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# The Influence of Surface Properties on Uptake of Oil into Complex Coacervate Microcapsules

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**Abstract**—A range of surfactants with different hydrophile-lipophile balance (HLB) values was selected to investigate the influence of interfacial properties on the uptake of oil droplets into complex coacervate microcapsules. The well characterized gelatin/acacia complex coacervate system was used in this study and the encapsulation of squalane, and oleic acid was investigated. The surfactants investigated were Span 85, Span 80, Span 40, egg yolk lecithin, and Tween 80. Combinations of surfactants were utilized to obtain intermediate HLB values. The percentage oil encapsulated was determined gravimetrically, based on the initial concentration and the amount extracted from the microcapsules. The aqueous interfacial tension values of the oils and oil/surfactant systems were measured using the Wilhelmy plate method. The interfacial properties were correlated to the percentage oil uptake by the coacervate phase. The relative hydrophobicity/lipophilicity of the oil influenced its uptake by complex coacervate droplets. The presence of surfactant affected oil uptake, depending on the HLB value of the surfactant or surfactant mixture. Uptake of squalane by the gelatin/acacia coacervates was found to be optimized by the addition of surfactants with HLB values in the range 2·5–6. The percentage uptake of oil decreased rapidly for systems prepared containing surfactants with HLB values outside this range. No correlation was observed between oil uptake by the coacervate phase and the interfacial tension of the oil and oil/surfactant systems with double-distilled deionized water.

Complex coacervation is a common method of microencapsulation (Bungenberg de Jong 1949; Nixon 1976; Nixon & Nouh 1978; Burgess & Carless 1985). This process is the separation of a mixture of two oppositely charged polyions into two distinct phases: a dense coacervate phase, which is relatively concentrated in the polyions and a dilute equilibrium phase (Bungenberg de Jong 1949). On dispersion of coacervate and equilibrium phases, coacervate droplets form which can be cross-linked or gelled to form microcapsules. The potential of coacervate systems to encapsulate materials was first noted by Bungenberg de Jong (1949), who showed that suspended organic liquid droplets or solid particles were taken up by coacervate droplets. Complex coacervate encapsulation of oils has been studied by a number of investigators (Jalsenjak et al 1987; Broda & Chuchracki 1987; Rozenblat et al 1989; Arneodo et al 1988).

The encapsulation of oils has been investigated for various reasons: to protect susceptible oils from oxidative decomposition; to protect volatile oils from evaporation; and to aid in the encapsulation of drug substances. Oil-soluble drugs can be dissolved and water-soluble drugs suspended in oil before encapsulation of the oil. The encapsulation of drugs is useful for the purposes of controlled and sustained drug delivery, taste masking, separation of incompatible substances (other drugs or excipients, such as buffers), protection of unstable drugs from the environment (moisture, light, heat, and oxidation), safe handling of toxic substances, conversion of liquids to solids, and aid in the dispersion of water-insoluble substances in aqueous media (Nixon 1976).

Characterization of the properties of oil-in-water (o/w) emulsion systems before complex coacervation is necessary to understand this encapsulation process. Investigations in our laboratory involving a wide range of materials have indicated that the surface properties of oils, in particular their relative hydrophobicity, significantly influence coacervate uptake. To investigate this encapsulation process the well characterized gelatin/acacia complex coacervate system and a series of oils and surfactants with differing HLB and interfacial tension values were selected: squalane, oleic acid, lecithin, Tween 80, Span 85, Span 80 and Span 40 (Table 1) (Martin et al 1983; King & Schwartz 1985). Squalane is a hydrocarbon with no hydrophilic groups and, therefore, no HLB value is available for this material. Oleic acid has an HLB value of 1. The HLB value of the surface-active material is a measure of its relative hydrophobicity/lipophilicity. The relative hydrophobicity/lipophilicity of the interface of oil droplets will affect their uptake into coacervate systems, as it has been established that coacervates take up relatively hydrophobic materials (Bungenberg de Jong 1949). Surfactants will adsorb at the interface of the oil droplets and change the relative hydrophobicity/lipophilicity of the interface.

A reduction in interfacial free energy ($\Delta E$) is considered to be a driving force in the coacervate droplet/core-material interaction (Donbrow 1992), and is influenced by the net interfacial area change ($\Delta A$) and the net interfacial energy change ($\Delta\gamma$), according to the equation:

$$\Delta E = \Delta\gamma \Delta A \quad (1)$$

It follows that increases in the interfacial area of the core material and the coacervate droplets will enhance uptake,

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Table 1. HLB values of surfactants used*.

| Surfactant | HLB value |
|---|---|
| Span 85 | 1·8 |
| Span 80 | 4·3 |
| Span 40 | 6·7 |
| Egg yolk lecithin | 8·0 |
| Tween 80 | 15·0 |

* Data obtained from Martin et al (1983) and King & Schwartz (1985).

and that the addition of surfactant will reduce uptake as the presence of molecules at the interface will decrease the y term. The coacervate (polymer-rich) phase must have a greater affinity for the core material than the equilibrium (dilute) phase in order for the coacervate phase to wet the core material. Wetting will be affected by the interfacial tension and the contact angle at the core material interface with the two coacervate phases, according to Young's equation:

$$\gamma_{CL1} = \gamma_{CL2} + \gamma_{L1L2} \cos\theta \quad (2)$$

where C is the core material, L1 is the equilibrium (dilute) phase, L2 is the coacervate phase, $\gamma_{CL1}$ is the interfacial tension between the core material and the equilibrium phase, $\gamma_{CL2}$ is the interfacial tension between the core material and the coacervate phase, and $\gamma_{L1L2}$ is the interfacial tension between the equilibrium phase and the coacervate phase.

Interfacial tension and HLB values were therefore selected as parameters to study oil uptake by the coacervate systems. The percentage encapsulation of various oils and oil/surfactant mixtures was investigated.

## Materials and Methods

Type A (acid-processed) gelatin was obtained from Gelatine Products Ltd, Sutton Weaver, Cheshire, UK. Acacia, silica gel (100 mesh) and fluorosil (100 mesh) were obtained from Fisher Scientific, USA. The optimum pH value for maximum coacervation was determined to be pH 3·9, using a micro-electrophoresis method (Burgess & Carless 1984). All gelatin and acacia solutions were deionized before coacervation by mixing with Amberlite resins IRA-400 and IR-120 for 30 min at 40°C (Janus et al 1951). Squalane, oleic acid, Span 85, Span 80, Span 40 and Tween 80 were obtained from Sigma Chemicals Co., USA. Egg yolk lecithin (egg phosphatide, egg lecithin) was obtained from Asahi Chemical Industry Co., Japan.

Single-distilled deionized water was used in the préparation of complex coacervate microcapsules. Deionized, double-distilled water from permanganate solution was used in the interfacial tension studies.

*Purification of oils*

Oils were passed three times through a column containing a 1:1 mixture of fluorosil (100 mesh) and silica gel (100 mesh) before use. Purification was checked by measurement of surface tension against time (4 h). No decay in the surface tension values indicated purity.

*Preparation of microcapsules*

Oil-in-water emulsions were prepared by dispersing the oil in deionized, 2% w/v acacia solutions at 40°C and 1300 rev $min^{-1}$ using a Fisher Dyna Mix (Fisher Scientific, USA) for 30 min. To these mixtures, deionized 2% w/v gelatin solutions were added, the pH was reduced to 3·9 by the addition of 0·2 M HCl and the stirring speed was reduced to 300 rev $min^{-1}$. The resultant coacervate systems were stirred at 40°C for 45 min and cooled to 5°C with continuous stirring to form microcapsules by gelling the coacervate phase. The microcapsules were isolated by decantation and washed once with distilled water, three times with methanol and once with isopropanol, all at 5°C. The microcapsules were then dried at room temperature (25°C).

Table 2. Surfactant mixtures used to obtain the different HLB values.

| Surfactant system | % | HLB value |
|---|---|---|
| Span 85 | 100 | 1·8 |
| Span 85 + Span 80 | 72 + 28 | 2·5 |
| Span 85 + Span 80 | 52 + 48 | 3·0 |
| Span 85 + Span 80 | 12 + 88 | 4·0 |
| Span 80 | 100 | 4·3 |
| Span 80 + Span 40 | 71 + 29 | 5·0 |
| Span 80 + Span 40 | 29 + 71 | 6·0 |
| Span 40 | 100 | 6·7 |
| Egg yolk lecithin | 100 | 8·0 |
| Egg yolk lecithin + Tween 80 | 93 + 7 | 8·5 |
| Egg yolk lecithin + Tween 80 | 71 + 29 | 10·0 |
| Egg yolk lecithin + Tween 80 | 43 + 57 | 12·0 |
| Tween 80 | 100 | 15·0 |

Surfactants, when used, were added before the emsulsification step to either the oil or water phase, depending on their relative solubilities, at a concentration of $1 \times 10^{-2}$ mol surfactant per litre of solution. Lecithin was added in the form of micelles to the water phase. In order to obtain intermediate HLB values, mixtures of surfactants were used at a concentration of $1 \times 10^{-2}$ mol total surfactant per litre of solution. The surfactants were mixed in different ratios on a molar basis, for ease of calculation of the HLB value of the mixture (Table 2).

*Determination of oil content*

Known quantities of washed, dry microcapsules (3·0 g) were mechanically crushed using a mortar and pestle for 5 min. The mortar was then scraped to remove microcapsules from the sides and this procedure was repeated thrice. The oil was extracted from the crushed microcapsules using 30 mL hexane. The crushed microcapsule/hexane mixture was filtered and after evaporation of the solvent the percentage oil encapsulated was determined gravimetrically. Five to ten millilitres hexane was used to wash the filter paper to remove any absorbed oil. Each encapsulation experiment was repeated thrice, and from each of the three batches, three different samples were analysed for oil content. Therefore, the reported values are the mean of nine determinations.

*Measurement of dynamic interfacial tension*

The interfacial tension values of the oils and oil/surfactant systems with double-distilled deionized water were monitored as a function of time using the dynamic Wilhelmy plate method. The apparatus consisted of a Cahn 2000 recording

microbalance sensor and control unit (Cahn Instruments, Inc., Cerritos, CA, USA), a water jacketed sample chamber, a circulating water bath with digital temperature control, a thermocouple for the sample chamber, a platinum Wilhelmy plate, an IBM PC-compatible data acquisition computer interfaced with an analogue-to-digital converter and a chart recorder. The temperature of the sample chamber was controlled to $\pm 0{\cdot}1$°C using a circulating-water bath and monitored using a thermocouple sensitive to $\pm 0{\cdot}1$°C. The humidity over the sample chamber was maintained at $68 \pm 2\%$ during the experiments.

The procedure for dynamic tension measurement was that of Yoon & Burgess (1992) as follows. The upper and lower phases were equilibrated for 31 min to the predetermined temperature in separate containers. The tension value was zeroed with the plate submerged in the upper phase, to account for the buoyancy of the plate, this procedure taking approximately 2 min to perform. The heavier aqueous phase was poured into the sample cell, and the completion of this step was taken as time zero, and the remainder of the procedure was completed in 2 min. The sample cell was raised slowly upward to the plate using a vibration-free motorized labstand connected to a variable voltage source until the entire plate was submerged in the heavier phase. The lighter oil phase was poured on the heavier phase. The sample cell was lowered until the plate was completely above the interface, breaking away from the interface and surrounded by the upper oil phase and the sample cell was raised slowly until the lower phase was attracted to the plate forming a meniscus around the plate. The last step reproducibly placed the bottom edge of the plate exactly at the interface and, hence, error due to the buoyancy of the plate or inconsistency of placement was eliminated.

The oil and oil/surfactant mixtures were shaken with water and the unstable mixtures were stored overnight (at least 16 h) to allow phase equilibrium. Preliminary studies established that equilibrium occurred within 16 h. The oil and water phases were then separated and the interfacial tensions were measured as a function of time at 25°C. The measured maximum and equilibrated pulling forces were plotted as a function of time on a calibrated strip chart. The interfacial tension was calculated from the pulling tension, the contact angle and the plate parameters using the following equations (Yoon & Burgess 1992):

$$\gamma = \frac{P_{eq}}{2\,(L + d)\cos\theta} \tag{3}$$

$$\theta = \cos^{-1}\left(\frac{P_{eq}}{P_{max}}\right) \tag{4}$$

where $\gamma$ is interfacial tension, $P_{eq}$ the equilibrium interfacial pulling force, L the width of the plate, d the thickness of the plate, $\theta$ interfacial contact angle and $P_{max}$ maximum interfacial pulling force.

Samples were prepared in triplicate and each sample was measured three times. Therefore, the reported values are the mean of nine determinations.

All glassware used was cleaned with a sulphochromic mixture and washed with double-distilled deionized water three times.

## Results and Discussion

*The effect of hydrophobicity on oil uptake into complete coacervates*

The percentage uptake of squalane and oleic acid by gelatin/acacia complex coacervates was investigated. These oils were selected as they constitute single chemical components rather than mixtures. The purity of the oils was ensured by passing them three times through a column containing a 1 : 1 mixture of fluorosil and silica gel before use. The oils were emulsified by high speed stirring (1300 rev $min^{-1}$) in the acacia solutions before the addition of gelatin, and subsequent coacervation. These emulsions were relatively unstable and small surfactant molecules were not added to aid the emulsification process. Oleic acid and squalane, which are relatively hydrophobic (oleic acid has an HLB value of 1 and squalane is a hydrocarbon with no hydrophilic groups and therefore no HLB value), had encapsulation efficiencies of $73 \pm 3{\cdot}1$ and $61 \pm 2{\cdot}2\%$, respectively. The relative encapsulation efficiency of these oils may be a result of their respective surface affinities or may be dependent on the stability of the emulsion systems prepared. Oil droplet coalescence and phase separation may have prevented a percentage of the oil being available for encapsulation, thus decreasing the measured uptake values compared with an ideally emulsified system.

*The effect of surfactant on percentage encapsulation of squalane*

Squalane was selected to investigate the effect of surfactant on the percentage of oil encapsulated. Surfactants with different HLB values were selected to determine the effect of HLB on the percentage encapsulation. Surfactant mixtures were used to obtain a wider range of HLB values (Table 2).

The addition of surfactants to coacervate/oil systems may affect oil uptake by two different mechanisms: improvement of the o/w emulsion stability resulting in a higher percentage of oil available as droplets suitable for uptake, or alteration of the oil droplet/coacervate phase interfacial tension and the relative interfacial hydrophobicity/lipophilicity of the oil droplets, both of which may affect uptake. Complex coacervates do not take up very hydrophilic materials (Bungenberg de Jong 1949).

The addition of individual surfactants and surfactant mixtures with HLB values in the range 2·5–6 resulted in high percentage encapsulation of squalane (93·2–99·6, Fig. 1). These percentage encapsulation values are much higher than those determined for squalane alone. The addition of surfactants and surfactant mixtures with HLB values of 6 or higher resulted in decreased percentage oil encapsulation. The addition of Tween 80, which has an HLB value of 15, resulted in no oil encapsulation, compared with squalane alone. The addition of surfactants and surfactant mixtures with HLB values below 2·5 resulted in decreased percentage oil encapsulation. These data are probably a consequence of the altered surface affinities of the oil droplets in the presence of surfactants, as all the surfactant systems studied resulted in relatively stable emulsions. None of the emulsions containing surfactants separated before or during the encapsulation process, as observed by optical microscopy. The tendency for the coacervate phase to take up the oil droplets will be reduced as the surface hydrophilicity of the droplets is

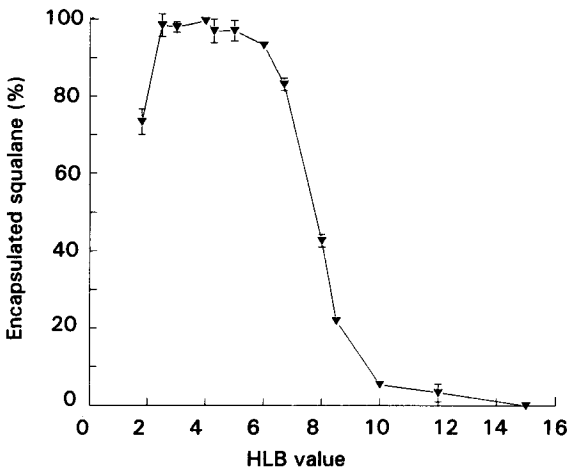


FIG. 1. Percentage w/w of squalane encapsulated by gelatin/acacia coacervates (2% w/v total polymer concentration, pH 3·9 and 40°C) as a function of the HLB value of the added surfactants. Means of nine trials are plotted ± s.d.

increased. Coacervate phases are hydrophobic and therefore will not have an affinity for apparently hydrophilic material.

*The effect of interfacial tension on oil uptake*

The dynamic interfacial tension values of oils and oil/surfactant systems were determined using the dynamic Wilhelmy plate method. The dynamic interfacial tension values decreased slightly with time in both systems. This appears to be due to an equilibration process at the Wilhelmy plate. In the case of pure oils the equilibrium interfacial tension values decreased with increasing hydrophilicity. The interfacial tension of the oils decreased in the presence of all the surfactants. All the surfactants give low interfacial tension values and although these values vary slightly, no direct correlation could be made between the interfacial tension values and the percentage oil encapsulation (Table 3). The variation in the effect of surfactants on oil uptake into coacervates indicates that the addition of surfactants does not simply affect uptake by altering the interfacial energy, as all the surfactants studied would then be expected to alter the percentage encapsulation of oil in a similar fashion. The addition of lecithin gave the lowest equilibrated interfacial tension value between squalane and water (1·1 dynes $cm^{-2}$), however, the percentage encapsulation of this system was only 5% w/w. This can be compared with the addition of Span 80 which resulted in an equilibrated interfacial tension value between squalane and water of 1·6 dynes $cm^{-2}$ and a percentage encapsulation of 97% w/w, and the addition of Tween 80 which resulted in an equilibrated interfacial tension value between squalane and water of 2·1 dynes $cm^{-2}$ and a percentage encapsulation of 0% w/w. It follows that the absorbed surfactant layer must change the surface characteristics (relative hydrophobicity/hydrophilicity) of the oil droplets either favourably or unfavourably and thereby affect uptake into the coacervates. The different hydrophobicities of the surfactants will affect the wetting of the oil by the coacervate phase. Although interfacial tension may affect uptake (eqn 1), this effect appears to be masked by the more dominant interfacial hydrophobicity/hydrophilicity parameter. The HLB values of the surfactants are representative of their relative hydrophobicity/hydrophilicity and this appears to be a better indicator of their uptake into complex coacervates.

Table 3. Relationship between interfacial tension values of oils and oil/surfactant systems against water (25°C) and the percent uptake of oil into coacervates prepared with these systems.

| System | Tension (dynes $cm^{-2}$) Mean* | Content of oil: (%, w/w) Mean† ± s.d. |
|---|---|---|
| Oleic acid | 3·3 | 6 ± 2·2 |
| Squalane | 10·9 | 73 ± 3·1 |
| Squalane + Tween 80 | 2·1 | 0 ± 0 |
| Squalane + lecithin | 1·1 | 5 ± 4·1 |
| Squalane + Span 80 | 1·6 | 97 ± 1·3 |
| Squalane + Span 85 | 1·3 | 73 ± 3·3 |

* No significant variability, n = 9. † n = 9.

*Conclusions*

The presence of surfactant in o/w systems affects oil uptake by complex coacervate droplets depending on the HLB value of the surfactant. The addition of surfactants with HLB values in the range 2·5–6 resulted in the maximum uptake of the emulsions into the coacervate microcapsules. Although surfactants with HLB values outside this range improve emulsion stability, they unfavourably change the surface properties of the oil droplets and hence prevent encapsulation. Reduction in interfacial tension, which occurs on the addition of surfactant, has been reported to increase uptake of core materials (Donbrow 1992). However, it appears from these studies that the percentage uptake of squalane oil is related to the relative hydrophobicity/hydrophilicity of the interface, rather than to the reduction in interfacial tension. The HLB values of the surfactants, which are related to the relative hydrophobicity of the molecules, appear to be good indicators of the effect of a surfactant or surfactant mixture on the uptake of oil into coacervate droplets.

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