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RESEARCH PAPER

Effect of Process Variables on the Microencapsulation of Vitamin A Palmitate by Gelatin-Acacia Coacervation

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ABSTRACT

Microcapsules of vitamin A palmitate were prepared by gelatin-acacia complex coacervation. The effects of colloid mixing ratio, core-to-wall ratio, hardening agent, concentration of core solution, and drying method on the coacervation process and the properties of the microcapsules were investigated. The microcapsules of vitamin A palmitate were prepared using different weight ratios of gelatin and acacia, that is, 2:3, 1:1, and 3:2 under controlled conditions. The other factors studied were 1:1, 1:2, and 1:3 core-to-wall ratios; 30, 60, and 120 min of hardening time; 2, 5, and 10 ml of formaldehyde per 280 g of coacervation system as a hardening agent; and 30%, 40%, and 50% w/w vitamin A palmitate in corn oil as a core material. The drying methods used were air drying, hot air at 40°C, and freeze-drying. The results showed that spherical microcapsules were obtained for all conditions except for 30 min of hardening time, which did not result in microcapsules. The optimum conditions for free-flowing microcapsules with a high percentage of entrapped drug were 1:1 gelatin-to-acacia ratio and 1:2 core-to-wall ratio when hardening with 2 ml formaldehyde for 60 min and using 40% w/w vitamin A palmi-

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tate in corn oil as the core concentration. In addition, drying the microcapsules by freeze-drying provided microcapsules with excellent appearance.
Key Words: *Complex coacervation; Gelatin-acacia; Microcapsules; Microencapsulation; Vitamin A palmitate.*

INTRODUCTION

Microencapsulation is a process of applying relatively thin coatings to small particles of solids or droplets of liquids (1–4). It has been practiced in the pharmaceutical industry for several years, and the literature on microencapsulation is extensive. There are many advantages of this useful technology. For instance, drugs are encapsulated for environmental protection, separation of incompatibles, taste masking, conversion of liquids to solids, reduction of gastric irritation, and prolonged or sustained release. Bakan (2) reported that microencapsulation of vitamin A palmitate could retard the rate of degradation from oxidation. Torrodo et al. (5) found that the microencapsulated vitamin A palmitate was able to prolong the release of vitamin A topically and thus enhanced the availability of vitamin A cream.

Microcapsules may be prepared by a number of methods, which include coacervation or phase separation, interfacial reactions, and miscellaneous mechanical methods (3,6). In this study, gelatin-acacia complex coacervation, a general method for microencapsulating oily materials (7), was used to prepare microcapsules of vitamin A palmitate. For complex coacervation, the experimental conditions largely determine the amount of complex coacervate and the properties of the microcapsules (8–15). The aim of the present study was to investigate the effects of process variables on the properties of vitamin A palmitate microcapsules.

EXPERIMENTAL

Materials

The materials used were vitamin A palmitate (Fluka, Buchs, Switzerland); type B gelatin, bloom strength 225 (Davis Gelatine, Botany, Australia); acacia BP 1993 (Wendt-Chemie, Hamburg, Germany); corn oil; 2-propanol; formaldehyde solution (34%–38% w/w, Ajax Chemicals, Sydney, Australia); 10% v/v acetic acid solution; and 20% w/v sodium hydroxide solution.

Preparation of Microcapsules

The solution of 30% w/w vitamin A palmitate in corn oil was emulsified in 10% w/w gelatin solution, pH 10 at 40°C. The emulsion was stirred at 200 rpm for 30 min. The 10% w/w acacia solution at 40°C was gradually added while stirring for another 30 min. Warm water (40°C) was added to dilute the colloidal concentration to lower than 3% (100 g of water per 30 g of colloidal solution). The pH of the mixture was adjusted to 4 by the dropwise addition of 10% v/v acetic acid solution. The mixture was cooled to 5°C while maintaining agitation. Formaldehyde was then added and mixed for various hardening times. To adjust the pH of the solution to 9, sodium hydroxide 20% w/v was added. After continued agitation for 30 min, the microcapsules were then separated by vacuum filtration and washed with 2-propanol to remove excess vitamin A palmitate. Finally, the microcapsules were dried by a suitable method.

The following variables affecting the coacervation process were investigated ($n = 2$): gelatin and acacia ratio (2:3, 1:1, and 3:2), core-to-wall ratio (1:1, 1:2, and 1:3), concentration of hardening agent (2, 5, and 10 ml of formaldehyde solution per 280 g of coacervation system), hardening time (30, 60, and 120 min), concentration of core solution (30%, 40%, and 50% w/w vitamin A palmitate in corn oil), and method of drying (air drying, hot air at 40°C, and freeze-drying).

Optical and Scanning Electron Microscopy

The morphologies and surface structures of the microcapsules were observed using an optical microscope (Olympus, Tokyo, Japan) and a scanning electron microscope (model S-2500, Hitachi, Tokyo, Japan).

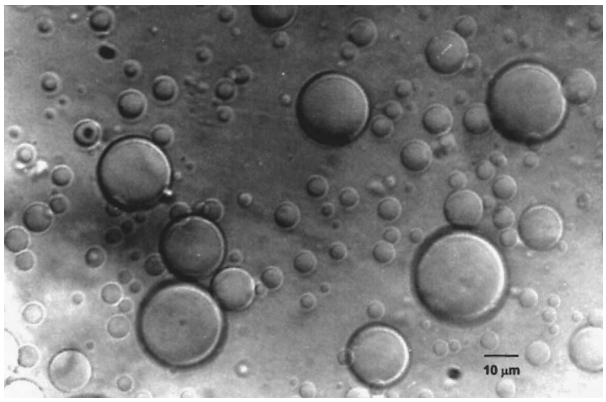
The particle size was determined using a microscope. At least 200 microcapsules were dispersed on a slide, and their diameters were measured using a suitable objective.

Determination of Encapsulated Drug

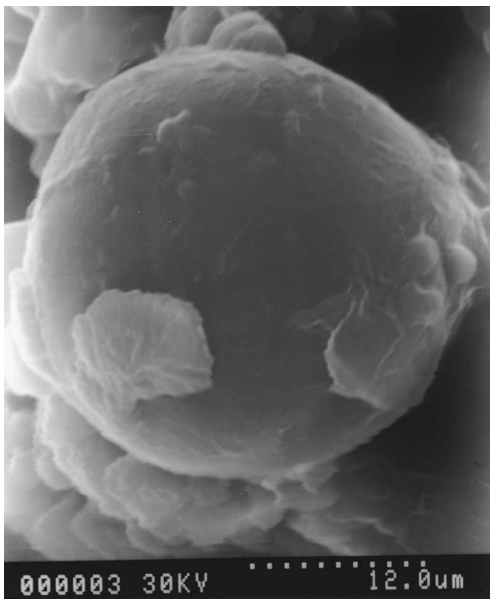
To determine the total drug content of the microcapsules, 100 mg of vitamin A palmitate microcapsules were accurately weighed and crushed with 2-propanol in the mortar. The suspension was then filtered to separate the shell fragments from the filtrate. The filtrate was diluted with 2-propanol to an appropriate concentration. The concentration of vitamin A palmitate was then determined by ultraviolet (UV) spectrophotometry (UV/Vis 918, GBC Scientific Equipment, Victoria, Australia) at 326 nm.

RESULTS AND DISCUSSION

Gelatin-acacia microcapsules that entrapped microdroplets of vitamin A palmitate in corn oil were prepared to convert the oily vitamin to solid powders. The microcapsules were collected as discrete, free-flowing powders. Figure 1 shows images of microcapsules obtained by optical and scanning electron microscopes. The particles were completely spherical with a colloidal layer of coacervate. The particle analysis revealed that the microcapsule size was in the range 20.2 to 29.5 μm; however, the variation in size was relatively large due to the difficulty in controlling the size of the liquid core droplets.



(a)



(b)

Figure 1. Microscopic photographs of the vitamin A palmitate gelatin–acacia microcapsules: (a) optical microscope (×500); (b) scanning electron microscope (×2500). The conditions for microencapsulation were 1:1 gelatin: acacia ratio, 1:2 core:wall ratio, 2 ml of formaldehyde/280 g of the coacervation system as the hardening agent, 60 min hardening time, and 30% w/w vitamin A palmitate in corn oil as the core material.

In this study, the effect of process variables on the appearance of the microcapsules and the efficiency of drug entrapment were evaluated. The results are shown in Table 1.

Effect of Gelatin-to-Acacia Ratio

The formulation using 2:3, 1:1, and 3:2 gelatin:acacia ratios yielded microcapsules with comparable size, percentage of drug content, and percentage of entrapped drug, as shown in Table 1. While the microcapsules prepared using either 2:3 or 3:2 gelatin:acacia weight ratios were aggregated powders, those prepared using 1:1 gelatin:acacia tended to be free-flowing powders. It was reported that the phase-separated, colloid-rich droplets in gelatin-acacia complex coacervation usually contained an approximately 1:1 ratio of the two colloids (3). As expected, the 1:1 gelatin:acacia ratio was suitable for the formation of vitamin A microcapsules, which were collected as discrete, free-flowing powders. At 2:3 and 3:2 polymer ratios, there was the presence of excess colloid after coacervation occurred, which could cause the aggregation of microcapsules during the collection process. This result is in accordance with that of Nixon and Agyilirah (16), who reported that equal amounts of gelatin and acacia produced the best microcapsules of phenobarbitone.

Effect of Core-to-Wall Ratio

The comparison of the properties of the microcapsules prepared using core-to-wall ratios of 1:1, 1:2, and 1:3 can also be seen in Table 1. It was observed during the microencapsulation process that, when using a 1:3 ratio, the system became lumpy and difficult to filter. Aggregation of microcapsules was also likely to occur. In contrast, the systems having 1:1 and 1:2 core-to-wall ratios had no difficulty in filtration, and free-flowing microcapsules were generally produced. Furthermore, the 1:2 ratio gave microcapsules with the highest percentage of entrapped drug, as seen in Table 1. The results substantially confirmed that the affinity of the core and the coating would be promoted at a 1:2 core-to-wall ratio.

Effect of Amount of Formaldehyde

The amount of formaldehyde had little effect on the morphologies of the microcapsules since the spherical

Table 1

Effect of Process Variables on the Properties of Vitamin A Palmitate Microcapsules

Gelatin: Acacia	Core: Wall	Formaldehyde (ml/280 g of Coacervate)	Hardening Time (min)	Particle Size (μm)	Drug Content (%)	Entrapped Drug (%)	Observation
Effect of gelatin-to-acacia ratio							
2:3	1:1	2	60	27.1 ± 11.9	11.35 ± 0.55	75.49 ± 3.56	Aggregation of microcapsules
1:1	1:1	2	60	25.1 ± 9.4	9.87 ± 0.85	65.61 ± 5.59	Free-flowing microcapsules
3:2	1:1	2	60	27.0 ± 9.5	9.59 ± 0.28	63.89 ± 1.97	Aggregation of microcapsules
Effect of core to wall ratio							
1:1	1:1	2	60	25.1 ± 9.4	9.87 ± 0.85	65.61 ± 5.59	Free-flowing microcapsules
1:1	1:2	2	60	25.5 ± 9.4	8.36 ± 0.36	83.43 ± 3.93	Free-flowing microcapsules
1:1	1:3	2	60	26.0 ± 8.3	4.99 ± 0.59	66.49 ± 7.96	Aggregation of microcapsules
Effect of amount of formaldehyde							
1:1	1:2	2	60	25.1 ± 9.4	8.36 ± 0.36	83.43 ± 3.93	Free-flowing microcapsules
1:1	1:2	5	60	28.1 ± 9.7	5.52 ± 0.88	54.89 ± 8.47	Free-flowing microcapsules
1:1	1:2	10	60	24.4 ± 7.7	7.12 ± 0.49	70.59 ± 5.30	Free-flowing microcapsules
Effect of hardening time							
1:1	1:2	2	30	—	—	—	No encapsulation
1:1	1:2	2	60	25.1 ± 9.4	8.36 ± 0.36	83.43 ± 3.93	Free-flowing microcapsules
1:1	1:2	2	120	25.9 ± 10.0	7.50 ± 1.50	74.52 ± 14.88	Free-flowing microcapsules

Note: The core concentration used in all formulations was 30% w/w vitamin A palmitate in corn oil.

and isolated microcapsules could be obtained for any different amount of formaldehyde used (2, 5, or 10 ml of formaldehyde/280 g of coacervate). However, the amount of formaldehyde seemed to influence the percentage of drug content and the percentage of drug entrapped by the microcapsules, as seen in Table 1. The percentage of drug content and the percentage of entrapped drug were highest when 2 ml of formaldehyde were used. In addition, the viscosity of the coacervate mixture was observed to be low, and filtration was easy for this condition. At higher amounts of formaldehyde, the viscosity of the vehicle was increased due to the cross linking between gelatin in the equilibrium colloid-poor layer caused by the excess formaldehyde (3).

In one study, it was shown that the amount of hardening agent affected the size of the microcapsules (12). As the amount of hardening agent increased, the size of the microcapsules increased since the hardened wall could prevent shrinkage during the dehydration process (12). However, this effect could not be observed in this study, and microcapsules of the same size were obtained when different amounts of formaldehyde were used.

Formaldehyde and glutaraldehyde are commonly used as hardening or cross-linking agents for a protein-polysaccharide complex coacervation system such as gelatin and acacia. Cross linking of coacervates is necessary to stabilize the coacervate emulsion droplets and hence form microcapsules (17). When collected, the microcapsules should be carefully washed to remove residual hardening agents since a trace amount of these agents can be harmful to health due to their toxicity.

Effect of Hardening Time

Table 1 also shows the properties of microcapsules prepared using different hardening times (i.e., 30, 60, and 120 min). A 30-min hardening time did not produce microcapsules, while 60- and 120-min hardening times yielded microcapsules of equal properties. Both were free-flowing granules with comparable percentage of drug content and percentage of entrapped drug. A hardening time of 30 min might not be enough to promote the cross linking of polymers on the surface. As a result, the microcapsules gelled on cooling and could not be separated by filtration. In this work, a hardening time of 60 min was suitable for the reaction of formaldehyde with the amino groups of gelatin, resulting in cross linking between the molecules of the product (18). The longer time of 120 min was not generally desirable since the microcapsule wall could deteriorate (19).

Effect of Core Concentration

The appearance, microscopic structure, and size of the microcapsules were similar when prepared using differ-

ent core concentrations (30%, 40%, and 50% w/w of vitamin A palmitate in corn oil). Figure 2 shows the effect of the core concentration on the percentage of drug content of the microcapsules when drying was by three different methods (air drying, hot air at 40°C, and freeze-drying). The use of a higher core concentration yielded a higher percentage of drug content in the microcapsule irrespective of the drying method. However, when considered in terms of the percentage of entrapped drug, the microcapsules dried by air drying or freeze-drying had the highest percentage of entrapped drug for a core concentration of 40% w/w vitamin A palmitate in corn oil (Fig. 3). In contrast, microcapsules dried by hot air at 40°C showed the lowest percentage of entrapped drug for the 40% w/w core concentration. This phenomenon seemed to be the effect of the drying method, which is discussed in the following section.

Effect of Drying Method

Although the sizes and shapes of the microcapsules dried by the three different methods were relatively similar, their physical appearances were obviously different. In general, the appearance of the microcapsules dried by air drying was that of a small yellow granule, while those dried by hot air at 40°C appeared to be brown and sheetlike. For the latter drying method, the leakage of oil usually occurred after storage. The microcapsules dried by freeze-drying showed the best appearance and were white powderlike granules.

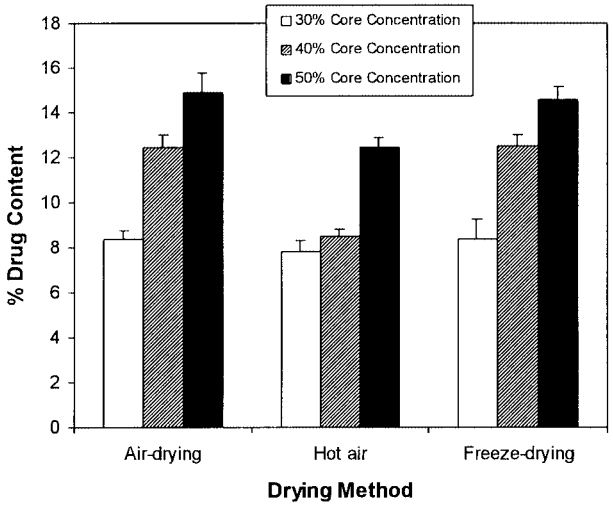


Figure 2. Effect of the core concentration on the percentage of drug content of vitamin A palmitate microcapsules dried by various drying methods.

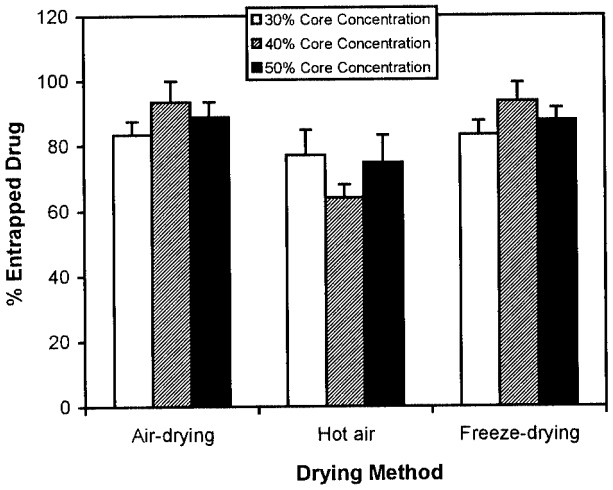


Figure 3. Effect of the core concentration on the percentage of entrapped drug for vitamin A palmitate microcapsules dried by various drying methods.

The percentage of drug content and the percentage of entrapped drug for the microcapsules that were dried by air drying were similar to the values obtained by freeze-drying, but were greater than the values from hot air drying (Figs. 2 and 3, respectively). The results might be because there was leakage of the microcapsule wall when hot air drying was employed. Although the percentage of drug content and the percentage of entrapped drug for the microcapsules dried by air drying were comparable to the values obtained by freeze-drying, the physical appearance of the microcapsules prepared by freeze-drying was better. Therefore, freeze-drying was the most suitable method for drying the microcapsules of vitamin A palmitate.

CONCLUSION

From the foregoing, it is obvious that process variables affected the properties of vitamin A palmitate microcapsules prepared by gelatin-acacia complex coacervation. The optimum conditions that provided free-flowing powders with a high percentage of entrapped drug were 1:1 gelatin-to-acacia ratio, 1:2 core-to-wall ratio, 2 ml of formaldehyde/280 g of the coacervation system as the hardening agent, 60 min of hardening time, and 40% w/w vitamin A palmitate in corn oil as the core material. The microcapsules should be dried by freeze-drying to produce the best appearance and a high percentage of entrapped drug.

The microencapsulation of vitamin A palmitate by gelatin-acacia complex is a promising method for converting an oily vitamin to solid powders for an aid to handling and ease of incorporation in preparations. In addition, protection of vitamin A palmitate with gelatin-acacia coating possibly prevents deterioration of this vitamin in the environment, thus enhancing its stability.

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