resulting in a higher proportion of drug to microspheres collected on the filter paper. As the drug encapsulation efficiency was calculated from the microspheres collected on the filter paper, the value was therefore greater than 100%.

HLB did not have a marked effect on the drug content as the difference between the highest (18.07% at HLB 4.5) and lowest (17.25% at HLB 5.0 and 6.0) values was less than 5% of the lowest value. However, it was observed that the drug content of the microspheres prepared at higher HLB of 5.0–6.0 was generally lower, possibly due to the greater loss of drug to the aqueous phase through solubilisation of the drug by the more hydrophilic surfactant. This was also noted in earlier studies where the drug encapsulation efficiency of a drug solution was found to be much lower than that of a drug suspension (Wan et al., 1991). A comparison of the drug content and mean size of the microspheres indicated no definite relationship, suggesting that size was not the primary factor affecting the drug encapsulation efficiency (Table 1). This was supported by microscopic examination which showed random distribution of relatively large drug particles in the microspheres.

In the drug release profiles, it was interesting to observe that the rate of drug release was enhanced by increasing HLB (Fig. 4). This was most likely due to the higher proportion of lipophilic surfactant in the surfactant mixture with

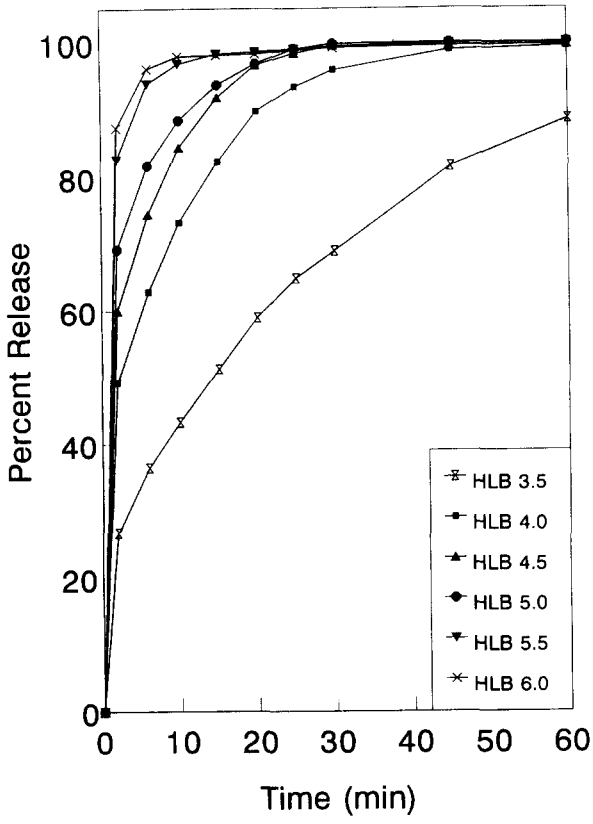

Fig. 4. Drug release profiles of microspheres.

a lower HLB. The surfactant being hydrophobic in nature was difficult to be removed from the microspheres during the washing process with distilled water. These surfactant molecules adsorbed on the surface of the microsphere would impede the diffusion of water into the microsphere during the drug dissolution study and retard the release of drug out of the microsphere. The much lower drug release rate of HLB 3.5 could also be attributed to the formation of clumps of microspheres which acted as aggregate units with longer diffusional pathways and subsequently reduced the rate of drug release.

Conclusion

The formation of discrete microspheres is dependent on the concentration of surfactants. Sufficient amount of surfactants should be present to prevent fusion of immature microspheres. The microspheres are less than 70 μm. The size distribution which is unimodal is significantly affected by HLB. Most of the microspheres are fairly spherical. The sphericity is affected by factors other than HLB. HLB does not produce a marked variation in the drug encapsulation efficiency. Microspheres prepared at high HLB show a slight decrease in drug content. However, the rate of drug release is enhanced by increasing HLB but the relationship is not directly proportional.

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