J. Pharm. Pharmacol. 1983, 35: 70–73
Received July 6, 1982

0022–3573/83/020070–04 $02.50/0

Physical factors affecting microencapsulation by simple coacervation of gelatin

OVAIS SIDDIQUI* AND HARRY TAYLOR

Department of Pharmacy, University of Otago, Dunedin, New Zealand

Gelatin has been coacervated at 60 °C using sodium sulphate. Interfacial tensions between coacervate and supernatant liquid, coacervate and two oils (with and without one of two drugs, clofibrate and chlormethiazole) and supernatant liquid and the oils (± drug) have been measured by a drop volume technique, in the presence and absence of one of three surfactants, cetrimide, sodium lauryl sulphate and hexadecyltrimethylammonium lauryl sulphate (double salt). Spreading coefficients calculated from tensions indicate that coacervate should spread readily over oil droplets in presence of double salt, less readily with cetrimide and spreading is unlikely in the presence of sodium lauryl sulphate. The sign of the charge on coacervate droplets and oil droplets was identified under different conditions and showed coacervate droplets and oil droplets have opposite charges except in the presence of sodium lauryl sulphate. Microcapsules were prepared using cetrimide or 'double salt' as emulsifier and release of drug measured. Those prepared with 'double salt' released more slowly than those prepared with cetrimide.

Green (1957) first demonstrated the microencapsulation of oil by coating droplets with a gelatin coacervate produced by treating aqueous gelatin solution with soluble salts (sodium sulphate or ammonium sulphate). A system of this type serves as a suitable basis for investigation of some of the factors involved in microencapsulation, namely adsorption of coacervate by substrate and its adhesion to the substrate, spreading of coacervate upon the substrate to form a complete coat and control of aggregation of coated particles. This work describes a study of these factors using simple coacervate of gelatin and two drugs presented as oily liquids.

MATERIALS AND METHODS

Materials

Gelatin alkali processed, Isoelectric pH 4·85 (40 °C), Bloom number 159, was supplied by Halewood Chemicals Ltd, Middlesex, U.K. (batch number 9909 L/N3596), cetrimide was from ICI Ltd, sodium lauryl sulphate from Dehydag Produkte, GDR; clofibrate was from Atromid S 500 capsules, ICI Ltd and Hemineurin capsules from Astra Chemicals Pty Ltd.

* Correspondence and present address—School of Pharmacy, University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania, Australia 7001.

Abstract from a thesis submitted by Ovais Siddiqui in partial fulfilment for the requirements of the degree of Master of Pharmacy at the University of Otago, Dunedin, New Zealand in September 1981.

Preparation of coacervate

To a 10% solution of gelatin (pH 4·85) maintained at 40 °C ± 1 °C was added dropwise with stirring sufficient 20% w/w sodium sulphate solution to produce coacervation (one half of the initial volume of gelatin solution). The phases were then allowed to separate into two layers at 40 °C. Either one of the surface active agents was dissolved in the gelatin solution, when required and coacervation was carried out following the above procedure.

Measurement of interfacial tension

A corrected drop volume method (Harkins & Brown 1919) was adopted using an assembly which was placed in a jacketted glass tube capable of maintaining the specified temperature throughout its length. The interfacial tension was measured between coacervate phase/supernatant liquid, coacervate phase/liquid substrate, with and without the presence of a surface-active agent. The drug was dissolved in the liquid substrate before the interfacial tension was measured. From the interfacial tension values obtained for different liquid pairs, the spreading tendency of the coacervate phase over the surface of the oil drops in the presence of supernatant liquid was determined by calculating the spreading coefficients by the equation of Davies & Rideal (1961).

Measurement of coacervate viscosity

The Haake-Rotovisko viscometer with plate and cone assembly (PK-1), with a slight modification,

was used to measure the coacervate viscosities. A cylindrical brass cup was used, at the base of which a hard glass disc was placed to avoid friction with the cone. The cup was placed on the plate and the cone was inserted into it, the cup was in close contact with the thermostatically controlled plate of the viscometer. Coacervate was poured into the brass cup with a small amount of supernatant liquid to prevent the coacervate becoming a gel on evaporation of solvent. The cup with the contents was allowed to attain temperature equilibrium by leaving it on the plate for 30 min before each measurement. The apparent viscosity of coacervate at 60 °C was calculated for different pH values.

Measurement of charge sign

Because of the high concentration of sodium sulphate, attempts to measure zeta potential of coacervate droplets produced only small and irregular voltages using a sedimentation potential technique. The theory of the suspension effect (Jenny et al 1950) was then applied to measure the sign of the charge on dispersed particles. Two calomel electrodes with leads connected to an operational amplifier with a gain of 200, were placed one at the top and one at the bottom of a sedimenting system. Coacervation was carried out and after a lapse of an hour, the coacervate was mixed in the supernatant liquid for 15 min. As the coacervate settled a potential between the two electrodes developed and was read by either a positive or a negative deflection in a 10 mV pen recorder connected to the amplifier. The coacervate was separated and the sign of the charge for the liquid pair of liquid substrate/supernatant liquid and liquid substrate/aqueous gelatin solution was measured following the above procedure. All the liquid pairs were again examined in presence of one of the surface active agents. The temperature of the system was kept at 60 ± 1 °C.

Preparation of 'double salt'

A lipophilic salt was prepared by double decomposition from solutions of cetrimide and sodium lauryl sulphate. Aqueous solutions of equal molar quantities of both cetrimide and sodium lauryl sulphate were mixed and warmed until the liquid became clear. The mixture was allowed to cool to room temperature (20 °C) and filtered. The filter cake was washed several times with water to remove sodium and bromide ions. A mixture of equal quantity of acetone and dichloromethane was used to dissolve the filter cake. Water in the organic solvent mixture was removed (dried $MgSO_4$) and on evaporation of solvent crystals were obtained, Found: C, 65·6; H, 12·3; N, 2·8; S, 6·3%; Calculated for $C_{29}H_{63}NO_4S$; C, 66·7; H, 12·2; N, 2·7; S, 6·2%.

Preparation of microcapsules

The preparation of microcapsules using the gelatin-water-sodium sulphate system was carried out using the method of Green (1957), modified to include ionic surface active agents. Cetrimide, 0·8%, or 'double salt' as emulsifying agent and 1% of sodium lauryl sulphate as dispersing agent were found to be appropriate for this particular microencapsulation process. The rate of cooling and stirring was kept constant. Approximately 6% of chlormethiazole base or 15% of clofibrate (with respect to liquid substrate) was dissolved in the liquid substrate. The temperature of the system was kept at 60 ° ± 1 °C.

An o/w emulsion of 10% gelatin solution (96 cm^3) and liquid substrate (24 cm^3) containing the drug was prepared using one or other surfactant (above) as emulsifier. The average radius of the droplets was seen to be around 10 μm. So that the gelatin carried an adequate negative charge 1 M NaOH, was added dropwise. A warm (60 °C) sodium sulphate solution (20% w/w) was added dropwise, over 2 h to bring about complete coacervation. Dispersing agent was then slowly added, over 15 min, and the mixture cooled slowly to 30 °C, then rapidly to 19 °C. The coacervated mixture was poured into three times its volume of 7% Na_2SO_4 solution at 5 °C and left 1 h. Stirring was at constant speed throughout.

The mixture was filtered and the filter cake was washed several times with water. The recovered filter cake was suspended in a formaldehyde–water (1:4 v/v) or formaldehyde–water (1:4 v/v pH 10) or formaldehyde–isopropanol (1:4 v/v) mixture. The formalization time was 1 h for all batches. The hardened microcapsules were filtered, washed with water and dried at room temperature (20 °C) or in a desiccator over silica gel under vacuum. Surface characteristics of microcapsules were examined using light and scanning electron microscopy. The average thickness of the microcapsule wall was estimated to be 1·4 μm.

In-vitro release measurements

An automated dialysis method of Barzilay & Hersey (1968) was used with slight modification permitting manual stirring to overcome the constant problem of agglomeration and insufficient exposure of the surface of microcapsule in the dialysis membrane. The temperature of the system was maintained at 37 ± 0·5 °C. The quantity of clofibrate or chlor-

methiazole released was measured spectrophotometrically at intervals. First order release rate constants were obtained from plots of log percentage remaining versus time (using the eventually constant amount released for calculation of 100%). Rate constants were divided by sample surface area g^{-1} (obtained approximately by calculation from microscopically measured average microcapsule diameter) to give 'standardized' release rate constants.

DISCUSSION

Interfacial tensions, and spreading coefficients calculated from them (Tables 1 and 2), imply that coacervate spreading over the oily substrate should be greatly favoured in the presence of the double lipophilic salt. With cetrimide, spreading coefficient values generally had small positive or negative values which imply a poor spreading tendency. In encapsulation runs in the presence of the two surfactants, however, there appeared to be no difference in the ease of microcapsule formation. The conditions in the two procedures were not identical. For interfacial tension measurements, coacervation in the presence of surfactant was completed before any part of the system came into contact with the oil phase. In the encapsulation runs oil was emulsified with gelatin solution before coacervation. In the absence of oil the 'double salt' (but not cetrimide) partitioned almost completely into the coacervate phase, as shown by the near zero interfacial tensions between coacervate and oil and the large values between supernatant phase and oil, which are little different from values obtained in the absence of surfactant. The 'double salt' dissolved readily in oils however, so that in encapsulation runs it would be present at the interface between supernatant liquid and oil droplets. The spreading coefficients would thus in practice be less positive than those listed in Table 2.

Table 1. Average values of interfacial tensions at medium concentration of surface-active agent for the coacervate phase and liquid substrate ($\gamma_{coac/oil}$ m Nm^{-1}) and supernatant liquid and liquid substrates ($\gamma_{sup/oil}$ m Nm^{-1}). Temperature 60 °C.

Liquid Pair	0·8% Cetrimide	0·8%* DS	1% Na LS
Coacervate phase/ liquid paraffin	7	*	7·1
Coacervate phase/liquid paraffin with clofibrate	4·5	*	5·9
Coacervate phase/liquid paraffin with chlormethiazole	5·5	*	6
Coacervate phase/maize oil	4·3	*	5·5
Coacervate phase/maize oil with clofibrate	4·7	*	5·2
Coacervate phase/maize oil with chlormethiazole	4·4	*	5·5
Supernatant liquid/liquid paraffin	5	27	8·8
Supernatant liquid/liquid paraffin with clofibrate	5·8	23·6	6·8
Supernatant liquid/liquid paraffin with chlormethiazole	4·8	20·4	11·4
Supernatant liquid/maize oil	4·2	17	10·5
Supernatant liquid/maize oil with clofibrate	5·8	18·4	11·2
Supernatant liquid/maize oil with chlormethiazole	5·1	15·9	14·6

*Values too small to measure for 0.8% double salt (DS)—coacervate flowed as continuous stream. NaLS = Na lauryl sulphate SA = surfactant.

Table 2. Spreading coefficients at 60 °C. (Abbreviations as in Table 1).

Liquid substrates	No SA	0·8% Cetrimide	DS	Na LS
Liquid paraffin	−2·2	−2	+27	+1·7
Liquid paraffin with clofibrate	+8·6	+1·3	+23·6	+0·9
Liquid paraffin with chlormethiazole	+0·4	−0·7	+20·4	+5·4
Maize oil	−2·8	−0·1	+17	+5
Maize oil with clofibrate	+2·8	+1·1	+18·4	+6
Maize oil with chlormethiazole	+8·6	+0·7	+15·9	+9·1

The flow properties of coacervates showed markedly pseudo-plastic behaviour. The apparent viscosity at 60 °C at the lowest shear rate used (62·4 s^{-1}) of 10% gelatin-w/w sodium sulphate coacervate at pH values of 3·85, 4·85, 5·85 were 8·8, 6·3 and 6·3 poise. At higher shear rates values were smaller but showed a similar pattern, that is a minimum between pH 4·85 and 5·85. The minimum should occur at the isoelectric point; pH 4·85, but this is a 40 °C value and moreover the high salt concentration should have some effect. Even at 60 °C the coacervate viscosity is high so that conditions during encapsulation need to be chosen so that coacervate can flow most easily over the oil droplet surface, that is the pH should be near to the isoelectric value.

It is considered that affinity between oil droplets and coacervate droplets would be greatest when the surfaces of the two phases carried opposite electric charges. The method eventually adopted to identify the sign of charge depended on the 'suspension effect' (Jenny et al 1950), which, like all double layer effects is considerably reduced in the presence of salts. Voltage readings obtained were always small,

but consistent and repeatable. The sign of charge obtained from gelatin at its natural pH, 5·4, and, as expected, (IEP = 4·85) the droplets carried a negative charge. This persisted in the presence of surfactant even when surfactant ion was positive, showing that the cetrimide ion with bromide counterion does not appear to concentrate markedly at the coacervate surface. When lauryl sulphate is the counterion, interfacial tension results show that the salt is present at the coacervate surface, but with both ions likely to be there in similar concentrations the overall effect on droplet charge will be small. Table 3 shows that coacervate droplets and oil droplets can be expected to carry opposite charges, except when the surfactant present is sodium lauryl sulphate. These results are consistent with behaviour during microencapsulation runs, which produced satisfactory microcapsules except when sodium lauryl sulphate was used as emulsifying agent.

Table 3. Measurement of charge-sign on dispersed particles in coacervating systems (60 °C). (Abbreviations as in Table 1).

Liquid pairs/SA (*-dispersed phase)	No SA	0·8% Cetrimide	0·8% DS	1% Na LS
Liquid paraffin*/gelatin solution	+	+	−	−
Maize oil*/gelatin solution	+	+	−	−
Coacervate phase*/ supernatant liquid	−	−	−	−
Liquid paraffin*/supernatant liquid	+	+	+	−
Maize oil*/supernatant liquid	+	+	+	−

Microencapsulation procedures were chosen to permit application of the above information. It was found that initial pH, and so presumably the negative charge on coacervate droplets, was important and for initial emulsions prepared with cetrimide, 1 M NaOH had to be added to bring the pH to about 6·80, otherwise the microcapsules formed gelatinous clumps during the coacervation step. Addition of NaOH was not needed when 'double salt' was the emulsifier. Presumably with cetrimide some surfactant entered the coacervate phase making its surface less negative. After coacervation was complete, and during subsequent stirring, the microcapsules formed tended to agglomerate. To increase the negative charge presumed to be present on the outer gelatin surface of the particles sodium lauryl sulphate was added at this stage. It was effective in preventing the agglomeration.

Of the hardening processes investigated, that using isopropanol as solvent had to be discarded, for although it produced the most free-flowing capsules, the solvent leached out nearly all the drug during the process. Madan et al (1976) noted loss of clofibrate to isopropanol but at a much slower rate.

The appearance of microcapsules in scanning electron micrographs was similar although those prepared with 'double salt' were consistently smoother than those prepared using cetrimide. Similarly capsules hardened by near-neutral formaldehyde were consistently smoother than those prepared under alkaline conditions. It is possible that the greater degree of cross-linking of gelatin in alkali caused greater distortion of the microcapsule wall.

The 'standardized' release rate constants obtained varied between $1{\cdot}1 \times 10^{-4}$ and $5{\cdot}6 \times 10^{-4}$ min^{-1} and are generally consistent with the microscopic appearance. Release rate constants obtained for microcapsules prepared using 'double salt' are lower than those for the corresponding product from cetrimide, as are those obtained from neutral hardening when compared with corresponding capsules that are alkali hardened. The 'double salt' appears to enhance the attachment of gelatin to oil droplet surface. This may be due to the presence in both surfaces of both positive and negative charges which can adopt complementary positions to enhance the overall attraction between oil and coacervate droplets. The values of standardized release rate constants are approximately 5–10 times greater than the 'standardized' release rate constant obtained from the $t_{1/2}$ value for microcapsules with a wall thickness of 1·4 μm (Madan 1981, his Fig. 6) attaining the capsule diameter of 200 μm reported previously (Madan et al 1976). The validity of such a comparison is however, doubtful because of the different kinetics applied.

Acknowledgements

One of us (O.S.) wishes to thank Mr Safiullah Siddiqui and Mr Wajahat Siddiqui for financial assistance to undertake this research.

REFERENCES

Barzilay, R. B., Hersey, J. A. (1968) J. Pharm. Pharmacol. 20: 232S–238S

Davies, J. T., Rideal, E. K. (1961) Interfacial Phenomena Academic Press, London, pp 24

Green, B. K. (1957) U.S. Patent 2,800,458

Harkins, W. D., Brown, F. E. (1919) J. Am. Chem. Soc. 41: 499–524

Jenny, H., Nielsen, T. R., Coleman, N. T., Williams, D. E. (1950) Science 112: 164–167

Madan, P. L., Madan, D. K., Price, J. C. (1976) J. Pharm. Sci. 65: 1476–1479

Madan, P. L. (1981) J. Pharm. Sci. 70: 430–433

Sliwka, W. (1975) Agew. Chem. Int. Ed. 14: No. 8, 539–549