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Microencapsulation of benzalkonium chloride

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Summary

A method based on the interfacial polycondensation of isocyanates has been used to prepare microcapsules containing an aqueous solution of benzalkonium chloride. Since the compound to be encapsulated is amphiphilic, particular attention was paid to the formulation of a starting system for encapsulation. To this end, we investigated the influence of the hydrophilic-lipophilic balance (HLB) of both the surfactants and the oil on the initial emulsion. Our results demonstrate that designing of the formulation in accordance with the HLB concept of Griffin (*J. Soc. Cosm. Chem.*, 1 (1949) 311–326) constitutes a good approach to the choice of the components of the microencapsulation system. The phase diagram constructed for a pseudo-ternary system showed that the area of the domain of the lipophilic continuous phase emulsion decreased with increasing concentration of benzalkonium chloride. This observation can be explained as being due both to the amphiphilic nature of the benzalkonium chloride and to modification of the aqueous solubility of the surfactants in its presence. The encapsulation yield of benzalkonium chloride was high (60%). The remaining 40% may either be present in the lipophilic phase or become incorporated into the shell of the microcapsules.

Introduction

The recent impact of AIDS, as well as that of other viruses, has created a serious problem among surgeons, and hospital and paramedical staff for which a solution is urgently required. The fear of contamination leads them to wear gloves (sometimes two or three pairs superposed) which become ineffective immediately upon the appearance of a tear in the latex film. The addition of some antiseptics could be a way of increasing the level of protection afforded. Examples of antiseptics known to be efficient against HIV are non-ionic detergents (Hicks et al., 1985) and quaternary ammonium compounds like benzalkonium chloride (Chermann et al., 1987). However, preliminary tests have shown that the use of such substances can involve severe drawbacks, particularly in the case of the latter type due to alterations being induced in the latex (Hutchin-

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son Co., 1988; unpublished results). In order to protect the latex, it was suggested that these substances should be contained in reservoirs such as microcapsules. Thus, microcapsules can act as coating systems which release substances only in the case where the latex film becomes ruptured, cut or pierced by a needle (Busnel and Argy, 1987).

The effectiveness of benzalkonium chloride against HIV has been demonstrated in aqueous solution (Chermann et al., 1987). Hence, in order to obtain a microencapsulated active compound, it is important that the preparation method should yield microcapsules containing an aqueous solution of benzalkonium chloride. Interfacial polycondensation has been reported to be suitable for preparing waterloaded microcapsules (Vivant, 1983). However, McGinity et al. (1981) demonstrated that formation of microcapsules did not occur in the presence of quaternary ammonium species when using this method. Interfacial polycondensation requires the formation of a stable water-in-oil (w/o) emulsion in order for the microcapsule wall to be produced. Quaternary ammonium species such as benzalkonium chloride possessing an amphiphilic nature tend to reside at the interface and result in the destabilization of the initial emulsion. This effect can lead to the formation of microcapsules being prevented and hence may be one of the reasons for the lack of success achieved previously.

In this study, we propose the use of a procedure based on interfacial polycondensation in the preparation of microcapsules from an aqueous solution of benzalkonium chloride. Our approach consists of formulation of the microencapsulation system as a stable w/o emulsion in line with the hydrophilic-lipophilic balance (HLB) concept of Griffin (1949). With this purpose in mind, our investigation was aimed at the determination of the influence of both the nature and the HLB of the surfactants and at the evaluation of the HLB value required for the lipophilic phase in the formation of microcapsules. Furthermore, we present a phase diagram for a pseudo-ternary system comprising surfactant/oil/benzalkonium chloride + water constructed for various concentrations of benzalkonium chloride.

Materials and Methods

Materials

The isocyanate used in this work was methylene diisocyanate (Desmodur® 44V20, Bayer, Puteaux, France). Polyoxyethylene sorbitan trioleate (Tween® 85, Sigma, St. Louis, U.S.A.), polyoxyethylene sorbitan monooleate (Tween® 80, Prolabo, Paris, France), sorbitan monooleate (Span® 85) and modified polyesters (Hypermer® A60, Hypermer® A394, Hypermer® A409, Hypermer® B246, ICI, Kortenberg, Belgium) were used as surfactants. Coconut oil-derived triglycerides C8–C10 (Miglyol®, Hülls, Germany) and xylene (Prolabo, Paris, France) were employed as lipophilic phases without further purification. Benzalkonium chloride (Barquat® MS 100) was kindly supplied by Lonza Co. (Puteaux, France).

Preparation of microcapsules

The preparation of microcapsules was carried out according to the method of interfacial polycondensation of isocyanate described previously (Pense et al., 1990). An aqueous phase (30 ml) containing the required amount of benzalkonium chloride was dispersed by rapid stirring (2500 rpm, Microvortex®, Grenier-Charvet, Rennes, France) in a lipophilic phase (200 ml) including the isocyanate (15 g) and a surfactant (7 g). On attainment of the desired droplet size, stirring was continued using a blade stirrer at low speed (350 rpm, Digital 2000, Heidolph, Germany) for the duration of polycondensation. Finally, the microcapsules were recovered by filtration, washed with cyclohexane and dried at room temperature. The quantities of each component used in the preparation are listed in Table 1.

TABLE 1

Typical composition for preparation of microcapsules of benzalkonium chloride

Lipophilic phase (%)		Aqueous phase (%)	
Mixture (xylene/ Miglyol®)	79.7	water	10.7
		benzalkonium chloride	1.2
Surfactant	2.4		
Isocyanate	6.0		

Pseudo-ternary phase diagram

The lipophilic and aqueous phases and surfactant were mixed in various proportions at room temperature in test tubes. After mixing, the nature of the continuous phase of the emulsion was determined by the dilution method. Estimation of the nature of the emulsion type (primary or multiple) was performed by light microscopy (Olympus, Paris, France).

Cloud point

The cloud point of a 0.5% aqueous solution of the surfactant Hypermer® A 60 in the presence of increasing amounts of benzalkonium chloride was determined. The measurement consisted of determination of the temperature corresponding to a rapid change in turbidity of the mixture (400 nm, Perkin-Elmer, Montigny, France). The temperature limit was 75°C.

Characterization of microcapsules

Microcapsules were examined by observation under both light and scanning electron microscopy (Leica, Rueil-Malmaison, France). The microcapsule size distribution was established using a Coulter® Counter TA II (Coultronics, Margency, France). Assay of the dosage of microencapsulated benzalkonium chloride was carried out according to an HPLC method (Meyer, 1980) after the chemical destruction of the microcapsule wall by treatment with DMF. The encapsulation yield of benzalkonium chloride was calculated according to the following formula:

$$\text{Yield (\%)} = \frac{\text{quantity of benzalkonium chloride in the microcapsules (determined by HPLC)}}{\text{quantity of benzalkonium chloride used in the preparation}}$$

Results and Discussion

Preliminary experiments were carried out with the microencapsulation system proposed by Vivant (1983) in which xylene constituted the lipophilic phase and Tween® 85 was used as the surfactant. In addition, we examined the effect of addition of benzalkonium chloride to this system at a concentration of 10% (w/v) in the aqueous phase and the use of a new polymeric surfactant: Hypermer® A60. The results show that microcapsules were formed in the absence of benzalkonium chloride. In contrast, in the presence of benzalkonium chloride, formation of microcapsules was observed only for a system containing Hypermer® A60 as surfactant. All systems leading to the formation of microcapsules corresponded to the presence of a lipophilic continuous phase emulsion during the early stage of preparation. Formation of this type of emulsion with the surfactant Hypermer® A60 is in accordance with Griffin's HLB concept. In fact, the HLB value for Hypermer® A60 is 6 and that required for xylene is 7: these are close to the range of HLB values for the formation of a w/o emulsion. When Tween® 85 (HLB = 11) was employed as the surfactant in the formulation of the microencapsulation system, a stable w/o emulsion could be obtained in the absence of benzalkonium chloride due to the large volume of oil as compared to that of water (Table 1). In the presence of benzalkonium chloride, microcapsules were not formed as a result of the inversion of the emulsion type.

In order to optimize the system and since the reactivity of isocyanates is well known to depend on the nature of the solvent (Wittberger and Morgan, 1959), we decided to alter the composition of the lipophilic phase. This was achieved via the addition of a synthetic triglyceride (Miglyol®), which was chosen because of its required HLB value of 6.6 being close to that for xylene. Microcapsules prepared in mixtures comprising xylene and Miglyol® in varying proportions showed differences in behavior during the washing and drying steps (Table 2). Very brittle microcapsules were obtained for a xylene content of the dispersing phase of 50% or above. The dry microcapsules recovered from a system containing 100% Miglyol® were agglomerated. Finally, microcapsules produced by systems with a high content of Miglyol®, within the range 60-85%, were sufficiently durable to be able to resist the washing and drying steps. These data demonstrate that

TABLE 2

Influence of composition of the lipophilic medium on properties of microcapsules

Lipophilic phase	Microcapsule batches
Xylene (100%)	very brittle
Xylene (80%)/Miglyol® (20%)	very brittle
Xylene (60%)/Miglyol® (40%)	very brittle
Xylene (50%)/Miglyol® (50%)	very brittle
Xylene (40%)/Miglyol® (60%)	readily isolated
Xylene (30%)/Miglyol® (70%)	readily isolated
Xylene (15%)/Miglyol® (85%)	readily isolated
Miglyol® (100%)	some agglomerates formed

the incorporation into such systems of an appropriate oil, selected on the basis of Griffin's HLB concept, does not prevent the formation of microcapsules. Batchwise differences observed between the microcapsules are attributable to a variation in the extent of isocyanate reactivity within the solvent mixtures (Arshady, 1989).

We have also investigated the effects on the preparation of microcapsules as a result of both the nature and the HLB of the surfactants. The surfactants used were either mixtures of Span® 85 and Tween® 80 in different proportions in order to result in HLB values spanning the range 2-12, or polymeric surfactants (Hypermer®) characterized by having differing HLB values (range: 5–9). In these experiments, the lipophilic phase consisted of xylene/Miglyol® (15:85) and the aqueous phase comprised a 10% aqueous solution of benzalkonium chloride. The results (Table 3) show that the type of emulsion and the morphology of the microcapsules differed according to the magnitude of the HLB of the surfactants. At low HLB values, agglomerated microcapsules resulted whereas at high values the instability of the emulsion prevented the formation of microcapsules. The optimal HLB values for obtaining microcapsules were determined to be between 5 and 8, i.e., within the range of HLB values required for the lipophilic phase. From Table 3 it may also be observed that the systems formed during the initial stages of the preparative procedure can exist in the form of a multiple emulsion characterized by the presence of a lipophilic continuous phase. Multiple emulsions were obtained with both classes of surfactants and were observed not to prevent microcapsules being formed.

The effect of benzalkonium chloride on formation of the system observed during the initial stage of preparation was monitored through the construction of a phase diagram for the pseudo-ternary system of composition Hypermer® A60/xylene-Miglyol® (15 : 85)/benzalkonium chloride + water. The resulting diagram (Fig. 1) indicates that the area of the lipophilic continuous phase emulsion undergoes modification to an extent that is governed by the amount of benzalkonium chloride present in the aqueous phase. As an illustration, 50% of water can become incorporated into a lipophilic continuous phase emulsion containing 10% Hypermer® A60 in the absence of benzalkonium chloride. In contrast, only 15% of an aqueous phase containing 10% benzalkonium chloride can become incorporated into a lipophilic continuous phase emulsion prepared using the same lipophilic system. The variation in the emulsion phase can be ascribed to both the amphiphilic nature of benzalkonium chloride and a change in the aqueous solubility of the surfactants in the presence of benzalkonium chloride. In fact, measurement of the cloud point of Hypermer® A60 demonstrates that increasing amounts of benzalkonium chloride give rise to an

TABLE 3

Influence of HLB values of a Span® 85/Tween® 80 mixture and of Hypermer® on emulsion type and formation of microcapsules

HLB of surfactants		Emulsion type	Microcapsule formation
Span® 85/Tween® 80			
	2	undefined	–
	3	w/o	–
	5	w/o	+
	8	w/o	–
	10	o/w/o	–
	12	o/w	–
Hypermer®			
B246	5	o/w/o	+
A60	6±1	o/w/o	+
A394	8±1	o/w/o	+
A409	9±1	undefined	–

increase in the value of the cloud point (Fig. 2), which corresponds to an enhancement in aqueous solubility of this surfactant and to an apparent increase in HLB. The fact that the behavior of the surfactants undergoes alterations in the presence of benzalkonium chloride can be attributed to the formation of mixed micelles of both species (Schott and Royce, 1984). Hence, when present in combination with benzalkonium chloride, the surfactants become more hydrophilic, thereby favoring the appearance of an aqueous continuous phase emulsion. Such an effect is manifested in the pseudo-ternary phase diagram as a reduction in the area of the lipophilic continuous phase domain (Fig. 1).

Microcapsules were characterized using samples prepared via an optimized system. The system consisted of a lipophilic phase containing a mixture of xylene/Miglyol® (30:70) and a polymeric surfactant (Hypermer® A60 or A409) and

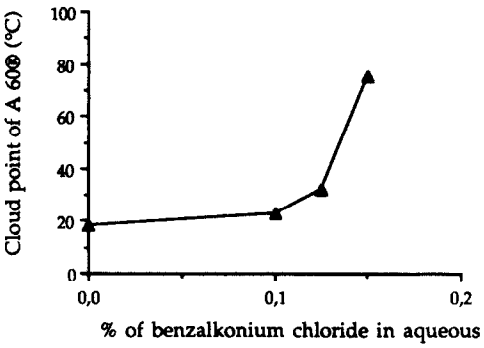

Fig. 2. Influence of concentration of benzalkonium chloride on the cloud point of Hypermer® A60 in aqueous solution.

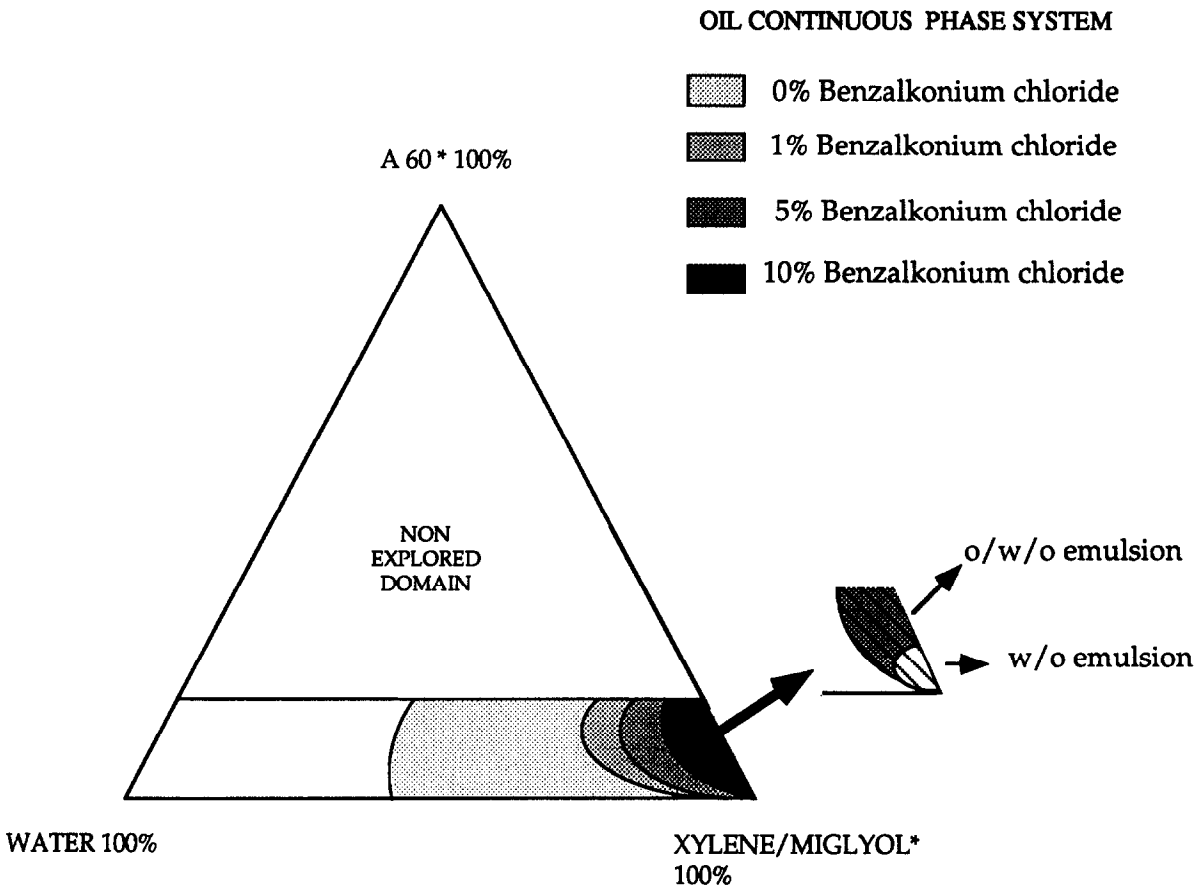

Fig. 1. Pseudo-ternary phase diagram of the system Hypermer® A60/xylene-Miglyol® (15:85)/benzalkonium chloride + water for different concentrations of benzalkonium chloride.

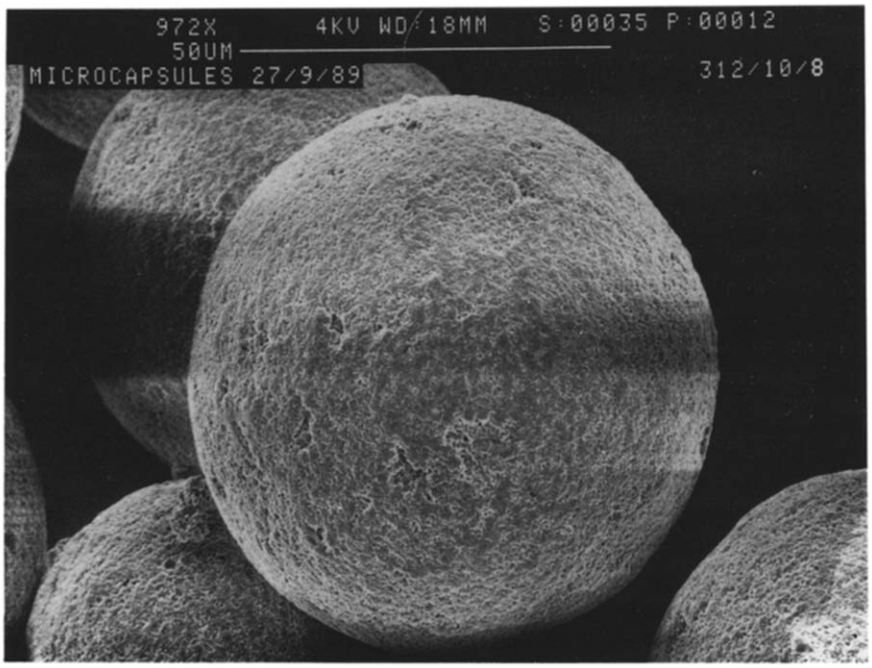

Fig. 3. Scanning electron photomicrograph of benzalkonium chloride-loaded microcapsules.

an aqueous phase with benzalkonium chloride as component. The microcapsules as observed under light microscopy are individualized, spherical and characterized by a thick shell. Scanning electron microscopy indicates their surface to be smooth and regular (Fig. 3). Table 4 lists the properties of the microcapsules studied here, e.g., mean diameter and encapsulation yield. The mean diameter of microcapsules prepared using a low concentration of benzalkonium chloride (1%) in the aqueous phase is less than that observed for a higher benzalkonium chloride level (10%). The difference can be explained on the basis of the amphiphilicity of benzalkonium chloride which has been shown to reduce the area of the domain of the lipophilic continuous phase emulsion. In fact, in the presence of 1% benzalkonium chloride, the microencapsulation system is located in the lipophilic continuous phase emulsion of the phase diagram (Fig. 1). Inspection under the microscope shows that the system corresponds to a simple w/o emulsion. This contrasts with the

TABLE 4

Properties of benzalkonium chloride-loaded microcapsules

Initial concentration of benzalkonium chloride (% w/v)	Surfactant type	Mean diameter (μm) ($\pm$S.D.)	Yield of microencapsulation of benzalkonium chloride (% w/v)
1	Hypermer® A60	10 $\pm$ 5	60
10	Hypermer® A60	50 $\pm$ 10	60
10	Hypermer® A394	50 $\pm$ 10	60

findings for the system in the case where 10% benzalkonium chloride was used; the location was determined as being in the domain of the multiple emulsion (o/w/o) (Fig. 1). Therefore, the size discrepancy between the two samples would appear to result from the simple emulsion droplets being smaller than globules of the multiple emulsion. The encapsulation yield of benzalkonium chloride was comparable among all the systems investigated, corresponding to 60% of the initial benzalkonium chloride content incorporated into the preparation medium. Two possible causes can be proposed in order to explain the loss of 40%. Firstly, benzalkonium chloride may partition between the lipophilic and hydrophilic phases during microcapsules preparation. However, the partition coefficients determined are indicative of the lack of diffusion of benzalkonium chloride into the lipophilic phase, irrespective of its composition (xylene/Miglyol®). Furthermore, such experiments were performed in the absence of surfactants in the lipophilic phase. Hence, the effect of surfactants on the partition coefficient of benzalkonium chloride was not taken into account. Alternatively, the hypothesis was put forward that an interaction takes place between benzalkonium chloride and the isocyanate during the reaction of polycondensation. This would lead to the incorporation of benzalkonium chloride into the microcapsule shell (Scher, 1979).

In conclusion, the microencapsulation of benzalkonium chloride could be achieved through the method of interfacial polycondensation, since the initial system was formulated in accordance with the concepts regarding the factors which govern the stability of emulsions.

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