J. Pharm. Pharmacol. 1982, 34: 687–691
Received March 15, 1982

0022–3573/82/110687–05 $02.50/0

Stabilization of water/oil/water multiple emulsions by polymerization of the aqueous phases

A. T. FLORENCE* AND D. WHITEHILL†

Department of Pharmacy, University of Strathclyde, Glasgow G1 1XW, U.K.

In order to improve the stability of w/o/w multiple emulsions which have isopropyl myristate as the non-aqueous 'oil' phase, either the internal aqueous phase of the multiple system or the secondary (outer or continuous) aqueous phase can be gelled. Production, by γ-irradiation, or cross-linked polyacrylamide or poloxamer gels in the aqueous phases of the emulsions leads to systems which have a greater intrinsic stability than untreated multiple emulsions. If the internal aqueous phase is gelled this prevents coalescence. When the continuous outer phase is gelled an opaque emulsion is produced in which the disperse w/o droplets are held in a hydrophilic polymer network from which the droplets are released on contact with water.

Multiple emulsions are complex, inherently unstable systems, which are unlikely to be commercially acceptable as drug delivery systems until problems of their instability in vitro and in vivo are solved. Despite this, there are few reports in the literature of attempts to improve their stability. In the work described here some effort was directed at improving stability by attempting to polymerize appropriate monomers in the aqueous phases of w/o/w multiple emulsions. The object was to reduce the potential for coalescence of the internal aqueous droplets or for their expulsion from the oil drops, or to prevent the coalescence of the oil drops themselves, the three major routes of breakdown in multiple emulsions (Davis 1976; Florence & Whitehill 1981).

Because the preparation of a w/o/w system is a two-stage procedure, it is possible to modify either the primary aqueous phase (which becomes the internal aqueous phase of the multiple system) or the secondary aqueous phase, which subsequently becomes the continuous aqueous phase. Each aqueous phase can be gelled by in situ polymerization reactions. Polyacrylamide has been used to demonstrate the technique, but less toxic systems are required for pharmaceutical use.

Al-Saden et al (1980 a,b) reported the gelation of aqueous solutions of poloxamer surfactants by the action of γ-irradiation. The poloxamers are relatively non-toxic poly(oxyethylene)-poly(oxypropylene) block copolymers, with the general formula

$$HO[C_2H_4O]_a[C_2H_6O]_b[C_2H_4O]_aH$$

Crosslinking of the surfactant molecules may be induced by simultaneous activation of two neighbouring molecules with the net result that the molecular weight of the polymer increases until a three-dimensional network is formed (gel-formation). Poloxamers with an ethylene oxide content of less than 70% were shown by Al-Saden et al (1980b) to degrade on irradiation. This was explained on the basis that a certain minimum chain length was required for gel formation. As the hydrophilic poloxamer compounds are surface-active (promoting o/w emulsification) oil-in-water emulsions may be prepared which contain the poloxamer in the continuous aqueous phase. After emulsification, the surfactant molecules can be crosslinked at the oil-in-water interface and in the continuous phase by γ-irradiation, forming a network of surfactant molecules which link the dispersed oil globules. Similarly, the poloxamer compounds can be used in the second emulsification step in the preparation of the w/o/w emulsion.

MATERIALS AND METHODS

Water was once-distilled from a glass still. Isopropyl myristate, used as the oil phase of the emulsions, was obtained from Croda Chemicals, Goole, Humberside. The non-ionic surfactants used were sorbitan monooleate (Span 80), polyoxyethylene sorbitan monooleate (Tween 80), manufactured by Honeywill Atlas, Carshalton, Surrey, and a polyoxyethylene (16·5) octyl phenol (Triton X-165) which was obtained from the Sigma Chemical Company, Poole, Dorset. The poloxamer compounds used were Pluronic F87 and Pluronic F88 poly(oxyethylene)-poly(oxypropylene), ABA

* Correspondence.
† Present address: School of Pharmacy, Robert Gordon's Institute of Technology, Aberdeen.

copolymers with 70 and 80% ethylene oxide content respectively which were obtained from Pechiney Ugine Kuhlmann, Bolton, Lancashire. Acrylamide and *NN'*-methylene-bis-acrylamide were both obtained from the Sigma Chemical Company. All materials were used as received.

Preparation of w/o/w multiple emulsions

The multiple emulsions were prepared by a two-stage emulsification procedure. The aqueous phase was emulsified in an equal volume of oil containing 5% (w/w) Sorbitan mono-oleate (Span 80) by means of a small vortex mixer (Whirlimixer, Fisons Scientific, Loughborough) to produce the primary water-in-oil emulsion. The w/o emulsion was re-emulsified in the same manner in an equal volume of water containing 2% (w/v) of Triton X165, a 3:1 mixture of Span 80 and Tween 80 or 5% 1:1 mixtures of Pluronic F87 and F88 as noted in the legends to the Figures, to produce the w/o/w emulsion. After preparation, the multiple nature of the emulsions was confirmed by microscopic examination.

Polymerization and gelation of the internal and external aqueous phases of w/o/w emulsions

Formation of poly(acrylamide) gels. Poly(acrylamide) gels were prepared from aqueous solutions of acrylamide and the crosslinking agent, *NN'*-methylene-bis-acrylamide. The total monomer concentration was 8% (w/v), and the concentration of crosslinking agent was either 5 or 25% of the total monomer concentration. The external aqueous phase of the w/o/w emulsions was gelled by emulsifying a w/o emulsion (prepared as previously described) in an equal volume of a second aqueous phase containing a surfactant mixture [Span 80/Tween 80 (3:1)] (2% w/v), acrylamide (6% w/v) and the crosslinking agent (2% w/v). After emulsification in the normal manner, the w/o/w emulsion was transferred to glass ampoules which were sealed under nitrogen. The samples were then exposed to γ-irradiation from a ^{60}Co source at doses of up to 0·25 Mrad.

The formation of poly(acrylamide) gel in the internal aqueous phase was accomplished in the following manner; a w/o emulsion was prepared by emulsifying the aqueous phase-acrylamide (8% w/v) and *NN'*-methylene-bis-acrylamide (2% w/v) in nitrogen-flushed water in an equal volume of the oil phase containing sorbitan monooleate (5% w/v). The w/o emulsion was sealed under nitrogen in a glass ampoule and exposed to γ-irradiation as described above. The resultant poly(acrylamide)-in-oil suspension was then re-emulsified in an equal volume of an aqueous phase containing various hydrophilic surfactants, resulting in a poly(acrylamide) gel-in-oil-in-water system.

Formation of poloxamer gels. Polymerization was carried out in the external aqueous phase of o/w or w/o/w emulsions according to the following procedure. The surfactant solutions were prepared by dissolving the poloxamer surfactants (2–10% w/v) in water prepared by flushing distilled water with nitrogen until saturated. Oil-in-water emulsions were prepared by emulsifying the oil phase in the poloxamer solution. Multiple w/o/w systems were prepared using the poloxamer solution as the hydrophilic secondary surfactant phase. After emulsification the samples were sealed under nitrogen in glass ampoules and exposed to various doses of γ-irradiation from a ^{60}Co source at a dose rate of 0·21 Mrad h^{-1}.

Rheology of irradiated poloxamer systems

Samples of w/o/w emulsion were examined using a cone and plate viscometer (Ferranti-Shirley) at 25 °C. Flow curves (plots of shear rate versus shear stress) were obtained from which the apparent viscosity (η_{app}) of the sample at various shear rate values could be calculated, as follows

$$\eta_{app} = \text{cone constant} \times \frac{\text{instrument reading} \times \text{range factor (poise)}}{\text{speed (rev min}^{-1})}$$

Stability assessment of irradiated poloxamer systems

The system chosen for this study was prepared according to the following formula: w/o emulsion: isopropyl myristate, containing 5% w/v Span 80 (70% v/v), distilled water (30% v/v); w/o/w emulsion water-in-oil emulsion (50% v/v), water, containing 10% (w/v) poloxamer (1:1 mixture Pluronic F87/F88) (50% v/v). The emulsions were sealed under nitrogen and exposed to γ-irradiation. Emulsion 1 was the control, emulsion 2 received 3·57 Mrad, emulsion 3, 4·83 Mrad and emulsion 4, 5·35 Mrad. Emulsions 2 and 3 were still fluid after irradiation but of much higher viscosity than the control, and emulsion 4 was just on the 'gel point'.

After preparation, the emulsion samples were stored at room temperature (20 °C) in glass ampoules. On preparation and after 1, 3 and 10 weeks, random samples were analysed by photomicrography. The ratio of 'filled' (multiple) to simple oil drops as well as the droplet size distributions of the simple and multiple (oil) drops and the internal aqueous droplets, were determined from photo-

graphic prints. The gelled emulsion was examined by gently stirring the sample in distilled water for 30 min. This allowed the gel network to break down slowly, releasing the drops. Samples of the resulting liquid were then examined in the usual manner. Flow curves of each system were also obtained in order to monitor any changes in the rheological properties of the emulsions.

RESULTS AND DISCUSSION

As the hydrophilic nature of the poloxamer surfactants prevented their use as stabilizers of the primary w/o emulsion, and as the more lipophilic members of the series degrade on irradiation, an alternative approach—a modified emulsion polymerization method based on the technique of Ekman & Sjoholm (1978) was used to gel the internal aqueous phase.

Initial experiments, in which a w/o/w emulsion containing the monomer and crosslinking agent initially in the internal aqueous phase was irradiated, were unsuccessful. It was found that release of the monomer and crosslinking agent into the external aqueous phase caused this phase to gel.

The problem of gelling the internal phase itself was solved by irradiating the primary w/o emulsion containing the monomer and crosslinking agent in the aqueous phase. The resulting poly(acrylamide)-in-oil dispersion was then redispersed in an aqueous phase containing hydrophilic surfactant to produce a w/o/w system containing a cross-linked poly(acrylamide) gel in the internal aqueous phase (Fig. 1a, b). This system has similarities to the gelatin microsphere/oil/water system described by Hashida et al (1977). The presence of polymorphic gel fragments can be clearly seen in Fig. 1a. Preliminary release rate experiments on these systems have been disappointing, no significant difference in the release of an iodide marker being found when release was compared to that from ungelled systems. It may be that other conditions are required to produce a more rigid gel. Stability of this system has not been assessed, although it is obvious from a knowledge of the mechanisms of breakdown of multiple emulsion systems (internal droplet coalescence and osmotic growth and shrinkage of the internal droplets, Florence & Whitehill 1981) that gelation of the internal phase blocks such routes of degradation.

The first method used to gel the external continuous phase was the production of a crosslinked poly(acrylamide)gel. Fig. 1c shows a photomicrograph of this system. Apart from varying the ratio and concentration of monomer and crosslinking agent, little control could be achieved over the reaction—rigid gels were produced resulting in immobile systems.

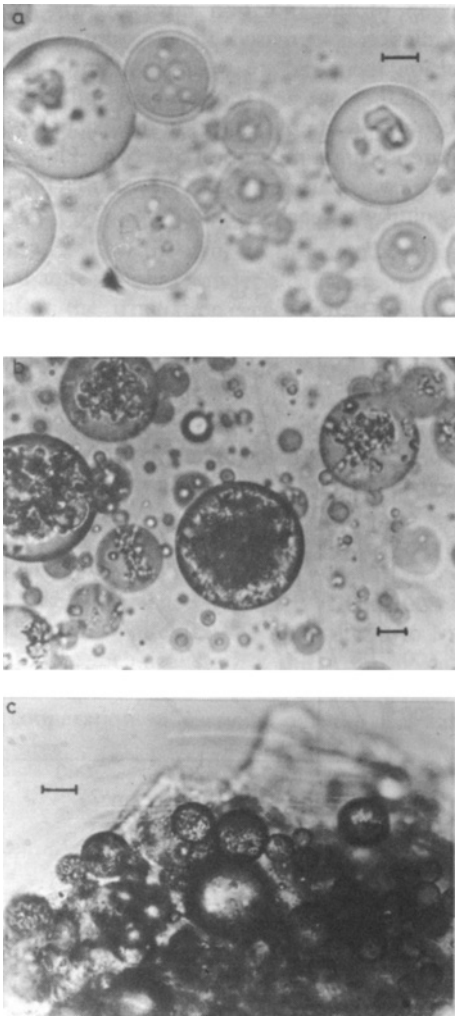

FIG. 1(a). Photomicrograph of a polyacrylamide gel/oil/water system prepared using Triton X165 (2% w/v) as surfactant for the second emulsification step. Bar = 5 μm. (b) Photomicrograph of a polyacrylamide gel/oil/water system prepared using a 3:1 mixture of 'Span 80'/Tween 80 (2% w/v) as surfactant for the second emulsification step. Bar = 10 μm. (c) Photomicrograph of the water-isopropyl-myristate-polyacrylamide gel system. Bar = 10 μm.

The poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) block copolymers were also used to gel the continuous aqueous phase. Poloxamers are used as the secondary hydrophilic surfactant in the preparation of the w/o/w system, and the finished emulsion is then irradiated. The polymerization reaction can be monitored by cone- and plate-viscometry. Fig. 2a shows the flow curve obtained for a water/isopropyl myristate/water emulsion as a

function of the radiation dose. As the dose of γ-irradiation is increased, the viscosity of the w/o/w emulsion increased up to a 'gel-point'. The 'gel-point' of the emulsion is dependent on the type and concentration of poloxamer. In the example shown, prepared using a mixture of 5% (w/v) Pluronic F87 and 5% (w/v) Pluronic F88 in the external phase, the 'gel-point' was reached at 4·2 Mrad (Fig. 2b).

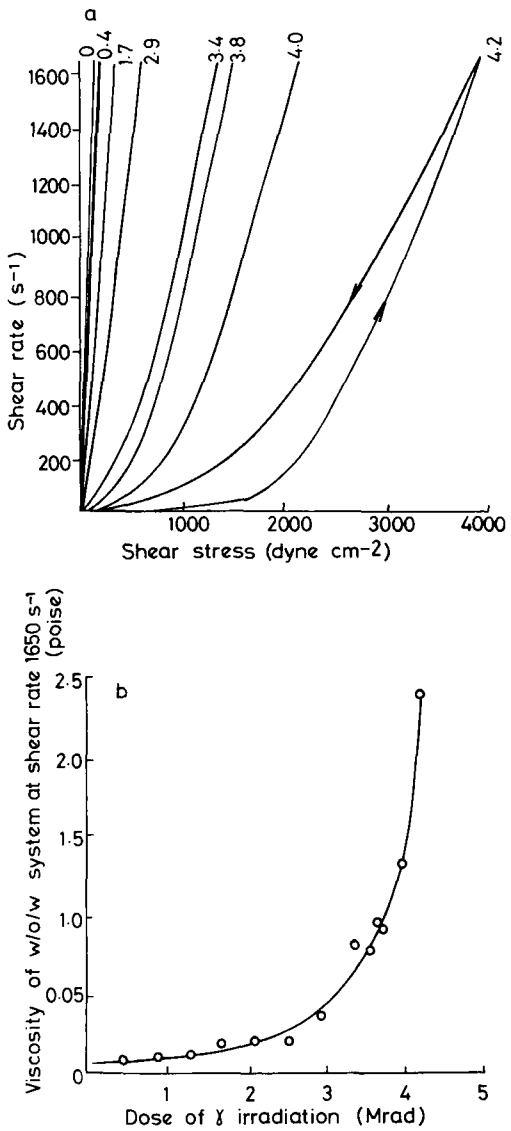

FIG. 2(a). Flow curve obtained using a cone-and-plate viscometer for a water-isopropyl myristate-water emulsion as a function of dose of γ-irradiation (shown in Mrad). (b) Viscosity of a w/o/w emulsion (at shear rate 1650 s^{-1}) stabilized with Pluronic surfactants (5% F87/5% F88) in the external aqueous phase as a function of dose of γ-irradiation.

The increase in viscosity shown by the flow curves indicates structure build-up in the external phase. The hysteresis loop exhibited by the gelled sample showed that the structure of the external phase was being broken down during the shearing process. These systems, which are water/oil/gel systems, do not cream. Fig. 3 is a photograph of a gelled w/o/w emulsion. The transparent gel shown for comparison, is composed of crosslinked poloxamer surfactant. It is not desirable, however, to expose the system to such a degree that a semi-solid emulsion system such as is shown in Fig. 3 is formed. Ideally,

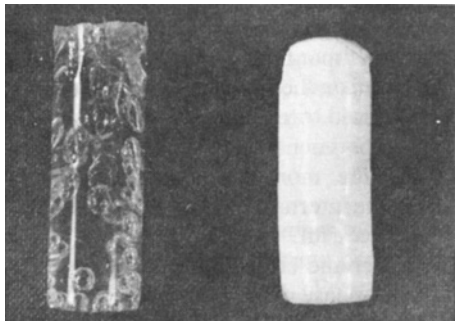

FIG. 3. Photograph of gelled poloxamer solution (10% w/v aqueous solution of a 1 : 1 mixture of Pluronic F87 and F88) (left) and a gelled w/o/w emulsion containing the same surfactant mixture in the external aqueous phase (right).

the system should be exposed until the 'gel-point' is approached. If this is done, the viscous w/o/w system will be stable to creaming but will release the multiple drops on mixing with water, due to the expansion of the gel on hydration. Stability studies on the w/o/w systems 1–4 showed each system exhibited a decrease in viscosity with time (Fig. 4). This is most likely to be due to changes in the gel structure, as the model system chosen for study contained a low percentage of 'multiple' drops, making a decrease in viscosity due to the loss of water from the internal aqueous phase unlikely. Photomicrographic studies showed that there was little change in the size of the multiple and simple oil drops or of the internal aqueous droplets of each system, although there was some evidence of multiple drop coalescence in the control system, not evident in the irradiated systems. The irradiated systems also appeared to be more stable to multiple drop rupture (Fig. 5). The ratio of multiple to simple oil drops in these particular emulsions was relatively low. The reason for this was the choice of surfactants and the relatively low primary aqueous phase volume used to minimize creaming during the long irradiation period in the reactor.

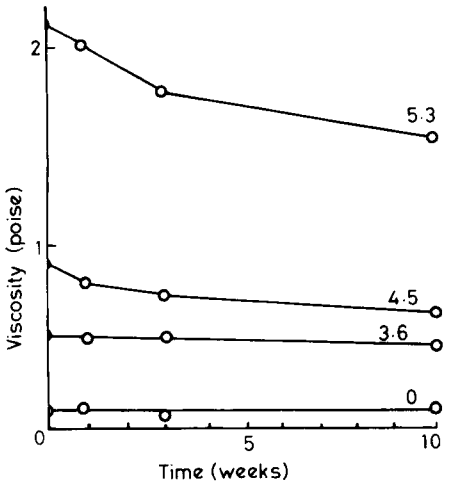

FIG. 4. Viscosity (at shear rate 1650 s^{-1}) of w/o/w emulsions exposed to different doses of γ-irradiation (shown in Mrad) over a storage period of ten weeks.

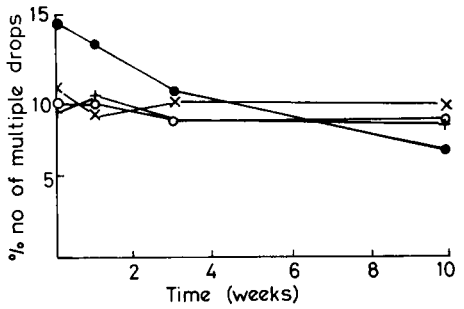

FIG. 5. Percentage number of multiple drops in w/o/w emulsions exposed to different doses of γ-irradiation (control) (●); 3·6 Mrad (○); 4·8 Mrad (×); 5·3 Mrad (+)—over a ten week storage period.

The main disadvantage of the use of γ-irradiation is that the drug has to be incorporated at the primary emulsification step and is therefore exposed to the γ-irradiation. It is possible, however, that the oil and surfactants may exert a protective effect on labile drugs but this has still to be investigated in these systems. The results of Al-Saden (1980) suggest that drugs solubilized in the core of poloxamer micelles are protected (to some extent) from the effects of irradiation.

It may, however, be possible to make use of the 'semi-permeable' nature of the oil layer in the w/o/w system and allow the drug to diffuse across to the internal aqueous phase under a concentration gradient after the irradiation process has been completed. Despite this problem the poloxamer systems are novel in that the technique makes use of a non-toxic surfactant, already present to stabilize the system, and the final product is sterile. A problem however with the poly(acrylamide) systems would be the possible toxicity arising from residual acrylamide monomer.

Acknowledgements

We thank the Science Research Council for the support of D. Whitehill. We are grateful to Mr J. Izatt and the technical staff of the Scottish Universities Research Reactor Centre, East Kilbride, for their cooperation in carrying out the irradiation procedures.

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