DEVELOPMENT OF SORBITAN MONOSTEARATE ORGANOGELS FOR CONTROLLED DELIVERY SYSTEMS

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Dated: May 14, 2011

CERTIFICATE

This is to certify that the thesis entitled "**DEVELOPMENT OF SORBITAN MONOSTEARATE ORGANOGELS FOR CONTROLLED DELIVERY SYSTEMS**" submitted by **Ms. MEENAKSHI SINGH** in partial fulfilment for the requirements for the award of Bachelor of Technology Degree in Biotechnology at National Institute of Technology, Rourkela is an authentic work carried out by him under the supervision of the undersigned.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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ABSTRACT

The study describes the development of sorbitan monostearate (Span 60) based organogels with mustard oil as the solvent. Different compositions of organogels were prepared by varying the concentrations of span 60. During the preparation of organogels, their microstructures were studied under compound light microscope. The formulated organogels were characterized by light microscopy, gel-to-sol transition temperature, long-term stability, pH, opacity measurements and hemocompatibilty studies. Metronidazole was incorporated within the organogels and its release behaviour was determined. The anti-microbial action of the drug-loaded organogels on *E. coli* was studied. The microscopy of the organogels suggests that a three-dimensional network of rod-like tubular aggregates of gelator is responsible for immobilising the solvent. Clusters of span 60 are visualised as dispersed in the liquid phase. The rate of formation of organogels and the release rate of metronidazole from the organogels was found to depend on span 60 proportions in the formulated organogels. The pH of the samples was found to be in the range of 6.4-7.1. The organogels were found to be hemocompatible in nature indicating their probable use as controlled delivery vehicles.

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1. INTRODUCTION

The word "gel" has been derived from the Greek word, *gelatus*, which means "to immobilise". In general, gels contain two components, one of which is a liquid and the other, a solid. The solid components form a three-dimensional networked structure which helps in immobilising the liquid component. The solid components are often regarded as gelators. Depending on the polarity of the liquid component, the gels may be regarded either as hydrogels (Polar phase) or organogels (Apolar phase). The immobilisation of the liquid within the three-dimensional network structure has been attributed to the surface active phenomena amongst the solid and the liquid phases.

As the name organogel suggests, organogels contain apolar solvents (e.g. kerosene oil, sunflower oil, mustard oil, mineral oil) as the continuous phase. Organogels may be developed by two mechanisms, namely, fluid-fibre mechanism and solid-fibre mechanism. The fluid fibre mechanism involves the development of reverse-miscellar structures when water is added to a solution of surfactant in apolar solvent. The reverse miscellar structures undergo modification when further amount of water is added to the system, and in turn, undergoes physical interaction amongst each other to give rise to a three-dimensional network structure. Since, the three dimensional structures which are formed are made up of basic structures which contain a polar liquid phase, the mechanism of formation of formation is regarded as fluid-filled fibre mechanism. The networked structures formed in turn, help in immobilising the apolar solvent. On the other hand, solid-fibre mechanism deals with the dissolution of solid organogelators in hot apolar solvent to give rise to a homogeneous solution. The solution, so obtained, is cooled down to room temperature. This results in the change in the solubility parameter of the organogelator, thereby, resulting in the precipitation of the organogelators. The precipitated organogelators start forming fibre-like structures which undergo interaction amongst each other to form a three-dimensional networked structure. The fibres, which are formed, are basically made up of organogelator molecules and do not contain any liquid phase. Hence, these type of organogels are said to be formed by solid-fibre mechanism. Similar to the fluid-fibre mechanism, the three-dimensional structure formed by the solid organogelators help in immobilising the liquid apolar phase.

Span 60 (sorbitan monostearate) has long been used in various cosmetic, food and pharmaceutical applications due to its ability to act as a structuring agent. It has been found that the span 60 based matrices have the ability to modulate the release property of the bioactive agents (e.g. oligopeptides, polypeptides, cyclosporin, and salicylic acid). The matrices of the span 60 based formulations are formed by solid-fibre mechanism. Their use in the above-mentioned industries has been increasing exponentially due to the easy formation of gelled structure, which have also been found to be thermodynamically stable for a prolonged period of time.

Mustard oil, obtained from the seeds of *Brassica nigra,* has been traditionally used in house-hold cooking. Apart from its utility in cooking, it has also been used in various clinical conditions due to its anti-microbial, rubefacient and anti-cancer activities. But, now-a-days, its use in clinical conditions is decreasing because of the difficulty in handling the oil, typically, spillage of the oil or breakage of the container. This may be overcome by structuring the mustard oil using a suitable organogelator. Hence, in the present study, attempts were made to use span 60 as the organogelator to modify the texture of the mustard oil. The gels which were developed were characterised thoroughly to figure out its suitability to be used as a drug-delivery vehicle.

2. REVIEW OF LITERATURE

2.1 OVERVIEW OF DRUG DELIVERY

Drug delivery refers to the application or, incorporation of pharmacologically-active agents (PAAs) to treat the symptoms of a disease. Drug delivery systems refer to the various methods and approaches used for the administration of PAAs to the targeted sites. Typically, pharmaceutical formulations consist of simple, fast-acting chemical compounds that are administered either non-invasively (non-invasive routes being oral, transdermal, nasal, rectal, vaginal and inhalational) or invasively[1], through injectables and implantable devices. The major drawback of conventional DDS is that the drug has to be administered at regular intervals of time in order to attain and maintain the *minimum effective concentration* (MEC) required in the systemic circulation to relieve the clinical manifestations of any given disease. The drug dosage at any given time should not cross the *maximum therapeutic level* (MTL), which defines the maximum concentration of the drug beyond which there is not much increase in the potency of the drug, though the risk of harmful side-effects considerably increases. Hence, the drug levels in the systemic circulation at any given time should be desirably maintained between the MEC and the MPL. This proves to be a challenging task because in most of the cases the concentration range between the MEC and the toxic dosage level is very narrow. In the typical DDS, the administered drugs deliver treatment continuously, rather than alleviating symptoms and preventing harmful outcomes solely as and when necessary. Also, in the current methods of drug delivery, many drugs' therapeutic effects are limited or reduced because of the partial loss of the drug due to biochemical degradation inside the body that occurs before they reach a desired target site.

In order to combat some of the drawbacks of typical DDS, scientists are actively developing controlled DDS, which maintain drug levels at the desired concentration levels, hence reducing the chances of under or overdose of the drug. The release profile of an ideal DDS should be flexible enough to adjust to any changes in the physiological requirements. The ultimate aim of all modern and recently developed drug delivery systems is to transport an incorporated drug or bioactive compound in its intact and active form to specifically targeted parts of the body

through a medium that can control the formulation's administration by means of either a physiological or chemical trigger.[2]

Intensive research in the last few years has proved that efficient and useful controlled release and delivery systems range from true nanosystems (e.g., patterned nanoparticles) to those in the micron range (e.g., ~100 μm polymer-coated beads). Delivery systems are divided into nano-, micro- and macro-delivery systems based on the size of the device used. Examples of nano-delivery systems include solid lipid nanoparticles, dendrimers, liposomes and microemulsions. Micro- delivery systems include micro-particles of varying compositions. Finally, macro-delivery systems include hydrogels, organogels, tablets and capsules.

2.2 GELS

Gels may be defined as an intermediate state of matter, comprised of both the liquid as well as solid components. A three-dimensional interconnected network of solid molecules or aggregates is responsible for immobilizing the liquid continuous phase[3]. Physical interactions or covalent bonding among the gelator molecules result in the formation of a three-dimensional network. Heating of the physical gels increases the thermal energy of the gelled systems leading to the destruction of the physical interactions (hydrogen bonds, Van der Waals interactions) among the gelator molecules responsible for holding the gel together in the stable form. The temperature at which the physical gels lose their structural integrity is known as the gel-to-sol-transition temperature (T_g). However, because the physical forces holding the gel together are re-activated as the gels are cooled down, hence, these gelled systems revert back to their original stable form as the temperature is lowered below their T_g [3]. The gel-to-sol-transition temperature is dependent on the properties of the gelator molecules- both physical as well as chemical, the naure of the solvent and the concentration of both. For the determination of Tg, various visual inspection methods like, bubble motion[4], inverted test-tube method[5], "dropping-ball" technique[6] have been reported. Chemical bonding among the gelator molecules gives rise to permanent gels, which do not undergo gel-to-sol transition even when their temperature is raised. Therefore, gels may be classified on the basis of the type of the nature of the bonds responsible for the three-dimensional network e.g. strong covalent bonds within the network give rise to stable chemical gels whereas physical gels are formed when weaker forces like hydrogen bonds and electrostatic and van der Waals interactions are responsible for

maintaining the solid network[3]. Moreover, if the classification is done on the basis of the liquid phase involved, gels can be broadly subdivided into *hydrogels* and *organogels*. Hydrogels have water as their dispersion medium whereas organogels have an organic solvent as the continuous liquid phase.

Organogels are semi-solid systems in which a three-dimensional network of gelator molecules or aggregates immobilise an organic liquid continuous phase, typically an apolar solvent or oil[7]. The skeleton of the gelled structure consists of either polymers or low molecular weight organogelators. These form a cross-linked structure either by physical or chemical interactions, thereby immobilising the organic phase within the network. Molecular interactions such as hydrogen bonding, metal coordination or dipolar interactions are responsible for the organogel structure[3]. Organogels are viscoelastic systems, having both viscous and elastic properties. Physical organogels, which behave like solids at lower shear rates, start flowing as the shear stress is increased. This flow is attributed to the weakening and subsequent disruption of the physical bonds holding the three-dimensional network together as the shear force increases, resulting in the *plastic flow behaviour* of such organogels.[8]

Some organogelators (e.g., lecithin) have been observed to undergo gelation in the presence of small amounts of water[8] (*fluid-fiber* containing organogels). The presence of trace amounts of water results in an exponential rise in the viscosity of the gelled systems as observed in the case of lecithin oraganogels- Addition of water to the apolar solution of lecithin increases the inherent viscosity by a factor of 10^4-10^6.[8, 9]. Entanglement of the fluid-fiber structures (tubular reverse micelles) result in the formation of rigid structures which might be responsible for the observed increase in viscosity. In *solid-fiber* containing organogels, a higher temperature is required for the dissolution of organogelator molecules in the apolar solvents. A decrease in temperature causes precipitation of the organogelator molecules and the subsequent physical interactions among the same result in the increased viscosity of the system.

Thermoreversibilty, a characteristic property of physical gels, is observed in case of organogels too. As discussed above, heating of the organogels increases the thermal energy of the gelled systems leading to the disruption of the physical interactions (hydrogen bonds, Van der Waals interactions) among the gelator molecules, and hence, it starts flowing. The temperature at which the organogels lose their structural integrity is known as the gel-to-sol-transition temperature

(T_g). However, because the physical forces holding the gel together are re-activated as the gels are cooled down, hence, these gelled systems revert back to their original stable form as the temperature is lowered below their T_g.

Under appropriate conditions of temperature and concentration, the gelator molecules undergo self-assembly (leading to aggregates formation) which subsequently gives rise to a three-dimensional solid network. The decrease in the total free energy of the system associated with the self-assembly of the organogelator molecules is responsible for the inherent thermostable nature of the organogels. Their thermostable nature has been utilised in the pharmaceutical industry by using organogels as sustained drug-delivery agents, where a longer shelf-life is required.[10]

3. MATERIALS AND METHODS

3.1 MATERIALS

Span 60 was procured from Loba Chemie, Mumbai, India. Nutrient agar was obtained from Microlife, Delhi, India. Metronidazole was a gift from Aarti drugs, Mumbai, India. Edible mustard oil was procured from the local market. Double distilled water was used throughout the studies.

3.2 PREPARATION OF ORGANOGELS

The organogels were developed by varying the proportions of mustard oil and span 60. The proportion of the span 60 was increased up to 23% of the total organogel amount. The samples were prepared by dissolving specific weight of span 60 in mustard oil, kept at 70 ^{o}C and stirred at 100 rpm until a homogeneous solution was obtained. The solution, so obtained, was allowed to cool down to 25 ^{o}C in a temperature controlled cabinet. The critical gelation concentration (CGC) of the span 60 was determined. The samples were stored at room temperature for further analysis.

3.3 MICROSCOPIC STUDIES

The samples of the organogels were analysed under compound light microscope (CH20i, Olympus India Pvt. Ltd., India). The organogels were heated to 70 ^{o}C and allowed to cool at room temperature. Sequential micrographs were taken till the temperature reached to room temperature. This study was conducted to understand the crystal growth of the organogels during the cooling of the hot homogeneous solution.

3.4 GEL-to-SOL TRANSITION

Gel-sol transition temperature was found out by incubating the organogels in a water-bath, whose temperature was varied between 30-70 ºC. The temperature of the water bath was increased with an increment step of 5 ^{o}C and the gels were kept at the corresponding temperature for 5 minutes. In order to determine any induced flow, the organogels were analysed by inverting test-tube method before further increasing the temperature of the water bath. The temperature, at which the gels started to flow, when the glass vials were inverted, was noted as the gel-sol transition.

3.5 STABILITY ANALYSIS OF THE ORGANOGELS

The stability of the pharmaceutical products may be carried out by incubating the samples at a particular environment for a longer time period. ICH guidelines of stability of pharmaceutical products indicates the storage of the products at 30°C ± 2°C/65% RH ± 5% RH for 6 months (intermediate accelerated stability test). Apart from the above, samples were also kept at 5 ^{o}C and 40 ^{o}C.

3.6 pH MEASUREMENT

The pH of the organogels was measured using a digital pH meter (ATC pH meter, MODEL 132E). The probe was kept in contact with the organogel samples and the corresponding pH reading was noted down.

3.7 OPACITY MEASUREMENTS

The organogels were heated at 60 ^{o}C and were subsequently cooled down at room-temperature. The turbidity of the solution was measured using colorimeter (EI-D10 Digital Photocolorimeter). The change in temperature of the solution was monitored using a digital thermometer (MexTech multithermometer) and the corresponding absorbance was recorded at 420 nm.

In a similar experiment, the turbidity of the organogels were analysed as a function of time.

3.8 HEMOCOMPATIBILITY

The hemocompatibility tests were done as per the modified ASTM protocol, which figures out the extent of hemolysis in the presence of the samples. The organogels were put in dialysis bags, which were put in 50 ml of saline solution for 30 min. 0.5 ml of the dialysate was used for the test. For this purpose, fresh goat's blood is collected in the presence of sodium citrate (anticoagulant). 8 ml of the citrated blood is diluted to 18 ml with saline solution. 0.5 ml of the diluted blood is taken in a centrifuge tube followed by the addition of the 0.5 ml of the test solution. The final volume was made up to 10 ml. For positive control, 0.5 ml of diluted blood is mixed with 0.01 N HCl and subsequently diluted to 10 ml. For negative control, 0.5 ml of blood is diluted to 10 ml with saline solution. The centrifuge tubes are incubated at 37 ^{o}C for 60 min. The % hemolysis is calculated as per the following formula:

$$\%\ Hemolysis = \frac{OD_{test} - OD_{Negative}}{OD_{positive} - OD_{Negative}} \times 100$$

If the % hemolysis is <5 then the material is considered as highly hemocompatible, a value <10 indicates hemocompatible whereas a value > 20 indicates non-hemocompatible.

3.9 ANTI-MICROBIAL STUDIES

Gram negative bacteria *E. coli* was used for antimicrobial study. Nutrient agar solid medium was used for the study. 1 ml of cell suspension (containing 10^{-6} to10^{-7} cfu/ml) in water was spread over the surface of the nutrient solid agar media. Wells of 9 mm diameter were made into the agar plates using a borer so as to accommodate 0.5 g of antimicrobial drug loaded organogel. The petri-dishes were incubated at 37 ºC for 24 h to allow the growth of the bacteria. The zone of inhibition was measured by using a ruler at the end of 24 h.

3.10 IN-VITRO DRUG RELEASE STUDIES

A two-compartment cell was used for the drug release study. The compartments were separated by the dialysis membrane (MW cutoff - 60 kDa, Himedia, Mumbai). The donor compartment contained 5 g of metronidazole loaded organogels while the receptor compartment contained 50 ml of water. Then the donor compartment was lowered to ensure that the dialysis membrane touched the receptor fluid, kept on stirring at 100 rpm. For the first 1h, the 50 ml water was completely replaced with fresh 50 ml water at an interval of 15 min. Subsequently, the

replacement of the water was done at an interval of 30 min until 6 h. A portion of the replaced water was kept for further analysis under UV visible spectrometer (Shimadzu UV 1601 r) at a wavelength of 277 nm. All the experiments were carried out in duplicates.

Attempts were also made to estimate what kind of model (e.g. zero-order, first-order and Higuchian models) the release pattern follows. The parameters (e.g. k and the determination coefficient r^2) were calculated for predicting the release model.

4. RESULTS AND DISCUSSION

4.1 PREPARATION OF ORGANOGELS

Accurately weighed span 60 was dissolved in mustard oil at 70 ^{o}C. The concentration of span 60 was varied from 1% (w/w) to 22 % (w/w) in order to determine the *critical gelling concentration* (CGC). CGC is defined as the minimum concentration of the organogelator required to immobilize the solvent. The hot span 60 solution was subsequently cooled down at room-temperature. As the temperature was decreased, there was a change in the solubility parameter of the span 60 molecules which resulted in the precipitation of the span 60 molecules in the oil continuous phase. The precipitation of the span 60 molecules made the solution cloudy. Depending on the concentration of the span 60 in oil, the solution either remained cloudy or formed an opaque solid-like structure. The samples were regarded as organogels, if the final product did not flow when the culture bottles were inverted[5] (Figure 1). The CGC of the span 60 to immobilize the mustard oil was found to be 17 % (w/w). Below 17 % (w/w) concentration of span 60, the final product was a turbid solution, which started flowing when the culture bottles were inverted (Figure 2). The apparent viscosity of the turbid solutions was found to be increased as the concentration of span 60 increased up to the concentration of 17 % (w/w). At 17 % (w/w) of span 60 concentration, the turbid solution started forming solid-like structure. All the gels were yellow in colour because of the inherent colour of mustard oil. They had a slight odour and were smooth and “oily” to touch. The composition of the organogels, which were used for further analysis have been tabulated in Table 1. It was observed that the organogels with higher proportions of span 60, attained the gel structure relatively quickly.

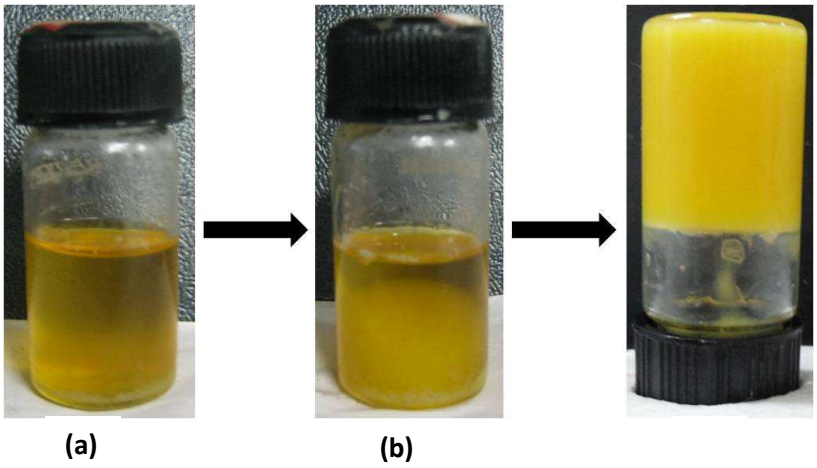


Figure 1: Gelation process of organogel containing 18% (w/w) span 60 in mustard oil:

(a) clear solution after heating; (b) uniform, cloudy suspension upon cooling and standing; (c) opaque, semi-solid gel upon further standing.

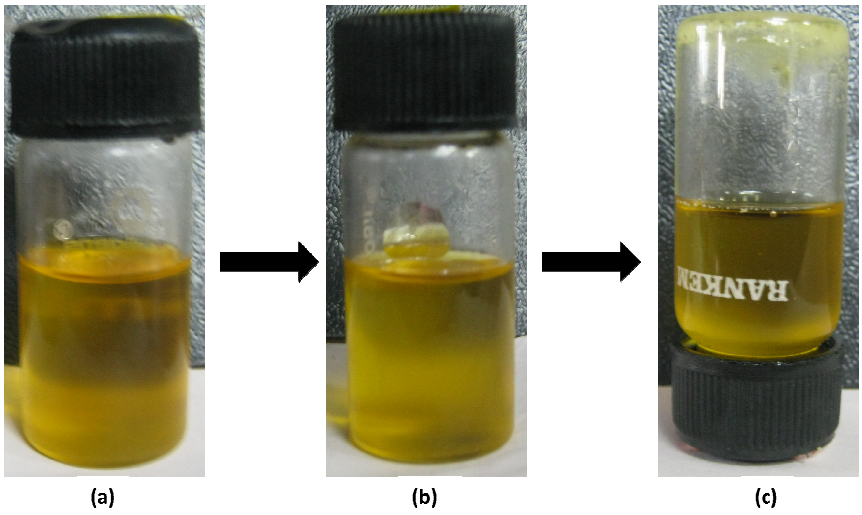


Figure 2: With 12%(w/w) SMS in mustard oil

(a) clear solution after heating; (b) turbid suspension upon cooling and standing; (c) Upon further standing, suspension flowing on inverting the culture bottle.

The composition of the organogels selected for further analysis is tabulated in Table 1. The formulated span 60 organogels have been shown in Figure 3.

Table 1: Composition of organogels selected for further analysis

Sample	Span 60 concentration (%, w/w)
A	17
B	18
C	19
D	20
E	21
F	22

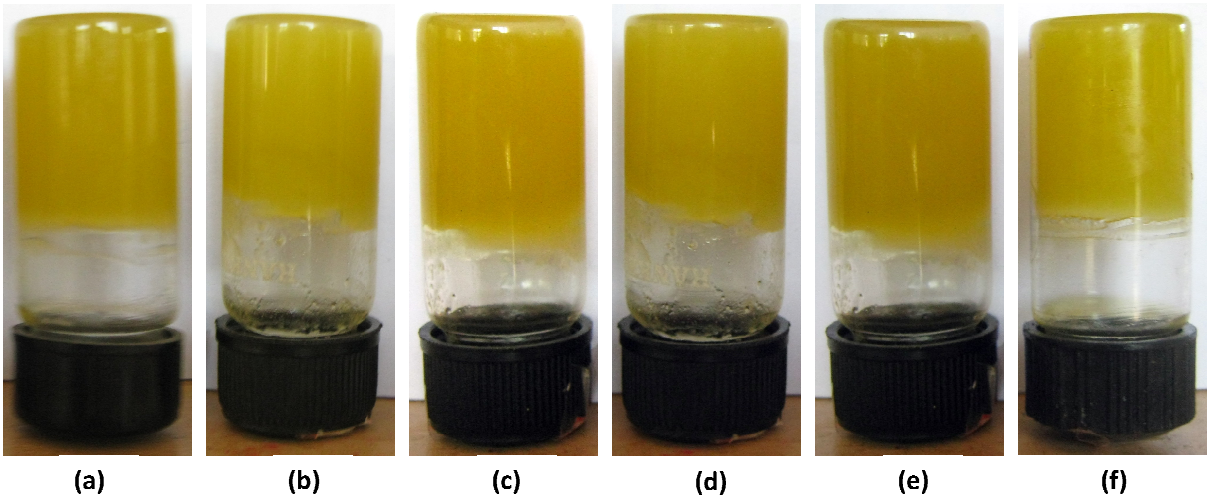


Figure 3: Span 60 based organogels:

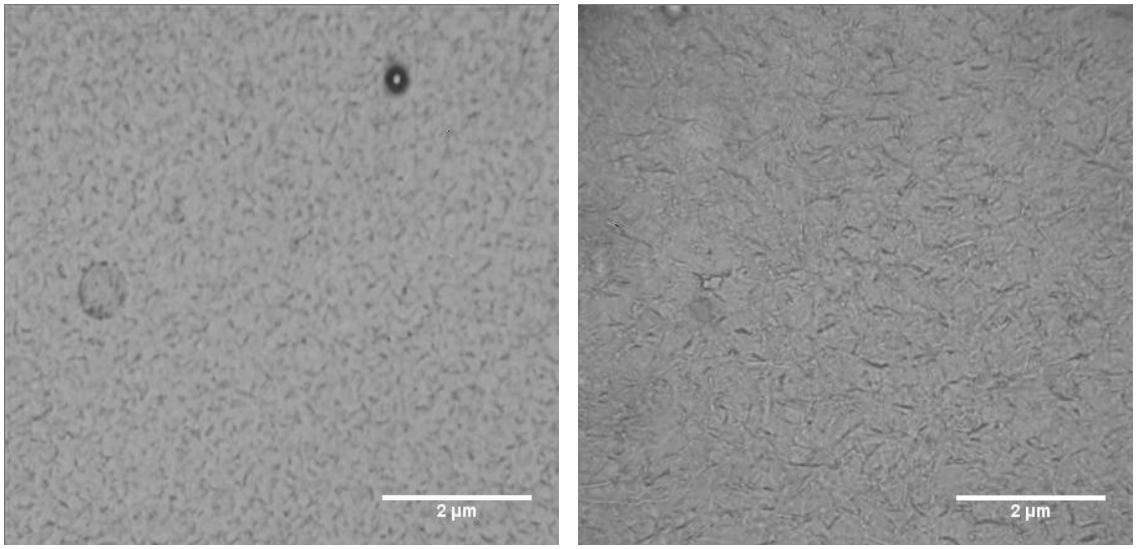
(a)A; 17%(w/w) (b)B; 18%(w/w) (c) C; 19%(w/w) (d) D; 20%(w/w) (e) E; 21%(w/w) (f) F; 22%(w/w)

4.2 MICROSCOPIC STUDIES

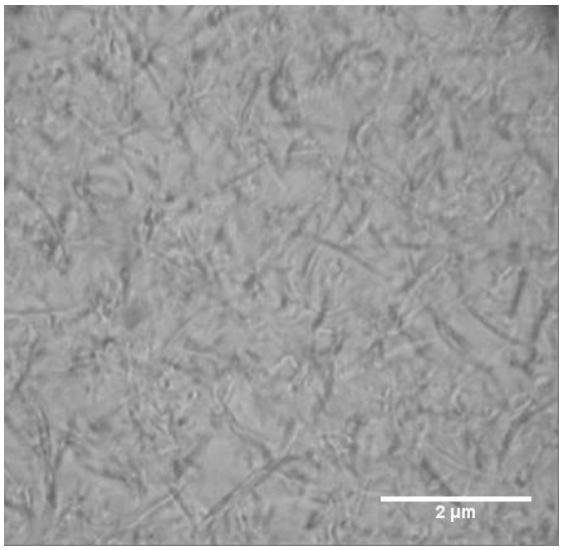
The process of gelation was monitored under a compound light microscope as the hot span 60 solution in mustard oil is cooled at room-temperature (figure 4). This was done to understand the underlying phenomena of gel formation. The micrographs showed the presence of gelator

molecules dispersed in the liquid phase. The gelator molecules self-assemble into aggregates as the samples cool down. These clusters of gelator molecules resemble *rod-like tubular structures.* These tubular aggregates continue associating with each other (figure 4), as the temperature decreases thereby resulting in the formation of a three-dimensional network. This solid skeleton of gelator aggregates is responsible for immobilising the liquid phase and giving rise to a gel.[3]

The effect of gelator concentration was also visualized on the cloudy solutions and the organogels. As the gelator concentration was increased up to the CGC, there was an increase in the tubular structures without any formation of the three-dimensional networked structures (Figure 5). As the gelator concentration was increased beyond the CGC, the density of the gelator aggregates or tubular structures forming the networked structure increased (Figure 6). This can be attributed to the increased number of gelator molecules in a given volume of oil.



(a) **(b)**



(c)

Figure 4: Organogel microstructure at different time points. Sequential micrographs (a−c) show the change in organogel microstructure: (A) Molten condition; t = 0 (B) At t = 1 min. (C) At t = 3 min.

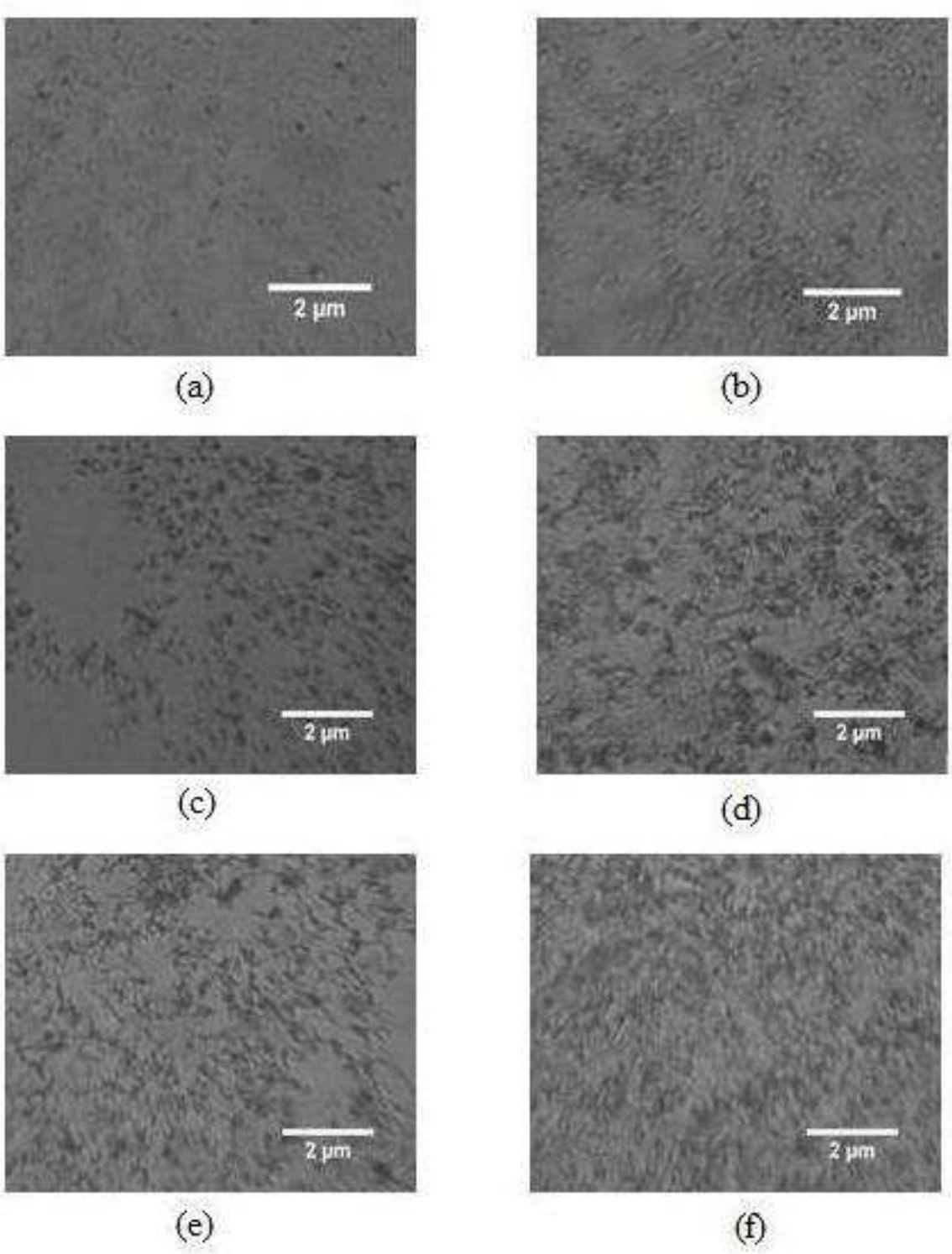


Figure 5: Microstructures for samples with SMS in mustard oil below CGC.

Span 60 conc. being (a) 2%(w/w) (b) 5%(w/w) (c) 8%(w/w) (d) 10%(w/w) (e) 12%(w/w) (f) 15%(w/w)

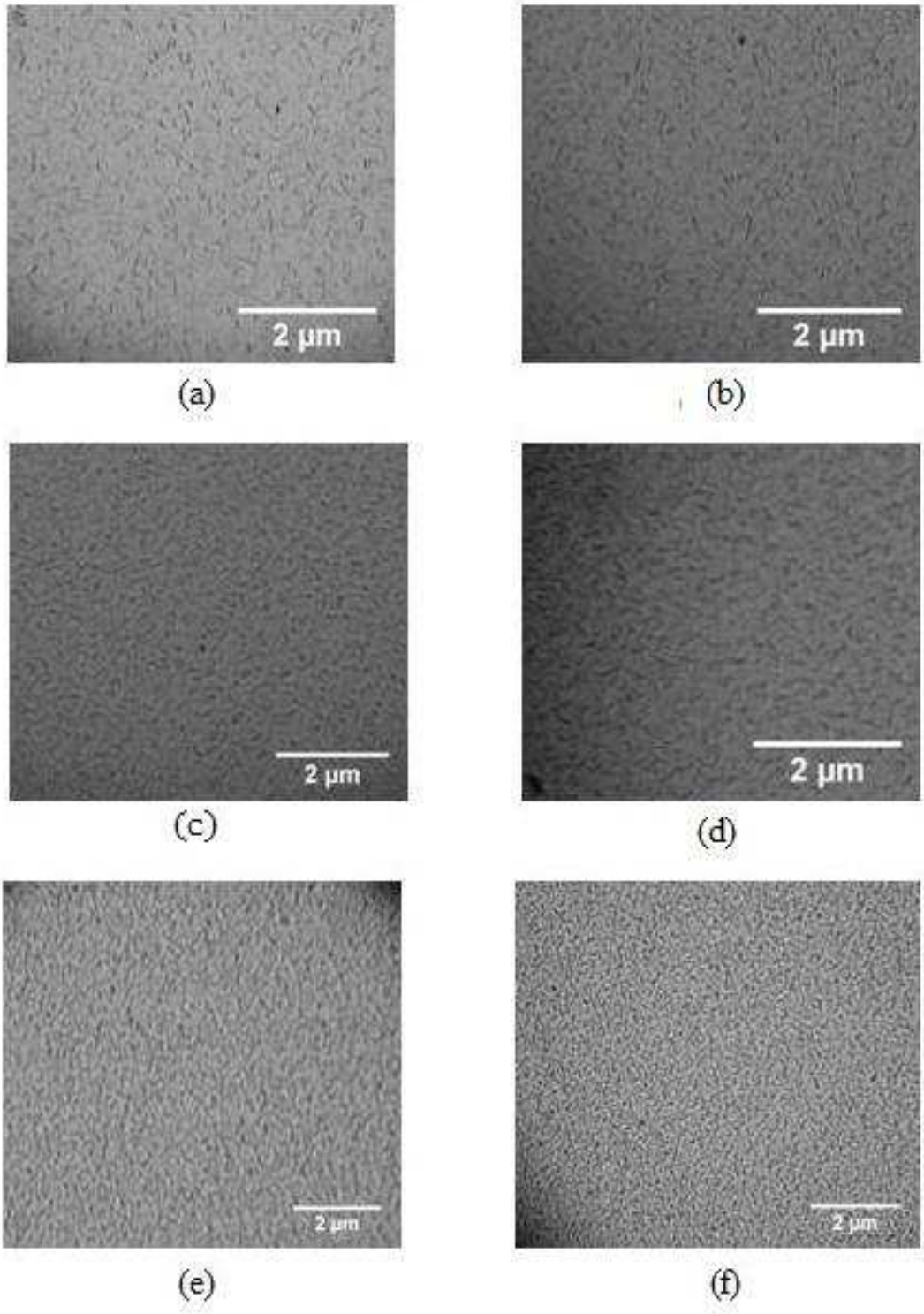


Figure 6: Microstructures for different organogel samples (a) A (b) B (c) C (d) D (e) E and (f) F.

4.3 GEL-SOL TRANSITION ANALYSIS

The organogels were subjected to increasing temperatures starting from 30 ºC. An increment of 5 ºC was made after 5 min incubation at the previous temperature. The samples were considered to have undergone gel-sol transition when they started to flow when the culture bottles were inverted.[5]

The rise in temperature results in the increase in surface active energy with a subsequent increase in the mobility of the self-assembled aggregates formed by the gelator molecules. With the further increase in temperature, the absorbed thermal energy interferes with the molecular interactions amongst the self-assembled aggregates, which are responsible for the three-dimensional network structure of the organogels. The subsequent disruption of the networked structure causes the system to flow freely (figure 7). The concentration of span 60 in the range of 17 % and 22 % (w/w) in the organogels did not affect the gel-to-sol transition temperature (table 2).

Table 2: Gel-to-Sol transition observations

Sample	Gel-sol transition temperature (^{o}C)
A	**50**
B	**50**
C	**50**
D	**50**
E	**50**
F	**50**

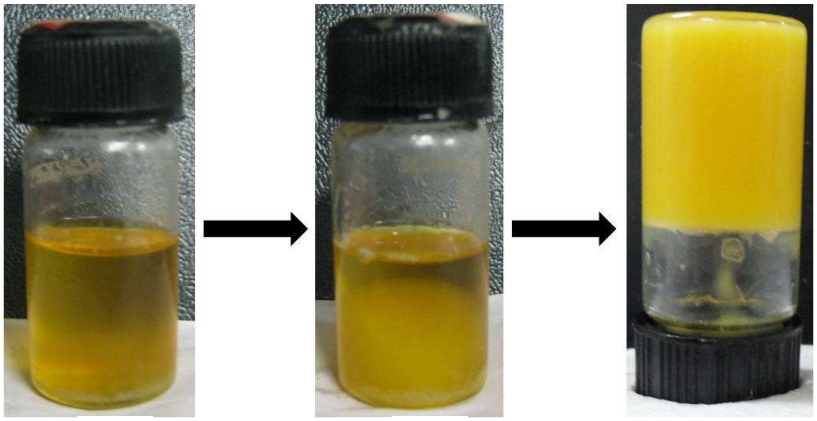


Figure 7: Gelation process of organogel containing 18% (w/w) SMS in mustard oil:

(a) clear solution after heating; (b) uniform, cloudy suspension upon cooling and standing; (c) opaque, semi-solid gel upon further standing**.**

4.4 GEL STABILITY ANALYSIS

The duration of the time period for which a gel remains intact (i.e. without the separation of the solid and the liquid phases) when stored in sealed vessels at room temperature is known as a gel's lifetime[3]. In order to determine the lifetime of the organogels under different environmental conditions, the samples were kept at 5 ^{o}C, 40 ^{o}C and at ambient temperatures. The observations of the study are tabulated in Table no. With the gel lifetime being less than a week, it was found that the storage of the organogels at 40 ^{o}C induced early destabilisation in the samples. The samples stored at ambient temperatures were found to possess intermediate stability whereas the samples stored at 5 ^{o}C were stable for longer periods of time (6 months, Refer Table 3). The results of the study indicate that, in order to prolong the shelf-life of the organogels, they should be preferably stored at lower temperatures. Their longer, stable lifetime will encourage their use as drug-delivery vehicles in the pharmaceutical industries.[11]

Table 3: Observations of the stability test

Sample	Destabilization time (days)		
	Ambient temperature	**At 5 ^{o}C**	**At 40 ^{o}C**
A	4 months	6 months	2 days
B	4 months	6 months	2 days
C	4 months	6 months	2 days
D	4 months	6 months	2 days
E	4 months	6 months	2 days
F	4 months	6 months	2 days

4.5 PH MEASUREMENT

The pH of the biomedical products is a significant characteristic of the formulations as these products are supposed to be in contact with the human tissues. Any variation from the physiological pH may lead to the irritation of the cells, which in turn might cause immunological reactions (e.g. redness, burning and itching of the skin in the applied area)[3]. For this reason, US pharmacopoeia has set pH standards for transdermal and topical formulations. The pH of the organogels was measured at room temperature by using electrode based digital pH meter. The pH values for all samples given in table 4. The tested samples had different concentrations of the span 60 and efforts were made to correlate the pH with the varying gelator concentrations. However, the pH of the samples did not follow any specific trend with respect to the organogelator concentration or any other component in the gel. The pH of the organogel samples was found to be in between 6.4-7.10, indicating their probable use in the formulation of the transdermal or topical products.

Table 4: pH values of organogels

Sample No	pH
A	6.78
B	6.42
C	6.85
D	7.07
E	7.10
F	6.54

4.6 OPACITY DETERMINATION

The changes in the absorbance of the organogels as the hot solutions were cooled at room-temperature have been shown in Figure 8. It was found that at the same temperature, the sample with higher concentration of span 60 showed a higher absorbance than that of the sample with lower concentration of the organogelator. Also, the sample with higher concentration reached the saturation value at a higher temperature indicating that the rate of precipitation of the span 60 is higher in those samples. This result supports the observation of quick gelling of the solvent as the span 60 concentration is increased.

The change in the absorbance of the hot solutions as a function of time has been shown in Figure 9. The results indicate that as the gelator concentration was increased, the absorbance of the solution was higher at a particular instance of time. This suggests that as the solutions are cooled at room-temperature, the rate of precipitation of the gelator is higher in samples with higher organogelators.

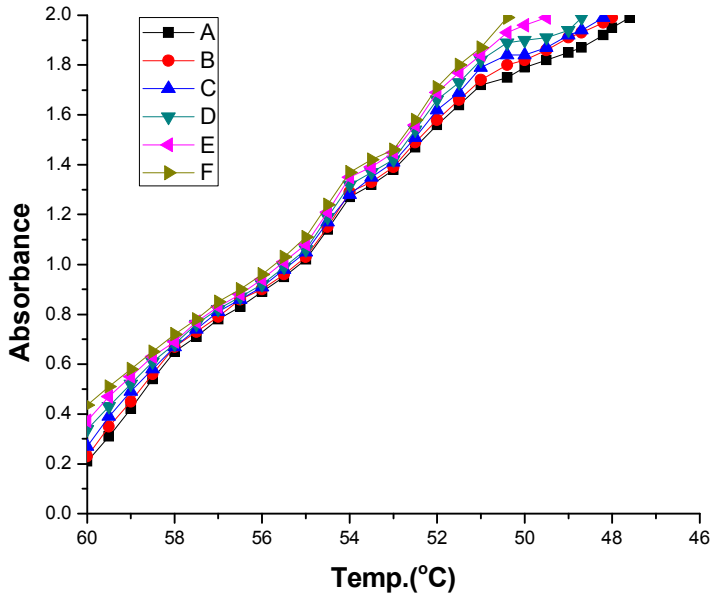


Figure 8: The change in the absorbance values of the different compositions of organogel samples as a function of temperature.

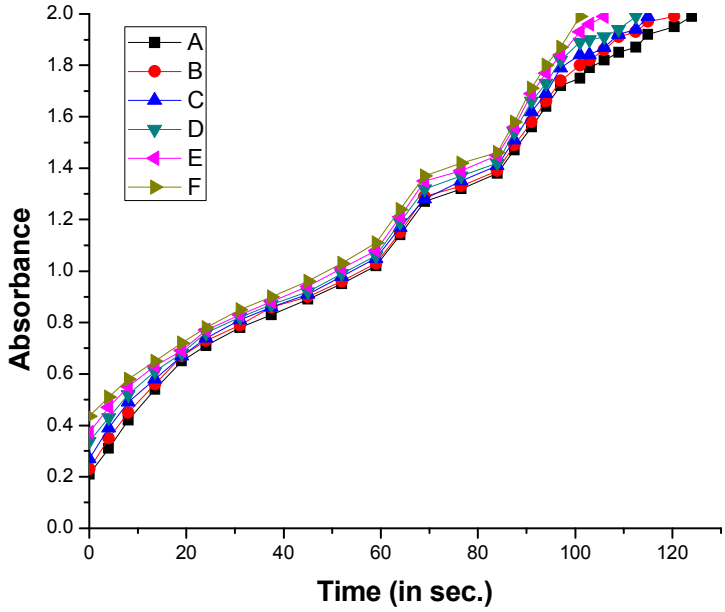


Figure 9: The change in the absorbance values of the different compositions of organogel samples as a function of time

4.7 ANTIMICROBIAL TEST

Metronidazole was incorporated within the organogel D, with concentration of span 60 in mustard oil being 20% (w/w). A bore of 9 mm diameter was made in the nutrient agar plates containing the specific microorganism, *E. coli*. The organogel without drug acted as a control loaded whereas the organogel loaded with drug served as the active sample. It was found that the bioactive agent was able to eliminate specific microorganism within a given area and did not allow the growth of the microorganism even after 24 h (Table 5). On the other hand, control organogel samples did not show any zone of inhibition. This indicates that the organogels may be tried as a controlled delivery system, where it may deliver the bioactive agent for a prolonged period of time.

Table 5: Zone of inhibition

Bioactive agent	**Zone of inhibition(Diameter, cm)**	
	E. coli	**Control**
Metronidazole, 1%(w/w)	1.8± 0.2	Nil

4.8 IN-VITRO DRUG RELEASE STUDY

The release profiles of the drug from the organogels have been shown in figure 9. The low CPDR value indicates the controlled release behaviour of the formulation and is reckoned to be the feature of amphiphilogels. The CPDR values were observed to decrease with increasing sorbitan monostearate concentration. The diffusional drug release is dependent on the solid skeleton network formed by the gelator molecules. As the gelator aggregates or fibers are responsible for the three-dimensional structured network that immobilises the liquid phase, it might be reasoned that excessive cross-linking with higher amounts of the gelator, act as a hindrance to the passage of the drug out of the matrix and hence decreases the drug release. Figure 10 shows the CPDR values as a function of time.

Table 6 shows the coefficients determined for different kinetic models of drug release. The release kinetics best-fit model indicated that the release of the drug from the organogels followed Higuchi Model kinetics indicating that the organogels may be used as controlled delivery systems.

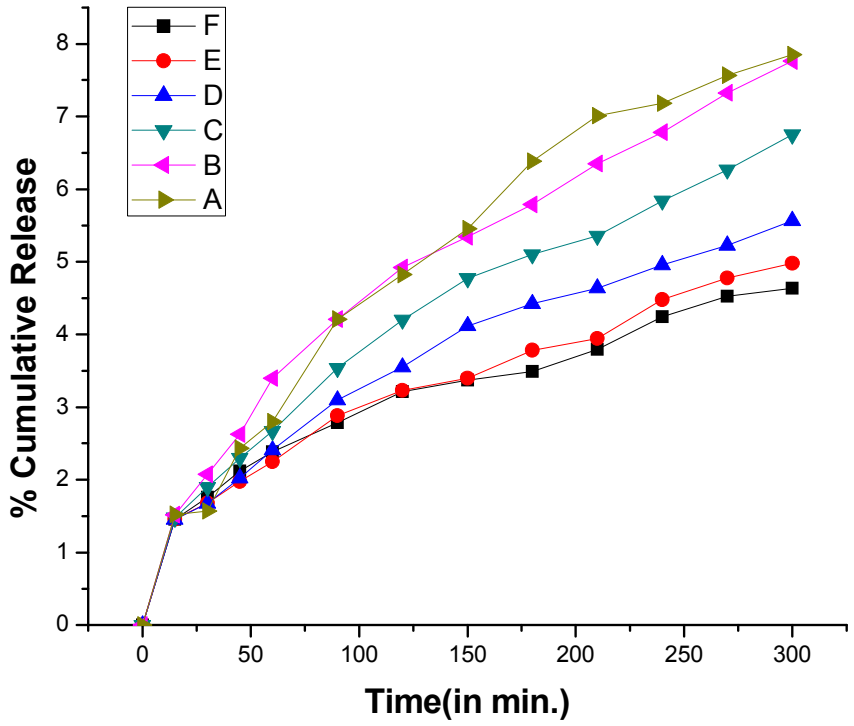


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Figure 10: CPDR values for different compositions of the organogel samples as a function of time

Table 6: Kinetics of drug release

Sample	Zero order		First order		Higuchi Model kinetics		Best-fit Model
	r^2	k	r^2	K	r^2	k	
A	0.9419	0.0252	0.8389	0.0028	0.9830	0.4949	Higuchi Model kinetics
B	0.9334	0.02247	0.808	0.0025	0.9968	0.454	Higuchi Model kinetics

C	0.9370	0.019	0.8453	0.0024	0.9960	0.389	Higuchi Model kinetics
D	0.9191	0.0161	0.85	0.0022	0.9961	0.321	Higuchi Model kinetics
E	0.9131	0.0138	0.8683	0.002	0.9925	0.2757	Higuchi Model kinetics
F	0.8782	0.01245	0.8340	0.0018	0.98672	0.2520	Higuchi Model kinetics

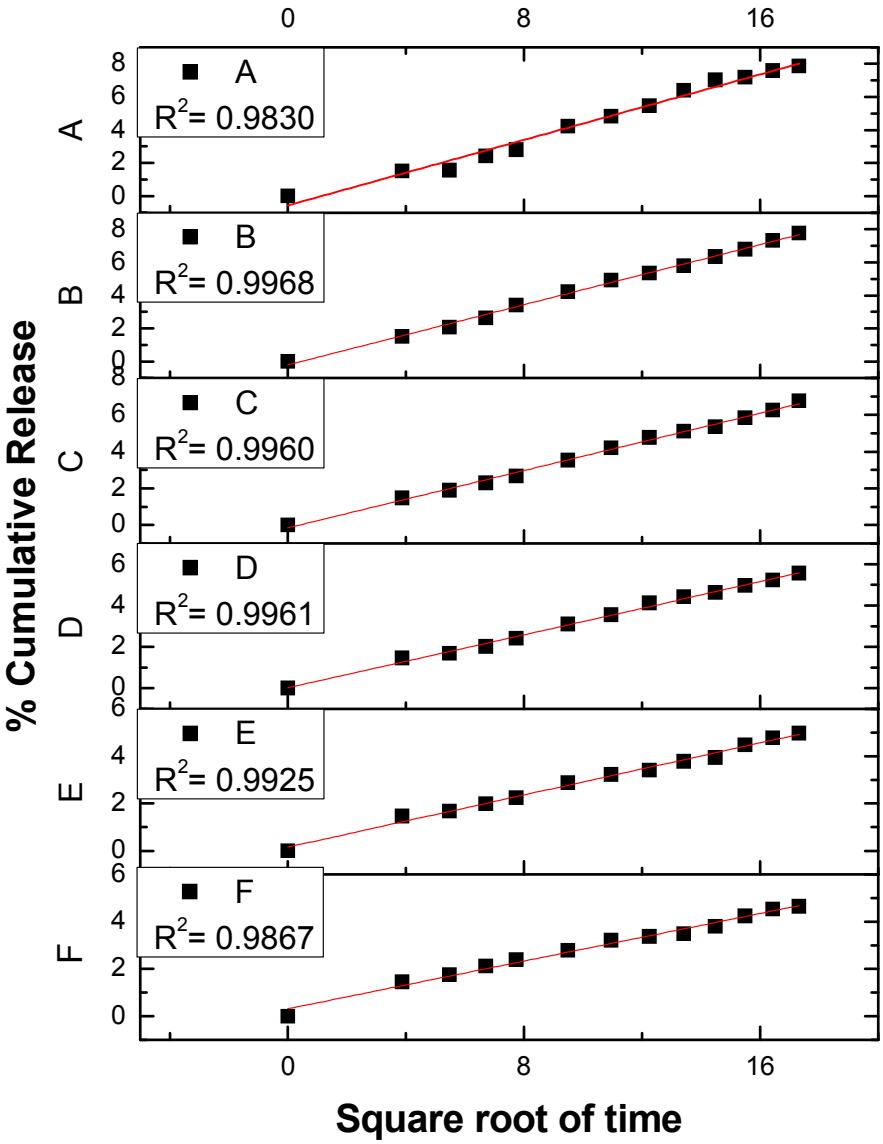

Figure 11: Higuchian-model kinetics for the different organogels samples (a) A (b) B (c) C (d)D (e)E (f) F.

4.9 HEMOCOMPATIBILITY

The hemocompatibility of the samples have been tabulated in Table 7. The results indicate that the samples are highly haemocompatible in nature indicating its biocompatibility. Hence the organogel samples may be tried as a drug delivery vehicle. Table 7 shows the percentage hemolysis values obtained for the various samples.

Table 7: Results of the Hemocompatibility test

Sample	% hemolysis
A	1.26
B	3.48
C	4.74
D	1.89
E	5.06
F	3.79

5. CONCLUSIONS

Organogels with varying proportions of sorbitan monostearate in mustard oil were prepared and their microstructures were successfully studied. The micrographs revealed the presence of *rod-like tubular structures* which are clusters of span 60, formed by the self-assembly of gelator molecules as the temperature of the organogel samples decreases. These aggreagates form a three-dimensional network which is responsible for immobilising the solvent. It was also observed that the gelation occurred sooner in samples with higher span 60 concentrations. The gel-to-sol transition temperature for the organogel samples was found to be 50^{o}C. The results of the gel stability studies indicate that, in order to have a longer shelf-life, the samples must be stored at cool places. The determination of opacity of the organogel samples confirm the microscopic observation that higher precipitation of gelator molecules occurs in samples with higher concentrations of the gelator. The formulated organogels were found to be highly hemocompatible. Metronidazole incorporated organogel samples showed anti-microbial studies against *E.coli* and it was found that the release of drug out of the gel matrix can be modulated by changing the span 60 concentrations.

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