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Non-ionic surfactant based organogels incorporating niosomes

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A novel anhydrous organogel was formulated as a potential delivery vehicle from a solution of two non-ionic surfactants, sorbitan monostearate (Span 60) and polyoxyethylene sorbitan monolaurate (Tween 20), in hexadecane at 60°C, which cools to a white, semi-solid, thermoreversible gel at room temperature. The gel comprises microscopic « clusters » of tubules and fibrils dispersed in the organic medium, as revealed by optical microscopy. The addition of an aqueous phase (water or niosome suspension) up to 17% v/v to the oil phase at 60°C produces water-in-oil (w/o) and vesicle-in-water-in-oil (v/w/o) systems, respectively. On cooling, the oil phase of these emulsions gels and surfactant rods, fibrils and some distorted droplets are observed. The release rate of hydrophilic solute from these gels was found to be lowest when a disperse system of spherical water droplets in the continuous oil phase is formed at high temperatures, compared to the faster release from the gel where the fibril structures act as nearly continuous aqueous channels running through the organic medium, providing a means of traversing the oil phase.

Un nouvel organogel anhydre a été mis au point à partir d'une solution de deux surfactifs non ioniques, le monostéarate de sorbitane (Span 60) et le monolaurate de sorbitane polyoxyéthyléné (Tween 20), réalisée à 60°C dans l'hexadécane, qui se transforme par refroidissement en un gel thermoréversible semi-solide blanc. A l'examen microscopique, il apparaît que ce dernier comporte des agglomérats de tubules et fibres dispersés dans le milieu organique. L'addition à 60°C d'une phase aqueuse (eau ou suspension de niosomes) jusqu'à 17% v/v à la phase huileuse entraîne la formation de systèmes eau dans l'huile (e/h) ou vésicules dans l'eau dans l'huile (v/e/h), selon la nature de la phase aqueuse employée. Au refroidissement, la phase huileuse de ces émulsions gélifie : des bâtonnets et des fibres de surfactifs et quelques gouttelettes déformées sont observés. La vitesse de libération du soluté hydrophile à partir de ces gels est plus faible avec un système dispersé (gouttelettes d'eau dans la phase continue aqueuse) formé à haute température qu'avec un gel dans lequel les structures apparentes des fibres forment des canaux continus à travers le milieu organique, offrant ainsi une possibilité de passage à la phase huileuse.

Key words : Organogel — Non-ionic surfactant — Controlled release — Vesicle-in-water-in-oil emulsion.

Mots clefs : Organogel — Surfactif non ionique — Libération contrôlée — Emulsion vésicule dans l'eau dans l'huile.

Non-ionic surfactants form a variety of aggregates from micelles to large vesicles, which can be used as vehicles for drug delivery. Controlled release from intramuscular and subcutaneous depots is possible with aqueous niosome suspensions, but there may be some advantages to exploring non-aqueous systems to provide for a slower dispersal of drugs. The design of non-aqueous delivery systems we have developed is a vesicle-in-water-in-oil system [1, 2] using sorbitan monoesters to prepare both vesicles and the final vesicle-in-water-in-oil emulsions. During recent detailed studies of these emulsions it was noted that sorbitan monostearate (Span 60), but not Span 80, causes the gelation of hexadecane at surfactant concentrations as low as 1% w/v (0.02 M), yielding a white semi-solid gel with a smooth texture and a variety of internal microstructures. At lower concentrations, e.g., 0.1% w/v, a network of « fibres » form within the organic solvent, the network becoming more dense with increasing surfactant concentration, until all the hexadecane is gelled. Gel formation in lecithin organogels has been said to occur through « *transient networks from entangled long, flexible and cylindrical reverse micelles* » [3-6]. In gelatin-containing microemulsion based organogels, aqueous channels coexisting with microemulsion-type droplets are thought to be responsible for the semi-rigidity of the gel [7, 8]. Sodium stearate swells in organic solvents [9] and there is much literature on stearates in organic solvents. We can locate no papers, however, systematically describing

organogels based on non-ionic surfactants, although the phase diagrams of mixed non-ionic surfactant systems in dodecane or mineral oils were elucidated some time ago and viscous inverse phases were identified [10]. Such organogel systems are described in this paper.

A second non-ionic surfactant, polyoxyethylene sorbitan monolaurate (polysorbate 20 or Tween 20) was added to optimize the gel formed. Tween 20 promotes the dissolution of Span 60 in hot hexadecane, possibly through mixed inverse micelle formation ; the resulting gel does not synerese upon storage. The microstructure of the organogel and the subsequent changes upon addition of increasing amounts of water have been investigated. Vesicle-in-water-in-oil emulsions were also prepared as potential controlled release systems for drugs and vaccines. Release of a model solute, 5,6-carboxyfluorescein, from v/w/o emulsions was compared with release from an aqueous solution, a niosome suspension and a water-in-oil emulsion, all at different temperatures.

I. EXPERIMENTAL

1. Materials

The non-ionic surfactants sorbitan monostearate (Span 60) and polyoxyethylene sorbitan monolaurate (polysorbate 20 or Tween 20) were purchased from Sigma (United Kingdom). Hexadecane was obtained from Fluka (United Kingdom). Cholesterol used in the preparation of niosomes and carboxyfluorescein used as model solute were purchased from Sigma and used as received. Solulan C-24 (poly-24-oxyethylene cholesteryl ether) was obtained from Amerchol (United States). Ultra high quality water was used throughout.

2. Preparation of niosomes

Non-ionic surfactant vesicles were prepared by the hand-shaking method. Three hundred micromoles of Span 60, cholesterol and Solulan C-24 in the ratio 45/45/10 was dissolved in 15 ml of chloroform in a 100 ml round-bottomed flask. Using a rotary evaporator, the organic solvent was then removed under reduced pressure and a thin lipid film formed on the flask wall, which was flushed with oxygen-free nitrogen for 10 min to remove all remaining chloroform and rehydrated with 6 ml of carboxyfluorescein solution while mechanically hand-shaken for 1 h at 60°C in a water bath. After cooling of the niosome suspension, free and entrapped carboxyfluorescein were separated by gel chromatography, niosomes being collected in the void volume.

3. Preparation of the anhydrous organogel

Span 60 (10% w/v) and Tween 20 (2% w/v) were dissolved in hexadecane at 60°C. A clear, colourless solution was obtained. On cooling at room temperature, the solution set to a gel.

4. Preparation of w/o and v/w/o emulsions

Variable amounts — up to 200 µl — of carboxyfluorescein solution or niosome suspension were added dropwise to 1 ml of the oil phase while vortexing. Both the aqueous and the oil phases were kept at 60°C. Opaque emulsions (w/o and v/w/o, respectively) were obtained. On cooling to room temperature, the emulsions gelled. The emulsions were defined by an R value, R being [water]/[surfactant], i.e., number of molecules per surfactant molecule. For release rate experiments, gels with R value of 44, i.e., 200 µl of aqueous phase/millilitre of oil phase. were used.

5. Light microscopy on gels

The organogels and the emulsions were observed using a light microscope. The stage was heated to follow changes in microstructure.

6. *In vitro* release studies

In vitro release of carboxyfluorescein from the four systems (aqueous solution, niosome suspension, w/o emulsion and v/w/o emulsion) was investigated, using a simple dialysis method. Size 3 (20/32") Visking tubing was soaked, 1.2 ml of each system pipetted into a Visking bag and the bag sealed. Each sample contained the same amount of carboxyfluorescein. The Visking bags were then immersed in 20 ml of Trizma buffer in universal bottles and constantly shaken in water baths at 4, 30, 37 and 60°C. At time intervals, 200 µl of recipient buffer was removed and fluorescence measured. Two hundred microlitres of fresh buffer was replaced in the universal bottles each time.

II. RESULTS AND DISCUSSION

1. Light microscopy on organogels

Organogels with R values of 0 to 44, representing 0 to 200 µl water in 1 ml gel, were examined by light microscope. *Figures 1 to 5* show the microstructure of some of the gels and illustrate the variety of surfactant structures within the gels, whose nature depends on both the water content and temperature as discussed above.

1.1. Anhydrous organogels

When hot, a clear solution is obtained. No visible microstructure is seen as the non-ionic surfactants dissolve in hexadecane, forming an isotropic phase of mixed reverse micelles containing Span 60 and Tween 20. This solution cools to a white semi-solid gel in which microscopic « clusters » made up of a very large number of small tubules seem to be evenly distributed in the solvent (*figure 1*). The « clusters » are fairly uniform in size and shape and some are linked to one another by fibrils. The tubular structures are responsible for

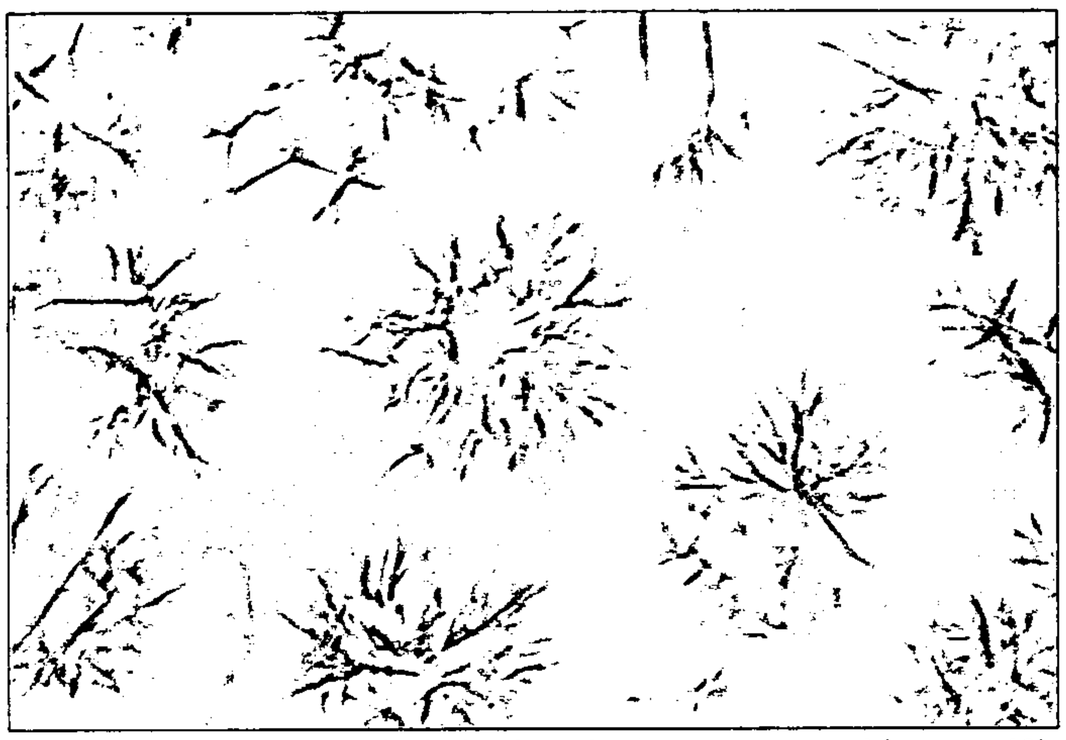

Figure 1 - « Clusters » of tubules in anhydrous organogel (mag. x 40).

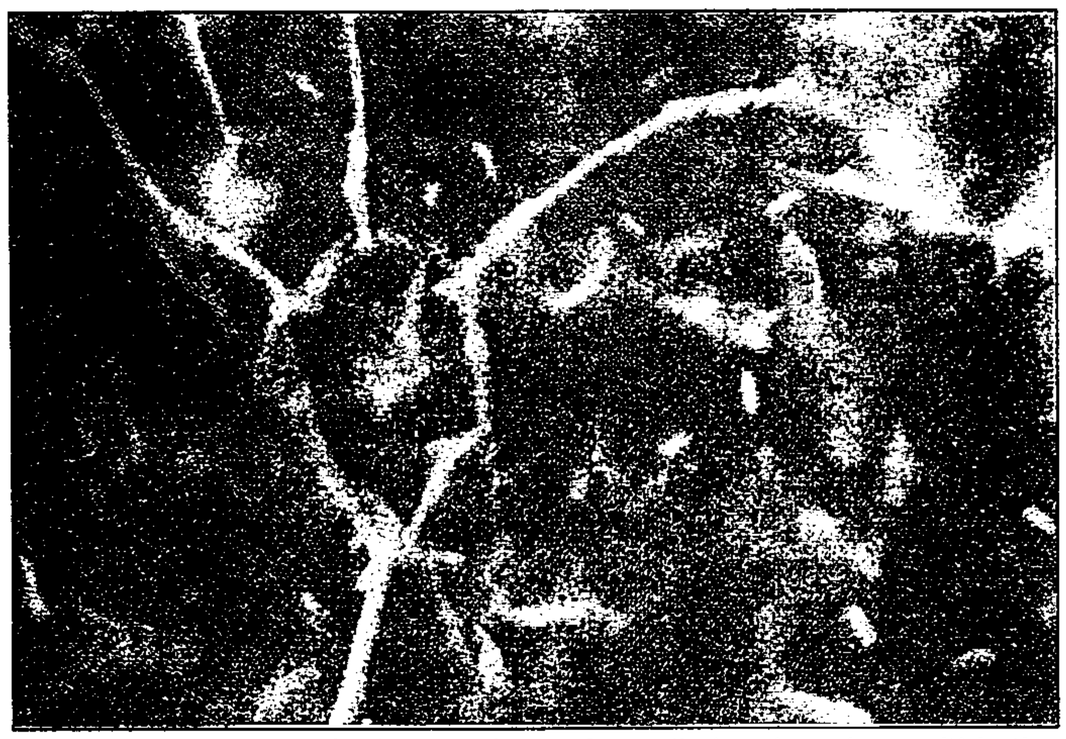

Figure 2 - Surfactant fibrils merging to form distinct junctions within the gel (200 µl of carboxyfluorescein solution in 1 ml oil phase) matrix (mag. x 80).

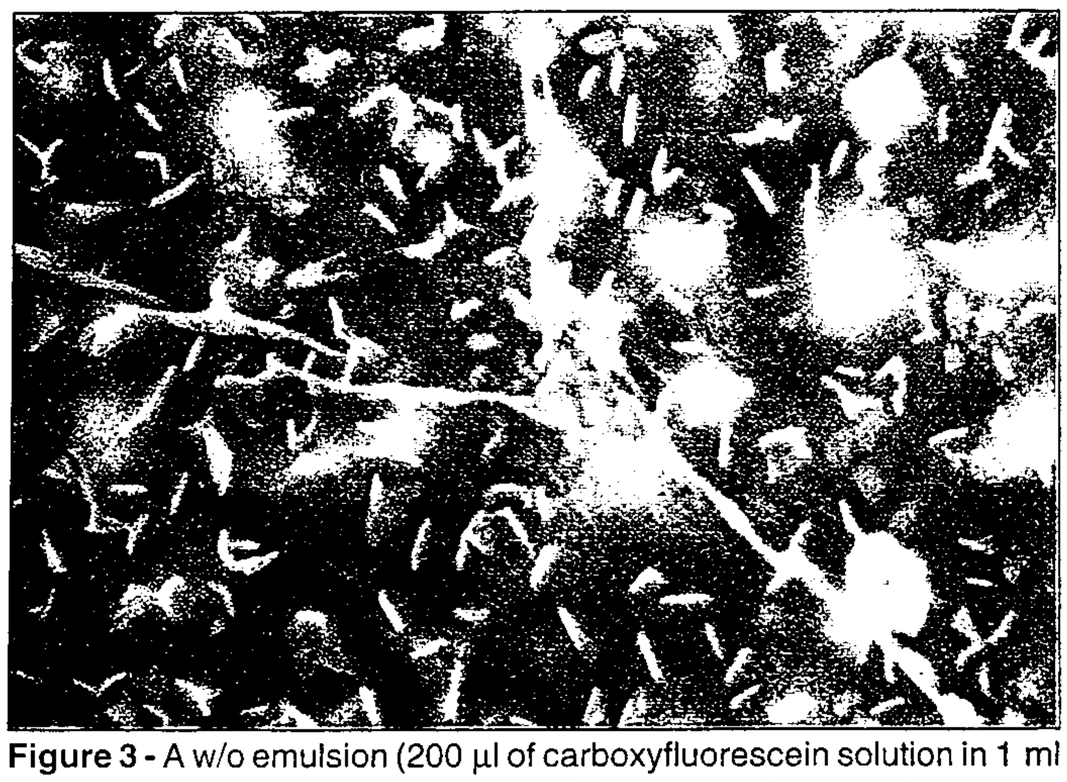

Figure 3 - A w/o emulsion (200 µl of carboxyfluorescein solution in 1 ml oil phase) that has gelled on cooling. The carboxyfluorescein solution has localized in rods, fibrils and distorted droplets (mag. x 40).

gelation by immobilizing the solvent, and are probably aggregates of reverse cylindrical micelles formed as a result of the reduced solubility of the surfactants in hexadecane on cooling. The tubules formed then appear to aggregate into « clusters ».

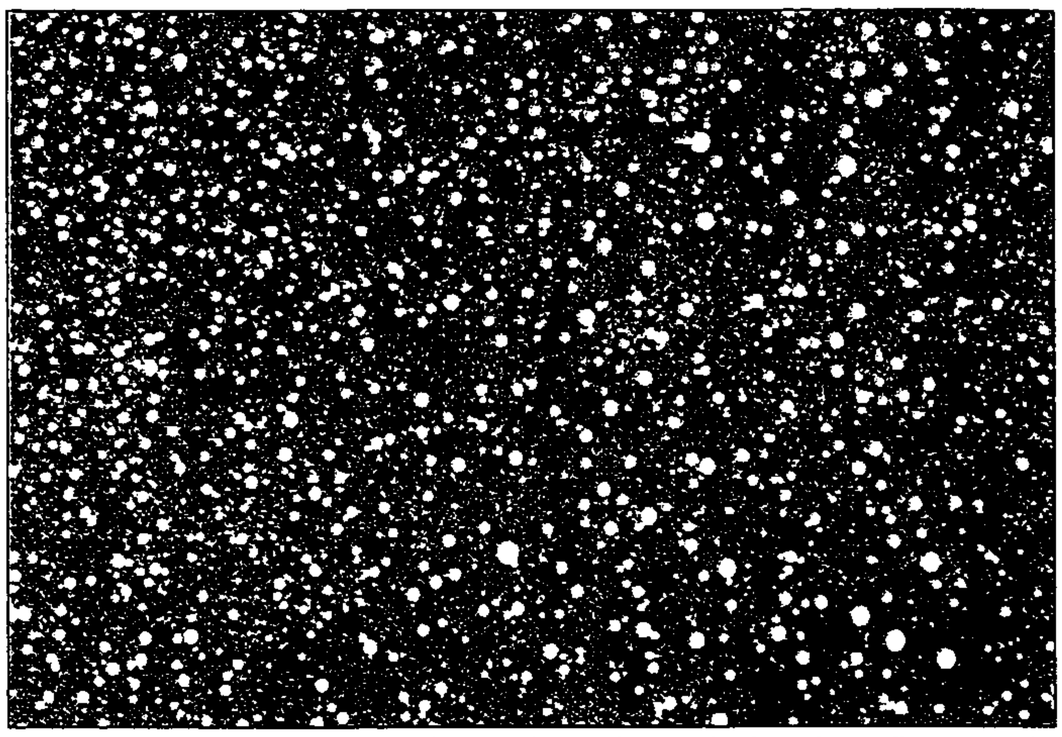

Figure 4 - A conventional w/o emulsion (200 µl of carboxyfluorescein solution in 1 ml oil phase) at high temperatures (mag. x 4).

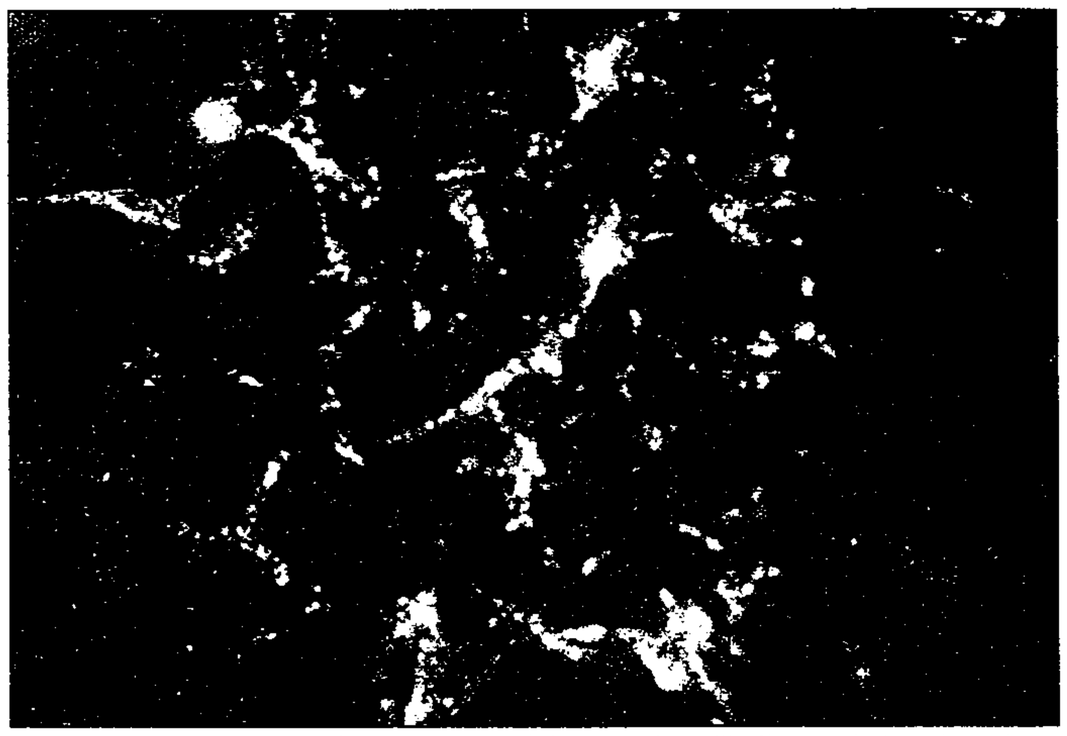

Figure 5 - Niosomes (size 300 nm) located in fibrils and droplets in a gelled v/w/o system after addition of 200 µl niosome suspension (composition : 300 µmol Span 60, cholesterol and Solulan C-24 in 45/45/10 ratio) in 1 ml of oil phase (mag. x 40).

1.2. Hydrous systems

As water is added to the gel, the clusters become smaller and more individual tubules appear to coexist with the remaining ones.The further addition of water results in the « clusters » disaggregating and individual tubules appearing almost exclusively. A negligible percentage of the added water will be dispersed in molecular form in the bulk organic solvent — most of it is expected to be found in water pools associated with the polar head groups of the surfactants [11, 12]. The location of the water pools is suggested by using carboxyfluorescein solution as the aqueous phase. Tubules fluoresce in the resulting gel showing the aqueous phase to be localized there.

Increasing the water content up to, for example, 100 µl water/millilitre oil phase, gives smooth cylindrical rods with tapered ends. Entanglement of these cylinders and end to end joining produce very long fibrils and distinct junctions in the gel matrix (*figure 2*). Fibril aggregates merging into microcrystalline nodes have been described before by Terech [13]. The further addition of water, up to 200 µl water/millilitre oil phase, gives some distorted aqueous droplets in addition to

tubules and fibrils. Presumably, the aqueous canals have become saturated with water and the excess accumulates in droplets bounded by an interfacial surfactant film (*figure 3*).

On heating the gels, a conventional water-in-oil emulsion is formed (*figure 4*). The surfactant aggregates dissolve in hexadecane at high temperature and form an amphiphilic layer at the interface between water droplets and the continuous oil phase. The system thus assumes the form of a normal water-in-oil emulsion with water droplets dispersed in a continuous oil phase.

1.3. Vesicle-in-water-in-oil formulations

In gels to which aqueous niosomes are added, tubules, fibrils and some distorted aqueous droplets are formed, with niosomes located in the tubular structures and in the droplets (*figure 5*).

On heating, a disperse system of spherical aqueous droplets (enclosing niosomes) in a continuous oil phase — a typical vesicle-in-water-in-oil system — is formed.

2. *In vitro* release studies

Figure 6 show the release profile of carboxyfluorescein with time from the four systems : aqueous solution, niosome suspension, w/o emulsion and v/w/o emulsion at 4, 30, 37 and 60°C. Solute diffuses from the samples through the Visking bag into the recipient buffer solution. The slowest release at temperatures 4, 30 and 37°C resulted from the niosome suspension. At these temperatures the v/w/o and w/o gels offer less resistance to transport of carboxyfluorescein, which diffuses along the aqueous channels within tubules and fibrils in the organic medium. Syneresis of the gels on prolonged shaking in contact with the recipient buffer also aids diffusion of the solute as some of the organic solvent barrier is removed. At 60°C, the gels melt to disperse systems (emulsions). Diffusion through the emulsions is thought to occur as in w/o/w emulsion : the solute is released from the vesicles, then diffuses through the aqueous phase, across the w/o interface and finally diffuses through the oil, probably mediated by inverse surfactant micelles [14]. This complex mode of transfer as well as the presence of the organic barrier cause a very slow rate of release of carboxyfluorescein from the emulsions at high temperatures.

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A novel non-ionic organogel was studied. It can exist as an anhydrous system or one containing an aqueous solution or suspension, e.g., of niosomes. The anhydrous gel was found to be stable for months at room temperature. Slow release of solutes from the gels can be exploited for the controlled release of drugs or antigens. The nature of the fibrillar structures which form the skeleton of the organogel containing the aqueous phase is being investigated, and their utility as vaccine adjuvants is under study. Acceptable alternatives to hexadecane are being sought.

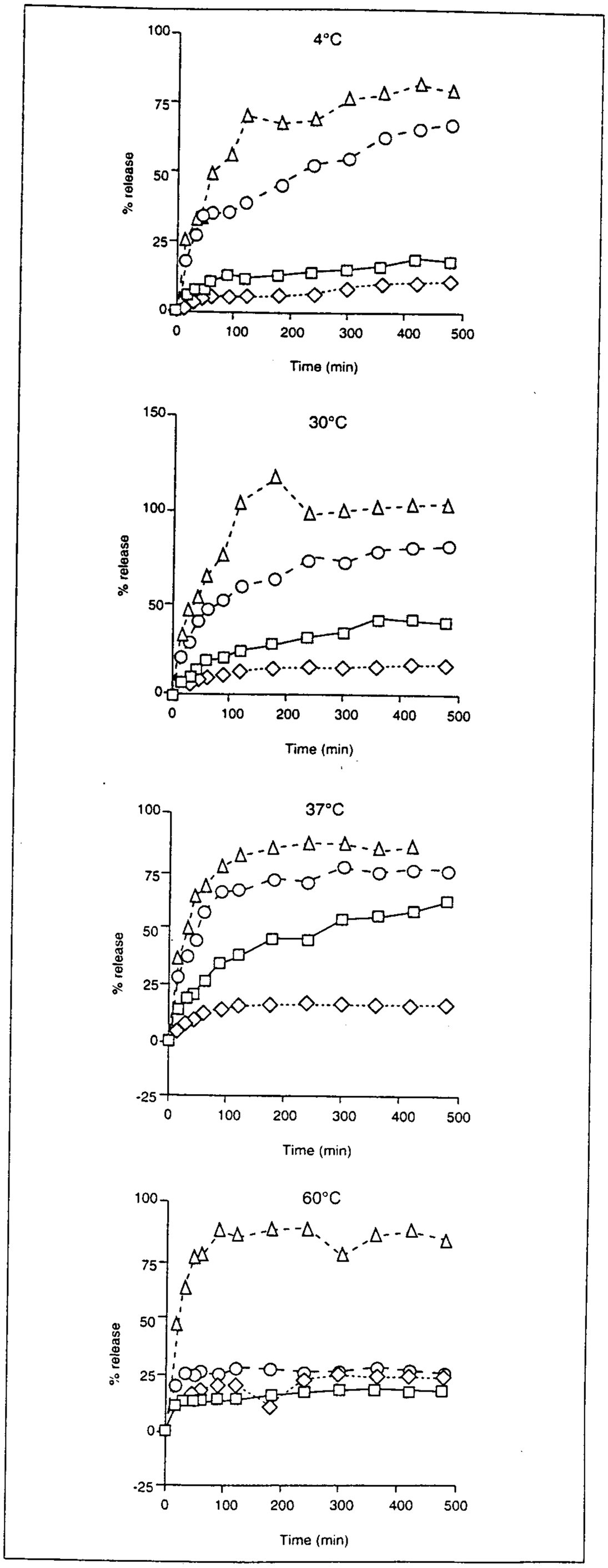

Figure 6 - Percentage release of carboxyfluorescein from v/w/o emulsion (squares), niosome suspension (rhombs), w/o emulsion (circles) and carboxyfluorescein solution (triangles) with time.

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