PREPARATION AND CHARACTERIZATION OF NOVEL SPAN 80: TWEEN-80 BASED ORGANOGELS FOR FOOD AND PHARMACEUTICAL INDUSTRIES

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE

OF

Mater of Technology

In

Biomedical Engineering

By

T. SUDHEEP KUMAR

Roll No. 209BM1015

June-2011

Department of Biotechnology & Medical Engineering

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Date: 06.06.2011

CERTIFICATE

This is to certify that the thesis entitled, **"Preparation And Characterization Of Novel Span 80: Tween-80 Based Organogels For Food And Pharmaceutical Industries"** submitted by **Mr. T. Sudheep Kumar** in partial fulfillment of the requirements for the award of Master of Technology Degree in **"Biomedical Engineering"** at the National Institute of Technology, Rourkela, Odisha is an authentic work carried out by him under my supervision and guidance.

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.

Dr. Kunal Pal

Project supervisor

Department of Biotechnology and Medical engineering

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T.SUDHEEP KUMAR

CONTENTS

ABBREVIATIONS

Abbreviation	Definitions
SM	Surfactant Mixture
GS	Gelator Solution
v/v	Volume by Volume
w/w	Weight by Weight
SO	Sunflower Oil
SA	Salicylic Acid
KDa	Kilo Dalton
XRD	X-Ray Diffraction Analysis
µm	Micrometer
Tgs	Gel-Sol Transition Temperature
FWHM	Full Width At Half Maxima
AUC	Area Under The Curve
FTIR	Fourier Transform Infrared Spectroscopy
CPR	Cumulative Percentage Release
TGA	Thermogravimetric Analysis
DTA	Differential Thermal Analysis
DC	Direct Current
PC	Phosphatidyl Choline

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Abstract

Tween 80-span 80 based organogels were prepared by fluid-filled structure mechanism by varying the composition of the organogels. The microstructures of the organogels were studied by light microscopy. The organogels were subjected to the accelerated stability test and time dependent stability test. The stable organogels were characterized by XRD, FTIR, and simultaneous DTA-TGA, pH and dc impedance measuring devices. Salicylic acid (model drug) was incorporated within the organogels and its release properties from the organogel matrices were studied. The antimicrobial efficiency of the salicylic loaded product was tested against *Bacillus subtilis*. The organogels were analyzed for biocompatibility using hemolysis studies. The microscopic studies indicated fluid-filled globular structures forming the gelled structures. The stability and the properties were found to be dependent on the proportion of the surfactant mixture (SM) and water. In general, when the ratio of SM: water was in the range of 1.3-1.6, the samples showed higher stability and improved properties. The release of SA from the organogels was found be combination of Fickian and non-Fickian kinetics. The samples showed good antimicrobial study and were found to be biocompatible in nature.

CHAPTER-01

INTRODUCTION AND OBJECTIVE

1.1. Introduction

Gels are defined as semi-solid preparations having both solid and liquid components within its structure. The solid component forms a networked structure, which results in the immobilization of the liquid component. Immobilization of liquid component within the networked structure of the solid component has been attributed to the interfacial tension amongst the solid and liquid components [1]. The liquid phase may either be polar or apolar in nature. If the liquid phase is polar in nature, then the gels may be regarded as hydrogels else as organogels [2]. The solid components are regarded as gelator [3]. Some of the organogelators (e.g. lecithin, span or tween) accommodate aqueous phase within itself to form fiber-like structures, which physically interacts amongst each other, resulting in the formation of a networked structure [4]. The organogels developed by this mechanism are usually non-crystalline, non-glassy and thermo-reversible in nature [4]. The apolar phase may either be mineral oil, organic solvent or vegetable oil. Of late, the research on organogels for applications in food, pharmaceutical and cosmetic industry has gained a tremendous momentum [4-7]. This may be attributed to the easy production techniques and inherent stability of the organogels [8]. Due to their easy spreadability, the organogels are becoming a vehicle of choice for cosmetics products and transdermal delivery systems [9]. In the present study, tween 80- span 80 mixture based organogels were prepared by fluid-filled structure mechanism and were characterized for their probable use as transdermal drug delivery vehicle.

1.2. Objective

To develop span 80-tween 80 mixture based organogels for probable use in pharmaceutical, nutraceutical and cosmetic industries.

CHAPTER-02

REVIEW OF LITERATURE

2. Review of literature

Based on physical properties, the organogels have been classified into solid matrix and fluid filled matrix organogels. The fluid matrix organogels consists of gels made from lecithin or Span or Tween. These lecithin or span or tween form a different kind of reverse micelles in presence of water and oil. These fluid matrix gels are transparent or opaque and thermoreversible in nature. These gels are also called as worm like or polymer like networks [10-13].

2.1. Lecithin organogels

Lecithin is a zwitter ionic phospholipid with two alkyl tails, which forms spherical or ellipsoidal reverse micelles when added to oil [14]. Lecithin is widely used in everyday life including human and animal food, medicine, cosmetics and industrial applications [15]. These are biocompatible in nature so can be used for longer time periods [16]. They can dissolve lipophilic, hydrophilic and ampiphilic moieties in them [17]. These are thermo reversible in nature. Up to 40° C they act as gels and above that temperature changes into solution state [18]. These organogels exhibit Newtonian fluid behaviour before gelling and exhibit maxwell's rheological behaviour after addition of polar phase [19].

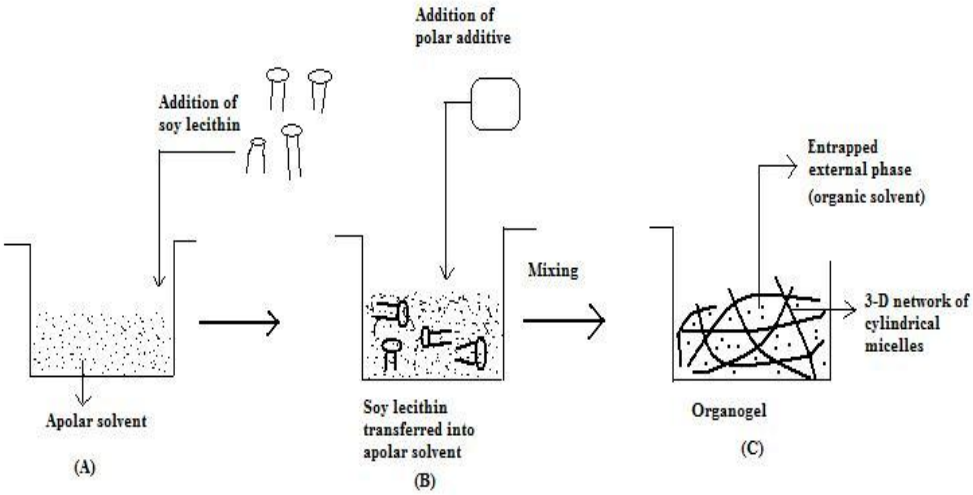


Fig 1: Diagram showing formation of lecithin organogels

Generally these lecithin organogels are the jelly-like phases, consist of a 3- dimensional network of entangled reverse cylindrical (polymer-like) micelles, which immobilizes the continuous or macroscopic external organic phase, thus turning a liquid into a gel [20]. Lecithin, when dissolves in non polar media alone, self-assembles in to reverse spherical

micelles at a concentration of ~0.01 mM [21]. Further, with the addition of water, this spherical reverse micellar state of lipid aggregates turns to form elongated tubular micelles (figure 1). Previous studies of reverse worm-like micelles indicated that two conditions are needed for substances to induce the growth of reverse worm-like micelles: The substance must have at least two functional groups that hydrogen-bond with the phosphate group of lecithin and must be slightly hydrophobic to penetrate into the reverse micellar layer [22]. Lecithin gels have been reported in a large number of solvents including linear, branched and cyclic alkanes, ethers and esters, fatty acids and amines. The amount of water added in order to achieve maximum viscosity of gels varies depending on solvent. Kaname and his co-workers developed organogels by using lecithin, sucrose fatty acid (SFE) ester and dodecane oil. In a mixed system of SFE and lecithin, the SFE binds to the phosphate group of neighbouring lecithin moieties, reducing the interface curvature of the molecular assembly and inducing the formation of reverse worm-like micelles. These reverse worm-like micellar regions could be enlarged by increasing lecithin concentration and the hydrophobicity of SFE. Ruggero Angelico and his co workers produced organogels by using lecithin/water/isopropylpalmitate [23]. The size and the shape of the micelles depend on the surfactant chain length, solvent structure, and the type of cosurfactant used and also on the concentration of the components. The formation of hydrogen bonding in lecithin organogels was studied by using different hydrocarbons like glycerol, formamide and ethylene glycol [19]. The ternary mixtures of lecithin, water, and hydrocarbon oil such as cyclohexane exhibit a rich phase behaviour, ranging from spherical micellar solutions (droplet-like aggregates), to giant worm-like tubular micelles, to a viscoelastic 3-D network of entangled worm-like micelles known as organogels [20, 24]. Electro rheological effects(ER) i. e increase in viscosity and dynamic shear moduli in lecithin by applying electrical field was studied by using oscillating rheology, polarizing microscopy, and electric birefringence [25]. The viscoelastic properties of lecithin organogels which included α- and β-anomers of alkylglucosides as well as their derivatives containing an alkyl chain of various length was also studied [26]. Poorly purified substances did not possess gel forming properties. When synthetic lecithins containing residues of saturated fatty acids used for the preparation of gels, the organogel formation is not observed [24]. The researchers have found that lecithin has capacity to form organogels in more than 50 items. This includes linear, branched and cyclic alkanes, ethers, esters, fatty acids and amines. The exceptions are represented by aromatic and chlorinated solvents. In their case the thickening effect is not observed with the addition of water because of its inclusion into micelles through a distinctive mechanism [21]. The

gelation of edible oil by a mixture of lecithin and sorbitan tri-stearate (STS) was studied. The two components individually in oil did not give structurant concentrations between 6% and 20% w/w. A synergetic effect was observed with their mixture at specific ratios of lecithin: STS between 40:60 and 60:40 when firm gels were obtained. The interaction of the two structurants was studied by varying concentration and ratio of lecithin: STS and evaluated through microscopy, texture analysis, X-ray diffraction (XRD), rheology and nuclear magnetic resonance (NMR) [27]. The effect of 1-butanol on the ternary structure of lecithin, water and triplamitate was observed. It was found that the lecithin organogels mixed with 1-butanol showed isotropic liquid phase with no cryatality [28]. The effect of added poly (ethylene glycol) monolaurate (PEGML) on the formation and properties of lecithin organogels composed of polymer-like micelles was studied by the methods of dynamic rheology and the Fourier transform IR spectroscopy. It was established that the addition of even small amounts of PEGML causes a significant decrease in viscosity, whereas the elastic properties of organogels remained almost unchanged. The desired viscoelastic property can be managed by modifying the various formulation components (i.e., selecting the type of organic solvent, concentration of gelator or cosurfactant, or the type or amount of polar agent), which significantly influence the structural stability and rheological behaviour of organogels. The increase in the gelator concentration leads to an increase in the viscosity and in turn the gel strength of a soy lecithin-IPP organogel matrix. The Spectral studies revealed that the PEGML molecules affect intermolecular hydrogen bonding during their incorporation into micelles. This leads to a decrease in the number of hydrogen bonds or their weakening and, as a result, to the disintegration of polymer-like lecithin micelles into shorter micellar aggregates [20]. The phase behaviour of a ternary system of lecithin/organic solvent/polar solvent is mainly governed by the concentration of polar solvent and lecithin. This is defined as n_w (i.e. molar ratio of polar solvent to lecithin). The phase behaviour of lecithin in n-decane employing water as the polar solvent has been discussed. At first, with the addition of water, the thickening effect is observed at a certain specific molar ratio of water to lecithin. After this threshold concentration, further addition of water leads to a sharp increase in the viscosity and the formation of organogel. The organogel state is maintained up to a particular molar ratio of water to lecithin, designated as n_{cr}. At the state where n_w is equal to n_{cr}, the maximum viscosity of organogel is achieved. On continuing the water addition above the n_{cr} (i.e., at $n_w > n_{cr}$) the 3-dimensional network collapses and separation of the homogenous organogel takes place in a 2-phase system consisting of low viscous liquid and a compact organogel or jelly-like phase [29]. The phase behaviour of organogels varies on

changing temperature conditions. The phase transition temperature (PTT) of a gel helps in optimizing the composition of gel. In one such study, concentration of gelator in a given LO (lecithion organogels) formulation was optimized by monitoring the PTTs of the organogel[30]. For the determination of PTTs, hot stage microscopy (HSM) and high sensitivity differential scanning calorimetry (HSDSC) have been reported to be useful. However, the inverse flow method, a simple technique based on visual observations has also been employed.

Table 1: n_{cr} values with different organic solvents employed in lecithin organogel systems composed of soyabean lecithin/organic solvent/water in 80:16:4 weight ratio [31]

Solvent	**n_{cr}**
1,7-octadiene	7
Butyl laurate	7
Cyclododecane	12
Cyclooctane	7
Dibutyl ether	6
Ethyl myristate	5
Isooctane	2
Isopropyl myristate	3
Isopropyl palmitate	3
n-hexadecane	1
n-hexane	3
n-octane	2
Trans-decalin	5
Tributylamine	2
Triisobutylamine	3

Nurettin Sahiner and his co workers produced lecithin organogels which contain acryl amide hydrogels in it. These hydrogel incorporated organogels are biocompatible and used for drug delivery applications. Angela Attar Nasseri and his co workers developed lecithin organogels by using isopropyl meristate for topical delivery of ketorolac. For any vehicle to be used for topical drug delivery applications, it is essential to study its rheological behaviour. The latter is important for its efficacy in delivering the molecules onto or across the skin site. The

critical parameters such as spreadability, adhesiveness (property related to bioadhesion on skin site) and gel consistency need to be modified in a favourable manner. There are two classes of lecithin organogels called as pluronic lecithin organogels (PLOs) and premium lecithin organogels (PrLO). The term pluronic refers to a series of non-ionic, closely related block copolymers of ethylene oxide and propylene oxide. These PLOs are made up of isopropyl palmitate, lecithin, water and pluronic F127. Generally these PLOs are opaque and yellowish in colour. Typically 22% (v/v) of oil phase is present in the PLOs [11]. The drugs methimazole and diclofenacare already incorporated into PLOs and used for topical route of drug delivery [32]. These PLOs are not thoroughly characterized and little is known about its physiochemical properties such as structure, rheology, stability and effect of drug incorporation etc. The drug Sumatriptan succinate was also entrapped successfully in pluronic lecithin organogels to treat nerve disorders. In this study formulations were developed with and without co surfactant pluronic F 127. The prepared organogels were evaluated for its appearance, organoleptic characteristics, and feel upon application, homogeneity, occlusivenes, washability, pH, viscosity, spreadability, gel transition temperature of formulations. The formulations were also evaluated for drug content, in vitro drug diffusion properties and skin irritation testing. The formulation containing pluronic showed greater spreadability and higher drug diffusion rate as compared to pluronic free organogel. Pluronic not only enhances the stability of organogel by increasing the viscosity but also increases the release of drug [33]. The premium lecithin organogels (PrLOs) are second type of lecithin organogels. Generally these are more temperature resistant than other gels. There was no skin irritation because these gels do not contain any pluronic derivatives. The research done on these PrLOs revealed that by using these gels bioavailability of drugs for the tissues has been increased. These gels are marketed generally in ready to use forms called as PrLO premixed gels. The bio active agents like diclofenac, ibuprofen, ketoprofen and progesterone are successfully entrapped in these gels and also applied intra dermally [34].

2.1.2. Mechanism of action of LOs

The barrier to transdermal delivery is the stratum corneum. The PLO disrupt the lipid layers of the stratum corneum without damaging them, as do harsher agents like dimethyl sulfoxide (DMSO), which dissolves the lipid layers. The PLO allow the medication to pass through the stratum corneum into the systemic circulation via the dermal-epidermal blood flow so that it is more likely to be absorbed.

2.1.3. Applications of lecithin organogels for topical drug delivery

The presence of organic and aqueous phase by means of a structurally well-defined micellar network of phospholipids, a large interfacial area, and the ability to entrap solutes within the gel matrix, along with long-term stability, makes them useful for a variety of applications. The topical applications of various drugs containing LO systems have been demonstrated to significantly enhance the skin permeation and absorption of both lipophilic and hydrophilic substances. The permeation enhancing effect of the lecithin gel was attributed to the vectoring properties of reverse micelles. It was suggested that the micellar entities being small in size and with hydrocarbon sheath might had been received by the skin barrier as hydrophobic entities, which were allowed for closer interaction with skin barrier leading to enhanced permeation of the drug molecules. Therapeutic compounds of different chemical and physicochemical background such as muscle relaxants, steroids, hormones, analgesics, antiemetics, cardiovascular agents, antithyroid drugs, and some macromolecules have been incorporated in the LOs with some very encouraging results. The enhanced skin penetration and site-specific delivery of bioactives into the deeper layers of skin has been achieved by employing organogels as topical vehicles. An organogel-based (LO or PLO) formulation containing extract of saw palmetto (having antiandrogen agent as a principal constituent) along with acylcarnitine and coenzyme Q has been reported as an effective formulation for the topical treatment of androgenic alopecia. The LOs have been found to be an excellent matrix for the delivery of a macromolecule with a molecular weight of 33,000 daltons. The PLO gel containing anti-inflammatory macromolecule bromelain (15%) along with capsaicin (0.025%) has been found to be an effective anti-inflammatory composition. Direct application of this PLO gel at the site of inflammation has been found to be useful for treating a variety of inflammatory indications. Willimann and co-workers investigated these systems for their role in trans-skin permeation of drugs by employing different organic solvents. The solubility of various drugs such as nifedipine, clonidine, scopolamine, and broxaterol was

noted to be increased in lecithin-IPP solution compared with the drug solubility in IPP alone, suggesting the solubility enhancing properties of the organogels. The IPP-based lecithin gel exhibited higher transdermal transport efficiency than that of cyclooctane lecithin gel. This difference in trans-skin delivery rate was due to the penetration enhancing property of the IPP. Bhatnagar and Vyas have investigated the trans-skin permeability of propranolol hydrochloride, a poorly permeable and water-soluble drug incorporated in LOs, across human cadaver skin. The LOs are alsoused for the topical administration of systemic hormones. The transdermal delivery of progesterone, incorporated in LOs, has been studied with an aim to minimize the bioavailability fluctuations associated with its preoral administration. Also, a transdermal formulation of testosterone has been successfully prepared by incorporating the therapeutically effective amounts of micronized testosterone in a PLO gel [34]. Angela attar nasseri and his co workers prepared lecithin organogels for topical application of Ketorolac Tromethamine (KT). They have prepared organogel by using lecithin and isopropyl myristate (IPM). The release profile of ketorolac was studied by using intact guinea pig skin and various artificial membranes. It was observed that as the lecithin concentration increased from 40 to 50 and then 60% w/w in formulations, a significant decrease in KT release was obtained. A remarkable increase in the drug release was also observed in formulations containing 6.5% w/w of KT compared to those containing 1% w/w of the drug. The optimized formulation of the organogel composed of 40% lecithin and 60% IPM (containing 0.6% w/w of water and 6.5% w/w of KT) showed the highest drug release rate. Increasing the water content of the organogels also resulted in an increase in KT release. The results have shown that KT could be incorporated at high concentrations into lecithin organogels and these systems could be considered as desirable drug delivery vehicles for water soluble drugs and are capable of providing an appropriate drug release rate. Sheikh and his co workers developed lecithin based organogels for topical delivery of aceclofenac drug. In this study they have prepared gel from lecithin, glacial acetic acid and water. These aceclofenal LOs have been characterized *invivo* and *invitro* environments. Findings of this study suggest that lecithin based organogels is able to provide desired anti-inflammatory action. *In vitro* skin permeation study demonstrated that ethyl oleate based organogel was effective in providing faster drug release. *In vivo* study confirmed the findings of *in vitro* study. The use of antibiotics in PLO has not been well documented clinically in humans. The Scientists also found that lidocaine, guaifenesin, and amitriptyline may break down PLO and make these gels thin [18, 35]. Most of the preparations that contain PLO should be stored at room temperature. If the active drug is not stable at room temperature, the gel must be refrigerated

and that can present problems because PLO may separate under refrigeration. Those preparations can be remixed by means of shear force to ensure micelle formation.The table 2 provides and 3 provide information about the drugs that are being incorporated by using lecithin organogels.

Table 2: Applications of lecithin organogels

S.NO	COMPONENTS	MODEL DRUG	APPLICATION
1	Lecithin, water and isopropyl palmitate	Scopolomins broxaterol	Treatment of asthma [18, 35]
2	Lecithin, sodium caprylate and sorbitan monooleate	Lidocaine	Treatment of neural disorders [36]
3	Lecithin, water and sorbitan monooleate	Digoxin cyclobenzaprin	Treatment of bruxism [37] For muscle relaxation
4.	Lecithin, water and isopropyl meristate	Methyl Nicotinate	Induction of erythema [38]
5	Lecithin, water, isopropyl meristate Pluronic F 127	Sumatriptan succinate	Treatment of nerve disorders [39]

Table 3: Commercially available pluronic lecithin organogels (Data obtained from Stafford pharmacy and home health care)

S.No	Therapeutic Category	Therapeutic Agents
1	Antiemetics	Dexamethasone, dimenhydrate, scopolamine
2	Muscle relaxants	Cyclobenzaprine, baclofen, buspirone
3	Neuropathy drugs	Clonidine, capsaicin, amitryptiline, gabapentin, phenytoin,
4	NSAIDs (non steroidal anti-inflammatory drugs)	Diclofenac, ibuprofen, ketoprofen, indomethacin,
5	Systemic analgesics	Acetaminophen, hydromorphone, morphine sulfate
6	Systemic hormones	Progesterone, testosterone

2.1.4. Applications of lecithin organogels for ophthalmic drug delivery

There are many types of dosage forms like eye drops, suspensions and ointments are available for treatment of ophthalmic problems. But in case of eye drops there is a disadvantage of washing out of eye drops due to eye tears. Therefore only some part of drug reaches target tissue. In suspensions usage one disadvantage is that rate of drug release depends on the dissolution of drug in the suspension mixture. So lecithin organogels can be used for ophthalmic drug delivery because they release the drug at steady rate and highly viscous so hard to wash out. We can incorporate variety of drugs(i. e lipophilic, hydrophilic an ampiphilic). Three lecithin organogel systems have been developed for ophthalmic drug delivery by using paraffin, isopropyl palmitate and cyclooctane. In this paraffin based lecithin organogels found to be safest compared to other two organogels [40].

2.2. Span and Tween based organogels

The surfactants are ampiphilic in nature by having both the hydrophilic and hydrophobic groups. The surfactants have the ability to transform into microstructures of spherical, rod or inverted micelles. The spherical micelles formed by surfactant has low viscosity while rod shaped micelles has higher viscosity properties. The rheological properties of surfactant depend on microstructure. The surfactants that are widely used for drug delivery are sorbitan mono state (Span 60) and sorbitan mono plamitate (Span 40). Multi component organogels have been developed by using non ionic surfactants as gelators like sorbitan monosterate. Some of the studies were done on these organogels to know the drug delivery efficiency. Murdan and his co workers have prepared surfactant based organogels by using sorbitan monosterate/polysorbate 20. First they have prepared hot (60°C) suspension of noisome (v/w) and this suspension was added to solution of sorbitan monostearate (v/v) by this water in oil emulsion was produced. Upon cooling this emulsion was turned into semi solid gel. It has also been established that the water-to-surfactant molar ratio (w= $[H_2O]$/[Surfactant]) is an important factor in controlling the formation of rod shaped reversed micelles. The formation of these reversed micelles can also enhanced by using co surfactants like polar solute with alcohol or amine. The structural evolution such as entanglement, micellar growth and branching can be predicted on the basis of the rheological behaviour and small-angle scattering techniques using X rays or neutrons which provide direct evidences of micellar growth. SANS data suggest that Na^+, Mg^{2+}, Ca^{2+} all form spherical droplets whilst Ni^{2+}, Co^{2+} and Zn^{2+} induce rod shaped aggregates [41]. The light microscopy results of these organogels

proved that the gel microstructure contains tubular gelator network in which the noisome suspension being entrapped tightly. Hence these scientists thought to entrap vaccines into these organogels. They have entrapped bovine serum albumin (BSA) and haemagglutinin (HA) as antigens (depots) into these gels. The immunogenicity studies of these gels have been conducted in vivo by applying these gels at intra muscular regions. The result shown there was a rise of antibody production against these two antigens. But the short lived nature of depot was observed, this might be due to the interactions of gel components with that of depot [42]. Another surfactant used for preparation of organogel was sodiumbis (2-ethylhexyl) sulfosuccinate (AOT). In this study AOT based gel was prepared by entrapping benzenediols (resorcinol derivatives). The NMR and FTIR studies done on these gels proved that hydrogen bonding between the surfactant molecules lead to the formation of mesh like structure and benzenediol forms binding with surfactant by using two carbonyl groups of surfactant. It was also proved that organogels prepared by using benzenediols were highly stable when compared to the gels prepared using phenol. This was because phenol uses only one carbonyl group of surfactant to from gel where as benzenediol uses both the carbonyl groups of surfactant to form stable gel [43]. The hydrophile-lipophile balance (HLB) for emulsification also plays important role in formation of either oil in water or water in oil emulsions. Generally HLB value of 6 results in formation of water in oil emulsion where as HLB value of 14 results in formation of oil in water emulsion. The applied shear conditions also have effect on formation of semi solid organogels. Under high shear, low internal phase emulsions formed using surfactant mixtures (span 80/tween 80). But at lower shear, high internal phase emulsions resulted. From these results, it appears that the emulsification method (applied shear and oil/water ratio) used can be of greater importance in determing the final emulsion type than the HLB values of the surfactants themselves [44]. The HLB value of the surfactant indicates the solubility of the surfactant. If the surfactant has lower HLB value then surfactant is more lipophilic or oil soluble or if the surfactant has higher HLB value then surfactant is more hydrophilic or water soluble. For the surfactant system several studies recommend that use a blend of at least two surfactants. This is due to mixtures of a low HLB and a high HLB surfactant give better coverage at the interface and a blend of two surfactants is typical.

Table 4: HLB values of Span 80/Tween 80 blends

HLB value	Span 80 (%)	Tween 80 (%)
4	100	---
6	83	17
8	65	35
10	46	54
12	28	72
14	9	91
15	---	100

Table 5: HLB values of some of surfactants

Name of the surfactant	HLB value
Sorbitan trioleate (Span 85)	1.8
Sorbitan tristearate (Span 65)	2.1
Sorbitan sesquioleate (Arlacel 83)	3.7
Glyceryl monostearate,	3.8
Sorbitan monooleate (Span 80)	4.3
Sorbitan monostearate (Span 60)	4.7
Sorbitan monopalmitate (Span 40)	6.7
Sorbitan monolaurate (Span 20)	8.6
Polyoxyethylene sorbitan tristearate (Tween 65)	10.5
Polyoxyethylene sorbitan trioleate (Tween 85)	11.0
Polyethylene glycol 400 monostearate	11.6
Polysorbate 60 (Tween 60)	14.9
Polyoxyethylene monostearate (Myrj 49)	15.0
Polysorbate 80 (Tween 80)	15.0
Polysorbate 40 (Tween 40)	15.6
Polysorbate 20 (Tween 20)	16.7

2.2.2. Applications of Span-tween based organogels in drug delivery

Non-ionic surfactants can be useful alternatives to naturally occurring surfactants, and polyoxyethylene Mono sorbitan *n*-acyl esters (Tweens), for example, have been reported to have minimal toxicity. Although there are some exceptions, the use of poly-oxyethylene sorbitan monooleate (Tween 80) appears acceptable for only oral or parenteral applications[9]. One of research done by murdan and his co workers revealed that the molecules present in non ionic surfactant (sorbitan mono stearate) based organogels has short half life period. In vitro results of this study gave the reason behind this unusual phenomenon. Actually when a surfactant based organogel is applied intra muscularly the outer surface of gel comes into contact with interstitial fluid present at that site. This fluid slowly causes breakdown sorbitan mono stearate tubules and then degradation of gel into smaller fragments. The surfactant tubules act as conduit for water penetration into gel. This leads to gradual erosion of gel into oil droplets from the gel mass. Actually fluid penetration and emulsification at gel surface are responsible for gel breakdown at injection site [45].

Table 6: Surfactant based organogels for cutaneous drug delivery (in vitro)

S.No	Organogel components	Model Drug	Used membrane
1	Tween 80, Span 80, IPM and water	8-Methoxsalen	Pig skin [46]
2	Tween 80, Span 20, IPM and water	Diphenhydramine hydrochloride	Human skin [6]
3	Tween 80, Span 80, IPM and water	Methotrexate	Pig skin [47]
4	Tween 21/81/85,Bis 2-silphosuccinate, IPM and water	Sodium salicylate	Pig skin [48]

Table 7: Surfactant based organogels for cutaneous drug delivery (in vivo)

S.No	Organogel components	Model Drug	Used animal
1	Tween 85, Targat, mono oleate, IPP and Glycerol	Bupranolol	Rabbit [49]
	Tween 85, Prolaxamer, IPP and Glycerol	Bupranolol	Rabbit [49]
2	Tween 85, IPP and Glycerol	Carazolol	Rabbit [49]
3	Tween 85, IPP and water	Hydro chloride trimolol	Rabbit [6]

The surfactants behave as permeation enhancers. The effects that are caused due to these organogels are investigated on shaved mouse as well as on human skin. In both cases, no significant increase of blood flow and in epidermal irritation was observed. Over all the organogels are regarded as safe [50].

CHAPTER-03

MATERIALS AND METHODS

3. Materials and Methods

3.1 Materials

Span 80 (sorbitan monooleate) was procured from Loba chemie, Mumbai, India. Tween 80 (polyoxyethylene sorbitan monooleate), rhodamine B and salicylic acid (SA, model drug) were procured from Himedia, Mumbai, India. Edible sunflower oil (SO) was purchased from the local market. Dialysis membrane (MW cut-off - 60 kDa) was purchased from Himedia, Mumbai, India. Double distilled water was used throughout the study.

3.2 Methods

A. Preparation of organogels

Span 80 and tween 80 were mixed thoroughly in the proportion of 1:2 ratio (w/w) to obtain the surfactant mixture (SM), which was used as gelator. Specified amount of the SM was added to the SO, kept on stirring on a magnetic stirrer. The above mixture (gelator solution, GS) was stirred for 20 min. Subsequently, water was added drop-by-drop to the GS using a burette until there was a formation of organogel or the total fraction of water has reached 80% of the volume of the GS-water mixture. Depending on the composition of the GS-water mixture, the system either formed gelled structures or remained as liquid mixtures. A ternary plot depicting the proportions of SM, water and SO was prepared to figure out the compositions, which formed organogels.

Samples for morphological studies were prepared in a similar manner using 0.01 % (w/v) aqueous rhodamine B solution as the polar phase. SA-loaded samples were prepared by dispersing SA into the SO, which was subsequently used for the development of organogels. The final concentration of SA in organogels was maintained at 1 % (w/w).

B. Characterization of organogels was carried out by using following methods

Microscopic study

To understand the mechanism of organogels formation, water was slowly added to the GS (95.83 %, w/w) until the formation of the organogel. The samples were observed under compound microscope (H600 AFL50, Hund, Germany) as the proportion of water was varied. The microstructures were analyzed using ImageJ software.

Gel-sol transition study

Gel-sol transition temperature (Tgs) was found out by incubating the organogels in a water-bath, whose temperature was varied from 30-70 ºC. The temperature, at which the gels started to flow, when the glass vials were inverted, was noted as the gel-sol transition [51].

Stability analysis of the organogels

The stability of the pharmaceutical products may be carried out either by thermocycling process or by incubating the samples at a particular environment for a longer period of time [52].

Alternate heating and cooling of emulsions for a particular period can help estimating stability of the emulsions [53]. Since the developed organogels contain dispersed aqueous phase in an oil continuous phase, they may be considered as water-in-oil emulsion gels. The organogels were alternatively incubated for 15 min at temperatures of 70^{o}C and -20^{o}C. The samples were regarded as stable samples, if the organogels withstood at least 5 cycles of thermocycling.

ICH guidelines of stability of pharmaceutical products indicates the storage of the products at 30°C ± 2ºC for 12 months (long-term stability) and 6 months (intermediate stability) [3]. The organogel samples were incubated at 30°C ± 2°C RH for 9 months to figure out any signs of destabilization.

X-ray diffraction study

Three stable samples and their corresponding SA-loaded samples were analyzed by XRD (XRD-PW 1700, Philips, Rockville, USA) using Cu-Kα radiation generated at 30kV and 20 mA. The samples were analyzed in the range of diffraction angle of 10º to 50º-2θ at a rate of 2° 2θ per min.

FTIR analysis

A representative blank organogel and its corresponding SA-loaded organogel were subjected to FTIR spectroscopy in the range of 450-4000 cm^{-1}. An ATR-FTIR spectroscope (AlphaE ATR-FTIR, Bruker, USA) was used for the study.

Thermal analysis

Thermal analysis of organogels was done by simultaneous thermogravimetric analysis (TGA) and differential thermal analysis (DTA) using H-Res/modulated TGA-DTA 2950 (TA instruments, USA) in the temperature range of 25^{o}C to 300^{o}C at a heating rate of 6^{o}C/min.

pH measurement

The pH of the organogels was measured using a digital pH meter (Model 132E, EI products, India). The pH was measured to figure out whether the pH of the organogels lie within the limits of skin pH [54].

Impedance measurement

DC impedance of the organogels were measured using an in-house developed DC impedance measuring unit. The impedance was measured both during the thermocycling stability test and on a time scale basis for the samples incubated at 30°C ± 2°C.

***Invitro* drug release studies**

A two-compartment cell was used for the drug release study. The compartments were separated by a dialysis membrane (MW cut-off - 60 kDa, Himedia, Mumbai). The donor compartment contained 5 g of SA loaded organogels while the receptor compartment contained 50 ml of water. The donor compartment was lowered to ensure that the dialysis membrane was in contact with 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 8 h. A portion of the replaced water was kept for further analysis under UV-vis spectrophotometer (Shimadzu UV 1601 r, Equipment for technology and science, Sanjose) at a wavelength of 294 nm. All the experiments were carried out in duplicates.

Antimicrobial test

Gram positive bacterium *Bacillus subtilis* **(**MTCC 121) was used for antimicrobial study. Nutrient agar solid (peptone-0.5%, beef extract-0.3%, agar-1.5% and Nacl-0.5% in 100ml of distilled water) was used as a culture media 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 drug loaded organogel. The petri-dishes were incubated at 37ºC 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.

Hemocompatibility test

The organogel was enclosed in dialysis tubing and kept in 50 ml normal saline at 37 ^{o}C for 30 min so as to allow the leaching of components from the organogels, if any. 0.5 ml of this solution is then diluted with 0.5 ml of diluted goat blood (prepared by diluting 8 ml of goat blood with 10 ml of normal saline) followed by the addition of 9 ml of normal saline and incubation at 37 ^{o}C for 1 h. Subsequently, the above suspension was centrifuged at 3000 rpm for 10 min. Similarly, positive and negative controls were also prepared. The normal saline containing the leached components in the test sample are replaced with 0.1 N hydrochloric acid and normal saline for positive and negative controls, respectively. The supernatant was siphoned off and analyzed at 545 nm using a UV-visible spectrophotometer. The % haemolysis was calculated from the following equation:

$$\% \, Hemolysis = \frac{OD_{Test} - OD_{Negative}}{OD_{Positive} - OD_{Negative}} \times 100$$

As per accepted norm, if the % hemolysis ≤ 5 and ≤ 10 for a test material then the sample is regarded as highly hemocompatible and hemocompatible, respectively.

CHAPTER-04

RESULTS AND DISCUSSIONS

4. Results and Discussion

4.1. Preparation of organogels

The organogels were prepared by dissolving the SM in SO (GS) followed by the addition of water. With the initial addition of water, the mixture turns into white turbid solution. As further amount of water was added, the samples either formed a gelled structure or remained as turbid solution. The samples were regarded as organogels, if the GS-water mixture did not flow when the culture bottles were inverted (Figure 2). The samples which formed gelled structures, released heat indicating an exothermic reaction as the gels were developed. A change of 5.3^{o}C was observed during the process. This indicates that the samples attain a low energy state as they undergo transition into gelled structures and attain a thermodynamically stable state. Depending on the composition, colour of the gels varied from white to pale yellow. All the gels were opaque in nature. They were having slight odour and oily to touch.

Figure 2: Organogel samples of different composition

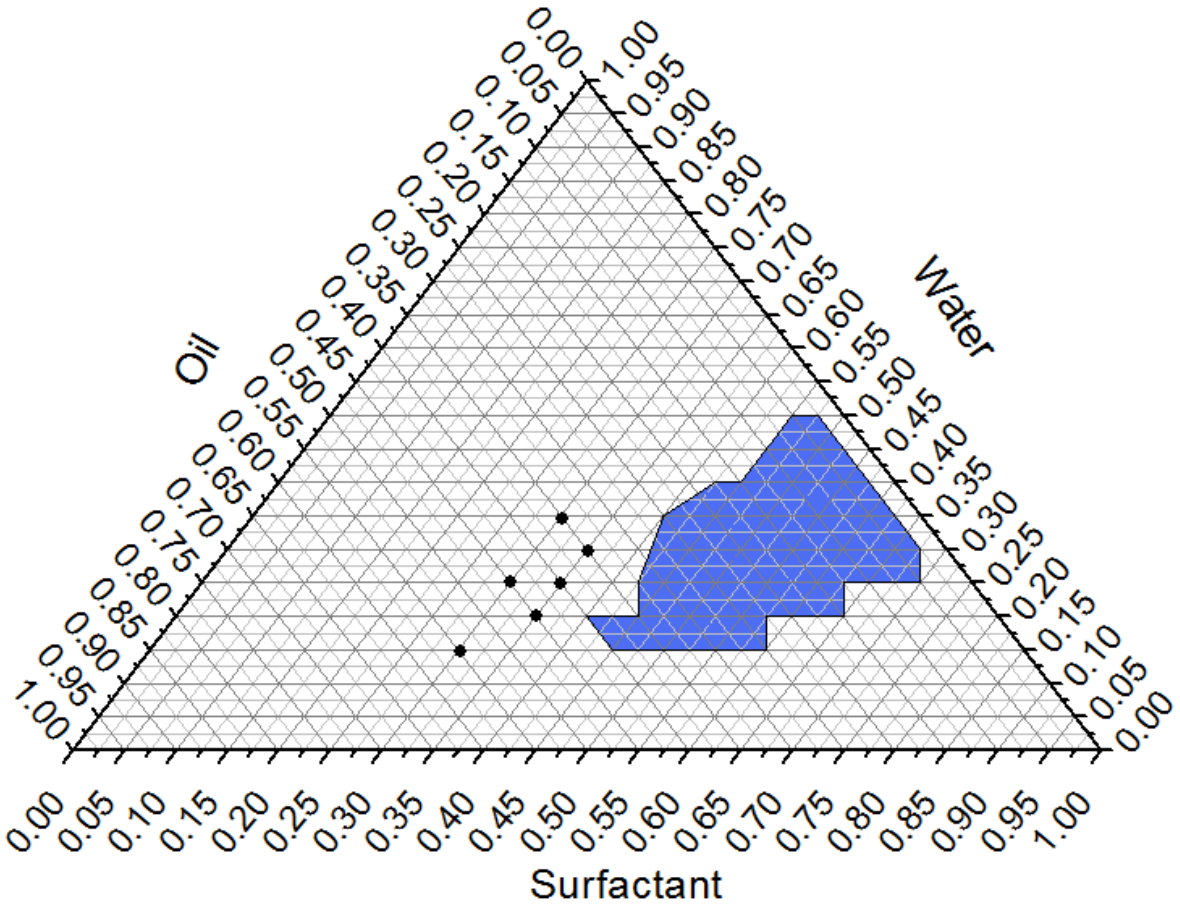


Figure 3: Ternary phase diagram of organogels

By varying the SM, oil and water composition, 780 sample points (approx.) were taken to figure out the compositions which lead to the formation of gels. Based on the experimentation, the compositions of gels were plotted in a triplot (figure 3). The gel fraction was found to be 16.6% (approx.). 15 compositions, throughout the gelled area, were selected randomly from the gelled compositions for in-depth analysis. Table 1 enlists the composition of the selected gels for further analysis.

Table 8: Composition of the gels used for further analysis

Sample	Surfactant (Fraction)	Water (Fraction)	Oil (Fraction)
A	0.30	0.25	0.45
B	0.35	0.15	0.50
C	0.35	0.20	0.45
D	0.35	0.25	0.40
E	0.40	0.25	0.35
F	0.425	0.40	0.175
G	0.525	0.15	0.325
H	0.625	0.20	0.175
I	0.575	0.40	0.025
J	0.50	0.35	0.15
K	0.525	0.4	0.075
L	0.550	0.35	0.1
M	0.525	0.30	0.175
N	0.70	0.25	0.05
O	0.525	0.35	0.125

4.2. Microscopic study

The microstructure of the GS was studied under microscope as different proportions of water were added to the GS (figure 4). The micrographs suggested that GS showed some irregular structures. As water was added to the GS, there was formation of globular structures. There was an increase in the number of the globular structures as the proportion of water was increased in GS. The morphological analysis of the globular structure indicated that there was a decrease in the size of the globular as the proportion of water was increased in the GS (figure 5). From the microscopic results it can be predicted that on addition of water in GS, there is a formation of globular reverse micellar structures having internal aqueous phase. These reverse micellar structures physically interacts with each other to form a three dimensional networked structure thereby resulting in the immobilization of the organic phase.

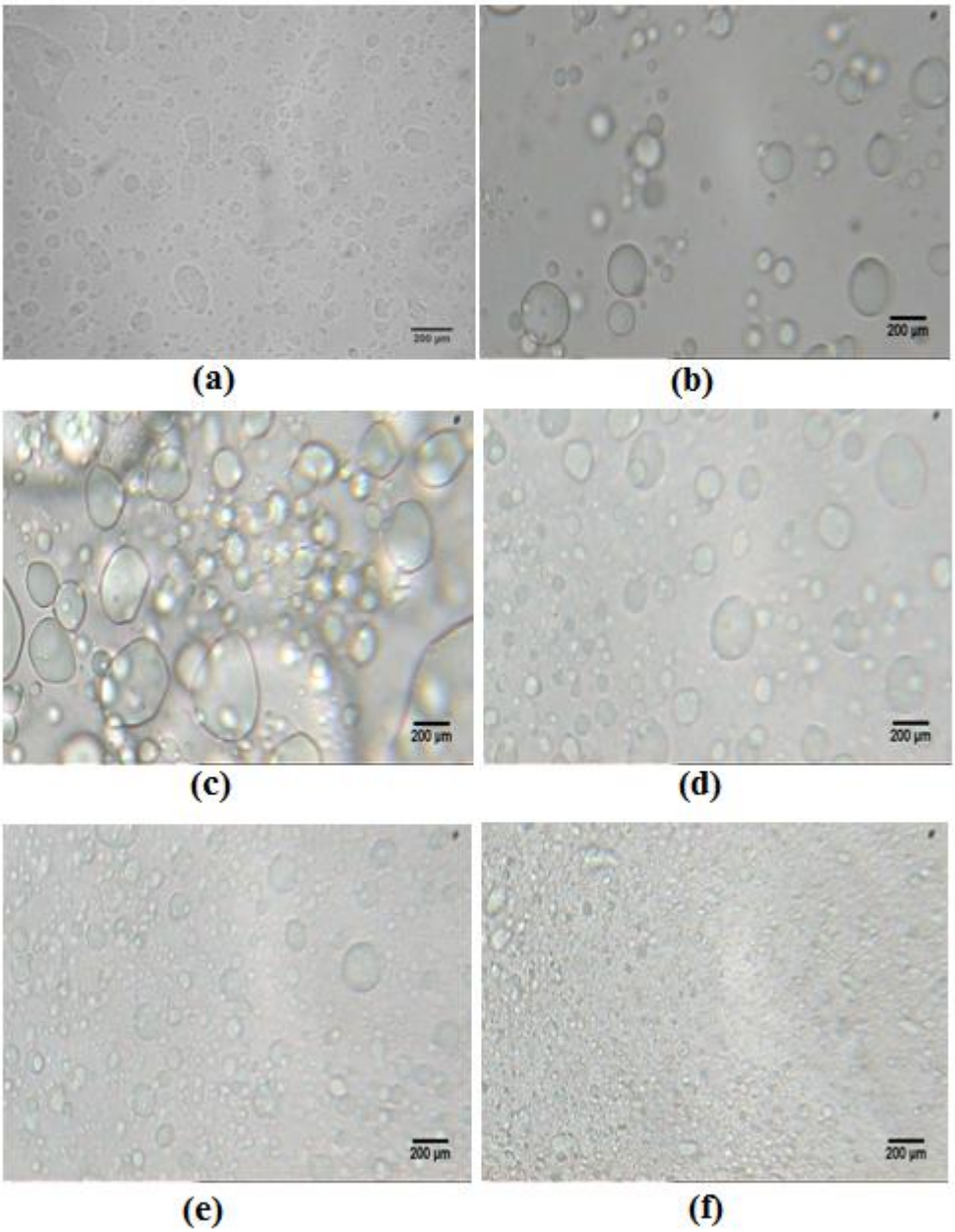
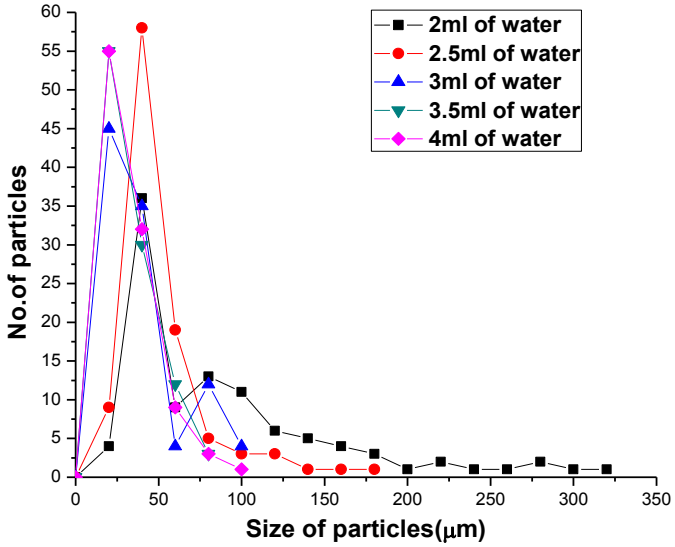
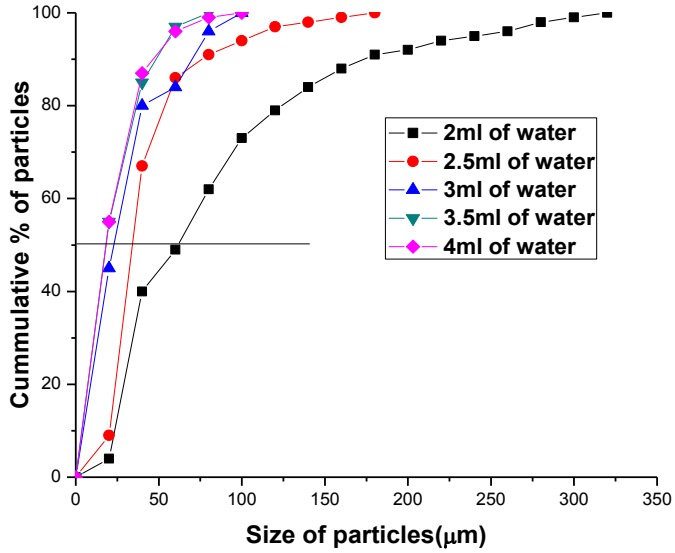


Figure 4: Microscopic study of GS with various proportions of water. a) 0.0 ml, (b) 2.0 ml, (c) 2.5ml, (d) 3.0 ml, (e) 3.5 ml, and (f) 4ml of water.



(a)



(b)

Figure 5: Frequency distribution of globular particles as the water proportions were varied. (a) Number of particles (b) Cumulative % of particles

4.3. Gel-sol transition analysis

The organogels were subjected to increasing temperature starting from 25ºC. An increment of 5ºC was made after 5 min of incubation at the previous temperature. The samples were considered to have undergone gel-sol transition, when they started to flow (figure 5) [53]. The gel-to-sol transition temperature of the organogels varied from 40^{o}C to 70^{o}C, depending on the composition of the organogels (Table 9). As the temperature increased, there was a corresponding increase in the surface free energy with the subsequent increase in mobility of the self-assembled structures formed by the gelators. With further increase in temperature, the interactions amongst the self-assembled structures gets reduced which leads to the disruption of networked structure, thereby causing the gelled system to flow freely [41].

In general, the gel-sol transition was found to be >60^{o}C for the samples having SM: water ratio in the range of 1.3 to 1.6 (w/w), with an exception of sample L. Any deviation from this leads to the decrease in the Tgs, indicating that the proportions of SM and water plays an important role in the formation of a thermodynamically stable gel.

Table 9: Results of gel-sol transition study

S. No.	Sample	Tgs (°C)
1	A	35
2	B, G, N	40
3	M	45
4	C, F, H, L	50
5	J, K	60
6	D, E	65
7	I, O	70

(a) **(b)**

Figure 6: Gel-sol analysis. (a) Sample E at 30º C and (b) Sample E at 65 º C.

4.4. Accelerated thermal stability study of organogels

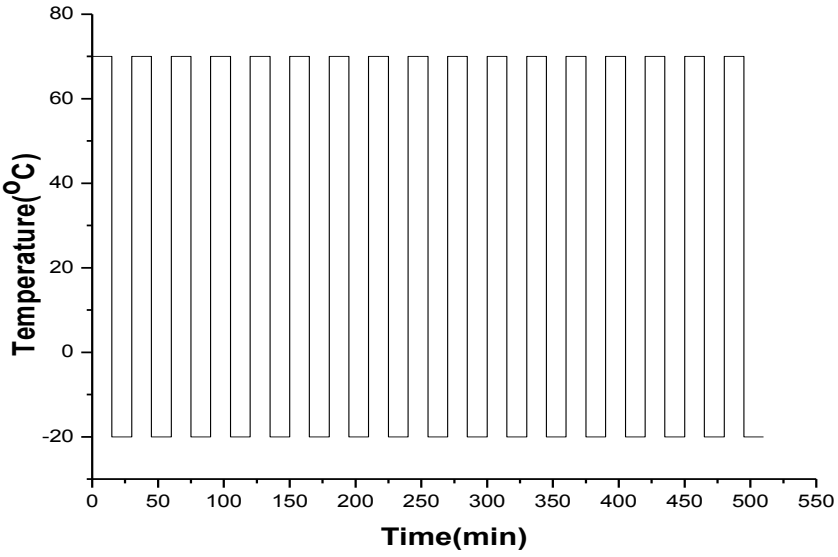


Figure 7: Temperature profile used for accelerated study

The accelerated stability testing of organogels were carried out as per the temperature profile given in the figure 7. The method employs continuous exposure of the samples to a freeze-thaw cycle at short intervals of time. The freezing temperature should be ≤ -5ºC whereas the thawing temperature is dependent on the type of formulation [55]. This method only provides a prediction and does not give us an absolute value. This has been attributed to the process of destabilization only during freeze-thaw cycles and not under storage conditions [55]. The

rupture of the surfactant layer in the presence of ice crystals at lower temperatures and or change in the physic-chemical property of the surfactant layer at higher temperatures leads to instability of organogels. In general, it is considered that the samples should withstand at least 5 cycles of freeze-thawing process. Thermocycling studies were done for all the 15 samples. From this study it was observed that samples E, I and L were stable even after 5 cycles of thermocycling indicating that these samples may be stable for a prolonged period of time (Figure 8). The results of the experiment have been tabulated in table 10.

Table 10: Results of accelerated thermal study

S.No	Sample	Stability
1	G, J, K, N, C	Destabilized within 1 cycle
2	B, F, H, O	Stable up to 2 cycles
3	A, D, M	Stable up to 4 cycles
4	E, I, L	Stable for >5 cycles

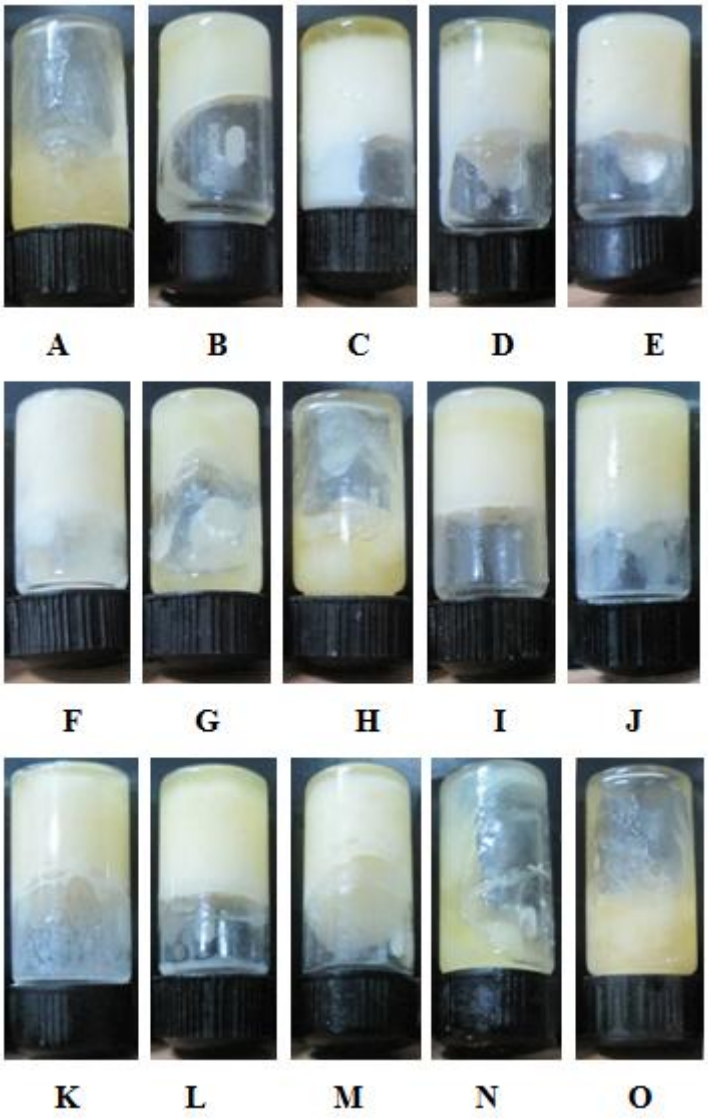


Figure 8: Samples showing the results of thermocycling

In a separate study, samples were kept at 30°C ± 2°C. Observations of the experiment have been tabulated in table 11. The stability of these organogels was manually checked at different intervals of time. The study indicates that depending on the composition of the organogels, the products may be stored at 30°C ± 2°C/65% RH ± 5% RH for the period varying from 6 months to 12 months. As per the ICH guidelines, the products withstanding the 30°C ± 2°C/65% RH ± 5% RH environment for 6 months are regarded as intermediate stable products while if they are stable for more than 12 months, they are regarded as long-term stable products [56]. The samples E, F and M may be regarded as intermediate stable products while the samples D, I, J, L and O were stable after 9 months of study and may be regarded as long-term stable products if they are found to be stable for 12 months. Compositional comparison of these intermediate and long term stable gels concludes that they are also having SM and water ratio in the range of 1.3 to 1.6 with the exception of F and M.

Table 11: Results of stability studies on time scale

S.No	Sample	Stability
1	G,H,N	Stable for 1 week
2	A,B,C	Stable for 3 weeks
3	K	Stable for 5 months
4	E,F,M	Stable for 7 moths
5	D,I,J,L,O	Stable for 9 months*

* stable after 9 months also

Based on the results of stability studies (Tgs, accelerated thermal stability and stability based on time scale), it was cleared that the stability of these organogels is highly dependent on their composition. It was confirmed that the organogels having SM and water ratio in the range of 1.3 to 1.6 were highly stable when compared to others. The samples E, I and L were selected for the further studies as they found to be stable after the for both stability tests.

4.5. X-ray diffraction analysis of organogels

The selected organogels (E, I and L) and their corresponding gels with SA (ED, ID and LD) were analyzed by XRD to understand the effect of drug on the crystallinity of the organogels (figure 9). The full widths at half maximum (FWHM) and area under the curve (AUC) values for all the samples have been tabulated in Table 12.

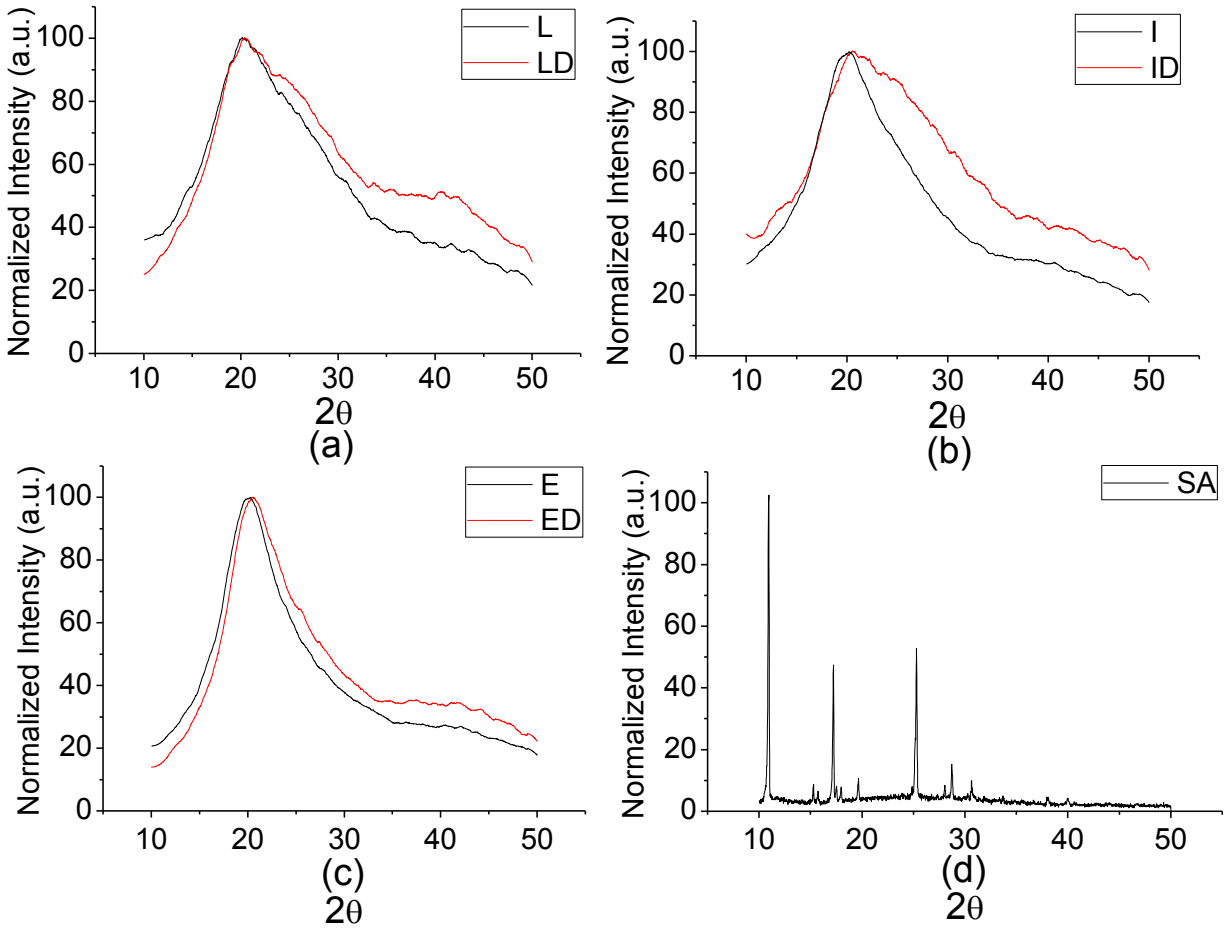


Figure 9: XRD analysis of the samples. (a) L, LD organogels (b) I, ID organogels (c) E, ED organogels and (d) Salicylic acid

Table 12: Values of FWHM and AUC for XRD study

S.No	Sample	FWHM	AUC
1	E	10.36	1652.11
2	ED	11.34	1772.10
3	I	13.74	1871.06
4	ID	20.64	2377.53
5	L	17.62	2108.91
6	LD	21.13	2339.21

Figure 9 shows the X-ray diffractograms of the organogel samples and SA. 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 very low crystallinity. The amorphous nature of the samples was in the order of E < I < L as evident from the FWHM values of 10.36, 13.74 and 17.62 for E, I and L samples, respectively. The amorphous nature has been related to the fluid matrix systems with no solid-fibrillar networks and absence of regular geometric structures. The crystalline fraction may be attributed to the presence of the bilayered inverse micellar tubular network formed by the surfactants upon addition of water [57]. 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 [58]. SA increased the amorphous nature, evident from the increased FWHM and AUC (table 12), of the gels thereby facilitating the easy diffusion of SA from the gel matrices [59].

4.7. FT-IR analysis

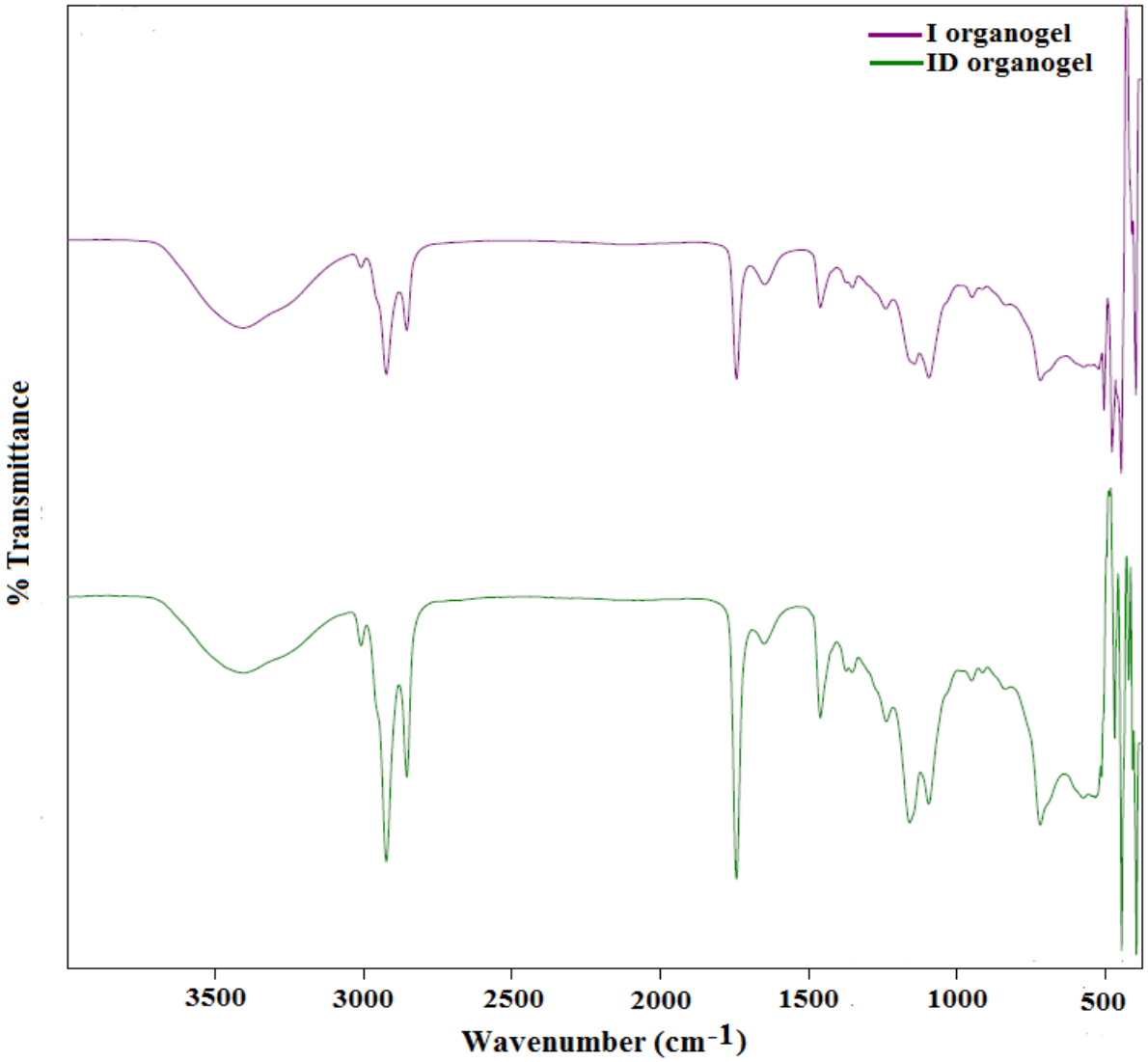
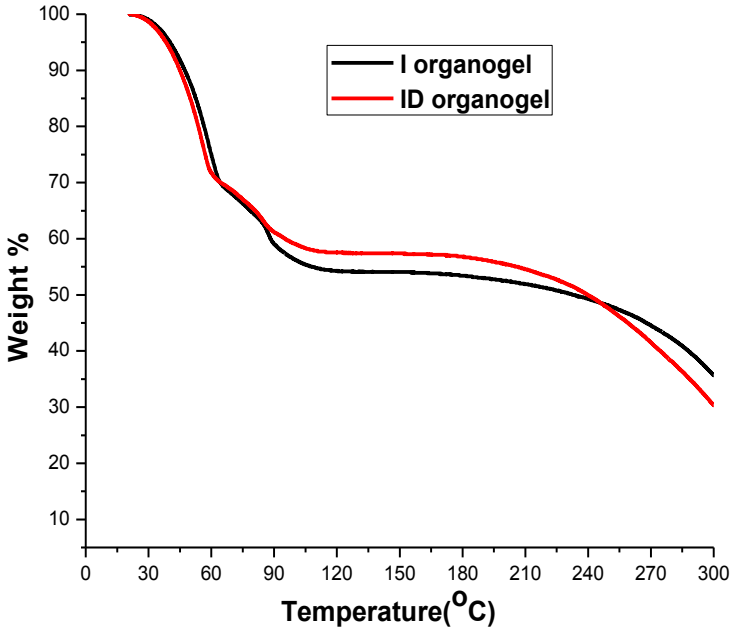


Figure 10: Graph showing the result of FT-IR analysis

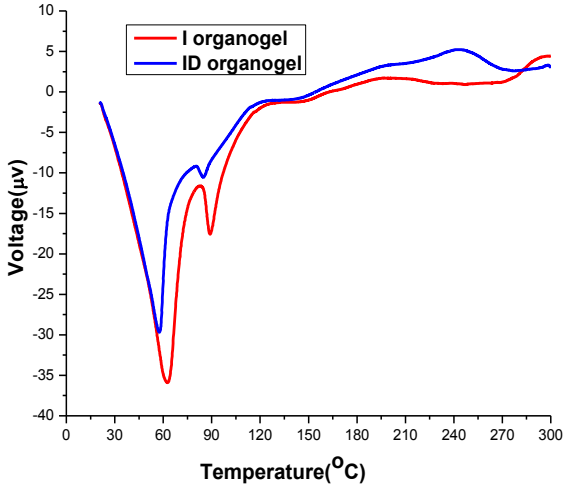
Fourier transform infrared spectroscopy indicates the presence of molecular interactions amongst the components present in a sample. Sample I was used as the representative blank organogel and sample ID was used as the drug loaded sample. The spectra of the samples I and ID organogels was found to be similar (figure 10). A shallow broad peak was observed in the range of 3,700 cm^{-1} to 3,100 cm^{-1} wave numbers in both the cases suggesting the presence of stretched hydrogen bonded O-H groups in the samples. This indicates that presence of intermolecular hydrogen bonds amongst the water and the surfactants resulting in the formation of inverse tubular structures, which upon extension and physical crosslinking yields fluid matrix gel [19]. The spectra of sample ID indicated additional peak at 572 cm^{-1} in

addition to the peaks at 468 cm^{-1} and 512 cm^{-1} which were also present in sample I organogel. This indicates the presence of the aromatic ring of the SA present in the organogel.

4.8. Thermal analysis



(a)



(b)

Figure 11: TGA-DTA thermograms of I and ID organogels (a) TGA (b) DTA

TGA thermograms of samples I and ID organogel have been shown in figure 11a. Organogels started losing their weight as the temperature was increased from room temperature to 100°C, indicates their gelled structure is being disturbed. About 40 to 45% weight loss occurred up to 100°C, which might be attributed to the evaporation of water. The percentage weight loss of gel is in accordance with the initial weight percentage of water in sample i.e. 40% (w/w) (see Table 1). Up to 62°C, steep decrease in weight loss was observed and with a subsequent gradual decrease in weight till 100 °C. Constant weight was maintained up to 240°C and then weight loss continued till 300°C [60]. DTA thermograms of samples I and ID organogels have been shown in figure 11b. The melting temperatures for I and ID organogels were observed at 62 ºC and 58 ºC, respectively. This supports the XRD observation that the addition of drug into the organogels resulted in the decreased physical interactions amongst the reverse-micellar tubules. The lesser the physical interactions, lesser will be the melting temperature of the gels. The complete evaporation temperatures of water were observed at 100ºC and 92ºC.

4.9. pH measurement

The pH of organogels was measured at 30ºC ± 2ºC by using electrode based digital pH meter. The pH values for all samples given in Table 13.

Table 13: pH values of organogels

Sample	pH±SD
A	6.69±0.012
B	7.13±0.016
C	7.24±0.011
D	7.10±0.013
E	7.01±0.016
F	6.80±0.017
G	7.27±0.010
H	7.25±0.011
I	7.15±0.009
J	7.15±0.090
K	7.23±0.010
L	7.20±0.011
M	7.37±0.080
N	7.31±0.090
O	7.20±0.010

The pH values of organogels varied in the range of 6.69 to 7.37. The pH of the organogels were in accordance to the USP guidelines for topical and transdermal formulations. According to USP the pH of gels or ointments whichever to be used for topical or transdermal applications should lie within the limits of normal skin pH of 4.5-7.4. If not so, immunological responses like redness, burning and itching of the skin in the applied area will results [54].

4.10. Impedance measurement

Table 14: Impedance values of organogels

SAMPLE No.	IMPEDANCE (IN OHMS) ±SD
A	0.105±0.021
B	0.227±0.023
C	0.128±0.015
D	0.091±0.009
E	0.092±0.013
F	0.062±0.012
G	0.230±0.017
H	0.162±0.011
I	0.075±0.008
J	0.075±0.006
K	0.063±0.005
L	0.076±0.013
M	0.08±0.007
N	0.128±0.015
O	0.078±0.008

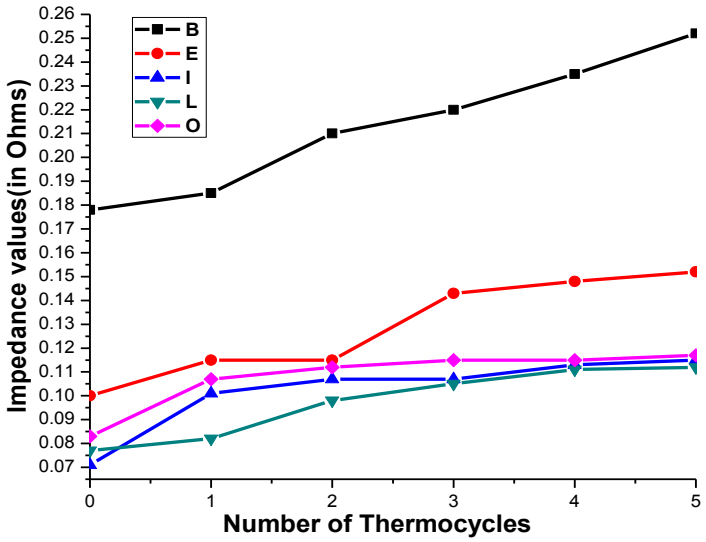


Figure 12: Graph showing plot between no. of thermo cycles Vs impedance values

The impedance of the organogels has been tabulated in table 14. The results indicate that there was a decrease in the impedance of the organogels as the proportions of water was decreased in the organogels.

The impedance analysis after every cycle of thermocycling indicated an increase in the impedance of the organogels. There was less change in impedance in case of stable organogels but there was a greater variation in the impedance for the destabilized organogels (figure 12). This may be attributed to the exclusion of oil from gel structure which results in the increment of the impedance of the organogel samples.

In a separate study, the impedance of the organogel samples B, E, I, L and O were measured on a daily basis for 21 days. The results indicated that the impedance of the samples E, I, L and O were relatively constant while the sample B organogel showed a wide variation in the impedance over a period of 21 days (figure 13). The variation in the impedance of the sample B organogel may be related destabilization. The increase in the impedance up to day 7 may be attributed to the leaching of the oil from the gelled structures whereas the subsequent decrease may be attributed to the destabilization of the reverse-micellar structures with the subsequent release of the aqueous phase. Since samples E, I, L and O were relatively stable for a longer duration, no drastic change in the impedance was observed [7].

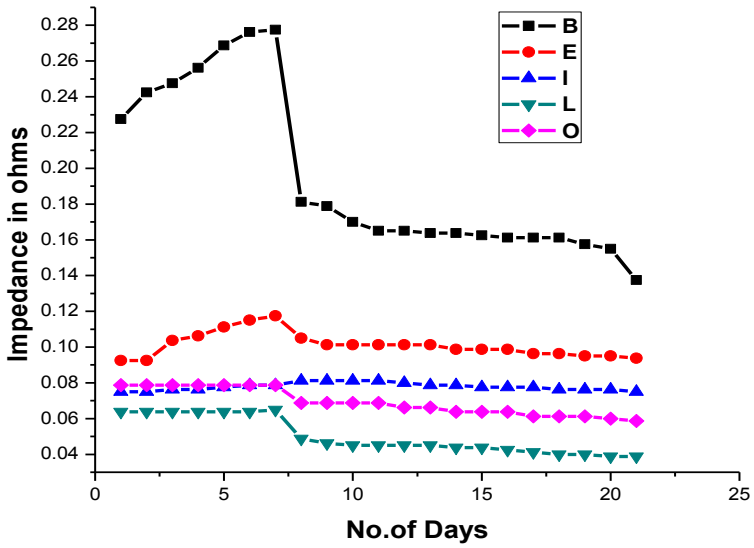


Figure 13: Graph showing plot between No. of days Vs Impedance values

4.11. Antimicrobial test

The sample ID organogel was used for antimicrobial test using *B. subtilis* as the test organism. The pure drug was taken as positive control and organogel without drug was taken as negative control. Table 15 shows the results of the test. It was found that SA was able to inhibit the proliferation of the microorganism within a given area and did not allow the growth of the microorganism even after 24 h. On the other hand, sample I did not show any zone of inhibition. This indicates that the organogels may be tried as matrices for controlled delivery system, where it may deliver the bioactive agent for a prolonged period of time.

Table 15: Results of antimicrobial test

Bioactive agent	**Zone of inhibition(Diameter, cm)**		
	B. subtilis	**Positive control**	**Negative control**
Salicylic acid(1%)	1.5±0.2	2±0.3	Nil

4.12. In vitro drug release study

The release profiles of the drug from the organogels have been shown in figure 13. The rate of release of SA was higher in sample ID organogel followed by samples LD and ED organogels (figure 14). The low CPDR value indicates the controlled release behaviour of the formulation and is reckoned to be the feature of amphiphilogels. In PC/Span 60/SO gels only 18% piroxicam drug was released even after 40h of in vitro drug release study [61]. The results indicate that as the amorphous nature of the organogels increased, there was a subsequent increase in the rate of release of the drug. As a matter of fact, the increase in crystallinity increases the crystallite domains, which results in the decrease in the diffusion of the drugs from the organogels and may explain the release profiles obtained for samples ED, ID and LD [62].

Table 16 shows the rate constants determined for different kinetic models of drug release. The best-fit model for release kinetics indicated that the release of the drug from the organogels followed Weibull Model kinetics (figure 15). This suggests that the organogels may be used as controlled delivery systems [63]. 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 [64].

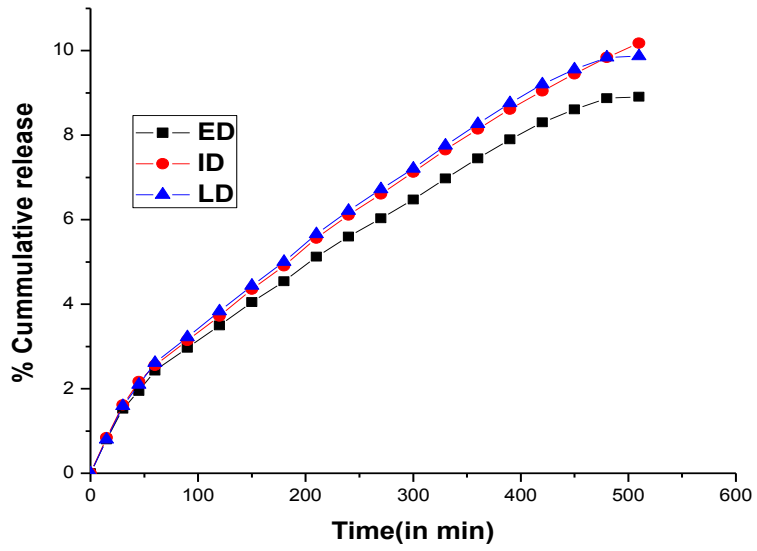
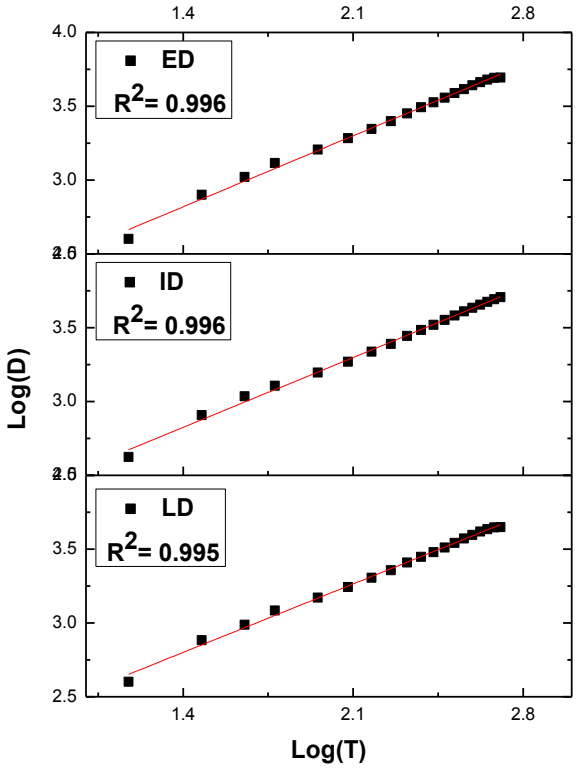


Figure 14: CPDR values for different compositions of the organogel samples as a function of time

Table 16: Kinetics of drug release

	Zero order		First order		Higuchi		Weibull	Korsmeyer-Peppas		Best-fit model
	r^2	k	r^2	k	r^2	k	r^2	r^2	n	
ED	0.979	8.726	0.830	0.001	0.971	0.379	0.996	0.996	0.662	Weibull; Fickian and Non fickian
ID	0.981	9.440	0.837	0.001	0.965	0.416	0.996	0.996	0.676	Weibull; Fickian and Non fickian
LD	0.980	9.335	0.820	0.001	0.968	0.420	0.995	0.995	0.687	Weibull; Fickian and Non fickian



Where, D is the drug dissolved in receptor medium and T is the time

Figure 15: **Weibull-model kinetics for the different organogels samples ED, ID and LD**

4.13. Hemocompatability test

The hemocompatability test was performed for samples E, I and L. The results of study have been tabulated in table 17. The results indicate that the organogels are highly hemocompatible in nature and indicating their probable biocompatibility [63].

Table 17: Results of hemocompatability test

Sample	% Hemolysis
E	4.74
I	1.58
L	3.48

CHAPTER-O5

CONCLUSION

5. Conclusion

The current study deals with the development of span 80-tween 80 based organogels. The organogels were formed by fluid filled globular structures which aggregated to form a matrix system. The stability of the organogels was found to be dependent on the SM and water proportions. The in vitro release studies indicated that the organogels may be used as matrix for controlled delivery systems. The amorphousity of the organogels played an important role in governing the release rate of the drug from the matrices. The release kinetics study indicated Weibull release kinetics and a combination of Fickian and non-Fickian release behaviour. The higher the amorphousity, higher was the drug release from the gels. The preliminary study suggests that the organogels were preliminary found to be hemocompatible. The results indicate that the developed matrices may be used as a vehicle for controlled delivery system.

CHAPTER-06

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