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Microencapsulation of theophylline in whey proteins: effects of core-to-wall ratio

S.J. Lee, M. Rosenberg *

Department of Food Science and Technology, University of California, Davis, CA 95616, USA

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Abstract

Effects of core-to-wall ratio ranging from 1:1.5 to 5:1.5 on formation, properties and core release from whey protein-based microcapsules containing theophylline were investigated. Microcapsules were cross-linked by glutaraldehyde-saturated toluene (GAST) in an organic phase. Size distribution of microcapsules, core content and core retention were affected by core-to-wall ratio. Proportion of small capsules was inversely related to core-to-wall ratio. Core content in microcapsules ranged from 6.7 to 65.7% (w/w) and core retention ranged from 16.8 to 85.4%. Outer topography and inner structure of microcapsules were influenced by core-to-wall ratio. Core release into simulated intestinal- and gastric-fluids was influenced by a combined effect of type of dissolution medium and core-to-wall ratio, through its influence on size, core content and structure of microcapsules. Results indicated that in order to attain a desired core content and release profile, the ratio of core-to-wall components, in suspensions consisting of whey proteins and theophylline, has to be carefully considered and adjusted.

Keywords: Core release; Microencapsulation; Theophylline; Whey proteins

1. Introduction

Whey proteins exhibit effective microencapsulating properties and have been indicated to be a suitable wall material for preparing water-soluble, spray-dried microcapsules for food applications (Moreau and Rosenberg, 1993; Rosenberg and Lee, 1993; Rosenberg and Young, 1993; Young et al., 1993a,b; Sheu and Rosenberg, 1995; Moreau and Rosenberg 1996; Rosenberg and Sheu, 1996; Rosenberg, 1997; Moreau and Rosenberg, 1998, 1999). Applications for whey proteins as wall material in microcapsules for controlled and/or sustained core release have been investigated only to a very limited extent. Heelan and Corrigan (1998) investigated encapsulation of different water-soluble core materials in whey protein-based wall system, cross-linked with aqueous glutaraldehyde. Preparation and some properties of whey protein-based, water-insoluble, model-lipid-containing microcapsules have been recently reported

* Corresponding author. Tel.: +1-530-7524682; fax: +1-530-7548145.

E-mail address: mrosenberg@ucdavis.edu (M. Rosenberg).

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(Lee and Rosenberg, 2000a,b). These microcapsules were prepared using techniques consisting of double emulsification and subsequent cross-linking with aqueous glutaraldehyde (Lee and Rosenberg, 2000b) or heat-induced gelation (Lee and Rosenberg, 2000a). Both approaches provided means to achieving high core retention and rendered the resulted microcapsule water-insoluble.

Microencapsulation of theophylline in whey protein-based capsules, cross-linked with glutaraldehyde-saturated toluene (GAST), has been recently reported by Lee and Rosenberg (1999). Their results indicated that core retention ($>$ 70%) was not affected by cross-linking conditions. Lee and Rosenberg (1999) indicated that drug release from the microcapsules was govern by diffusion-driven phenomena and did not involve solubilization or erosion of the wall matrix.

Wall-to-core ratio has been reported to significantly affect formation, properties, and drug-release characteristics of microcapsules consisting of different wall materials and theophylline (Pongpaibul et al., 1988; Thanoo et al., 1992; Latha and Jayakrishnan, 1994; Wu et al., 1994). Results of these studied indicated increase in capsule size and core retention with increase in core-to-wall ratio that also resulted in a wall-material-specific effect on core release. Effects of wall-to-core ratio on formation and properties of microcapsules consisting of whey proteins and water-soluble drug have not been reported yet. Objectives of our research were therefore to investigate the influence of core-to-wall ratio on formation, physical properties, and core release characteristics of GAST-cross-linked whey protein-based microcapsules containing theophylline, as a model water-soluble drug.

2. Materials and methods

2.1. Wall and core materials

Whey protein isolate (WPI) containing 95.6% protein (Davisco Foods International, Inc., Eden Prairie, MN) was used as wall materials. Theophylline (Sigma Chemical Co., St. Louis, MO) was used as a model water-soluble core.

2.2. Preparation of microcapsules

Microcapsules were prepared using a modification of the method reported by Lee and Rosenberg (1999). Core-in-wall suspensions (CIWS) were prepared by dispersing 1.6, 3.2, 4.8, 6.4, or 8.0 g of theophylline in 12 g of 20% (w/w) WPI solution (pH 7.0), prepared in de-ionized water. The resulting CIWS had a core-to-wall solids mass ratio of 1:1.5, 2:1.5, 3:1.5, 4:1.5 and 5:1.5, respectively, and were denoted A, B, C, D, and E, respectively. In all cases, microcapsules were prepared using 12 g of CIWS and cross-linking was carried out for 1 h at 25°C and 250 rpm using 30 ml of GAST. In all cases, a pre-cross-linking dispersing stage of 2 min (250 rpm, 25°C) was used. Cross-linked microcapsules were treated as described by Lee and Rosenberg (1999).

The proportion of large capsules (diameter >700 μm), medium-size capsules (diameter 450–700 μm) and small microcapsules (diameter <450 μm) was determined as described by Lee and Rosenberg (1999). Microcapsules were denoted identical to the CIWS used (A–E).

2.3. Theophylline content and retention

Theophylline content of microcapsules included in each size category, total core content and core retention were determined using the procedures reported by Lee and Rosenberg (1999). Theophylline retention was expressed as the ratio (in %) of core content found in microcapsules to a theoretical core content, assuming 100% core retention during the microencapsulation process.

2.4. Core release

Core release, from 5.5 mg microcapsules, into enzyme-free simulated intestinal fluid (SIF) or enzyme-free simulated gastric fluid (SGF) was investigated at sink conditions, at 37°C and 100 rpm as reported previously by Lee and Rosenberg (1999).

2.5. Structure analysis

Outer topography and inner structure of microcapsules were investigated by SEM using the procedures previously reported by Rosenberg and Young (1993) and by Lee and Rosenberg (1999).

2.6. Statistical analysis

Experiments were replicated and analyses were carried out in triplicate ($n = 6$). The significance of the results (at $P < 0.05$) was tested by t-test or ANOVA using the SigmaStat software (Jandel Scientific Software, San Rafael, CA).

3. Results and discussion

3.1. Structure and size distribution of microcapsules

Outer topography and inner structure of microcapsules prepared with the different CIWS is presented in Figs. 1 and 2A,B and in Figs. 2C–F, respectively. The spherical microcapsules (150 μm to ~1 mm in diameter) exhibited some surface cracks, the occurrence of which was promoted with increase in core-to-wall ratio. Cross-linking conditions were much milder than those indicated by Lee and Rosenberg (1999) to promote formation of surface crack and thus the formation of these features, in microcapsules investigated in the present study, was attributed to effect of wall-to-core ratio. Surface of core-containing microcapsules exhibited irregular structural features that represented footprint of surface-core crystals that were removed from the surface of the cross-linked capsules during the washing stage (Lee and Rosenberg, 1999).

The thickness of wall matrix layers separating core domains decreased with increase in the core-to-wall ratio (Figs. 2C–F and 3). It could thus be suggested that at the constently maintained wall solution composition (and amount) and cross-linking conditions, the fragility of the matrix increased with increase in core-to-wall ratio. This, and the increase in number of core crystals embedded, at different orientations, in the wet wall matrix have probably led to a higher mechanical stress development during the drying stage of the process. Such stress development could explain the apparent increase in surface cracks formation with increase in core-to-load ratio. Results (Fig. 3B,C) indicated that surface cracks did not propagate deeply into the microcapsules but were limited to the outer layers of the wall matrix, thus supporting the above assumption regarding their origin.

The number of core crystals that were embedded in the voids-free dense wall matrix of (mostly small) microcapsules prepared with CIWS A and B (Fig. 2C and D, respectively) was significantly lower than that in capsules prepared with CIWS C–E (Figs. 2E,F and 3) thus, indicating poor core retention. The absence of empty core domains in these capsules (Fig. 2C and D) indicated that the significant core loss occurred prior to the cross-linking stage. This was also supported by our visual observations made during microencapsulation that indicated significant core losses from CIWS A and, to a lesser extent, from CIWS B into the organic phase, prior to cross-linking.

In general, the number of core crystals embedded in wall matrices was proportionally related to the core-to-wall ratio. Results (2C–F and 3) indicated that in all cases where core was retained in microcapsules, its crystals were embedded throughout the wall matrix, similar to what has been reported by Lee and Rosenberg (1999). Inner surfaces of voids from which core crystals had fallen out during specimen preparation were smooth (Fig. 3E,F) and thus indicated that core crystals were physically entrapped in the protein-based matrix.

Size distribution of microcapsules was significantly affected by core-to-wall ratio (Fig. 4). The proportion of large microcapsules increased with increase in the core-to-wall ratio. Microcapsules prepared with CIWS A and B exhibited similar size-distribution ($P > 0.05$) that differed ($P < 0.05$) from that of microcapsules prepared with any other CIWS. About 95% of the microcapsules prepared at core-to-wall ratio 1:1.5 and 2:1.5 (CIWS A and B) were smaller than 450 μm. In contrast, 85% of the microcapsules prepared at core-to-wall ratio of 5:1.5 (CIWS E) were larger

than 700 μm. The proportion of large and medium-size capsules in batches prepared with CIWS D was 7.5 and 1.9 times higher than that in batches prepared with CIWS C, respectively. Similar ($P > 0.05$) proportions of small- and medium-size microcapsules were found in batches prepared with CIWS D. These proportions were ~1.9 times smaller than those observed in batches prepared with CIWS C.

Effect of core-to-wall ratio on size distribution of microcapsules could be attributed to the significant increase in viscosity of CIWS with increase in the core-to-wall ratio that was observed by us. This increase was due to formation of aggregates

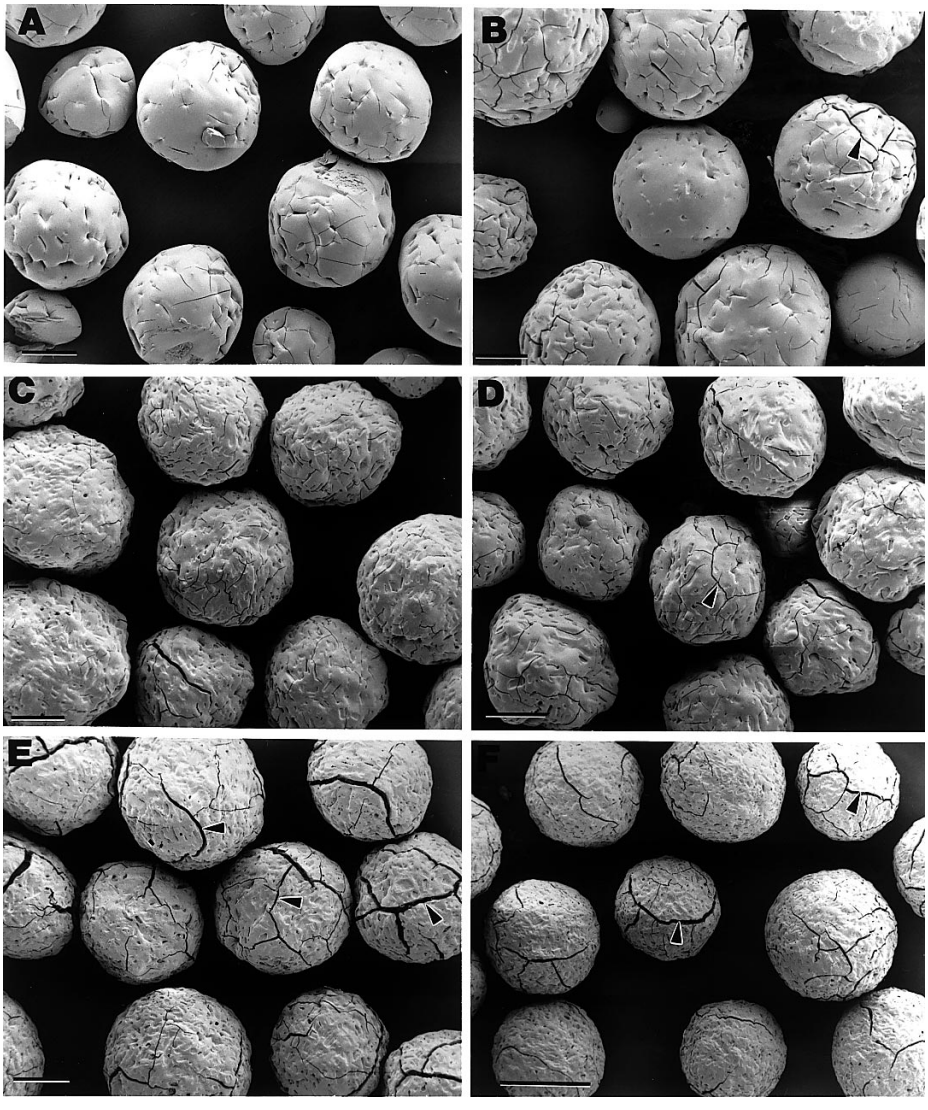

Fig. 1. Outer topography of theophylline-loaded whey protein-based microcapsules. Micrographs A, B, and D: small-size microcapsules prepared with CIWS A, B, and C, respectively. Micrographs C and E: medium-size capsules prepared with CIWS C and D, respectively. Micrograph F: large capsules prepared with CIWS D. Arrowhead, surface cracks. Bar = 100 μm (A, B), 200 μm (C–E), 500 μm (F).

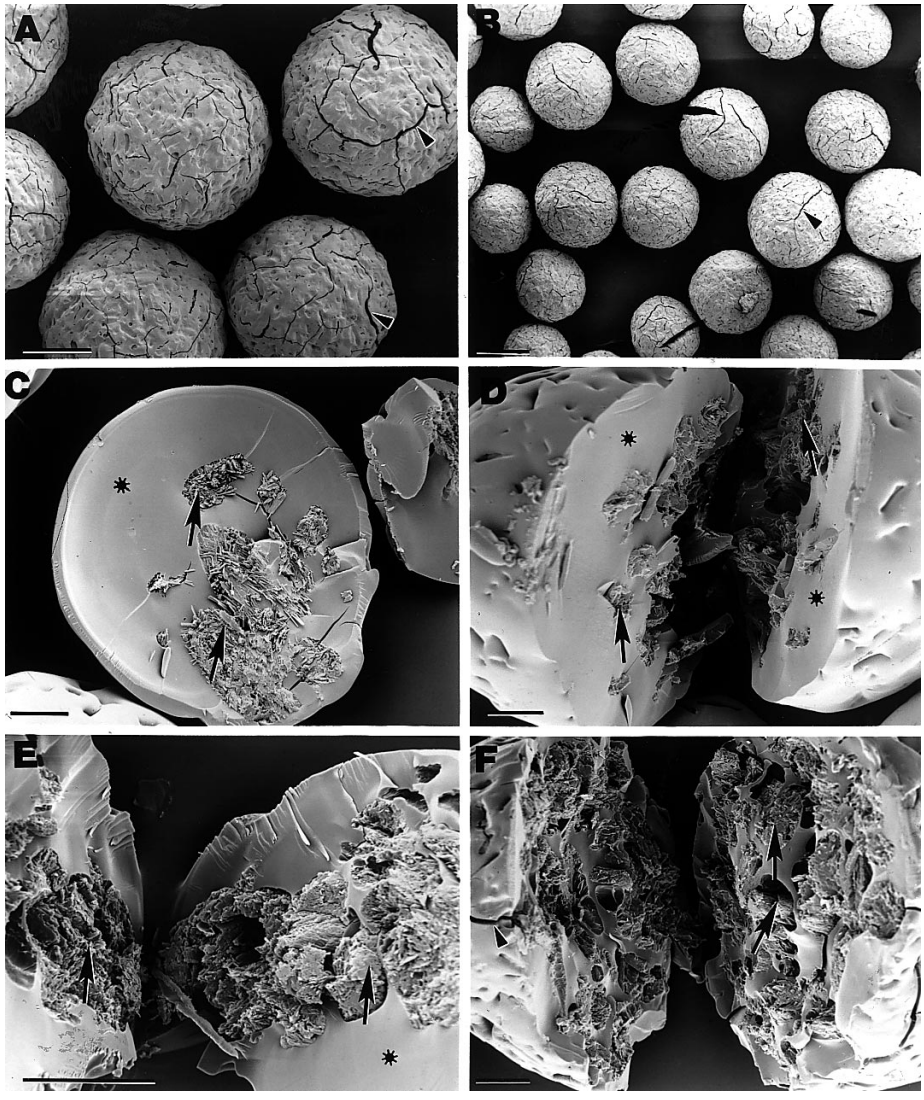

Fig. 2. Outer topography of medium-size and large microcapsules prepared with CIWS E (micrographs A and B, respectively) and inner structure of small capsules prepared with CIWS A, B, C, and D (micrographs C–F, respectively). Arrowheads, surface cracks; arrows, core crystals; asterisk, wall matrix. Bar = 50 μm (C–F), 200 μm (A), 500 μm (B).

consisting of core crystals held together by the whey protein solution. Results suggested that below a certain core load, viscosity of CIWS, and probably cohesive forces in this suspension, were too low and thus core crystals were not maintained within the wet CIWS droplets. Similar effects of core content on viscosity of CIWS and consequently on capsules size have been reported previously for other CIWS (Pongpaibul et al., 1988; Latha and Jayakrishnan, 1994; Wu et al., 1994; Katti and Krishnamuri, 1999). At a given stirring conditions prior to cross-linking, formation of core crystals aggregates and thus increase in viscosity of CIWS resulted in a significant increase in proportion of larger CIWS droplets. Core content in these droplets (wet, uncross-linked capsules) was higher than that included in wet capsules prepared at a lower core-to-wall

ratio. Judging by the proportion of medium- and large-size capsules, a core-to-wall ratio of 3:1.5 seemed to be critical in the above respect. These results were supported by the above-discussed structure analysis.

The extent to which core-to-wall ratio affected size distribution of microcapsules in our study, differed significantly from that reported for other theophylline-containing systems. For example, Latha and Jayakrishnan (1994) reported, for theophylline-containing GAST-cross-linked casein-based capsules, that proportion of large, medium-size and small capsules in batches prepared with CIWS containing 66, 60 and 50% theophylline was 42, 48 and 9.6%; 30.7, 63.8 and 5.5%; and 14.6, 70 and 15.4%, respectively. Results of Latha and Jayakrishnan (1994) indicated increase in proportion of large capsule with in-

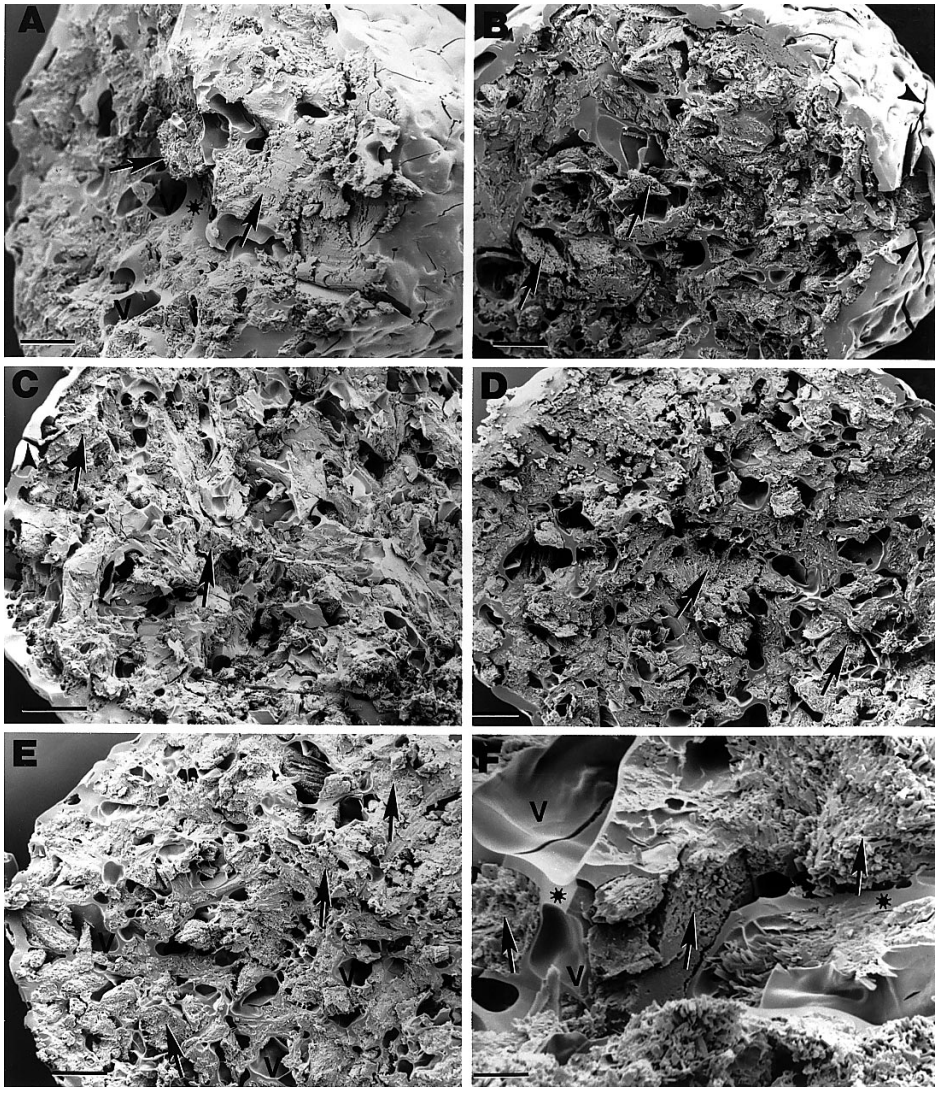

Fig. 3. Micrographs A, B and D: inner structure of medium-size capsules prepared with CIWS A, B and D, respectively. Micrographs C, E and F: inner structure of capsules prepared with CIWS D, E and F, respectively. Arrowheads, surface cracks; arrows, core crystals; asterisk, wall matrix; V, empty core domains (see text). Bar = 10 μm (F), 50 μm (A, B, D, and F), 100 μm (C).

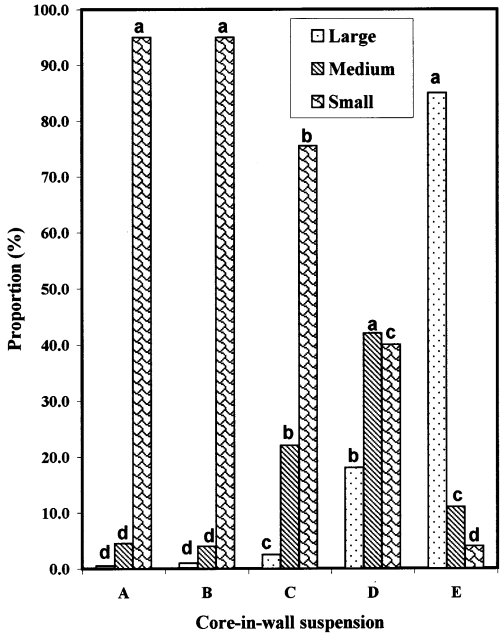

Fig. 4. Proportion of small (< 500 μm), medium-size (500–700 μm) and large (> 700 μm) microcapsules in microcapsule batches with different CIWS. For a given size category, bars denoted with different lowercase letters are significantly different ($P < 0.05$).

crease in core-to-wall ratio, however, regardless of this ratio, most of the microcapsules were of medium size (400–700 μm). Differences between our results and those of Latha and Jayakrishnan (1994) could probably be attributed to significant differences between the viscosity of 20% casein and that of 20% WPI solutions and hence differences in the viscosities of resulting CIWS.

3.2. Core content and retention

Total core content of washed and dried microcapsules increased ($P < 0.05$) with core-to-wall ratio of the CIWS (Table 1) and ranged from 6.7 to 65.7% (w/w). Except for large and medium-size capsules prepared with CIWS E, core content in microcapsules prepared at a given core-to-wall ratio was proportionally related to microcapsule size (Table 1). For example, core content of medium-size microcapsules prepared at a core-to-wall ratio of 3:1.5 (CIWS C) was 25% higher than that in the small microcapsules prepared with the same CIWS; core content in large microcapsules prepared at a core-to-wall ratio of 4:1.5 (CIWS D) was 7.5% and 25.2% higher than that in medium-size and small microcapsules, respectively.

In general, core retention during microencapsulation increased with core-to-wall ratio in the CIWS and was affected by size-distribution of microcapsules (Fig. 5). The overall lowest core retention (16.75%) was obtained with CIWS A and the highest core retention (85.41%) was obtained with CIWS E. For microcapsules prepared with CIWS C-E, where no core losses to the dispersing solvent were evident, core losses could be mainly attributed to effects of the washing stage (Lee and Rosenberg, 1999). In these cases, core retention reflected the effects of core-to-wall ratio on core content included in wet microcapsules and on capsules size and thus on their surface-to-volume ratio.

Table 1
Theophylline content in microcapsules prepared at different core-to-wall ratios[a]

CIWS (core-to-wall ratio)[b]	Theophylline (%, w/w)			Overall content of Theophylline (%, w/w)
	Large[c]	Medium	Small	
A (1:1.5)	–	–	6.7^{D}	6.7^{E}
B (2:1.5)	-	–	14.8^{C}	14.8^{D}
C (3:1.5)	–	53.5^{B}	42.7^{B}	44.0^{C}
D (4:1.5)	57.1^{B}	53.0^{B}	45.5^{B}	50.7^{B}
E (5:1.5)	66.2^{A}*	64.3^{A}*	57.9^{A}	65.7^{A}

[a] ABCD — given columns followed by different superscript letters are significantly different ($P < 0.05$).
[b] See text for composition of CIWS.
[c] Large-, medium- and small-size microcapsules.
* A given row followed by an asterisk is not significantly different ($P > 0.05$).

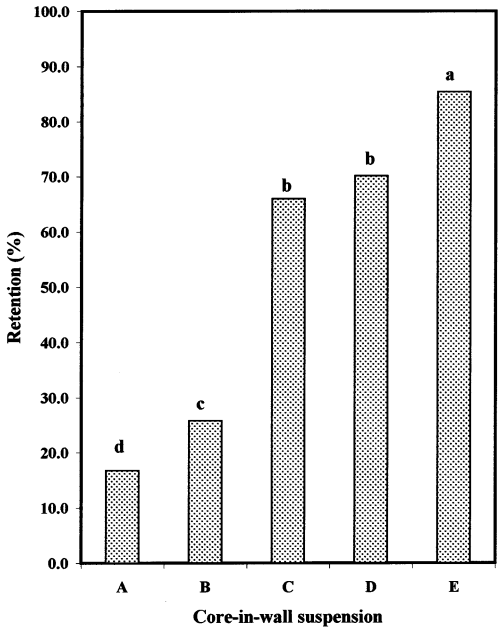

Fig. 5. Core retention during microencapsulation obtained with different CIWS. Bars denoted with different lowercase letters are significantly different ($P < 0.05$).

The initial core load in CIWS B was twice that included in CIWS A however, the effect of initial core load on core retention was relatively small and $\sim 75\%$ the drug was lost, most of it probably prior to cross-linking. The difference between core content included in CIWS C and B was identical to that between CIWS A and CIWS B, however, core retention obtained with CIWS C was 2.5 times higher than that obtained with CIWS B. Proportion of medium-size capsules prepared with CIWS C was 5.5 times higher than that in capsules prepared with CIWS B. Results obtained with CIWS C could thus be attributed to the fact the core losses to the dispersing solvent (prior to cross-linking) did not occur and to the overall smaller surface area of the microcapsules that promoted core retention during washing. High core retention (70–85.4%) was obtained at initial core-to-wall ratio $> 3{:}1.5$ and could be mainly attributed to the effect of core-to-wall ratio on size of microcapsules and thus on the surface-to-volume ratio of the microcapsules. The latter significantly effected diffusion-governed core losses during the washing stage of the microencapsulation process. Similar effect of microcapsules size distribution on core retention could explain core retention obtained with CIWS D and CIWS E (Figs. 4 and 5).

Decreasing the wall-to-core solids from 1:0.66, in CIWS A, to 1:3.33, in CIWS E, did not adversely affect core retention. Core retention obtained with CIWS A and C was lower than, and that obtained with CIWS E was slightly higher than that reported (for comparable initial core load) by Thanoo et al. (1992). Theophylline retention obtained with CIWS B and C was lower than that that was reported, for comparable core load, by (Latha and Jayakrishnan, 1994) In all cases, core retention obtained in our study was significantly higher than that reported by Heelan and Corrigan (1998).

3.3. Theophylline release from microcapsules

Core release from microcapsules was time-dependent and was affected by a combined influence of type of dissolution medium, core content and size of microcapsules (Figs. 6–8). Core release into SIF was significantly faster than that into SGF (Figs. 6–8) and thus reflected differences in the extent to which capsules swelled in the two media (Latha and Jayakrishnan, 1994; Heelan and Corrigan, 1998; Lee and Rosenberg, 1999). For example, complete core release from large microcapsules prepared with CIWS D and CIWS E into SGF was 1.4 and 1.5 times slower than that into SIF, respectively (Fig. 6). The differences between rates of core release into the two dissolution media were inversely related to the initial core-in-wall ratio in CIWS and thus to the ultimate core content in microcapsules. This reflected the influence of core-to-wall ratio on the proportion of wall components, per unit mass of microcapsules, that was available for interaction with the dissolution medium and thus on swelling.

Results obtained with small microcapsules (Fig. 8) indicated that although rate of core release was affected by core content, the influence of the surface area-to-volume ratio on core release was more profound than that of core load. In general, core release from small microcapsules was com-

pleted, in most cases, within less than 1h, thus, suggesting no practical potential for these capsules.

Core release from large and medium-size capsules prepared with CIWS C and CIWS D into SIF and SGF was ~12 and 33% shorter than that from medium-size capsules prepared with CIWS E, respectively (Fig. 7). These results reflected the influence of ~20% difference in core content between these microcapsules (Table 1). Similarly, core content of large microcapsules prepared with CIWS E was ~16% higher than that of large capsules prepared with CIWS D and the time needed for complete core release from the former was ~50% longer than that from the latter.

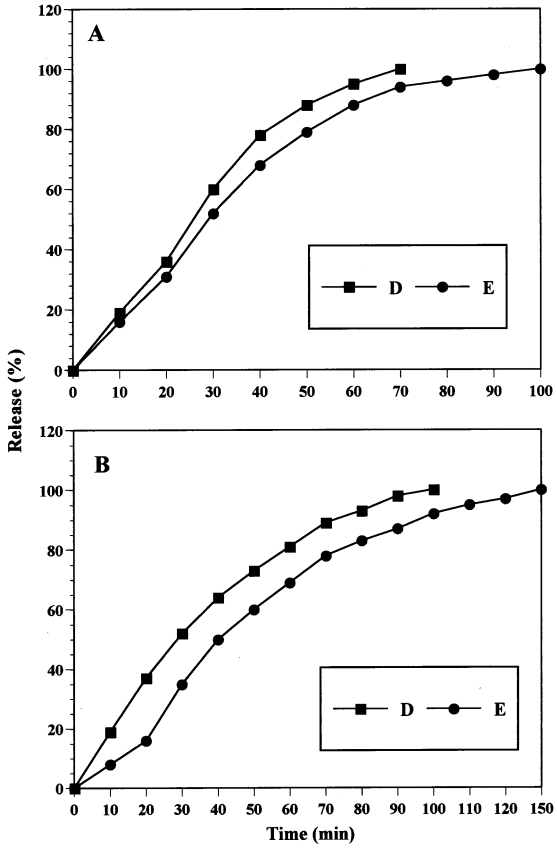

Fig. 6. In vitro core release from large microcapsules prepared with CIWS D and E into enzyme-free SIF (A) and enzyme-free SGF (B) at 37°C.

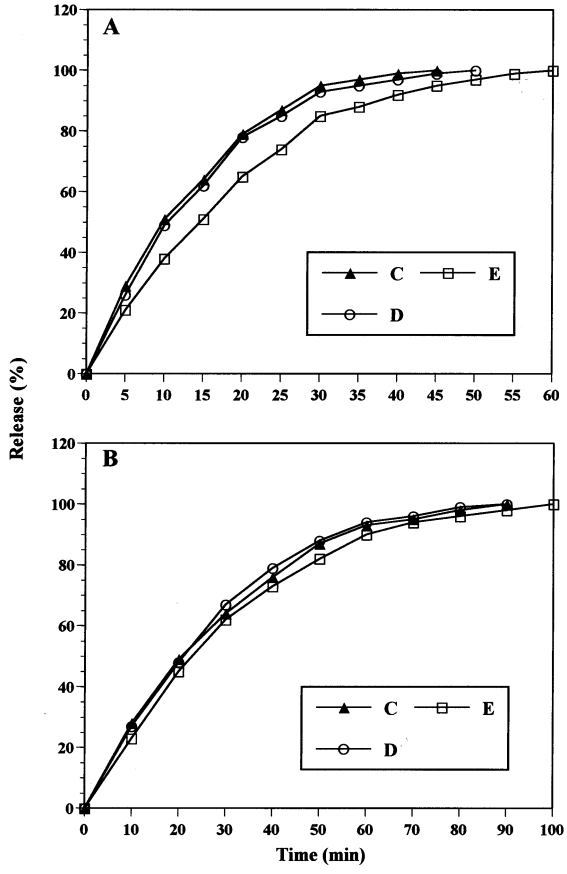

Fig. 7. In vitro core release from medium-size microcapsules prepared with CIWS C–E into enzyme-free SIF (A) and enzyme-free SGF (B) at 37°C.

Microcapsules of different size, prepared from a given CIWS, exhibited different core release profiles that reflected the influence of core-to-wall ratio on rate of core release through its influence on both core content and particle size. For example, core content of large microcapsules prepared with CIWS E was 14% higher than that of small capsules prepared with the same CIWS (Table 1). The time needed for complete core release from the large capsules into SIF and SGF was 2.5 and 2.15 times longer than that from the small microcapsules. However, studying core release from small and medium-size microcapsules prepared with CIWS C indicated that although core content of the latter was 25% higher than that of the former, core release from the small capsules was

only 1.3–1.5 times faster than that from the medium-size capsules (Figs. 7 and 8).

Overall, the influence of core content on rate of core release was much more significant in large capsules than in smaller microcapsules. This result suggested that the influence of the surface area–to-volume ratio on rate of core release was more significant than that of core content. The relationships between core-to-wall ratio and core release identified in our study were similar to those reported by Pongpaibul et al. (1988), however, for comparable core-to-wall ratios, rate of core release observed in our study was faster. Our results agreed with those reported by Wu et al. (1994) who reported on theophylline release from ethylcellulose-based capsules. Our results differed from

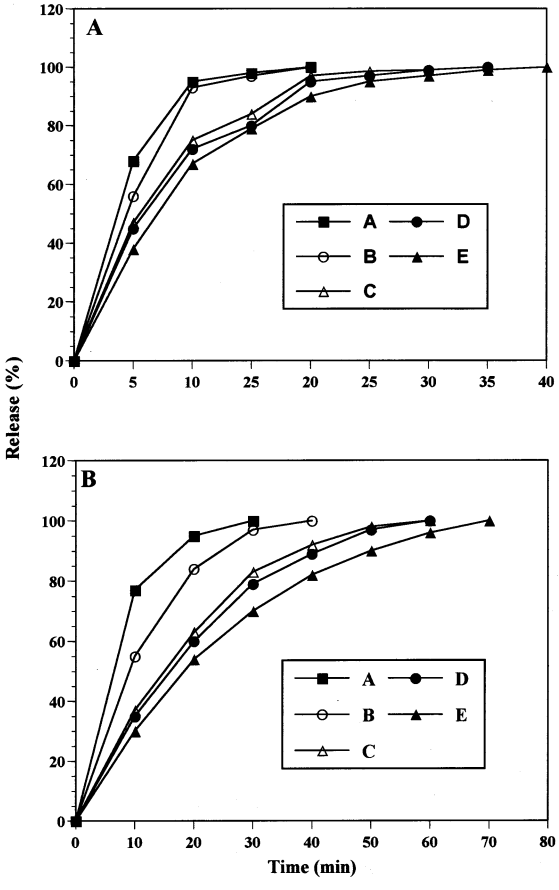

Fig. 8. In vitro core release from small microcapsules prepared with CIWS A–E into enzyme-free SIF (A) and enzyme-free SGF (B) at 37°C.

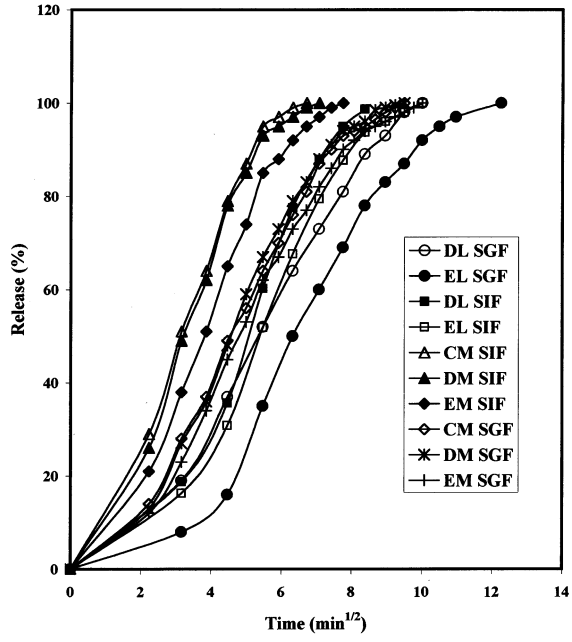

Fig. 9. Theophylline release (at 37°C) vs. square root of time in SGF or SIF from medium-size (M) and large (L) microcapsules prepared with CIWS C–E. In all cases, mean values ($n = 6$) are presented.

those of Thanoo et al. (1992) who reported that release of theophylline from GAST-cross-linked chitosan capsules was not affected by core-to-wall ratio. Effect of core-to-wall ratio on core release observed in our study was more significant than that reported by Latha and Jayakrishnan (1994) for theophylline release from casein microcapsules.

Higuchi plots (Higuchi, 1963) prepared with results obtained with large and medium-size microcapsules (Fig. 9) indicated that in all cases, plots were characterized by two linear segments, which differed in their slope, followed by a non-linear segment, where the Higuchi model was no longer valid. The first linear segment described release of 10–20% of total core content and reflected the initial hydration and swelling of microcapsule. Differences between the slopes of the two linear segments of the release plot obtained with SGF were larger than those obtained with SIF and were more pronounced for large capsules

than for smaller ones. This could be attributed to the significant effect of both type of dissolution medium and microcapsules size on swelling. Following the swelling stage, core release into both SIF and SGF could be described by the linear function of the Higuchi model ($R^2 = 0.96–0.98$) that was valid during up to 80–95% of the time needed for complete core release. Differences in slope of the second linear segment of the plots obtained with different microcapsules could be attributed to effect of both core-to-wall ratio and type of dissolution medium on core release. Deviation from linearity that was observed during final stages of core release, indicated effects of liquid-filled pores and empty core domains on mass transport phenomena that governed final stages of core release (Lee and Rosenberg, 1999).

With increase in core-to-wall ratio, the number and overall volume of core domains per unit mass of microcapsules increased. This could have led, in potential, to an early and more profound effect of liquid-filled empty core domains on core release profile. However, this was apparent only with large microcapsules prepared with the highest core-to-wall ratio (CIWS E). Results thus indicated that regardless of core-to-wall ratio, similar phenomena governed core release.

4. Conclusion

Physical and functional properties of whey protein-based microcapsules containing theophylline were significantly affected by the core-in-wall ratio. Core retention and microcapsules size increased with increase in core-to-wall ratio. Outer topography and inner structure of microcapsules were also influenced by core-to-wall ratio. Results indicated that through its effects on structure, size and core content of microcapsules, core-to-wall ratio significantly affected rate of core release from microcapsules. However, regardless of core-to-wall ratio, drug release was governed by the same diffusion-driven phenomena.

References

Heelan, B.A., Corrigan, O.I., 1998. Preparation and evaluation of microspheres prepared from whey protein isolate. J. Microencap. 15, 93–105.

Higuchi, T., 1963. Mechanism of sustained action medication. J. Pharm. Sci. 52, 1145–1149.

Katti, D., Krishnamuri, N., 1999. Preparation of albumin microspheres by an improved process. J. Microencap. 16, 231–242.

Latha, M.S., Jayakrishnan, A., 1994. Glutaraldehyde cross-linked bovine casein microspheres as a matrix for the controlled release of theophylline: in-vitro studies. J. Pharm. Pharmacol. 46, 8–13.

Lee, S.J., Rosenberg, M., 1999. Preparation and properties of glutaraldehyde cross-linked whey protein-based microcapsules containing theophylline. J. Controlled Release 61, 123–136.

Lee, S.L., Rosenberg, M., 2000a. Whey protein-based microcapsules prepared by double emulsification and heat gelation. Food Sci. Technol. J. (LWT) 33, 80–88.

Lee, S.J., Rosenberg, M., 2000b. Preparation and some properties of water-insoluble, whey protein-based microcapsules. J. Microencap. 17, 29–44.

Moreau, D.L., Rosenberg, M., 1993. Microstructure and fat extractability in microcapsules based on whey proteins or mixtures of whey proteins and lactose. Food Struct. 12, 457–468.

Moreau, D.L., Rosenberg, M., 1996. Oxidative stability of anhydrous milkfat microencapsulated in whey proteins. J. Food Sci. 61, 39–43.

Moreau, D.L., Rosenberg, M., 1998. Porosity of whey protein-based microcapsules containing anhydrous milkfat measured by gas displacement pycnometry. J. Food Sci. 63, 819–823.

Moreau, D.L., Rosenberg, M., 1999. Porosity of microcapsules with wall systems consisting of whey proteins and lactose measured by gas displacement pycnometry. J. Food Sci. 64, 405–409.

Pongpaibul, Y., Maruyama, K., Iwatsuru, M., 1988. Formation and in-vitro evaluation of theophylline-loaded poly(-methyl methacrylate) microspheres. J. Pharm. Pharmacol. 40, 530–533.

Rosenberg, M., 1997. Milk derived whey protein-based microencapsulating agents and a method of use. US Patent 5 601 760.

Rosenberg, M., Lee, S.Y., 1993. Microstructure of anhydrous milkfat/whey proteins emulsions. Food Struct. 12, 267–274.

Rosenberg, M., Sheu, T.Y., 1996. Microencapsulation of volatiles by spray drying in whey protein-based wall systems. Int. Dairy J. 6, 273–284.

Rosenberg, M., Young, S.L., 1993. Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat — structure evaluation. Food Struct. 12, 31–41.

Sheu, T.Y., Rosenberg, M., 1995. Microencapsulation by spray-drying ethyl caprylate in whey protein and carbohydrate wall systems. J. Food Sci. 60, 98–103.

Thanoo, B.C., Sunny, M.C., Jayakrishnan, A., 1992. Cross-linked chitosan microspheres: preparation and evaluation as a matrix for the controlled release of pharmaceuticals. J. Pharm. Pharmacol. 44, 283–286.

Wu, J.-C., Chen, H.-R., Chen, H., 1994. Studies on properties of ethylcellulose microcapsules prepared by emulsion non-solvent addition method in the presence of non-solvent in polymer solution. J. Microencap. 11, 519–529.

Young, S.L., Sarda, X., Rosenberg, M., 1993a. Microencapsulating properties of whey proteins. 1. Microencapsulation of anhydrous milk fat. J. Dairy Sci. 76, 2868–2877.

Young, S.L., Sarda, X., Rosenberg, M., 1993b. Microencapsulating properties of whey proteins. 2. Combination of whey proteins with carbohydrates. J. Dairy Sci. 76, 2878–2885.