Water-in-Sorbitan Monostearate Organogels (Water-in-Oil Gels)

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Abstract □ Novel multicomponent organogels containing an aqueous phase are described, and some properties which influence their potential as delivery devices for hydrophilic drugs and vaccines are discussed. The gel is produced by preparing a hot water-in-oil (w/o) emulsion using sorbitan monostearate, a nonionic surfactant which is also the organogelator, as the principal emulsifying agent. On cooling at room temperature, the w/o emulsion sets to an opaque, semisolid, thermoreversible organic gel. Cooling the emulsion results in a reduced solubility of the sorbitan monostearate in the oil, with a corresponding decrease in solvent–surfactant affinities, causing surfactant self-assembly into aggregates. The microstructure of the w/o gel is seen by light microscopy to consist of a network of tubules and fibrils (containing the aqueous phase) dispersed in the organic medium. X-ray diffraction and freeze-fracture studies suggest that the tubular aggregates in the w/o gel are made up of surfactant molecules arranged in inverted bilayers and that the aqueous phase is accommodated within these inverted bilayers, bound by the polar headgroups of the surfactant molecules. The presence of water in the tubular skeleton of the organic gels results in the establishment of percolating electroconductive aqueous channels in the organogel. Increasing the water content of a w/o gel causes the surfactant tubules to swell with a corresponding increase in conductivity until the tubules are saturated. Further increase in the water content results in the excess water accumulating in droplets within the organic medium and a decrease in conductivity as the gel integrity is compromised. The w/o gels (containing a model antigen, radiolabeled bovine serum albumin, in the aqueous phase) have demonstrated depot properties after intramuscular administration to mice, entrapped antigen being released over a period of days.

Introduction

Interest in organic gels has increased, and a number of applications of these nonaqueous systems are being investigated.[1] Examples include transdermal drug delivery devices,[2] biosynthetic media,[3−8] moisture sensors,[9,10] synthetic templates,[11] purification and separation media,[12] and carriers for liquid crystals.[13] We previously reported the gelation of organic solvents by the nonionic surfactant, sorbitan monostearate.[14] These are opaque, thermoreversible semisolids whose microstructures consist of interconnected tubular aggregates, which, as suggested by freeze-fracture and X-ray diffraction measurements, are assemblies of the gelator molecules arranged in bilayers.[15]

The nonionic surfactant-based organogels, comprising pharmaceutically acceptable excipients (sorbitan monostearate, polysorbate 20, and a biodegradable oil, e.g., isopropyl myristate) may have potential as delivery devices for hydrophobic and hydrophilic drugs and vaccines. The hydrophobic active entity can be dissolved or dispersed in the sol phase at high temperatures before gel formation on cooling. This ensures a homogeneous distribution of the active ingredient in the organogel. A hydrophilic substance (dissolved in an aqueous medium) may also be incorporated in the organogel, by adding the aqueous phase to the organic solution at high temperatures (60 °C). A water-in-oil (w/o) emulsion is thus produced, where discrete aqueous droplets, bounded by surfactant films, are dispersed in the continuous external oil phase. On cooling, the w/o emulsion is converted to the gel state i.e., a w/o organogel is produced. We have previously reported the preparation of organogels where the aqueous phase was a niosome suspension, such that a vesicle-in-water-in-oil (v/w/o) gel is produced.[14,16] An active agent such as dissolved or suspended drug or antigen can thus be delivered in vivo within a nonaqueous medium. The latter may present a barrier to the diffusion of the active after in vivo administration, and it was speculated that a w/o gel may act as an intramuscular/subcutaneous depot for hydrophilic drugs and vaccines. In turn, the addition of the aqueous phase alters the organic gel. In this paper we report on the effects of the addition of an aqueous phase on sorbitan monostearate organogels. The gelation mechanism, the location of the aqueous phase in the w/o gel, and the maximum amount of aqueous phase which can be incorporated in the gel are explored. The in vivo release rate of a model hydrophilic antigen from a w/o gel was investigated, and an aqueous solution and a w/o emulsion (liquid state) were used as controls.

Materials and Methods

Sorbitan monostearate, 5,6-dicarboxyfluorescein, and bovine serum albumin (BSA) were purchased from Sigma (UK) while polysorbate 20, hexadecane (analytical grade), and isopropyl myristate (analytical grade) were from Fluka (UK). ^{125}I used to radiolabel BSA was from Amersham Pharmacia Biotech (UK). All were used as received, except for hexadecane and isopropyl myristate which were dried at room temperature overnight in a vacuum oven (Gallenkemp, UK) to ensure the absence of moisture which might affect gelation. Ultrahigh quality (double distilled) water was used throughout. In-bred male Balb/c mice (weighing approximately 20 g) were from Bantin & Kingman, Hull, UK.

Organogel Preparation—Sorbitan monostearate (10% w/v) and polysorbate 20 (2% w/v) were weighed into a vial, and the organic solvent (hexadecane or isopropyl myristate) was added. The mixture was heated to 60 °C in a water-bath and a transparent organic solution was produced. After the latter sol was cooled at room temperature, an opaque, thermoreversible semisolid gel was obtained.

Preparation of w/o Gel—The aqueous phase (water/5,6-dicarboxyfluorescein solution/radiolabeled bovine serum albumin solution) was added dropwise to the oil phase (the gel prepared

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above) while vortexing, both phases being at 60 °C. A w/o emulsion was obtained. On cooling at room temperature, the w/o emulsion gelled to an opaque, thermoreversible semisolid.

Preparation of w/o Emulsion—The aqueous phase (radiolabeled bovine serum albumin solution) was added dropwise to the oil phase (10% w/v sorbitan monooleate + 2% w/v polysorbate 20 in isopropyl myristate) while vortexing, both phases being at 60 °C. A water-in-oil (w/o) emulsion was obtained.

Light Microscopy—A light microscope (Nikon Microphot-FXA, Japan) with an attached camera (Nikon, FX-35DX, Japan) and a hot-stage (Linkam TC 93, UK) was used to analyze the gel microstructures.

X-ray Diffraction Studies—X-ray data on hexadecane organogels were collected on a Siemens D500 diffractometer (in the Department of Crystallography, Birkbeck College) equipped with a copper tube and a quartz primary-beam monochromator giving a wavelength $\lambda = 1.54056$ Å. The X-ray tube was run at 45 kV and 30 mA. Samples were mounted in a flat-plate specimen holder and flattened with a glass microscope slide. The sample was spun about an axis normal to its flat-plate surface, and diffraction patterns were measured using a scintillation detector. The data were obtained for the 2θ range 1−35° in steps of 0.05° at 10 s per point. A w/o gel containing 48 μL water per milliliter of gel was studied to investigate the effect of the added water on the surfactant organization in the tubular aggregates, and an anhydrous gel was used as control.

Freeze Fracture Investigations—The gel (containing 167 mL water/mL of hexadecane organogel) was sandwiched between copper plates and then quickly frozen using liquid propane (−180 °C). The frozen samples were loaded in a holder under liquid nitrogen, transferred to a Balzers BAF 400, and fractured at −150 °C. The frozen planes were replicated with platinum (2 nm) at an angle of 45° and with carbon (20 nm) from an angle of 90°. The replicas were then cleaned with distilled water, mounted on copper grids, dried, and examined using a transmission electron microscope.

Conductivity Measurements—A Wayne−Kerr conductivity bridge was used to measure the conductance of hexadecane gel samples containing increasing amounts of water. Two carbon electrodes (diameter 5 mm, 5 cm apart) were used while a constant voltage from a DC source was applied. Five conductance readings were taken for each gel sample, and the mean was calculated.

Intramuscular Administration—The presence of a depot property was investigated by measuring the amount of a model radiolabeled antigen, bovine serum albumin (BSA), which remains at the site after intramuscular injection. A biodegradable oil (isopropyl myristate) was used as the organic solvent in the w/o gel. An aqueous radiolabeled BSA solution and a w/o emulsion were used as controls. At time $t = 0$, each group of mice (15 mice/group) was injected intramuscularly with 50 μL of a formulation (each mouse received the same amount of radiolabeled BSA, around 108 Bq). Three mice from each group were killed at different times, t, postinjection, the injected leg was amputated, and the radioactivity in the leg was measured using a mini-γ counter. The count obtained was expressed as a percentage of the initial total radioactivity at time $t = 0$.

The injection technique was very important to ensure reproducibility, especially for the w/o gel; the latter was taken up into a glass syringe when hot and thus in the liquid state. The sol phase was then allowed to cool and set to the gel state (observed visually) before intramuscular administration. Both w/o gel and w/o emulsions were administered using glass syringes; plastic syringes were avoided as their rubber plunger tips absorb the oily vehicle and swell such that the force needed to expel and thus administer syringe contents would be dramatically increased, and the formulation would be altered through loss of oil.

Results and Discussion

Water-in-Oil Gel Formation—A w/o gel is prepared by first forming the w/o emulsion (the sol phase) at high temperatures: the aqueous phase at 60 °C is added dropwise to the oil phase (oil solution of the nonionic surfactants, sorbitan monostearate, and polysorbate 20), while vortexing. A w/o emulsion is produced, where the aqueous droplets, bound by surfactant interfacial films, are dispersed in the continuous external oil phase (Figure 1a). The hydrophilic nonionic surfactant, polysorbate 20, was included in the organosol prior to emulsion preparation, as it increases the gel lifetime[15] and enhances emulsion stability by forming mixed surfactant films with the sorbitan monostearate molecules at the w/o interface. On cooling, the w/o emulsion gels to an opaque, thermoreversible semisolid. Cooling results in a decrease in the solubility of the sorbitan monostearate gelator in the oil and consequently lowered solvent−gelator affinities. As a result, the surfactant molecules self-assemble into tubular aggregates (Figure 1b) which join and interact with one another. Junction points are established (Figure 1b), and a three-dimensional network is formed which immobilizes the oil phase. The fluorescent aggregates in Figure 1b indicate that the aqueous phase (in this case, fluorescent, 5,6-dicarboxyfluorescein solution), previously bound by the surfactant interfacial layer at 60 °C, is incorporated into the surfactant tubules upon gel formation. The w/o gel is physically stable for months at room temperature.

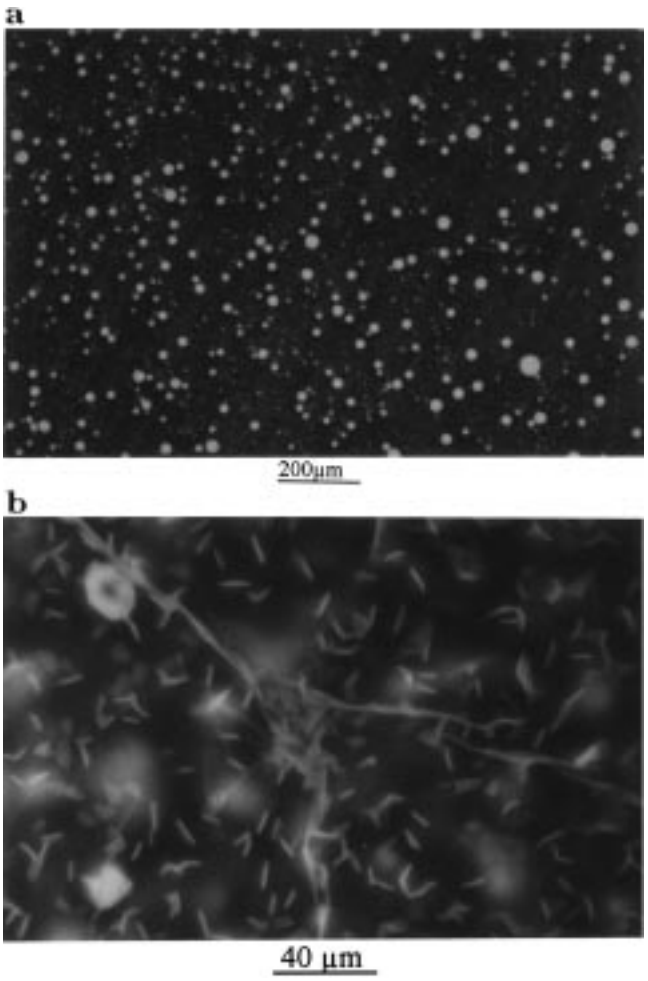

Figure 1—(a) The microstructure of a w/o emulsion at 60 °C: aqueous droplets (fluorescent CF solution) dispersed in the continuous oil phase. (b) The microstructure of a w/o gel at 25 °C: tubules and fibrils incorporating the aqueous fluorescent CF solution in the organic medium. Junction nodes are responsible for the integrity of the gel skeleton.

The w/o gel is thermoreversible: on heating it melts to a liquid w/o emulsion, as an increase in temperature causes a corresponding increase in the gelator solubility in the oil phase and the tubular aggregates disassemble. The w/o emulsion can, in turn, be cooled to the gel state. The gelation temperature (T_g) at which the w/o gel melted to the emulsion was found to be 41−44 °C by hot-stage microscopy.

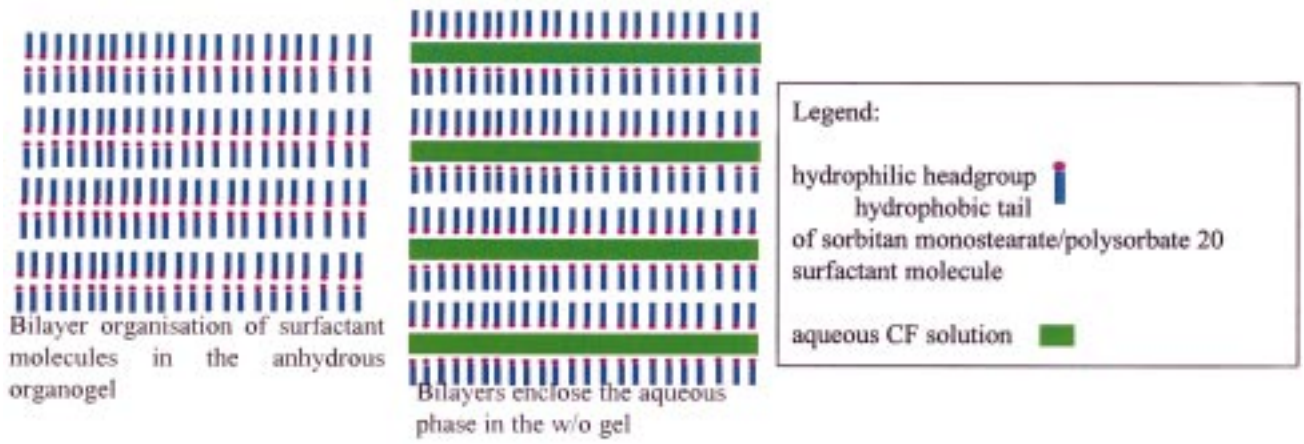

Figure 2—Schematic diagram shows the suggested location of aqueous phase within bilayers, bound by surfactant headgroups. The structures here are highly idealized.

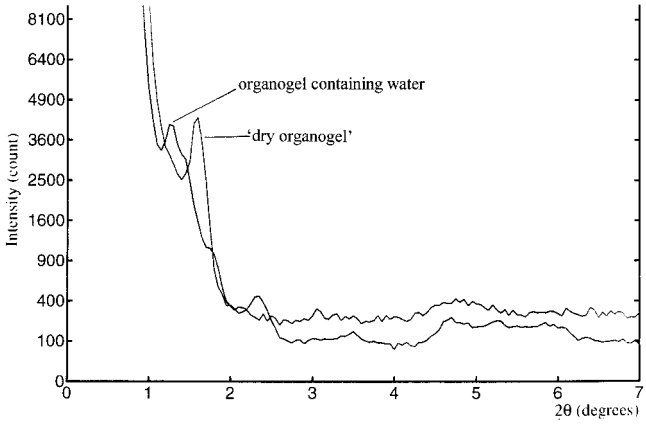

Figure 3—X-ray diffraction measurements show an increase in bilayer width upon the incorporation of an aqueous phase in the organic gel ($\downarrow \theta \equiv \uparrow$ distance).

Location of the Aqueous Phase within Surfactant Tubules—The tubular aggregates in sorbitan monostearate organogels are thought to be composed of surfactant molecules arranged in multiple inverted-bilayers (Figure 2). Such an arrangement was suggested by freeze-fracture investigations which revealed the presence of bilayers, and X-ray diffraction measurements which showed peaks at 5.9 and 0.4 nm. These values (relating to the bilayer width and the distance between two adjacent surfactant molecules, respectively) were found to correlate with the theoretical calculated values, 5.91 and 0.46 nm, obtained using the software QUANTA & CHARm (unpublished results).

Figure 1b shows the aqueous phase to be located within the tubular surfactant aggregates in the w/o organogel. The aqueous phase is most likely accommodated within the surfactant bilayers as schematically shown in Figure 2. The water molecules will also hydrogen bond with the surfactant headgroups, which may further stabilize the w/o gel. The water-in-bilayer hypothesis is supported by X-ray diffraction measurements which show an increased bilayer width, from 5.9 to 6.9 nm when water is incorporated in the gel (Figure 3). Freeze fracture microscopy also shows that the bilayered nature of the organogel is retained in the w/o gel (Figure 4).

Water in Tubules: Effect on Gel Microstructure and Electrical Conductivity—Water-in-oil gels containing increasing amounts of aqueous phase were prepared and examined using light microscopy to determine first the effect on gel microstructure and second, the maximum

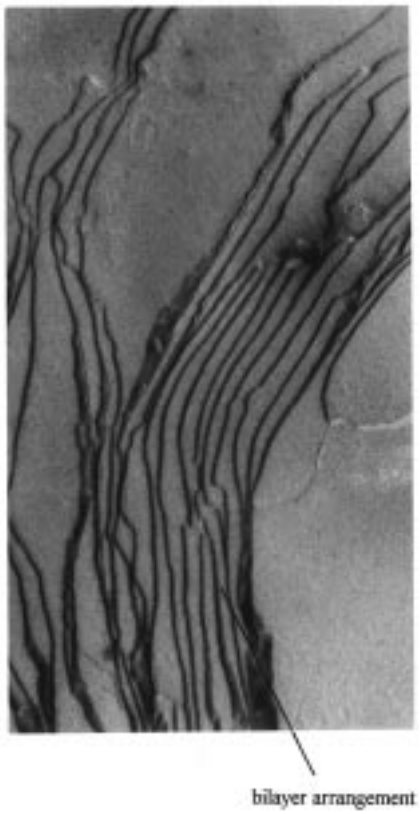

Figure 4—Freeze-fracture micrograph shows a bilayered arrangement in a w/o gel sample.

amount of aqueous phase that can be incorporated in sorbitan monostearate/hexadecane organogels. Upon the addition of increasing amounts of water, the star-shaped clusters of surfactant tubules (Figure 5a) break up gradually, initially coexisting with individual tubules until all the clusters break and individual tubules and fibrils are seen in the medium. Further addition of water results in swollen tubules as their bilayers enclose increasing volumes of water (Figure 5b). Swelling of tubules is, however, a limited process as the bilayers can only enclose a certain amount of water while retaining their integrity. Excess water added after the saturation point has been reached, accumulates in droplets, bound by surfactant films (Figure 5, parts c and d). Addition of excessive amounts of water finally results in gel breakdown as aggregate integrity is lost, the surfactant molecules being in interfacial films

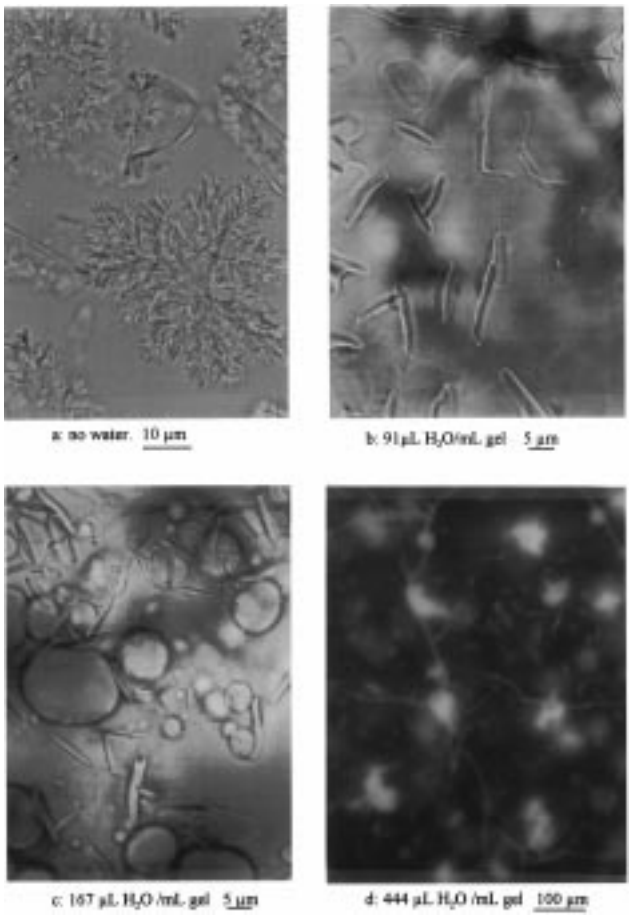

Figure 5—The changing microstructure of the w/o gel containing increasing amounts of water.

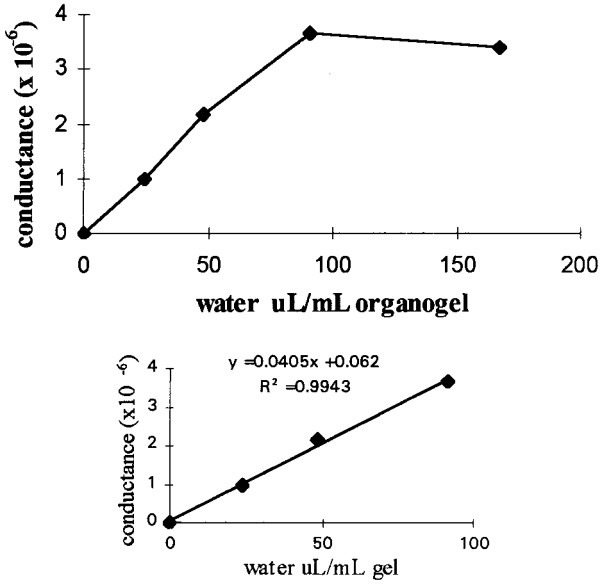

Figure 6—The electrical conductivity (1/Ω) of w/o gels containing increasing amounts of water (μL/mL organogel).

around the water droplets and in monomeric or spherical micellar form in the bulk oil.

The changing microstructures of the w/o gels is reflected in the electrical conductivity changes (Figure 6). The presence of water in the interconnected surfactant tubules results in the establishment of electroconductive aqueous channels in the organic gel. Electrical conductivity increases linearly with increasing amounts of water until a

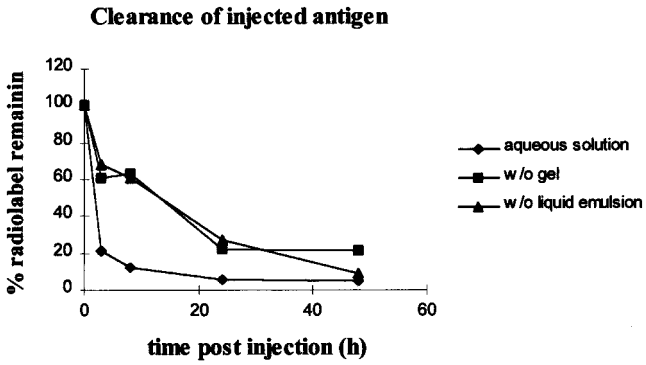

Figure 7—The clearance rates of radiolabeled model antigen after intramuscular administration in a w/o gel and controls, a w/o emulsion, and an aqueous solution.

peak is reached after which the conductivity decreases. The peak lies between 91 and 167 μL of water/mL of organogel and corresponds to the saturation level of water in surfactant bilayers in the tubules. This tallies with the light micrographs in Figure 5 which also indicate a saturation point between 91 and 167 μL water/mL of organogel. After the saturation point is reached, the distorted water droplets containing the excess aqueous phase cause a deterioration of the gel structure and integrity. This interferes with the electrical conductivity of the gel and the latter decreases, as part of the surfactant molecules are involved in interfacial films around the water droplets and fewer surfactant tubules providing aqueous channels are available for electrical conduction.

The Water-in-Oil Gel: A Delivery System for Drugs and Vaccines? The water-in-oil gel is a formulation in which hydrophilic drugs and vaccines may be administered in vivo, in an organic vehicle. Such incorporation may confer certain advantages to the active entity, e.g., the organic gel may present barriers to diffusion, such that after administration, the active entity, incorporated within the surfactant bilayers in the organogel is slowly released from the w/o gel, and a depot effect may be achieved.

To determine any depot effect after intramuscular injection, we measured the clearance rate of a model antigen (bovine serum albumin) administered in a w/o gel to mice. Figure 7 shows the clearance rates (defined as the rate at which injected antigen disappears from the site of injection) of BSA from a w/o gel and from the controls, aqueous solution, and w/o emulsion. After injection of the aqueous BSA solution, almost all the BSA is cleared within 8 h. On the other hand, the w/o emulsion and the w/o gel release the antigen slowly over a period of days. The clearance rate of antigen from the w/o emulsion is similar to that from the w/o gel except at 48 h postinjection, where the w/o gel is superior as a depot. After 48 h, 20% of the injected antigen is still present at the injection site. Gelling of the oil phase, though apparently not important in the initial stages of antigen clearance, seems to confer some advantage in increasing the duration of the antigen at the injection site.

After injection, the organic gel probably remains at the site of injection and assumes, like oils, a pea-shaped depot,[17] unlike aqueous formulations which spread along the muscle fibers. The local interstitial fluid then penetrates into the gel mass via the interconnected tubular network. This invasion of the interstitial fluid into the gel mass slowly breaks down the gel into smaller fragments. At the same time, emulsification occurs at the gel surface between the oil and the interstitial fluid and oil droplets bud off from the gel. The model hydrophilic solute is thus released as the gel slowly breaks into smaller fragments and erodes. This suggested release mechanism is based on

in vitro investigations where an aqueous solution was made to contact with a gel mass. This gel disintegration after administration explains why the depot effect achieved by the organogel is more transient than anticipated. A short depot effect may, however, be sufficient for certain applications, e.g., as immunoadjuvants, where a short depot action is thought to be effective in enhancing the immune response to antigens.[18]

Conclusions

We have reported the gelation of water-in-oil emulsions when a gelator, sorbitan monostearate, is used as the emulsifying agent. The w/o gels are thermoreversible semisolids whose microstructure consists of interconnected tubular aggregates within which the aqueous phase is trapped. The latter aqueous phase is believed to be entrapped within the inverted surfactant bilayers, bound by polar headgroups in the tubules. The presence of water in the surfactant tubules allows electrical conduction through the gel, conductivity being proportional to the aqueous content in the gel. The w/o gels enable the delivery of hydrophilic active entities within an organic medium, and we have demonstrated an in vivo depot over several days, but their longevity is compromised by the access of water to the system by percolation.

References and Notes

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