SPAN-60 BASED ORGANOGELS AS PROBABLE MATRICES FOR TRANSDERMAL/TOPICAL DELIVERY SYSTEMS

The thesis submitted to

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF TECHNOLOGY IN BIOMEDICAL ENGINEERING

by

PATIL VINAYAK VASANTRAO

Roll no-209BM1008

Under the guidance of

Prof. Dr. S. S. Ray.

Research supervisor

Prof. Dr. Kunal Pal.

Co-Supervisor

DEPARTMENT OF BIOTECHNOLOGY & BIOMEDICAL ENGINEERING,
NATIONAL INSTITTUTE OF TECHNOLOGY,
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Dedicated to my family

Department of Biotechnology and Medical Engineering,

National Institute of Technology Rourkela

Date: 02.06.2011

CERTIFICATE

This is to certify that the thesis entitled "**Span-60 based organogels as probable matrices for transdermal/topical delivery systems**" submitted by **Mr. Vinayak Vasantrao Patil**, for the partial fulfillment of the requirements for the degree of M.Tech, embodies the bonafide work done by him in the final year of his degree under the supervision of Dr. Kunal Pal, Assistant Professor, and Dr. S. S. Ray, Assistant Professor, Department of Biotechnology and Medical engineering, National Institute of Technology, Rourkela, Odisha. The thesis or any part of it has not been submitted earlier anywhere for any degree or diploma or any other qualification.

(Dr. Kunal Pal) (Dr. S. S. Ray)

Co-Supervisor Research supervisor

ACKNOWLEDGEMENT

I would like to extend my gratitude and my sincere thanks to my honorable, esteemed supervisor **Prof. Dr. Kunal Pal**. He is not only a great teacher with deep vision but also and most importantly a kind person. I sincerely thank for his guidance and encouragement. I am glad to work with him. My special thanks go to **Prof. Dr. S. S. Ray** for his valuable guidance & motivation.

I would like to extend my heartfelt gratitude to research scholars of Biomaterials & Instrumentation laboratory, **Ms. Beauty Behera and Mr. Saisatish Sagiri,** whose ever helping nature and suggestions has made my work easier by many folds.

I would like to thank all my friends, my classmates especially Sudheep, Bibhu, Vicky, Pradnya, Sandhya, and Subrat for their constant moral support, suggestions, ideas, and the thoughtful discussions we had. I have enjoyed their presence so much during my stay at NIT, Rourkela.

I would like to thank all those who made my stay at Rourkela an unforgettable and rewarding experience.

Last but not least I would like to thank my parents, who were always caring for me throughout my journey till date. They rendered me enormous support during the whole tenure of my stay at NIT Rourkela.

Patil Vinayak Vasantrao

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ABBREVIATIONS

Abbreviation	Definition
GRAS	Generally Regarded As Safe
v/v	Volume by Volume
w/w	Weight by Weight
Span-60 (SMS)	Sorbitan Monostearate
KDa	Kilodalton
XRD	X-Ray Diffraction Analysis
mm	Millimeter
D/W	Distilled Water
SO	Sunflower Oil
SMS-SFO gel	Organogel composed of Sorbitan Monostearate and Sunflower oil
CGC	Critical Gelation Concentration
Tg	Gel-Sol Transition Temperature
CPR	Cumulative Percentage Release
AUC	Area Under The Curve
FWHM	Full Width At Half Maxima

ABSTRACT

The current study describes the development of span-60 based organogels using sunflower oil (SO) as the apolar solvent. The organogels were analyzed for their stability. Subsequently, the stable organogels were characterized by gel-sol transition studies, microscopic analysis, opacity measurement, FTIR spectroscopy, XRD analysis, thermal analysis (using simultaneous TGA-DTA and DSC) and pH measurement. Salicylic acid (SA), model drug, was incorporated within gels and their *in vitro* release behavior and antimicrobial efficiency against *E. coli* and *B. subtilis* were studied. To ascertain the biocompatibility of the gels, hemocompatibility tests were conducted. The stability tests indicated that the gels were inherently stable when stored below 25°C. The gel-sol transition study indicated that as the concentration of the gelator was increased, there was a subsequent increase in the transition temperature. This was also confirmed by the instrumental thermal analysis studied. Microscopic analysis indicated that the solid fibers, formed by the clusters of needle-shaped gelator particles, form the backbone of the organogels structure. Opacity measurements suggested that the rate of gelation of the organogels is higher in organogels with higher gelator concentration. FTIR spectroscopy indicated the presence of hydrogen bonding in both blank and SA-loaded organogels. XRD studies showed that there was a change in the crystallinity of the samples and the change was dependent on the composition of the organogels. The pH of the organogels was found to be within the normal human skin pH range. The release studies indicated that the release of SA from the organogels occurred by Higuchian kinetics and were able to restrict the growth of *E. coli* and *B. subtilis* efficiently. The organogels were found to be hemocompatible in nature. Based on the results, the developed organogels may be tried as a drug carrier for transdermal and/or topical formulations.

Chapter 1

Introduction and objective

1.1 Introduction

In the recent years, semisolid products have gained much importance in the food, nutraceutical, pharmaceutical and cosmetics industries. They have been used either as gels, lotions, creams, ointments and jellies. The method of preparation of these products is very tedious and complicated. Apart from this, there is a great concern associated with the long-term stability of most of these products due to which there is a reduced shelf-life of these products. The semisolid preparations having both solid and liquid components in its structures have been regarded as gels [1]. Gel-based semisolid products have been found to be more stable than other types [2]. In general, gel-based products may be categorized either as hydrogels or organogels, depending on the polarity of the liquid component. Hydrogels have water as the liquid component while organogels have apolar solvent (e.g. hexane, isopropyl myristate, sunflower oil and corn oil) as the liquid component. Amongst the gel-based products, the use of organogels based products is increasing, which may be attributed to the easy method of preparation and inherent long-term stability of these products [3]. Depending on the mechanism of the formation of the three dimensional skeleton, which help in immobilizing the apolar phase, the organogels are further categorized as fluid-filled structure and solid-fiber based organogels [4].Till recent past, most of the organogel based products were developed using non-biocompatible components. But with the advancement in the pharmaceutical, food, nutraceutical and cosmetics industries, various organogel based products are being developed using biocompatible components and have in use for human consumption [5-6].

Sunflower oil (SO) obtained from dried kernels of *Helianthus annuus* is valued, healthy, and widely available commonly used edible oil. SO is rich in vitamin E. Presence of many anti-oxidant and anti-carcinogenic species in SO has been reported [7-10].

In the present study, attempts were made to develop and characterize SO based organogels, using span-60 as the organogelator, for probable application in controlled drug delivery.

1.2 Objective

Development and characterization of span-60 based organogels for probable application in controlled drug delivery systems.

Chapter 2

Review of literature

2.1 Introduction to gels

Gels are the semisolid viscoelastic systems. They are said to be the systems easy to identify then to define. We use many gels in our day to day life, such as soaps, shampoo, toothpaste, hair gel and cosmetics, as well as contact lenses and gel pens etc. Gel can either be natural gel or artificial or synthetic gel [2]. They may be considered as the intermediate between solid and liquid state of matter. Gels are considered to be at the interface between "complex fluids" and phase-separated states of matter [4]. Despite higher concentration of liquids in the composition of gels, they exhibit the properties of solids. Gels may also be considered as dispersions in which solid is a continuous phase and liquid is a discrete or discontinuous phase.

The gels can be dried and obtained in solid form. Depending upon the method of drying gels may be classified as either aerogel (formed by replacing the liquid phase with air), cryogel (obtained by freeze drying), and xerogel (obtained by using conventional drying method) (figure 1) [2].

There are 2 major types of gels, i) Hydrogels and ii) Organogels. The classification is based on nature of liquid, that the gel immobilizes. Hydrogels contains high amount of water in there composition. Whereas gels in which, the immobilized liquid is organic solvent are termed as organogels (figure 1).

Organogels are generally formed by immobilization of various liquids within 3-dimensional network, formed by self assembly of fibers, formed of molecules called as gelators [4]. The network stops the flow of liquid by altering the surface tension of the liquid. It is the network of the gelator which gives gel a structure and stickiness. Interaction between gelator molecule to form aggregate and hence the fibers might be either covalent (chemical) or simply physical. Chemical gels are thermally irreversible whereas gels formed by weak non-covalent interactions (physical gels) are reversible [2].

Due to involvement of complex mechanism behind bonding interactions and formation of network, it's difficult to predict the ability of any molecule to gel the specific solvent. Hence research in the field of gels is largely based on serendipitous discoveries of gelators through blind screening. Many of the aspects of organogels are still poorly understood [4, 11].

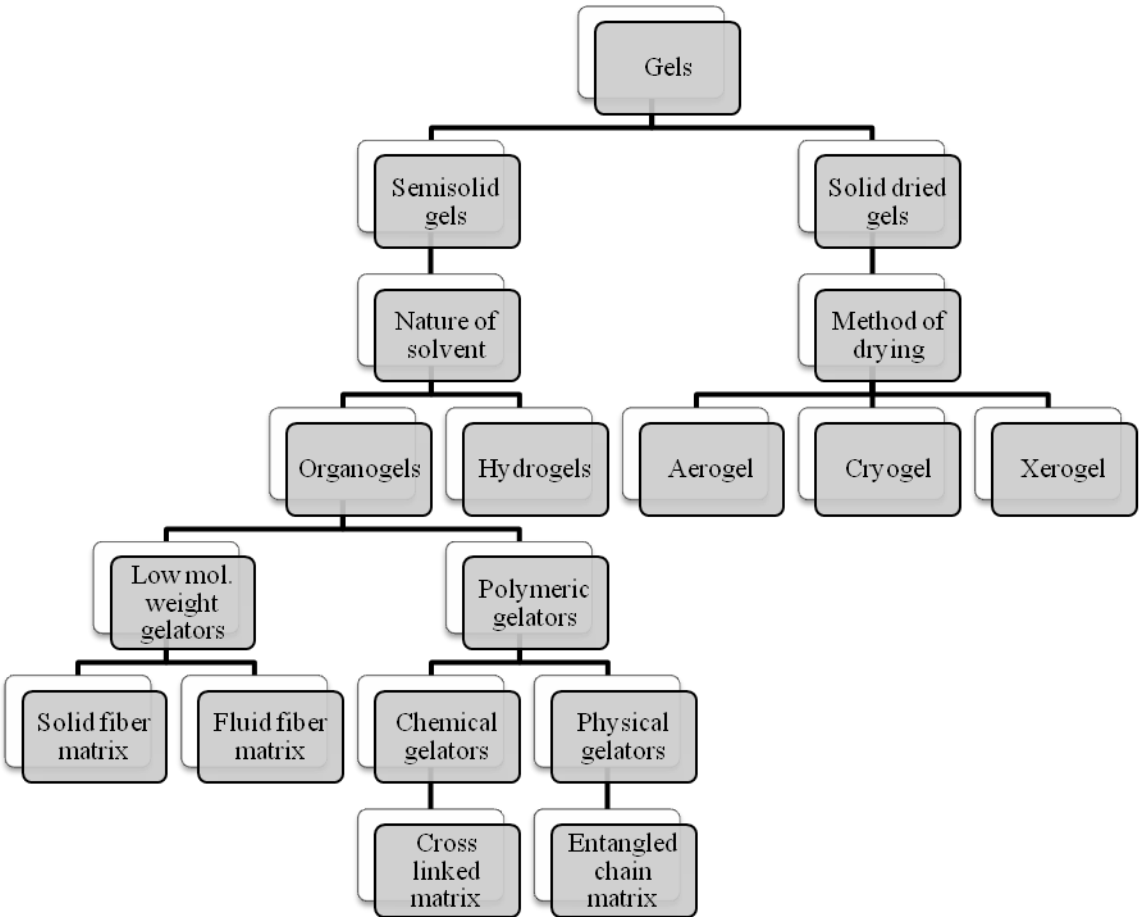


Figure 1: Classification of gels [2, 5]

A minimum concentration of gelator required to gel the solvent is known as Critical gelation concentration (CGC). Below CGC concentration the resulting system exhibits flow properties and behaves as a liquid.

Stability of organogels depends on concentration of gelator, presence of aqueous phase, storage temperature, and properties of solvent. Organogels shows thermo-reversible behaviour. As temperature increases the interaction between gelator molecules decreases and organogels forms a liquid state, but as the temperature decreases the interactions between gelator molecules are restored and organogels obtain there original form[12].

Organogels are reported to have many applications in Pharmaceuticals, nutraceuticals, food, cosmetics, and preservation of arts etc.

Organogels do have some advantages over conventional drug delivery system, which includes longer shelf-life, no need of sophisticated instruments, less chances of batch to batch variations, ease of preparation and thermo-reversible nature of the organogels-based formulations [3]. The ability of the organogels to accommodate both hydrophilic and hydrophobic compounds within its structure has also widened the scope of use of organogels in various delivery systems. The research on biocompatible and edible organogels has added a new dimension in the food and pharmaceutical industries because of the easy preparation of the organogels [5]. The ability of the organogels to tailor the release of the solute molecules incorporated within its structure is keeping the researchers keen to develop new controlled drug delivery systems. Edible oil organogels have unique physical, functional and nutritional properties[6].

Most of the organogels developed till-date consists of toxic solvents (like cyclohexanes, n-octane, kerosene etc) hence their human applications may create serious problems. Unfortunately, scarce toxicological information about many commonly used organogelators also restricts the use of organogels in drug delivery [5]. Hence more stress is being given on the development of biocompatible organogels which are based on generally regarded as safe (GRAS) materials. Edible oils can serve as better organic solvents for human use.

2.2 Mechanism of gel formation

A gel can be divided into primary, secondary, and tertiary structure like a protein to understand the mechanism of gel formation. Primary structure (A^0 to nm scale) is composed of unidirectional aggregation of gelator molecules. The secondary structure (nm to µm scale) is nothing but the morphology of the aggregates like micelles, vesicles, fibers, ribbons [13-14] or sheets. Whereas tertiary structure of a gel (µm to mm scale) involves the interaction of individual aggregates to form gel network [2].

As stated earlier, organogels are formed by 3-dimensional network of intertwined fibers [11]. Fibers may be either fluid filled hollow fibers or solid fibers. The mechanism of formation of both is illustrated in figure 2 and figure 3 respectively.

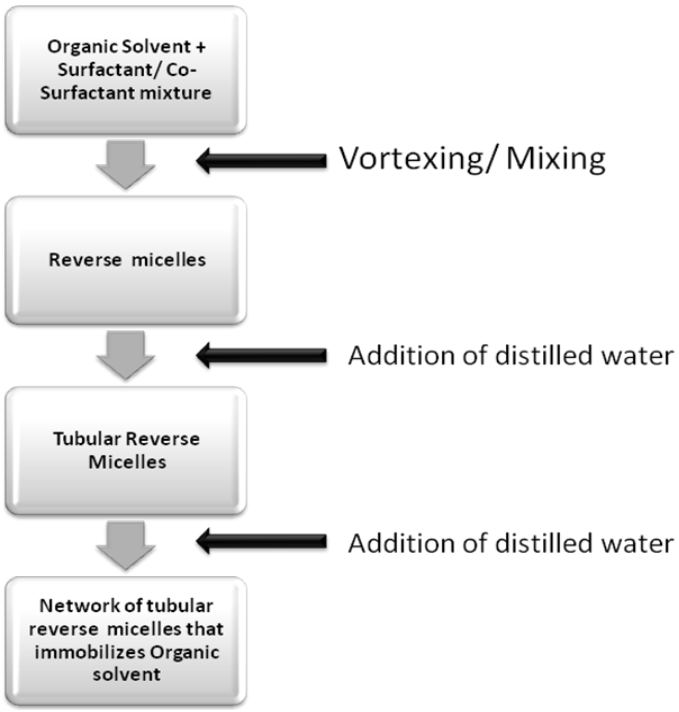


Figure 2: Method of formation of organogels by fluid-filled fiber mechanism [5, 15]

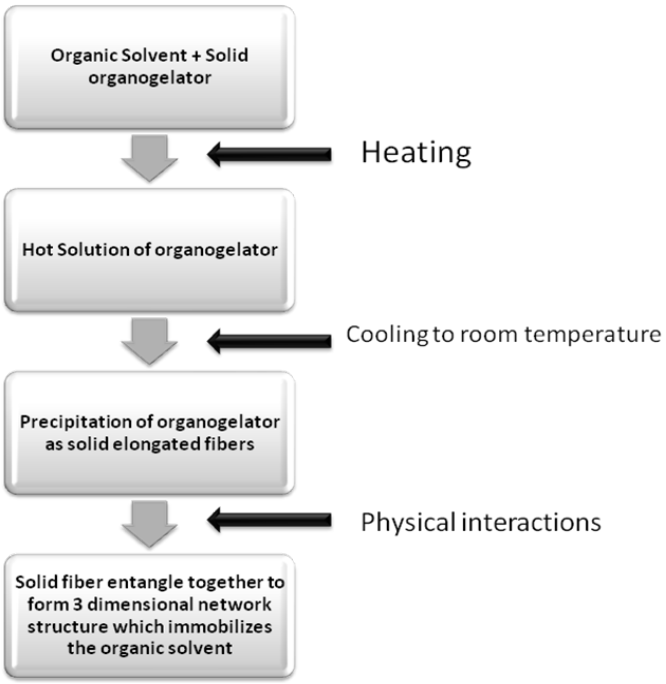


Figure 3: Method of formation of organogels by solid fiber mechanism [5, 15]

Gels prepared by using LMW organogelator are stabilized by solid fibers. They are generally prepared by dissolving gelator in organic solvent at higher temperature and subsequent cooling at room temperature (figure 3). During cooling process three situations are possible i.e. a highly ordered aggregation giving rise to crystals, or a random aggregation resulting in an amorphous precipitate or an aggregation process intermediate between these two, which gives rise to a gel (figure 4).

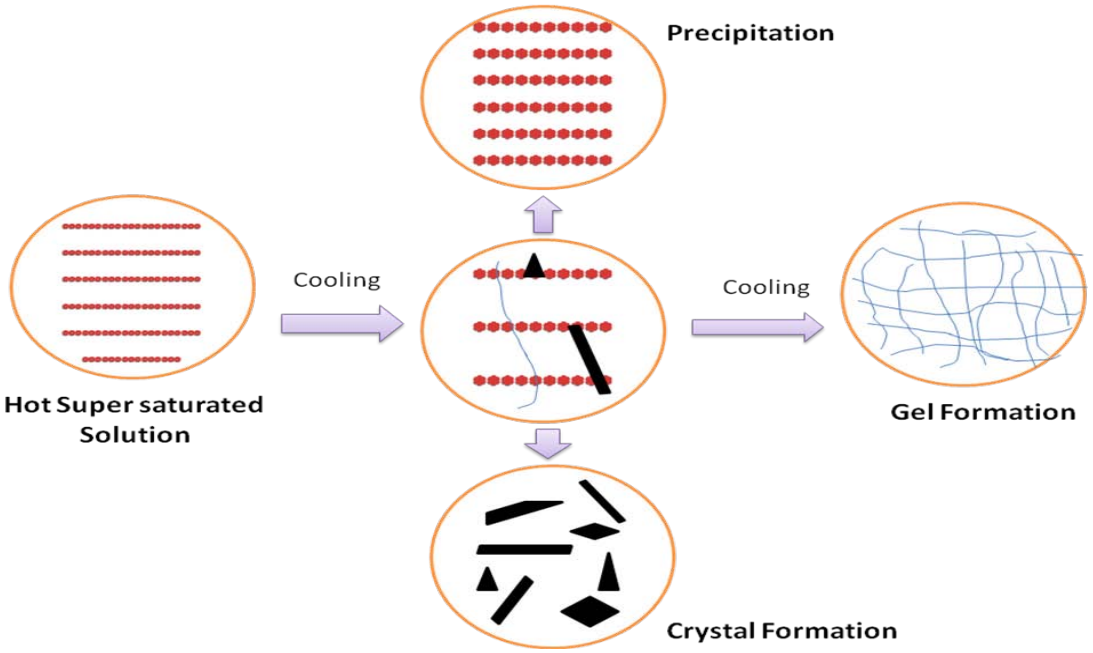


Figure 4: Various possible aggregation modes of gelator molecules [2]

The physical gels formed by LMW organogelators are stabilized by relatively weak inter-chain interactions such as hydrogen bonding [16], van-der Waals forces, metal coordination [17], and π-π-stacking [11]. These organogelators forms fibers, strands, tapes or helix via unidimensional growth of the molecules [2, 5, 11].

In case of cinnamic acid salt gelators it has been investigated that, prerequisite for the one-dimensional (1D) growth of the gel fibrils is mainly governed by the 1D hydrogen-bonded networks involving the ion pair. Whereas all the non-gelators show either two- (2D) or zero-dimensional (0D) hydrogen bonded assemblies involving the ion pair [11].

Specialized type of heat set gel has also been developed using ß-CD and Para substituted aniline with Lithium chloride in N, N-Dimethylformamide. These gels are thermo-reversible in nature. They give clear solution at the room temperature but solidify and turn into gel on heating to higher temperature [18].

2.3 Classification of organogelators

Organogelators may be differentiated as one component organogelators and two component organogelators. Two component organogelators are dependent on one or more other compounds to gel the organic liquid. One component organogelator posses a ability to gel the organic solvent alone, without help or addition of any other component [2].

Organogelators are broadly classified into 2 categories depending upon molecular weight of gelator [5]

i) **Low Molecular Weight (LMW) organogelators (LMWOG) and**
ii) **Polymeric organogelators.**

2.3.1 Low Molecular Weight (LMW) organogelators

Many classes of low molecular weight compounds form stable and efficient gels with various organic solvents at low concentrations. They are characterized by their good solubility in organic solvents upon heating and smooth gelation at low concentration [16]. Gels formed using LMW organogelators are also termed as supra-molecular gels. In recent years they have got more attention due to great structural variety and diversity range they offer for a selector [2]**.**

Depending upon the major driving force involved in molecular aggregation LMW organogelators can be classified as either, i) Hydrogen bond based organogelators or ii) Non hydrogen bond based organogelators [11].

a) Amygdalin based organogelator

Amygdalin has worked as good gelator for broad range of solvents such as nonpolar hexanes to polar aqueous solutions. These supermolecular hydrogels were demonstrated as, an enzyme triggered drug-delivery model for hydrophobic drugs [19].

b) Carbohydrate base organogelator

Trehalose, α-D-glucopyranosyl-(1-1)-ß-D-glucopyranoside is an alpha-linked disaccharide. It is obtained by fungi, plants, and invertebrate animals and it has been extensively used in the food, pharmaceutical, and cosmetic industries. It is used to gel many organic solvent at very low concentrations (0.04% w/v) [19].

c) Amino acid based organogelators

Simple cyclo(dipeptides)s are consisting of many amino acids and possesses remarkable gelation ability for many organic liquids such as, edible oils, glyceryl esters, alcohols, and aromatic molecules [16].

d) Cinnamic acid based organogelators

Organic salts prepared from dicyclohexylamine and substituted of non-substituted Cinnamic acid act as good gelators for organic liquid. They are capable of selective gelation of oil from oil/water mixture. Dicyclohexylammonium 4-chlorocinnamate, 3-chlorocinnamate, 4-bromocinnamate, 3-bromocinnamate, 4-methylcinnamate are some of the examples [11].

e) Soyabean lecithin based organogelators

Soyabean lecithin was gelled by addition of water and extensively employed for the drug delivery [20].

2.3.2 Polymeric organogelators

Polymeric organogelator acts in similar way as that in case of LMW organogelators. Polymeric organogelators also immobilizes the organic solvent by formation of network

structure formed by physical interactions between polymer molecules. Polymers may be either linear, hyperbranched or star shaped polymer [5]**.**

Organogelators based on photochromic dihydroindolizine (DHI) system were developed by chemical synthesis. Controlled delivery of drug from these polymeric organogels can be achieved by using temperature, acidity, or light, as an external stimuli. These novel materials can be also used as photoreponsive materials and in nanotechnology [21].

Polymeric micelles made up of self assembly of amphiphillic molecules have been used in oral drug delivery. These micelles increases solubility of hydrophilic compounds in oil and hence can be used in preparation of anhydrous peptide products [22].

Smart polymeric gels have been extensively used as a stimuli sensitive drug delivery in various biomedical applications like sensors and actuators [23]. They involve polymers that respond to change in common physiological triggers including change in pH, electrolyte concentration. Recent work has demonstrated an ability of the specific polymers to respond to antigen-antibody interaction, glucose, and enzymes [24]. Hence organogels made up of smart polymers is area with wide scope in biomedical applications.

Novel pH sensitive copolymer gelators have been prepared for controlled drug delivery. Dipyridamole was used as model drug and its controlled release rate was obtained by optimizing, polymer concentration, polymer molecular weight, temperature and pH of the solution [25].

Table 1: Some commonly used organogels

Organogelator used	**Organic solvent gelled**
L-alanine derivatives	Various pharmaceutical grade vegetable and synthetic oils [26]
Cholesterol	Liquid Paraffin [27]
Sorbitan tristearate + Lecithin.	Sunflower oil [28]
Phytosterol + Oryzaznol	Various Edible oils [29]
TAG, DAG, MAG, FA, Waxes	Various Edible oils [29]

12-Hydroxystearic acid (12-HAS).	Soyabean oil or capric/caprylic triglyceride [30]
ß-Cyclodextrin (ß-CD) Para substituted anilines (ps-An)	Lithium Chloride in N, N- Dimethyl formamide [18]
Triazine functionalized with α-amino acidic appendages.	Haloalkanes, and aromatic solvents [31]

2.4 Characterization of organogel

Following various methods have been used for the characterization of organogels,

Test for gelation/Determination of Critical Gelation concentration (CGC)

Inverted tube or inverted vial method is the most common method to confirm the gelation. In this method the weighed amount of organogelator is taken into a vial with weighed amount of organic solvent. The vial is then closed properly to create a pressure inside and so as to increase the boiling point of the liquid. The vial is carefully heated to a optimum temperature so as to melt its content completely. Then the vial is allowed to cool for sufficient time before inverting it. After inversion absence of flow indicates the formation of gel [2, 32].

Gel-Sol transition temperature (Tg)

Gel-sol transition temperature is the temperature below which gel doesn't shows any distinct flow property. Gel loses its structural integrity at the temperatures above Tg. It is one of the important characteristic of organogel. It can be determined with either glass ball drop method [33], bubble motion [34], or by simple tube inversion method [32, 35]. Tg depends on physical and chemical properties of organogelator and solvent, as well as there interaction (either physical or chemical). It increases with the rise in the gelator concentration [11]. Thermal stability of

organogels can be accessed by plotting Tg against gelator concentration. Permanent gels formed by the chemical interaction between the large polymeric molecules don't show gel-sol transition.

Analytical methods

Analytical techniques like FT-IR, NMR Spectroscopy, X-Ray diffraction [13] analysis have been employed for the characterization of organogels. These methods provide valuable information regarding molecular interaction during aggregation of organogelator molecules. FT-IR gives profitable information regarding Hydrogen bonding. The presence of intermolecular hydrogen bonding can be confirmed by NMR spectroscopy [16, 18].

Shape of fibrillar network of organogelators can be studied by small-angle neutron scattering (SANS) technique [14].

Information about morphology, specific interactions, internal mobility of the constituents, and molecular organization of organogels can be obtained by using, NMR measurement which involves magic angle spinning (MAS) in the solid-state NMR, spin relaxation times, nuclear overhauser enhancements (NOE), or multiple-quantum (MQ) spectroscopy, the pulse field gradient (PFG) technique and magnetic resonance imaging (MRI) [36].

2.5 Pharmaceutical applications of organogels

Skin acts as an effective barrier for most of the drugs except nitroglycerine scopolamine, nicotine, clonidine, fentanyl, estradiol, testosterone, Lidocaine, and oxybutinin. Hence topical formulation which will enhance permeability of drug and reduce the side effects is always a need of formulation [37]. Many organic substances like lipids act as a penetration enhancer and hence give an additional edge to the organogel formulations prepared from them. Organogels have been investigated successfully as a dermal pharmaceuticals [38].

Severe gastric irritation caused due to oral administration of aceclofenac can be avoided by topical transdermal drug delivery [39]. It's a choice of drug for osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Ethyl oleate based lecithin organogels (EO/Lecithin) were used for topical delivery of aceclofenac. They have found to be more effective than conventional hydrogels. The histopathological studies also proved the safety of the system [40]. Aceclofenac

was also formulated in the form of microemulsion for topical application [41]. Microemulsion have disadvantages that it need a large amount of surfactant and cosurfactants for stabilization of nanodroplets, poor viscosity and spreadabillity. On the other hand Lecithin organogels doesn't require addition of any additional surfactant or penetration enhancer, as lecithin serves both the purposes. Organogels are having better viscosity and spreadability than microemulsion.

Soyabean lecithin organogels shows a faster rate of transdermal drug delivery of scopolamine and broxaterol as compared to conventional patches [42]. It has found to improve skin penetration of Diclofenac and Indomethacin when used with isopropyl palmitate [43]. Piroxicam an effective NSAID has been successfully incorporated into lecithin gels [44]. Ketorolac Tromethamine could also be incorporated into lecithin organogels in high amounts [45].

Organogels have been extensively used as controlled drug delivery systems as summarized in table 2.

Table 2: Organogels used in controlled or sustained delivery of drugs

Organogelator used	**Organic solvent gelled**	**Model drug used**	**Pharmacological activity or category of the drug**
12-HAS (Hydroxystearic acid)	Soybean oil	Ibuprofen	NSAID [46]
Isostearyl alcohol	Isostearyl alcohol, Propylene glycol	Haloperidol	Transdermal drug delivery [47-48]
Stearyl acrylate	Oleyl alcohol	Indomethacin.	NSAID [49]
N-stearoyl L-	Vegetable	Leuprolide	LH-hormone releasing

alanine (m)ethyl esters	oils and Biocompatible hydrophilic solvent		hormone agonist in prostate cancer, endometriosis and precocious puberty [50]
PG (Propylene glycol) or GP1 (dibutyllauroylglutamide)	Terpene (Limonene)	Haloperidol	Anti-Psychotic and as a transdermal patch [47]
Pluronic lecithin organogels	Water	Morphine	topical analgesic for cancer pains [51]
Modified tyrosine organogelator	Safflower oil	Rivastigmine	Acetylcholinesterase Inhibitor in Alzheimer's disease [52]
Gelatin containing microemulsion based gel	Isopropyl myristate and Tween 85	Sodium Salicylate	Topical Drug delivery through iontophoresis [53]
Poly(N-isopropylacrylamide)	Oleyl alchohol and water	Indomethacin	Temperature dependent pulsatile drug release system [54]
Egg lecithin Span 40 Cholesterol	Alchohol Water	Propranolol derivatives	Non selective ß–blocker for hypertension [55]

Sodium deoxycholate Tween 80			
Water	Lecithin Isopropyl palmitate Cycloocta ne	Scopolamine Broxaterol Estradiol Amino acids Peptides	As a bronchodilator in asthma [42]
Water	Lecithin Iso-octane	Propranolol	Anti-hypertensive [56]
Water	Lecithin	Tetra-benzamidine	Anti-tumor [57]
Water	Soyabean lecithin	Methyl nicotinate	Topical application [58]
Sorbitan monostearate	Sweet almond oil, alkanes like hexane, decane, vegetable oils, etc.	Propranolol, Cyclosporin	antihypertensive and immunosuppressant [27]
Lecithin	Various organic	Diclofenac	Analgesic [59]

	solvents		
Glyceryl fatty acid esters	Mygliol®	Ethinyl Estradiol, Piroxicam	Orally bioactive estrogen [49], NSAID [60]
Soya Lecithin	Isopropyl Mysristate	Ketorolac Tromethamine, cyclobenzaprine+ketoprofen +diazepam	NSAID [45] Anti-Psychotic [61]
Soybean Lecithin	Isopropyl palmitate	Diclofenac, Indomethacin.	Analgesic [43]
bis-(4-stearoylaminophenyl) methane (BSAPM).	Propylene carbonate	Ferrocene, Ferricenium	Anti-cancer [62]
N-stearine-N0-stearyl-L-phenylalanine	i-propyl myristate, Tween 80, propylene glycol and water	Sodium Salicylate	Anti-bacterial [63]
1,3:2,4-di-O-benzylidene-D-Sorbitol (DBS)	Propylene Glycol	5-Flurouracil (5-FU)	Antifungal [64]
Span 60	Hexadecane	-	-[65]

2.6 Miscellaneous applications of organogels

Smart gels which show novel response to photochemical, thermal or metallic response have been developed [66-67].

Development of biomaterials based soft materials will be a need of time [19]. Polymeric gels have found many industrial applications such as food, cosmetics [68], athletic shoes, preservation of arts [69], and chromatography.

LMWOGs have also been found to be promising structure-directing agents (templates) to make helical transition- metal oxides [70] and silica [71], to make microcellular materials [72], and in a CO2-based coating process [72] to make dye-sensitized solar cells [73].

Selective water gelation property of some organogelators has been used in containment of oil spills. Organogelators can be useful in easy disposal of used edible oil in family kitchens [74].

Extended unidirectional packing and long range intermolecular interactions make organogels a potential candidate in the research of molecular-based ferromagnetism, linear-anisotropic energy transfer, charge carrier transporting, and light harvesting [75].

Organogels has been used in the lubricant industry from 1970’s [4]. They are applied for the gelation of flammable solvents [76]. Organogels have been successfully used as a substitute for edible saturated trans fats [6].

Extractant impregnated organogels have been successfully employed for the separation of metal ions from their aqueous solutions [77].

The Triton-X100 based quaternary W/O microemulsion organogels consisting of Triton-X100, water, 1-hexanol and n-hexane, were utilized in immobilization of lipase enzyme obtained from *Candida rugosa* [78]. Immobilization of *Mucor Javanicus* lipase enzyme in Gelatin based microemulsion gel formed with tween-85 and sodium bis(2-ethylhexyl) sulfosuccinate (AOT) was also carried out successfully [79].

Chapter 3 Materials and methods

3. Materials and Methods

3.1 Materials

Span-60 (sorbitan monostearate) was purchased from Loba chemie, Mumbai, India. Salicylic acid (SA) was purchased from Sara fine chemicals, India. Edible refined sunflower oil (SO) was purchased from the local market. Dialysis tubing (MW cutoff: 60 kDa) was purchased from Himedia, Mumbai, India. All experimental studies were carried out using double distilled water.

3.2 Methods

3.2.1 Preparation of organogels

Organogels of various compositions were prepared by dissolving specified amount of span-60 in SO, kept in a water bath maintained at 60^{o}C and stirred at 500 rpm, until a homogeneous clear solution was obtained. The proportion of span-60 in SO was varied from 1- 25 % (w/w). The hot solution, so obtained, was allowed to cool down at room-temperature (RT) so as to allow gel formation. The samples were regarded as organogels, if upon cooling, the solution mixture failed to flow when the culture bottles were inverted [2, 40]. The minimum concentration (critical gelation concentration; CGC) of span-60 required for gelation of the apolar phase was determined. Drug loaded organogels were prepared by dispersing SA (a model drug) in SO. All the samples were kept at room-temperature for further analysis.

3.2.2 Organoleptic evaluation

Freshly prepared organogels samples were observed for their color, odour, taste, appearance and texture.

3.2.3 Accelerated stability studies

The accelerated stability test was done by thermo-cycling method. In short, freshly prepared organogel samples were subjected to repeated freezing-thawing cycles, by keeping them

alternatively at temperature -20°C and 60°C, respectively, for 15 minutes each. The study was continued up to 8 hours (i.e. 16 freezing- thawing cycles). Samples were analyzed visually after each 15 min for any destabilization indicator. A sample may be regarded as stable, if it can withstand at least 5 cycles of thermo-cycling.

3.2.4 Stability studies on time scale

Organogel samples were stored at ambient temperature, 5°C and 40°C and were observed at regular intervals for any signs of destabilization for a period of 9 months.

3.2.5 Gel-Sol transition studies

Organogels were incubated at various temperatures in the range of 30°C and 60°C in a constant temperature water bath. An increment of 5°C was made after 5 min incubation at previous temperature. Samples were analyzed by inverted test-tube method after each incubation period [2, 40]. The temperature at which samples started flowing was recorded as gel-sol transition temperature (Tg).

3.2.6 Microscopic studies

A compound optical microscope (Olympus CH20 i) was used for analyzing the microstructure of the organogels. Attempts were made to understand the mechanism of the organogel formation by varying the proportions of span-60 and analyzing their microstructures.

3.2.7 Opacity measurement

The organogels were heated at 70^{o}C and were subsequently cooled down at room-temperature. The turbidity of the solution was measured using colorimeter (EI-D10 Digital Photo colorimeter). The change in turbidity of the solution was monitored at 400 nm using digital photo-colorimeter (EI Instruments India. model 312) either as a function of time or temperature.

3.2.8 Fourier Transform Infra Red Spectroscopy

Infrared spectroscopy of the samples was carried out by using ATR-FTIR instrument (Alpha-E by Brucker, USA). The raw materials, representative blank organogel and representative SA-loaded organogel were scanned in the range of 3500 cm^{-1} to 500 cm^{-1} to understand the interactions amongst the components of the organogel [80-81].

3.2.9 XRD analysis

The samples were subjected to XRD analysis using Philips, XRD-PW 1700, Rockville, USA. Cu-Kα was used as the source, which was operating at 35 KV and 30 mA. Samples were scanned in the range of 10° to 50° 2θ at a rate of 2° 2θ per min.

3.2.10 Thermal analysis

Thermal properties of organogels were studied using simultaneous thermo-gravimetric analysis-differentials thermal analysis (TA 60WS thermal analyzer, Shimadzu, Japan) and differential scanning calorimetry (DSC; STA 449C Jupiter, Netzsch, Germany). For simultaneous TGA-DTA analysis, samples were heated from 27°C up to 250°C, at a rate of 6°C/min. For DSC analysis, the samples were subjected to heating in the temperature range of 29°C and 80°C, at a rate of 2.5°C/min.

3.2.11 Antimicrobial evaluation

Gram negative bacteria *Escherichia Coli* and gram positive bacteria *Bacillus Subtilis* were used to analyze the antimicrobial activity of the organogel. Solid nutrient agar was used as the media. 1 ml of broth cell suspension containing 10^{-6} to10^{-7} cfu/ml was introduced into petriplates. Spread plate method was employed for the inoculation of the media. Wells of diameter 9 mm were created inside the media using a borer so as to accommodate 0.5 gm of organogel loaded with drug. Plates were then incubated at 37°C, for 24 h. The zone of inhibition was measured using scale after completion of incubation period.

3.2.12 In vitro drug release studies

A two-compartment cell was used for drug release study. Accurately weighed 1 g of organogel containing SA was introduced inside donor compartment. The compartments were separated by

the dialysis membrane (MW cutoff - 60 kDa, Himedia, Mumbai). Receptor compartment contained 50 ml of double distilled water (receptor fluid). Donor compartment was lowered so that the dialysis membrane was in contact with the receptor fluid, kept on stirring at 60 rpm, at 37°C. For the first 1h, 50 ml of receptor fluid was completely replaced with fresh 50 ml water at an interval of 15 min. Subsequently, the replacement of the receptor fluid was done at an interval of 30 min up to 8 h. 5 ml of replaced fluid was kept for further analysis under UV visible spectrophotometer (Systronic 2203) at a wavelength of 277 nm and the rest was discarded. All the experiments were carried out in duplicates. Data obtained was analyzed by applying various drug release kinetic models, so as to find out best fit model.

3.2.13 pH measurement

The pH of organogel samples were detected by using digital ATC pH meter (EI instruments, model no- 132E). The pH of the organogels was measured by bringing the probe of the pH meter in contact with the samples. The pH of topical drug delivery preparations should lie in the range of 4.5-7 (skin pH), to avoid irritation to the skin [82].

3.2.14 Hemocompatibility test

The hemocompatibility test was carried out to find out the extent of hemolysis in the presence of organogel samples. The organogels were put in dialysis bags, which were then immersed in 50 ml of saline solution for 30 min. 0.5 ml of the dialyzate was used for the test. For this purpose, fresh goat's blood is collected in the presence of sodium citrate (anticoagulant). 8 ml of citrated blood was diluted to 18 ml of saline solution. 0.5 ml of the diluted blood was taken in a centrifuge tube followed by the addition of the 0.5 ml of the test solution. Final volume was made up to 10 ml by addition of normal saline. For positive control, 0.5 ml of diluted blood was mixed with 0.5 ml of 0.1 N hydrochloric acid and subsequently diluted to 10 ml. For negative control, 0.5 ml of blood was diluted to 10 ml with saline solution. Centrifuge tubes were incubated at 37°C for 60 min. Tubes were subjected to centrifugation at 3000 rpm for 10 min to separate the cell content. Optical density (OD) of supernatant was determined at 545 nm using

spectrophotometer (Systronic 2203). The % hemolysis was calculated as per the following formula:

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

Chapter 4

Results & discussions

4.1 Preparation of organogels samples

Span-60 was dissolved in SO, which was kept at 60°C and kept on stirring at 500 rpm, so as to obtain a clear homogenous solution. As the temperature of solution is lowered, span-60 starts precipitating out of the SO due to the change in the solubility parameter. The precipitated span-60 crystals start growing in size as fibers. These fibers physically interacts with each other to form a three dimensional networked structure [11]. Clear solution firstly turns into cloudy solution and finally forms yellowish-white or white gels (figure 6). After the culture bottles were cooled to room-temperature, the bottles were inverted and observed for any flow. The samples were regarded as organogels, if they did not flow [2, 40]. The CGC of the organogelator was found to be 18 % (w/w). The organogels used for further analysis have been tabulated in table 3.

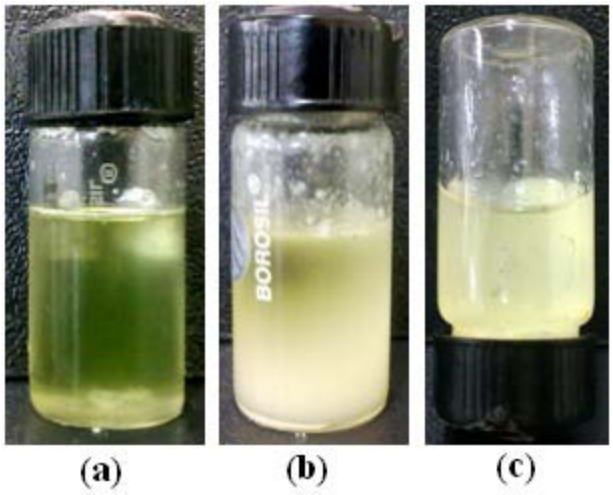


Figure 5: Solution of 15 % (w/w) span-60 in SO (a) clear solution after heating at 60°C; (b) turbid suspension upon cooling and standing; (c) Upon further cooling and standing, suspension flowing on inverting the culture bottle.

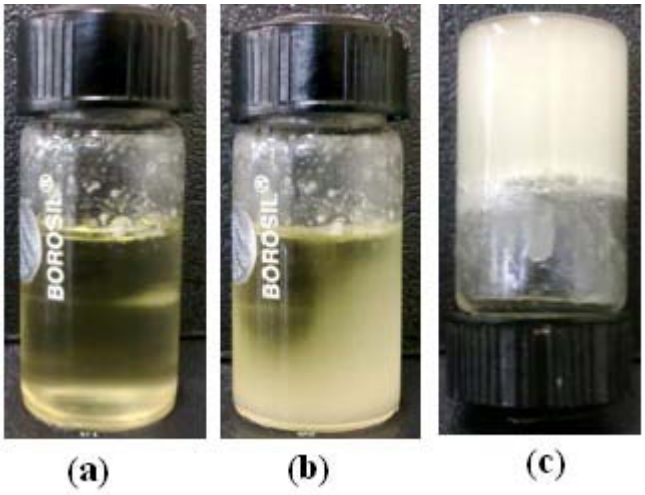


Figure 6: Gelation process of organogel containing 18% (w/w) span-60 in SO; (a) clear solution after heating; (b) uniform, cloudy suspension upon cooling and standing; (c) opaque, semi-solid gel upon further standing.

Table 3: Composition of the organogels used for further analysis

Sample code	Concentration of various components (w/w)	
	Span-60	**SO**
A	18	82
B	19	81
C	20	80
D	21	79
E	22	78
F	23	77
G	24	76
H	25	75

4.2 Organoleptic evaluation

The samples were found to be yellowish-white in color. Sample A lost its consistency when agitated with hand. As the concentration of the organogelator was increased, the consistency of the products increased. Incorporation of SA slightly improved the consistency of the sample A. All the samples were found to be oily to touch and were having gritty nature. The blank organogels were having a bland taste whereas SA-containing organogels were having slightly acidic taste.

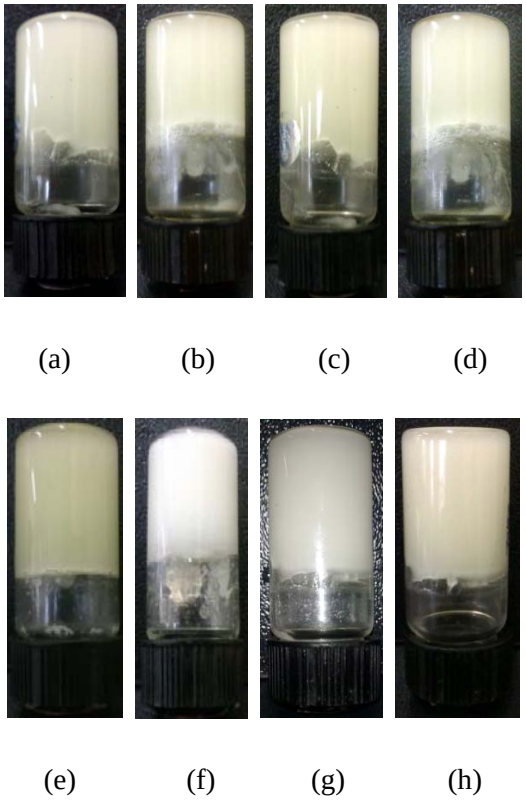


Figure 7: Span-60 based organogels; (a) A, (b) B, (c) C, (d) D, (e) E, (f) F, (g) G, and (h) H

4.3 Accelerated stability studies

Accelerated thermal stability test was carried out by thermo-cycling method. The method involves incubation of the samples alternatively at higher (60°C) and lower (-20°C) temperatures. The study helps in predicting the mechanism of destabilization of the samples either due to the change in the physio-chemical properties at the extreme conditions. At higher

temperatures, the chances of oxidative changes are predominant which may alter the stability of the samples whereas at lower temperatures, the formation of solidified structures may alter the physical interactions amongst the sample components responsible for the formation of the networked structures. This method only gives an prediction about the stability [83]. In general, it is expected that the samples should withstand at least 5 cycles of freeze-thawing process to be regarded as stable samples [84]. All the samples (mentioned in table 3) were found to be stable for more than 16 cycles without any signs of destabilization.

4.4 Stability studies on time scale

The duration of the time period for which a gel maintains its integrity, without any separation of the solid and the liquid phases, when stored in sealed vessels at a given environmental condition helps in predicting the shelf-life of the product at the given condition [15]. In order to determine the lifetime of the organogels under different environmental conditions, the samples were kept at 5°C, 40°C and at ambient temperatures (25°C). The observations of the study are tabulated in table 4. The results indicate that when the samples are stored at 40°C, the samples get destabilized within 6 weeks time whereas the samples kept at 5°C and room-temperature were found to be stable during the experimental period of 32 weeks (8 months). The results suggests that the shelf-life of the samples may be prolonged if they are stored at lower temperatures and may be used as drug-delivery vehicles in the pharmaceutical industries [85].

Table 4: Results of stability studies on time scale

Sample	**Destabilization time (weeks)**		
	Ambient temperature	**At 5°C**	**At 40°C**
A	32	32	4
E	32	32	5
F	32	32	6
H	32	32	6

4.5 Gel-sol transition

The organogels were subjected to increasing temperatures starting from 30°C to 60°C. An increment of 5°C 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 [86].

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 8). Gel-sol transition temperature of organogels was found to be dependent on gelator concentration. The concentration of span-60 in the range of 17 % and 23 % (w/w) in the organogels did not affect the gel-to-sol transition temperature (table 5). Gel-sol transition temperature (Tg) of the organogel containing 24 % and 25 % (w/w) span-60 was found to be more than that of others. This may be attributed to the requirement of more heat energy required for the disruption of the more densely packed network structure as the gelator proportion is increased.

Table 5: Results of gel-sol transition

Sample	**Gel-sol transition temperature**
A, B, C, D, E, F	55°C
G, H	60°C

Figure 8: Gel-sol transition; (a) Sample A at 30º C and (b) Sample A at 55 º C.

4.6 Microscopic studies

The variation in the microstructure of the organogels was studied as the gelator proportion in the oil was changed (figure 9). The results showed the presence of needle shaped crystals of span-60 in SO when 5 % (w/w) gelator concentration was used span-60 (figure 9). As the concentration of the gelator was increased, these clusters aggregated to form fiber-like structures. The density of these fiber-like structures increased with the increase in the gelator concentration. As the CGC was reached, these fiber-like structures were found to form networked skeleton which helped in the immobilization of the SO.

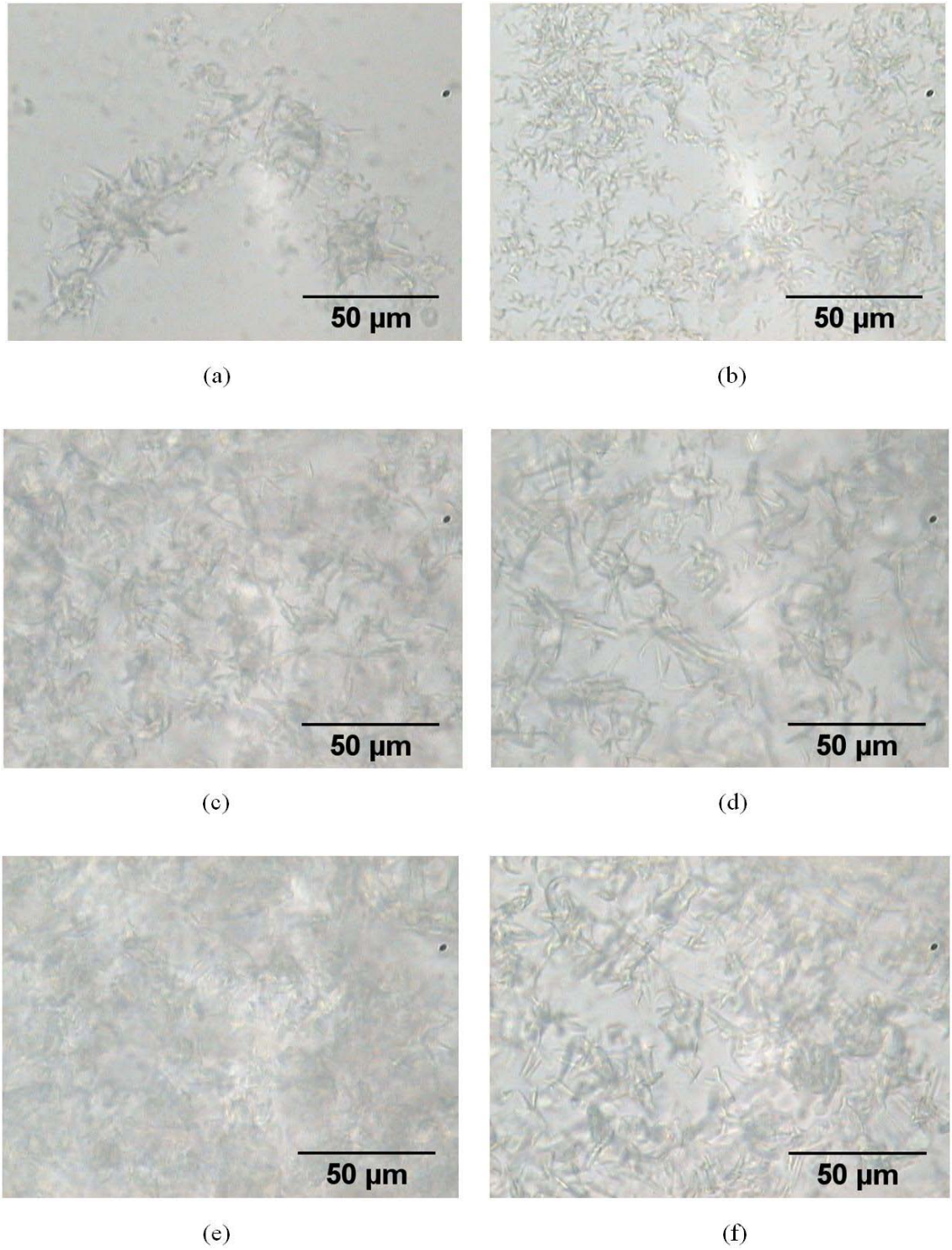


Figure 9: Micrographs of span-60/SO mixtures at RT. (a) 5 % (w/w), (b) 10 % (w/w), (c) 15 % (w/w), (d) 18 % (w/w), (e) 20 % (w/w), (f) 22 % (w/w)

4.7 Opacity measurement

The changes in the absorbance of the organogels as the hot solutions of span-60/SO were cooled at RT have been shown in figure 10. 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. As the concentration of the gelator was increased in the sample, the quicker the saturation in the OD was observed. This indicates that the rate of precipitation of the span-60 increases as the concentration of the span-60 is increased. This result supports the observation of relatively quick gelation of samples with higher concentration of span-60. Sol-gel transition was found out by linear curve fitting of the absorption points in the linear region (figure 10). The point of intersection of the linear curves was taken as the sol-gel transition. The sol-gel transition was found to be 58.59^{o}C, 55.13^{o}C, 55.09^{o}C and 54.47^{o}C for samples A, E, G and H, respectively.

The change in the absorbance of the hot solutions as a function of cooling time has been shown in figure 11. 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 proportions of organogelators.

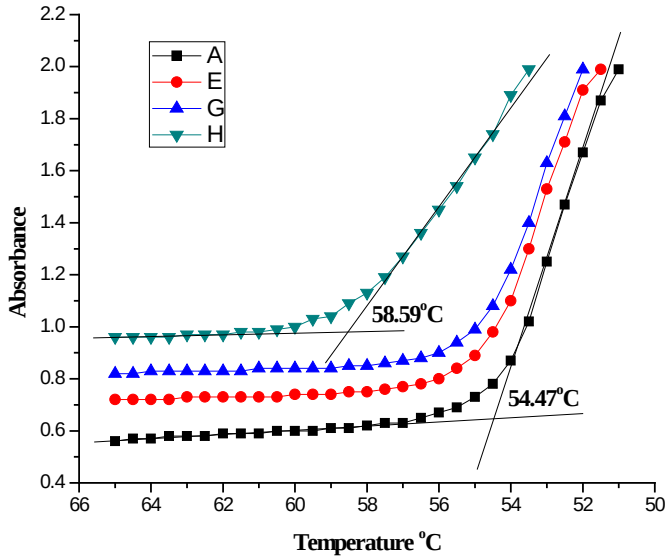


Figure 10: The change in the absorbance of the hot solutions of span-60/SO as the same cooled down at RT w.r.t. temperature.

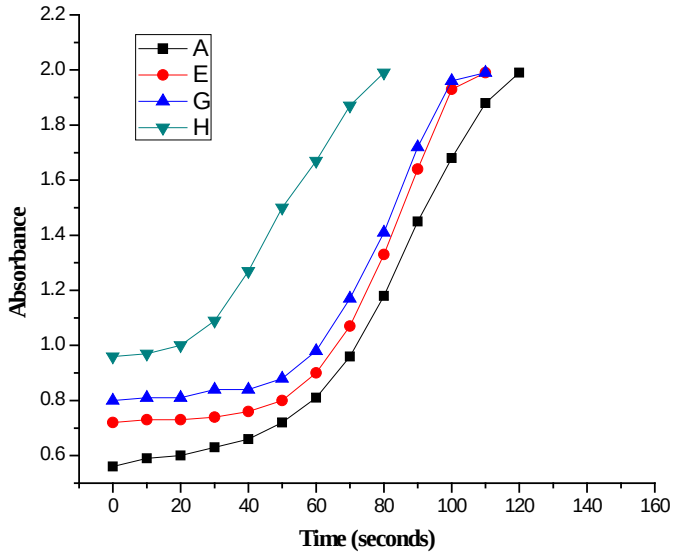


Figure 11: The change in the absorbance of the hot solutions of span-60/SO as the same cooled down at RT w.r.t. time.

4.8 FTIR analysis

Sample A and AD were taken as the representative samples. Figure 12 shows the FTIR spectrogram of span-60, SO, sample A and sample AD. FTIR spectroscopy indicates the presence of molecular interactions amongst the components present in the sample. The spectra of the A and AD samples were found to be similar. SO also showed a similar spectra as that of sample A and AD except the absence of the broad peak in the range of 3,700 cm^{-1} to 3,100 cm^{-1}. This suggests the presence of stretched hydrogen bonded O-H groups in the samples A and AD indicating the presence of intermolecular hydrogen bonds which play an important role in formation of solid fiber organogels [13]. Peaks observed for sample AD were more intense than that of sample A which might be attributed to the presence of SA within its structure. The characteristic functional groups stretches indicate the presence of alkane (3008 cm^{-1}, and 2922 cm^{-1}), N-methyl (2853 cm^{-1}), saturated ester (1743 cm^{-1}) and C-O stretch (1160 cm^{-1}) in the raw materials and the samples [81, 87].

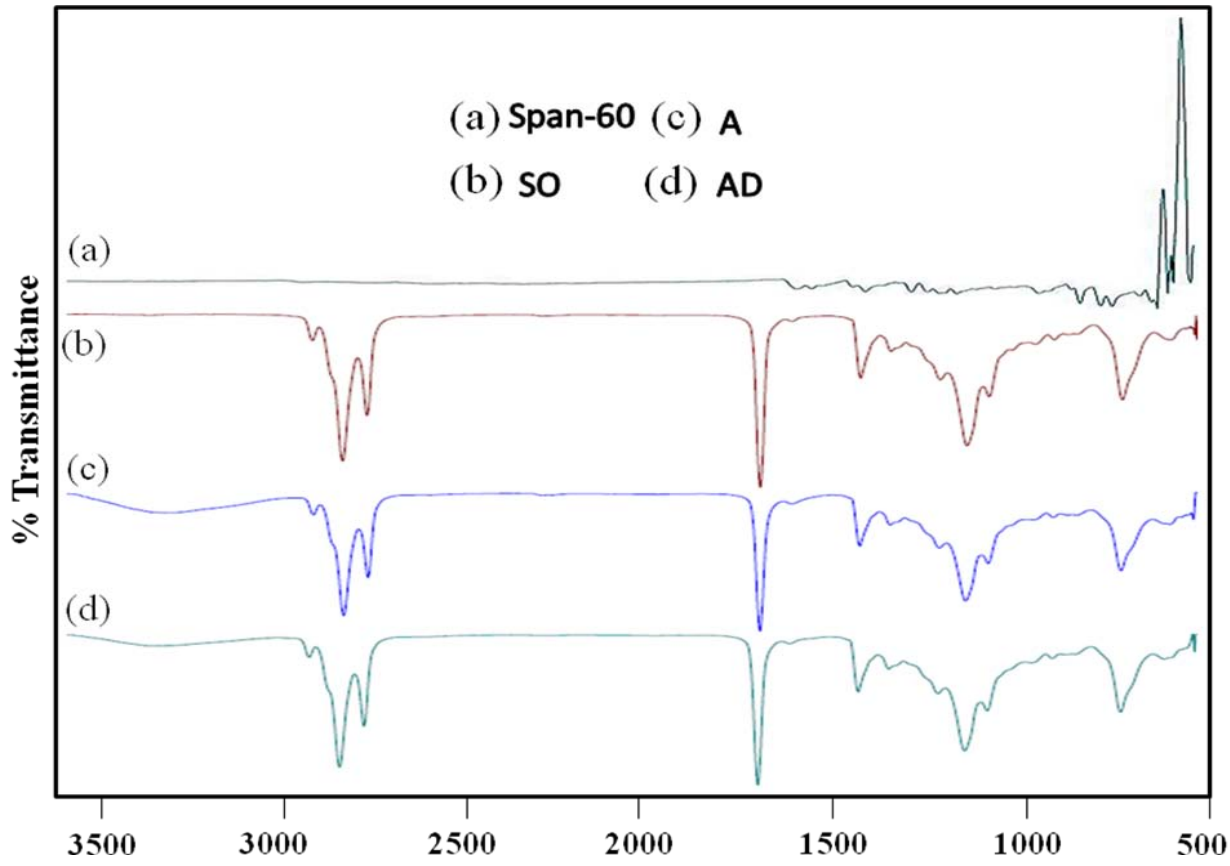


Figure 12: Graph showing results of FT-IR analysis; (a) span-60, (b) SO, (c) A, and (d) AD

4.9 XRD analysis

The A and H organogel samples were taken as the representative samples. The effect of incorporation of SA and thermo-cycling on the crystallinity of the samples was studied by XRD analysis. The XRD profiles have been shown in figure 13. The full widths at half maximum (FWHM) and area under the curve (AUC) for the analyzed samples have been tabulated in table 6.

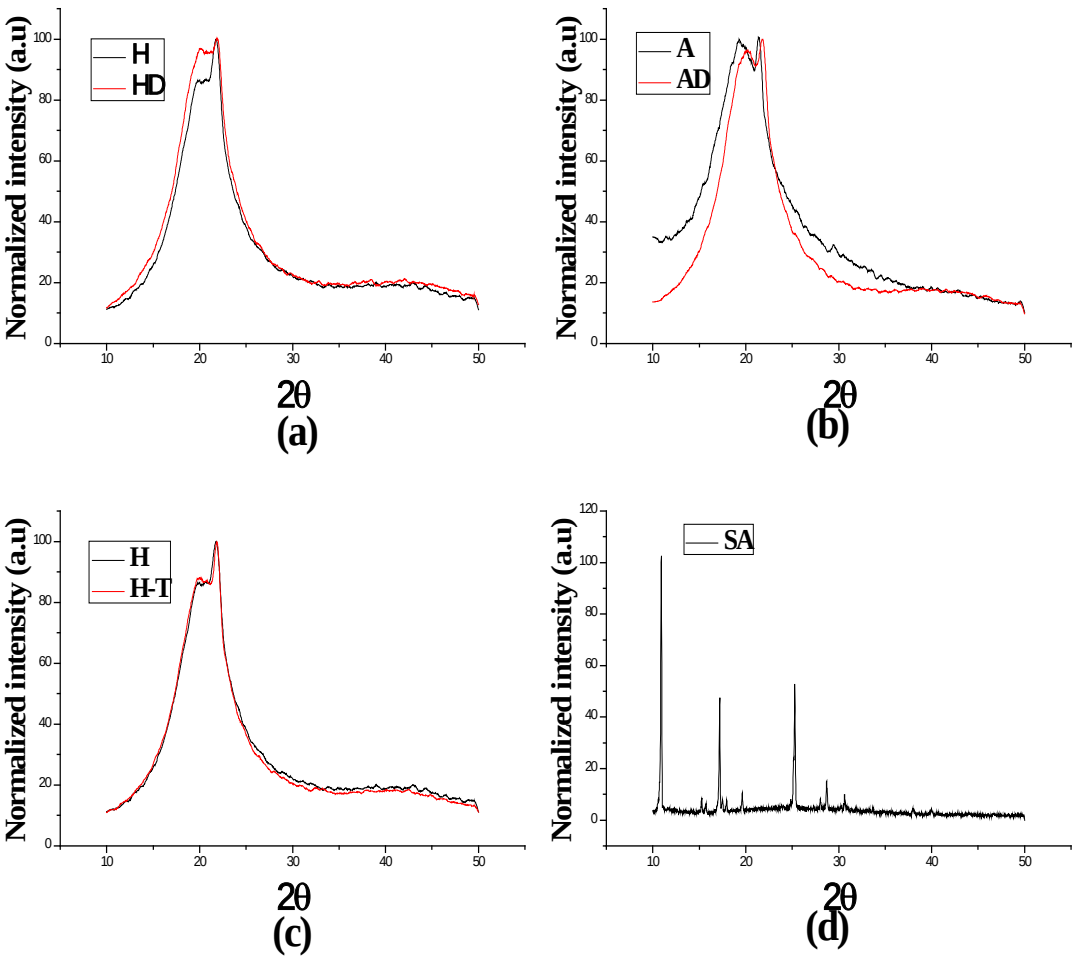


Figure 13: XRD data of the organogel samples; a) H and HD b) A and AD c) H and H-T and d) SA

Table 6: Values of AUC and FWHM for XRD studies

Sample	AUC	FWHM	Sample	AUC	FWHM	Sample	AUC	FWHM
H	431.53	6.24	**A**	687.45	9.00	**H**	431.53	6.24
HD	556.06	6.92	**AD**	532.82	6.72	**H-T**	473.16	6.24

The presence of a single broad peak at 20° 2θ for all the blank organogels and drug loaded organogels indicate amorphous dominant nature of the samples with low crystallinity. Crystallinity of organogels may be attributed to the presence of network structure made up of solid fibers of span-60 formed in SO. SA showed three sharp peaks at 10°, 18° and 30° 2θ indicating its crystalline nature. But as SA was incorporated in the gels no peaks corresponding to SA was found. This can be explained by the solubility of SA in the oil fraction of gels [88]. SA used in the composition of organogel got uniformly dissolved in the SO which is entrapped within the gelator network. Hence, the crystals of SA are not available responsible for the characteristic peaks. Incorporation of SA slightly decreased the crystalline nature of the sample H evident from the increased FWHM and AUC (table 6). On the other hand, the crystallinity of the sample A increased with the incorporation of the SA. This explains the improved consistency of the sample A as SA is incorporated. The XRD profile of the sample H indicated that there was not much change in the crystallinity of the samples even after accelerated thermal stability tests (sample HT).

4.10 Thermal Analysis

The simultaneous DTA-TGA thermogram profile of the sample A has been shown in figure 14. DTA thermogram showed a melting endotherm of sample A at 58.34ºC. A subsequent exothermic peak corresponding to weight loss, obtained from TGA thermogram, was observed at ~196 °C. This might be attributed to release of heat energy due to the degradation of the sample components.

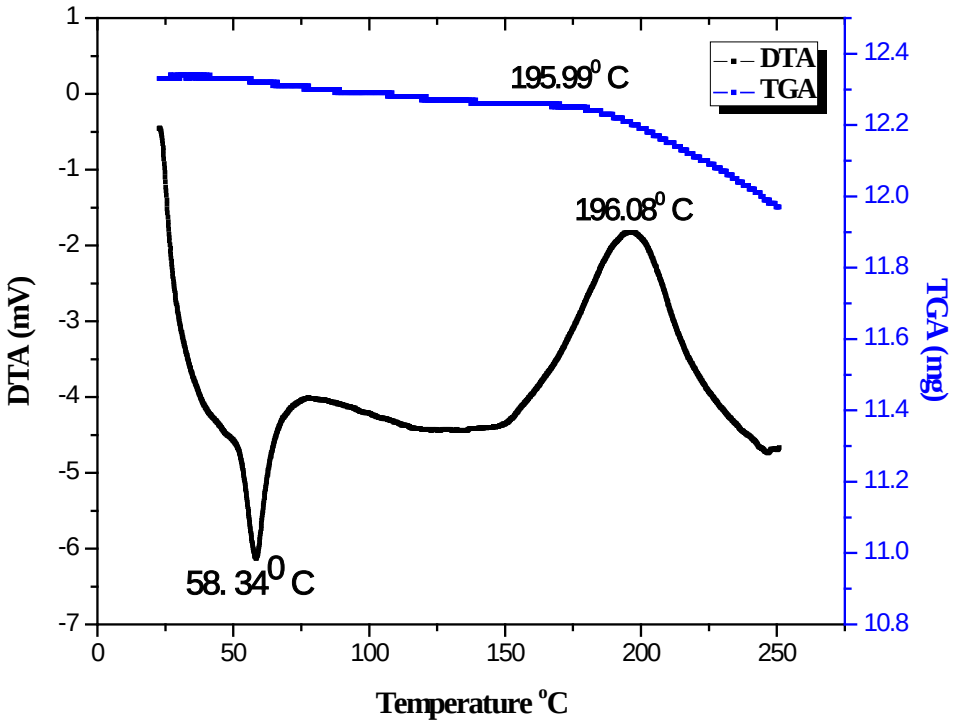
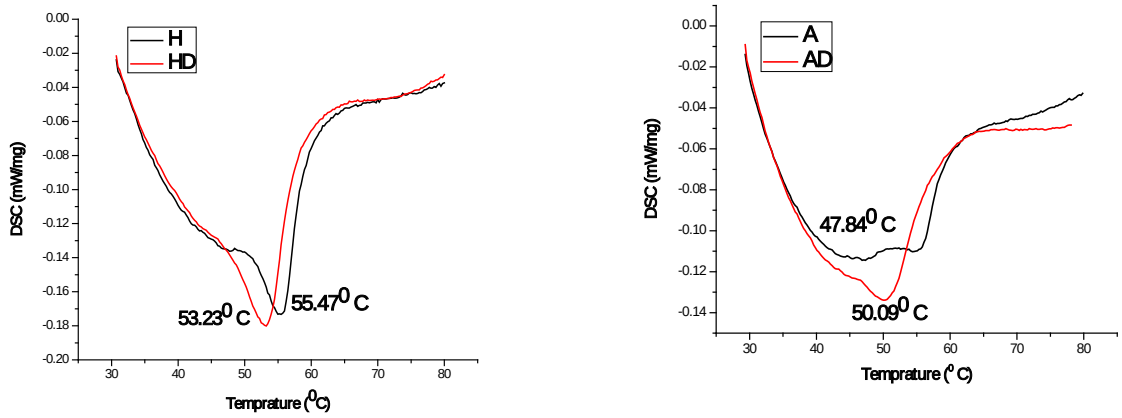


Figure 14: Simultaneous TGA-DTA thermogram of organogel A

The samples H, A, HD and AD were subjected to thermal analysis, using a differential scanning calorimeter, in the temperature range of 29°C to 80°C. Characteristic endothermic peaks were observed in DSC thermograms of H, HD, A and AD. The results indicate that with the increase in the gelator concentration, there was a subsequent increase in the melting endotherm. The melting endotherm of sample AD was found to be higher than the sample A whereas the melting endotherm of sample HD was found to be lower than sample H, figure 15. This may be explained by the XRD results, which suggested that the crystallinity of the sample AD was higher than sample A and the crystallinity of sample HD was lower than sample H. In general, higher the crystallinity, higher is the melting temperature [89].



(a) (b)

Figure 15: DSC thermogram of organogels; a) H and HD, b) A and AD

4.11 Antimicrobial evaluation

The sample A acted as a negative control whereas the sample AD was used as the test samples for the antimicrobial efficacy of the formulation against *E. coli* and *B. subtilis*. The zone of inhibition was measured after 24 h of incubation of the microbes in the presence of the organogels. The antimicrobial activity after the incubation period has been shown in figure 16 and the results have been tabulated in table 7. The sample AD was able to inhibit the growth of both the microorganism even after 24 h in the neighborhood zone (table 7). On the other hand, the sample A did not show any zone of inhibition. This suggests that the antimicrobial activity of the sample AD is due to the SA present in the formulation.

Table 7: Results of Antimicrobial screening test.

Bioactive agent	**Zone of inhibition (diameter; cm)**		
	E. coli	***Bacillus subtilis***	**Control**
Salicylic Acid 1 % (w/w)	1.1 ± 0.2	1.1 ± 0.3	nil

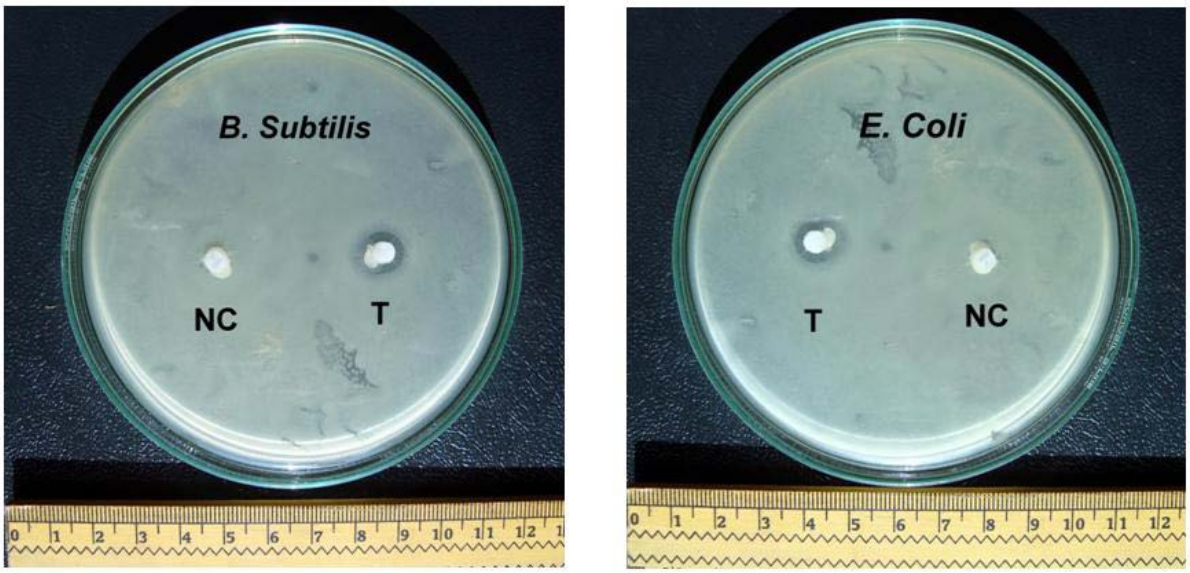


Figure 16: Effect of samples A and AD on the microbial growth; (a) *B. subtilis*, and (b) *E. coli*. T= Test (sample AD) and NC= negative control (sample A).

4.12 In vitro drug release studies

Release of drug from a formulation depends upon its solubility and partition coefficient in oil and water [45]. In vitro drug release study help predicting the drug release profile and drug release kinetics. This may help in correlating the drug activity when administered in the physiological system The type of release pattern from a formulations can be predicted by

applying various drug release kinetic models, e.g. zero order, first order, Higuchi, Hixon-Crowell, Weibull, Korsemeyer-peppas etc. [90-92].

The release profiles of the drug from the organogels have been shown in figure 17 as a function of time. The amounts of drug release from the AD, DD, and HD samples were found to be 40 % (approx.), 38 % (approx.) and 36 % (approx.), respectively, at the end of 8 h. This suggests that as the amount of gelator is increased there is subsequent reduction in the drug release rate. This indicates that drug release is dependent on the solid skeleton network formed by the gelator molecules [5, 93].

Table 8 shows the drug release rate constant (k) and coefficient of determination (r^2) 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 (figure 17 and table 8), indicating that the organogels may be used as controlled delivery systems. Korsmeyer-Peppas model was used to figure out the fickian constant ‘n’. The n value was found to be in between 0.5 and 0.7, suggesting that the release mechanism was a combination of both fickian and non-fickian kinetics.

It can be concluded from the above that the A, D and H samples may be used as a matrix for controlled release, which have been quite often associated with the solid fiber organogels [5, 93].

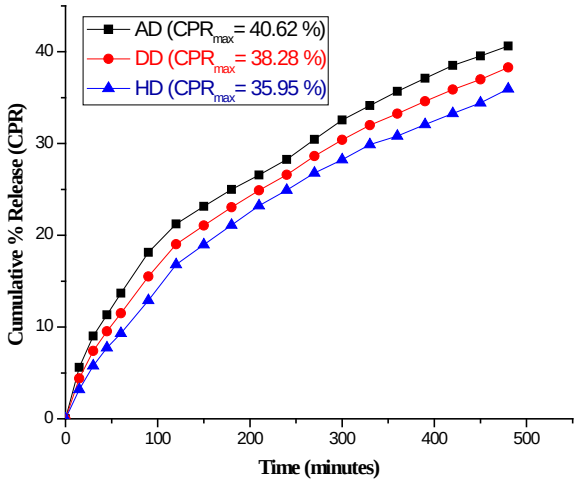


Figure 17: CPR values for different compositions of the organogel samples as a function of time

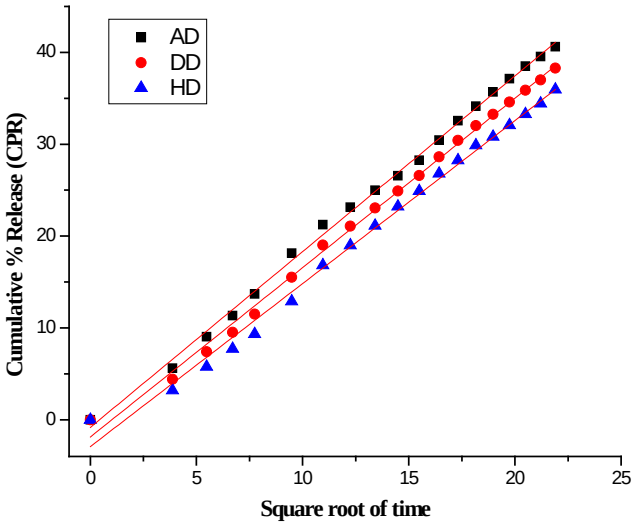


Figure 18: Higuchian-model kinetics for the different organogels samples; (a) AD, (b) DD, and (c) HD

Table 8: Kinetics of drug release

Sample	**r^2, k and n values for in vitro drug release kinetics**											
	Zero Order		**First Order**		**Higuchi Model**		**Hixon-Crowell Model**		**Weibull model**		**Korsmeyer peppas Model**	
	r^2	**K_0**	**r^2**	**K_1**	**r^2**	**k_H**	**r^2**	**k_{HC}**	**r^2**	**K_W**	**r^2**	**n**
AD	0.922	0.001	0.0390	$5.422E^{-4}$	**0.998**	1.914	0.019	$8.598E^{-4}$	0.846	1.119	0.995	0.592
DD	0.940	0.001	0.111	$9.669E^{-4}$	**0.997**	1.844	0.110	$8.691\ E^{-4}$	0.868	1.131	0.996	0.602
HD	0.949	0.001	0.045	$5.898E^{-4}$	**0.993**	1.775	0.025	$9.923E^{-4}$	0.895	1.147	0.988	0.619

4.13 pH measurement

The pH values of the organogel samples were found to be in the range of 5.5 and 6.7 (table 9), which is in close to skin pH 4.5-7 [82, 94-97]. This indicates that the samples may not cause any irritation to the skin and hence can be used for topical applications.

Table 9: pH values of organogel samples

Sample Code	pH
A	5.90 ± 0.30
B	5.83 ± 0.42
C	6.50 ± 0.15
D	6.70 ± 0.20
E	5.50 ± 0.30
F	5.70 ± 0.15
G	6.30 ± 0.30
H	6.10 ± 0.30

4.14 Hemocompatibility test

The %age hemolysis of goat RBCs was measured in the presence of the sample leachant and has been tabulated in table 10. The results suggested that all the organogel samples were highly hemocompatible (% hemolysis < 5 %) and may be regarded as biocompatible in nature [98].

Table 10: Results of the hemocompatibility test

Sample	% Hemolysis
A	0.85
AD	1.25
H	0.98
HD	1.32

Chapter 5 Conclusion

The study reports the successful development of span-60 and SO based organogel. Microscopic studies indicated that small needle-shaped clusters aggregated to form fibers, which underwent interaction amongst each other to form a networked structure. The networked structure helped in the immobilization of the SO. Stability studies indicated the stable nature of the developed organogels. The antimicrobial studies carried out using SA loaded samples suggested that the formulations may be used as antimicrobial organogels. In vitro studies indicated prolonged release of SA from the organogel matrix and the release of the SA from the organogel followed Higuchian kinetics. The pH of the organogels suggested that the organogels might be non-irritant in nature. The organogels were found to be biocompatible in nature. In short, the organogels developed may be tried as a matrix for controlled drug delivery.

Chapter 6 Bibliography

1. Esch, v., et al., *Design and Application of Self-Assembled Low Molecular Weight Hydrogels.* European Journal of Organic Chemistry, 2005: p. 3615–3631.
2. Maity, G.C., *Low Molecular Mass Gelators of Organic Liquids.* Journal of Physical Sciences, 2007. **11**: p. 156-171.
3. Murdan, S., *Organogels in drug delivery.* Expert opinion on drug delivery, 2005. **2**(3): p. 489-505.
4. Terech, P. and R.G. Weiss, *Low Molecular Mass Gelators of Organic Liquids and the Properties of Their Gels.* Chemical Reviews, 1997. **97**(8): p. 3133-3160.
5. Vintiloiu, A. and J.-C. Leroux, *Organogels and their use in drug delivery -- A review.* Journal of Controlled Release, 2008. **125**(3): p. 179-192.
6. Hughes, N.E., et al., *Potential food applications of edible oil organogels.* Trends in Food Science & Technology, 2009. **20**(10): p. 470-480.
7. Tarrago-Trani, M.T., et al., *New and Existing Oils and Fats Used in Products with Reduced Trans-Fatty Acid Content.* Journal of the American Dietetic Association, 2006. **106**(6): p. 867-880.
8. Racette, S.B., et al., *Phytosterol-Deficient and High-Phytosterol Diets Developed for Controlled Feeding Studies.* Journal of the American Dietetic Association, 2009. **109**(12): p. 2043-2051.
9. Holliday, R. and K. Phillips, *Health Benefits of the Sunflower Kernel.* Cereal Foods World, 2001(46(5)): p. 205-208.
10. Chandrashekar, P., B.R. Lokesh, and A.G. Gopala Krishna, *Hypolipidemic effect of blends of coconut oil with soybean oil or sunflower oil in experimental rats.* Food Chemistry, 2010. **123**(3): p. 728-733.
11. Dastidar, P., et al., *Structure–Property Correlation of a New Family of Organogelators Based on Organic Salts and Their Selective Gelation of Oil from Oil/Water Mixtures.* Chem. Eur. J, 2004. **10**: p. 5311-5322.
12. Rogers, M.A., A.J. Wright, and A.G. Marangoni, *Crystalline stability of self-assembled fibrillar networks of 12-hydroxystearic acid in edible oils.* Food Research International, 2008. **41**(10): p. 1026-1034.
13. Wang, X., et al., *Reversible organogels triggered by dynamic K+ binding and release.* Journal of Colloid and Interface Science, 2011. **353**(2): p. 412-419.
14. Terech, P., et al., *Structural variations in a family of orthodialkoxyarenes organogelators.* Journal of Colloid and Interface Science, 2006. **302**(2): p. 633-642.
15. Murdan, S., G. Gregoriadis, and A.T. Florence, *Novel sorbitan monostearate organogels.* Journal of pharmaceutical sciences, 1999. **88**(6): p. 608-614.
16. Hanabusa, K., et al., *Low Molecular Weight Gelators for Organic Fluids: Gelation Using a Family of Cyclo(dipeptide)s.* Journal of Colloid and Interface Science, 2000. **224**: p. 231-244.
17. Kishimura, A., T. Yamashita, and T.J. Aida, *J. Am. Chem. Soc.* Vol. 127. 2005.
18. Zhao, W., et al., *Heat-set supramolecular organogels composed of [beta]-cyclodextrin and substituted aniline in N,N-dimethylformamide.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2011. **374**(1-3): p. 115-120.
19. Vemula, P.K. and G. John, *Crops: A green Approach toward Self-Assembled Soft Materials.* Accounts of Chemical Research, 2008. **41**(6): p. 769-782.
20. Scartazzini, R. and P.L. Luisi, *Organogels from lecithins.* J Phys Chem., 1988. **92**: p. 829-833.
21. Ahmed, S.A.-M. and J.-L. Pozzo, *Photochromism of dihydroindolizines Part IX: First attempts towards efficient self-assembling organogelators based on photochromic dihydroindolizines and N-acyl-1,[omega]-amino acid units.* Journal of Photochemistry and Photobiology A: Chemistry, 2008. **200**(1): p. 57-67.
22. Gaucher, G., et al., *Polymeric micelles for oral drug delivery.* European Journal of Pharmaceutics and Biopharmaceutics, 2010. **76**(2): p. 147-158.

23. Chaterji, S., I.K. Kwon, and K. Park, *Smart polymeric gels: Redefining the limits of biomedical devices.* Progress in Polymer Science. **32**(8-9): p. 1083-1122.
24. Roy, D., J.N. Cambre, and B.S. Sumerlin, *Future perspectives and recent advances in stimuli-responsive materials.* Progress in Polymer Science. **35**(1-2): p. 278-301.
25. Taktak, F.F. and V. Bütün, *Synthesis and physical gels of pH- and thermo-responsive tertiary amine methacrylate based ABA triblock copolymers and drug release studies.* Polymer, 2010. **51**(16): p. 3618-3626.
26. Motulsky, A., et al., *Characterization and biocompatibility of organogels based on l-alanine for parenteral drug delivery implants.* Biomaterials, 2005. **26**(31): p. 6242-6253.
27. Almeida, I.F. and M.F. Bahia, *Evaluation of the physical stability of two oleogels.* International Journal of Pharmaceutics, 2006. **327**(1-2): p. 73-77.
28. Pernetti, M., et al., *Structuring edible oil with lecithin and sorbitan tri-stearate.* Food Hydrocolloids. **21**(5-6): p. 855-861.
29. Pernetti, M., et al., *Structuring of edible oils by alternatives to crystalline fat.* Current Opinion in Colloid & Interface Science, 2007. **12**(4-5): p. 221-231.
30. Lukyanova, L., et al., *Preparation and evaluation of microporous organogel scaffolds for cell viability and proliferation.* Colloids and Surfaces B: Biointerfaces, 2010. **79**(1): p. 105-112.
31. Maffezzoni, R. and M. Zanda, *Stable organogels derived from triazines functionalized with chiral [alpha]-amino acid derivatives.* Tetrahedron Letters, 2008. **49**(35): p. 5129-5132.
32. Shaikh, I.M.e.a., *Aceclofenac Organogels: In Vitro and In Vivo Characterization.* Current Drug Delivery., 2009. **6**: p. 1-7.
33. Tan, H.e.a., *Thermoreversible gelation of atactic polystyrene solutions.* Macromolecules, 1983. **16**(1): p. 28-34.
34. Stein, S.B. and H.Z. Angew, *Math. Mech*. Vol. 80. 2000.
35. Macosko, C.W., *Rheology:Principles, Measurements and Applications.* 1994, New York: VCH Publisher. 1011-1013.
36. Shapiro, Y.E., *Structure and dynamics of hydrogels and organogels: An NMR spectroscopy approach.* Progress in Polymer Science. **In Press, Corrected Proof**.
37. Prausnitz, M.R., S. Mitragotri, and R. Langer, *Current status and future potential of transdermal drug delivery.* Nat. Rev. Drug Dis, 2004. **3**: p. 115-124.
38. Kumar, R. and O.P. Katare, *Lecithin Organogels as a Potential Phospholipid-Structured System for Topical Drug Delivery: A Review.* AAPS PharmSciTech, 2005. **6**(2): p. E298-E310.
39. Luigi, T., et al., *Aceclofenac cream versus piroxicam cream in the treatment of patients with minor traumas and phlogistic affections of soft tissues: a double-blind study.* Curr. Ther. Res, 1995. **56**: p. 702-712.
40. Shaikh, I.M., et al., *Aceclofenac Organogels: In Vitro and In Vivo Characterization.* Current Drug Delivery., 2009. **6**: p. 1-7.
41. Yang, J.H., K. Young, and K.M. Kim, *Preparation and evaluation of aceclofenac microemulsion for transdermal delivery system.* Arch. Pharm. Res., 2002. **25**: p. 533-540.
42. Willimann, H., et al., *Lecithin organogel as matrix for transdermal transport of drugs.* Journal of Pharmaceutical Sciences, 1992. **81**(9): p. 871-874.
43. Dreher, F., et al., *Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport.* Journal of Controlled Release, 1997. **45**(2): p. 131-140.
44. Agrawal, G.P., et al., *Preparation and characterization of reverse micelle based organogels of piroxicam.* Pharmazie, 2004. **59**: p. 191-193.

45. Zia, H.A., et al., *Lecithin-Stabilized microemulsion-Based organogels for topical application of ketorolac tromethamine. II. in vitro release study.* Iranian Journal of Pharmaceutical Research, 2003. **2**: p. 117-124.
46. Iwanaga, K., et al., *Characterization of organogel as a novel oral controlled release formulation for lipophilic compounds.* International Journal of Pharmaceutics, 2010. **388**(1-2): p. 123-128.
47. Lim, P.F.C., et al., *Limonene GP1/PG organogel as a vehicle in transdermal delivery of haloperidol.* International Journal of Pharmaceutics, 2006. **311**(1-2): p. 157-164.
48. Kang, L., et al., *SMGA gels for the skin permeation of haloperidol.* Journal of Controlled Release, 2005. **106**(1-2): p. 88-98.
49. Tokuyama, H. and Y. Kato, *Preparation of thermosensitive polymeric organogels and their drug release behaviors.* European Polymer Journal, 2010. **46**(2): p. 277-282.
50. Plourde, F., et al., *First report on the efficacy of l-alanine-based in situ-forming implants for the long-term parenteral delivery of drugs.* Journal of Controlled Release, 2005. **108**(2-3): p. 433-441.
51. Paice, J.A., et al., *Morphine Bioavailability from a Topical Gel Formulation in Volunteers.* Journal of Pain and Symptom Management, 2008. **35**(3): p. 314-320.
52. Bastiat, G., et al., *Tyrosine-based rivastigmine-loaded organogels in the treatment of Alzheimer's disease.* Biomaterials, 2010. **31**(23): p. 6031-6038.
53. Kantaria, S., G.D. Rees, and M.J. Lawrence, *Gelatin-stabilised microemulsion-based organogels: rheology and application in iontophoretic transdermal drug delivery.* Journal of Controlled Release, 1999. **60**(2-3): p. 355-365.
54. Tokuyama, H. and Y. Kato, *Preparation of poly(N-isopropylacrylamide) emulsion gels and their drug release behaviors.* Colloids and Surfaces B: Biointerfaces, 2008. **67**(1): p. 92-98.
55. Namdeo, A. and N.K. Jain, *Liquid crystalline pharmacogel based enhanced transdermal delivery of propranolol hydrochloride.* Journal of Controlled Release, 2002. **82**(2-3): p. 223-236.
56. Bhatnagar, S. and S.P. Vyas, *Organogel-based system for transdermal delivery of propranolol.* Journal of Microencapsulation, 1994. **11**(4): p. 431-438.
57. Nastruzzi, C. and R. Gambari, *Antitumor activity of (trans)dermally delivered aromatic tetra-amidines.* Journal of Controlled Release, 1994. **29**(1-2): p. 53-62.
58. Bonina, F.P., et al., *Effects of phospholipid based formulations on in vitro and in vivo percutaneous absorption of methyl nicotinate.* Journal of Controlled Release, 1995. **34**(1): p. 53-63.
59. Mahler, P., et al., *Double-blind, randomized, controlled study on the efficacy and safety of a novel diclofenac epolamine gel formulated with lecithin for the treatment of sprains, strains and contusions.* Drugs under experimental & clinical research, 2003. **29**: p. 45-52.
60. Pénzes, T., et al., *Topical absorption of piroxicam from organogels--in vitro and in vivo correlations.* International Journal of Pharmaceutics, 2005. **298**(1): p. 47-54.
61. Friedman, M.L., NY), *Treatment of bruxism*. 2003: United States.
62. Feng, G., et al., *Cyclic voltammetry investigation of diffusion of ferrocene within propylene carbonate organogel formed by gelator.* Electrochimica Acta, 2008. **53**(28): p. 8253-8257.
63. Feng, G., et al., *Gelation of microemulsions and release behavior of sodium salicylate from gelled microemulsions.* European Journal of Pharmaceutics and Biopharmaceutics, 2009. **71**(2): p. 297-302.
64. Wang, H., et al., *Host-guest interactions of 5-fluorouracil in supramolecular organogels.* European Journal of Pharmaceutics and Biopharmaceutics, 2009. **73**(3): p. 357-360.

65. Murdan, S., G. Gregoriadis, and A.T. Florence, *Gelation of hexadecane with span 60: a potential organogel-based controlled release system.* European Journal of Pharmaceutical Sciences, 1996. **4**: p. 158-158.
66. Murata, K., et al., *New cholesterol-based gelators with light- and metal-responsive functions.* Journal of the Chemical Society, Chemical Communications, 1991(24): p. 1715-1718.
67. Dawn, A., et al., *Studies on a new class of organogelator containing 2-anthracenecarboxylic acid: Influence of gelator and solvent on stereochemistry of the photodimers.* Organic & Biomolecular Chemistry, 2009. **7**(21): p. 4378-4385.
68. Johnson, K. and B. Wilkins. *Organogel formulation technology for the 21st century*. 2008 [cited 2011 01/03/11]; Available from: http://www.organotech.com/.
69. Carretti, E., L. Dei, and R.G. Weiss, *Soft matter and art conservation.* Soft Matter, 2005. **1**: p. 17-22.
70. Kobayashi, S., et al., J. Am. Chem. Soc., 2002. **124**: p. 6550 –6551.
71. Jung, J.H., et al., J. Am. Chem. Soc., 2000. **122**: p. 5008-5009.
72. Shi, C., et al., Science, 1999. **286**: p. 1540 –1543.
73. Kubo, W., et al., Chem. Commu, 2002: p. 374-375.
74. Bhattacharya, S. and Y. Krishnan-Ghosh, Chem. Commu, 2001: p. 185-186.
75. Tsutomu, I. and S. Shinkai, *Dye-based organogels: stimuliresponsive soft materials based on one-dimensional self-assembling aromatic dyes.* Topics in Current Chemistry, 2005. **258**: p. 119-160.
76. Abdallah, D.J. and R.G. Weiss, *Organogels and Low Molecular Mass Organic Gelators.* Advanced Materials, 2000. **12**(17): p. 1237-1247.
77. Nii, S., et al., *Extractant-impregnated organogel for capturing heavy metals from aqueous solutions.* Separation and Purification Technology, 2010. **73**(2): p. 250-255.
78. Dave, R. and D. Madamwar, *Candida rugosa lipase immobilized in Triton-X100 microemulsion based organogels (MBGs) for ester synthesis.* Process Biochemistry, 2008. **43**(1): p. 70-75.
79. Nagayama, K. and M. Imai, *Enhanced activity of Mucor javanicus lipase in polyoxyethylene sorbitan trioleate containing microemulsion-based organogels.* Journal of Molecular Catalysis B: Enzymatic, 2005. **34**(1-6): p. 44-50.
80. Anand, A. and M. Arora, *Insturmental methods of chemical analysis*. 2007, Mumbai: Himalaya Publishing House.
81. Brucker. *Guide for Infrared spectroscopy.* 2011 [cited 2011 05/04/11]; Available from: http://www.brukeroptics.com/downloads.html.
82. Bhatia, V.N. and R.H. Barber, *The effect of pH variations of ointment bases on the local anesthetic activity of incorporated ethyl aminobenzoate. I. Hydrophilic ointment U. S. P.* Journal of the American Pharmaceutical Association, 1955. **44**(6): p. 342-343.
83. Lieberman, H.A., M.M. Rieger, and G.S. Banker, *Pharmaceutical dosage forms--disperse systems*. Vol. 2. 1998: M. Dekker.
84. Gooch, J.W., *Emulsification and Polymerization of Alkyd Resins*. 2002: Kluwer Academic/Plenum Publishers.
85. Sobczuk AA, Tamaru S, and S. S., *New strategy for controlling the oligothiophene aggregation mode utilizing the gel-to-sol phase transition induced by crown-alkali metal interactions.* Chem Commun (Camb). 2011. **47**(11): p. 3093-5.
86. Talukdar, M.M., et al., *Rheological characterization of xanthan gum and hydroxypropylmethyl cellulose with respect to controlled release drug delivery.* Journal of pharmaceutical sciences, 1996. **85**(5): p. 537-540.
87. Coates, J., *Interpretation of Infrared Spectra, A Practical Approach*, in *Encyclopedia of Analytical Chemistry*, R.A. Meyers, Editor. 2000, John Wiley & Sons Ltd: Chichester. p. 10815-10837.

88. Yu, H. and C. Xiao, *Synthesis and properties of novel hydrogels from oxidized konjac glucomannan crosslinked gelatin for in vitro drug delivery.* Carbohydrate Polymers, 2008. **72**(3): p. 479-489.
89. Markovic, N., M. Ginic-Markovic, and N.K. Dutta, *Mechanism of solvent entrapment within the network scaffolding in organogels: thermodynamic and kinetic investigations.* Polymer International, 2003. **52**(7): p. 1095-1107.
90. Costa, P. and J.M. Sousa Lobo, *Modeling and comparison of dissolution profiles.* European Journal of Pharmaceutical Sciences, 2001. **13**(2): p. 123-133.
91. Nanjwade BK, et al., *Formulation and Characterization of Hydralazine Hydrochloride Biodegraded Microspheres for Intramuscular Administration.* Journal of Bioanalysis & Biomedicine, 2011. **3**(1): p. 032-037.
92. Shoaib Harris M, et al., *EVALUATION OF DRUG RELEASE KINETICS FROM IBUPROFEN MATRIX TABLETS USING HPMC.* Pakistan Journal of Pharmaceutical Sciences, 2006. **19**(2): p. 119-124.
93. Yang, Y., et al., *Properties of topically applied organogels: rheology and in vitro drug release.* Asian Journal of Pharmaceutical Sciences, 2008. **3**(4): p. 175-183.
94. Schmid-Wendtner, M.H., *PH and Skin Care*. 2007: Abw Wissenschaftsverlag.
95. *Ayushveda*. [cited 2011 01/04/11]; Available from: http://www.ayushveda.com/beautytips/makeup-tips/skin-ph.htm.
96. Skin Biology [cited 2011 01/04/11]; Available from: http://www.skinbiology.com/skinhealth&aging.html.
97. Yosipovitch G and H. J., *The importance of skin pH. Skin & aging.* HMP Communications 2003. **11**(3): p. 88-93.
98. Pal, K., A. Banthia, and D. Majumdar, *Biomedical evaluation of polyvinyl alcohol–gelatin esterified hydrogel for wound dressing.* Journal of Materials Science: Materials in Medicine, 2007. **18**(9): p. 1889-1894.