World Journal of Science and Research. 4(2): 01-06 (2019)

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research

Research Article Chemistry

FORMULATION AND CHARACTERIZATION OF LECITHIN AND SUNFLOWER OIL AND ANTI-MICROBIAL ACTIVITY

B. Priyanga and R. Mamani*

PG Department of Chemistry Bon Secours College for Women, Nationally Accredited with 'A' grade by NAAC Affiliated to Bharathidasan University, Tiruchirappalli, Thanjavur - 613006. Tamil Nadu, India

ABSTRACT

The current study deals with the development of Lecithin and Sunflower oil based organogels. Lecithin and Sunflower oil based organogels were prepared by fluid-filled structure mechanism by varying the composition of water. The microstructures of the organogels were studied by light microscopy. The organogels were subjected to the accelerated temperature study. The stable organogels were characterized by pH. The antimicrobial efficiency of the Lecithin organogel product was tested against *Escherichia colia*, *Staphylococcus aureus* and *Candida albicans*. The results of the present study demonstrated that 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 water proportions. The fluorescence behavior also differs in different composition of water. The UV Visible spectrum studies indicated that the organogels may be used as matrix for controlled delivery systems. The viscosity of the gel directly proportional to the water added. The preliminary study suggests that the organogels were found to be antimicrobial activity.

***Citation*:** B. Priyanga and R. Mamani. (2019). Formulation and characterization of lecithin and sunflower oil and anti-microbial activity. *World Journal of Science and Research*. 4(2): 01-06.

Article Info:

Received on 18th March 2019
Accepted on 19th April 2019
Online April 2019

Keywords:
Organogels, Lecithin Sunflower oil, Fluorescence behavior, viscosity, antimicrobial activity ,

Corresponding author
R. Mamani,
Assistant Professor PG and Research Department of Chemistry, Bon Secours College for Women Thanjavur - 613006,

INTRODUCTION

Traditionally, organogel systems are applied topically when the active agent is oil-soluble or penetration into the deeper skin layer is required. Surfactants act as penetration enhancers that alter the membrane bilayer structure and thus reduce the diffusion barrier and enable the drug to penetrate deeply into skin. Since the discovery of simple gelator molecules, organogels have attracted increasing attention. These novel formulations can be used in small quantities without further additives, resulting in more biocompatible products. A wide variety of organogels have been developed by researchers and classified based on the nature of the organogelators, such as LO, gelatin-stabilized organogels, limonene GP1/PG organogel, non-ionic surfactant based organogels and polyethylene organogels (Sahoo *et al.*, 2011). Among these

organogels, LOs are a unique micellar system composed of a non-polar oil phase, an aqueous polar phase, and a surfactant phase, which is derived from soy or egg lecithin. Self-assembly of lecithin molecules in non-aqueous media into reversed giant cylindrical micelles occurred when small amounts of water, glycerol, or formamide are added. LO was first introduced by Scartazzini and Luici in 1988. They observed that the addition of small amount of water into non-aqueous solutions of naturally occurring lecithin caused a sudden rise in the viscosity. The chemical name of lecithin is 1,2-diacyl-sn-glycero-3- phosphatidylcholine (Shchipunov *et al*.,2001).

Organogels preparations for external application to skin have gained much demand, since it is easily absorbed through the skin layers. In general, organogels are thermodynamically stable in nature and have been explored as matrices for the delivery of bioactive agents. In the last decade, interest in physical organogels has grown rapidly with the discovery and synthesis of a very large number of diverse molecules, which can gel organic solvents at low concentrations.In the present study, Lecithin - Sunflower oil mixture based organogels were prepared. The prepared organogels were characterized and evaluate their biological application. The following are important objectives are

MATERIALS AND METHODS

Materials

Lecithin was procured from Loba chemie, Mumbai, India. Sunflower oil Dialysis membrane was purchased from Himedia, Mumbai, India. Double distilled water was used throughout the study.

Preparation of Lecithin and Sunflower oil organogels

Lecithin and Sunflower oil 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 (0.5ml, 1.5ml, 3ml, 6ml, and 9ml) 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.

Characterization of organogels was carried out by using following methods

Thermal analysis of organogels was done by simultaneous thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) in the temperature range of 25ºC to 100ºC at a heating rate of 6ºC/min (Jeong *et al*., 2002). The pH of the organogels was measured using a digital pH meter. Microscopic study: To understand the mechanism of organogels formation, water was slowly added to the GS until the formation of the Lecithin and Sunflower oil and Tween organogel. The samples were observed under high power microscope as the proportion of water was varied. 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 20-80 ºC. The temperature, at which the gels started to flow, when the glass vials were inverted, was noted as the gel-sol transition (Shaikh *et al*., 2009). The viscosity of all the gel formulations was determined by using Oswald's viscometer. The viscometer is dried and the same volume of the liquid under examination is taken and the process is repeated as before. Let the time of flow be t2. Then

$$\frac{\eta_1}{\eta_2} = \frac{d_1 t_1}{d_2 t_2}$$

Where d1 and d2 are the densities of the two liquids and η1, and η2 are the coefficient of viscosities of the two liquids (one of which is water). The viscosity of the liquid can be easily calculated. The organogel was examined under visible UV-Visible spectrum. The antimicrobial activity was performed by well method (NCCLS, 1993; Awoyinka *et al.*, 2007). The microbial strains employed in the biological assays were Gram – **positive** bacteria: *Staphylococcus aureus* (MTCC 3160), and Gram – **negative** bacteria: *Escherichia coli*, (MTCC 732). The fungus of *Candida albicans* (MTCC 183). Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India. A loop full of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Organogel for the experiment: The organogel as 30µl was used for the experiment. Standard solution as Chloramphenicol (Bacteria) and Fluconazole (Fungi) (25mg/ml distilled water- 30µl) used to compare the organogel. Antibiogram was done by well method. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. Measurement of zone of inhibition:

RESULTS AND DISCUSSION

The organogels were prepared by dissolving the surfactant mixture in sunflower oil

followed by the addition of water. With the initial addition of water, the mixture turned 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 surfactant–sunflower oil–water mixture did not flow when the culture tube were inverted.

Organogel

The organogel were prepared by dissolving the Lecithin and Sunflower oil 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 organo gels, if the GS-water mixture did not flow when the culture bottles were upright. The samples which formed gelled structures, released heat indicating an exothermic reaction as the gels were developed. This indicates that the samples attain a low energy state as they undergo transition into gelled structures and attain a thermodynamically stable state.

pH and Temperature of Lecithin and Sunflower oil organogel

The pH value of organogel is 4.5 to 5.5 and the temperature range is 27 ºC for Lecithin and Sunflower oil gel (Table 1). The pH and temperature 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 and temperature is 25 to 35 ºC If not so, immunological responses like redness, burning and itching of the skin in the applied area will results (Scartazzini *et al*., 1955).

Table 1 pH and Temperature of Lecithin and Sunflower oil organogel

S.No	Water (ml)	pH	Temperature (ºC)	Color
1	0.5	4.81	27	Greenish
2	1.5	4.59	27	Greenish Yellow
3	3	4.72	27	Light Yellow
4	6	5.23	27	Creamy Yellow
5	9	4.73	27	Creamy White

Gel-sol transition analysis

The samples were considered to have undergone gel-sol transition, when they started to flow. The gel-to-sol transition temperature of the Lecithin and Sunflower oil organogel from 86 to 92ºC depending on the composition of the organogels (Table 2). 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 (Murdan, 1999).

Table 2: Gel-sol transition study of Lecithin and Sunflower oil organogel

S.No	Water in (ml)	Temperature	Color
1	0.5	86	Yellowish Green
2	1.5	88	Light Yellow
3	3	89	Light Green
4	6	90	Greenish White
5	9	92	White

Microscopic study

The microstructure of the GS (Gel-Sol) was studied under microscope as different proportions of water were added to the GS. 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 (Table 3). 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 macular structures physically interacts with each other to form a three dimensional networked structure thereby resulting in the immobilization of the organic phase.

Table 3: Morphological analysis of the globular structure in Lecithin and Sunflower oil organogel with various proportions of water

S.No	Water in (ml)	Microscopic structure
1	0.5	Very small globular
2	1.5	Decreased globular numbers
3	3	Small globular
4	6	Medium globular
5	9	Large globular

Fluorescence behavior of Lecithin and Sunflower oil organogel

Fluorescence is the phenomenon exhibited by various chemical constituents present in the organo gel. One of the important feature of fluorescence is that UV light induces a fluorescent nature in many products where fluorescence is not seen in natural day light. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many products, which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some drugs are often assessed qualitatively in this way and it is an important parameter of drug evaluation (Kokashi *et al*., 1957). In the present study to analyzed in fluorescence behavior of Lecithin and Sunflower oil organogel represented in table 4.

Table 4: Fluorescence behavior of Lecithin and Sunflower oil based organogel

S.No	Water (ml)	Visible Light	Short UV	Long UV
1	0.5	White	Light green	Violet
2	1.5	White	Yellowish green	Violet
3	3	White	Light green	Violet
4	6	White	Yellowish green	Violet
5	9	White	Pale green	Violet

Viscosity of Lecithin and Sunflower oil organogel

Lecithin and Sunflower oil 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. The size and the shape of the micelles depend on the surfactant chain length, solvent structure, and the type of co-surfactant used and also on the concentration of the components. The ternary mixtures of Lecithin and Sunflower oil, 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 (Shchipunov, 1996; Aliotta, 2000; Angelico *et al*., 2000).

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 behavior of organogels. The increase in the gelator concentration leads to an increase in the viscosity and in turn the gel strength of a Lecithin and Sunflower oil organogel matrix. In the present study to observe to increase the viscosity by adding water which is helpful enhance the drug delivery to target (Table 5).

Table 5: Viscosity of Lecithin and Sunflower oil organogel

S.No	Water in (ml)	Viscosity
1	Water	1
2	0.5	1.0005
3	1.5	1.1004
4	3	1.1191
5	6	1.1238
6	9	1.1401

Ultraviolet/Visible (UV/VIS) Spectroscopy

Absorption spectroscopy is usually performed with molecules dissolved in a transparent solvent. The absorbance of a solute depends linearly on its concentration and therefore absorption spectroscopy is ideally suited for quantitative measurements. The wavelength of absorption and the strength of absorbance of a molecule depend not only on the chemical nature but also on the molecular environment of its

chromophores. Absorption spectroscopy is therefore an excellent technique for following ligand-binding reactions. Spectroscopic measurements are very sensitive and nondestructive, and require only small amounts of material for analysis (Brown, 1980). UV/Vis spectroscopy is in its simplest form, a sample is placed be Sunflower a light source and a photo detector, and the intensity of a beam of light is measured before and after passing through the sample.

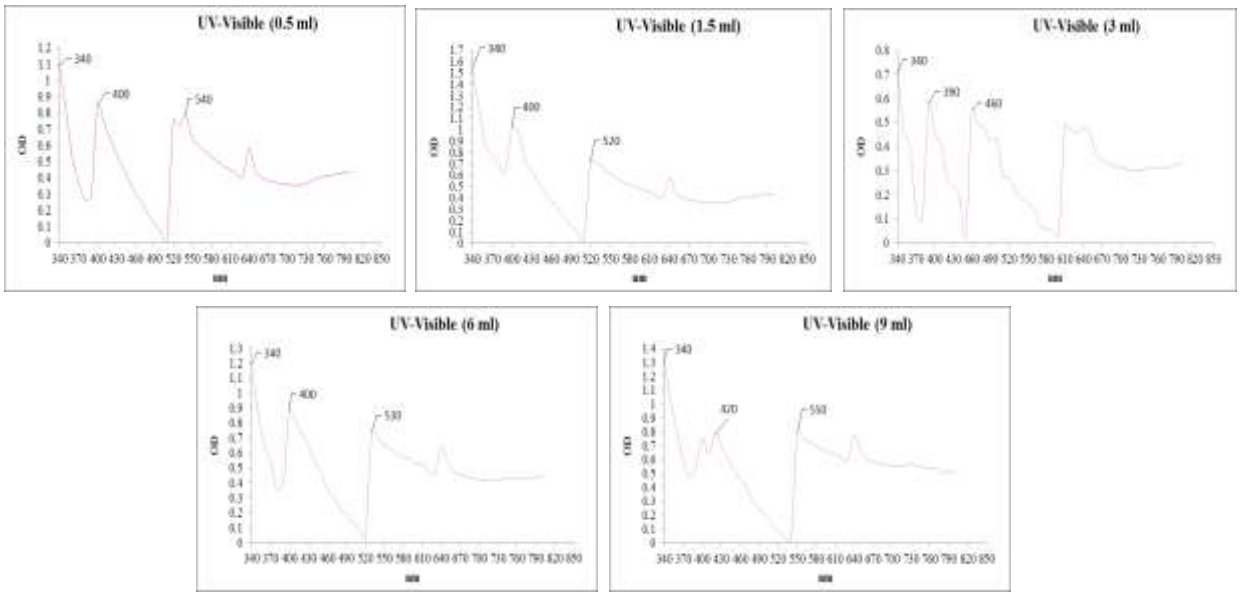

Figure 1: UV Visible absorption spectrum of Lecithin and Sunflower oil based organogel with various proportions of water. a) 0.5 ml, (b) 1.5ml, (c) 3 ml, (d) 6 ml and (e) 9 ml of water.

The UV-VIS profile (Figure 1) of the organogel was studied at a wavelength range of 340 to 800 nm. Three major bands were recorded at 340, 400 and 540 nm for 0.5 ml, 340, 400 and 520 nm for 1.5 ml, 340, 390 and 460 nm for 3 ml, 340, 400 and 530 nm for 6 ml and 340, 420 and 550 nm for 9 ml. The broad absorption peak ranges from 330–600 nm suggest that this gelator could provide efficient organogel upon mixing with water. The compounds with absorption at 390 nm in order to identify the molecular structures that result in light absorption like that of brown carbons (Xue-Qing Li 2006).

Antimicrobial test

The organogel was used for antimicrobial test using *E. coli*, *S. aureus* and *C. albicans* as the test organism. Table 6 shows the results of the test. It was found that organogels 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. The antibacterial activity was differing in various concentration of water added. The antibacterial activity was directly proportional to the water concentrations. Higher water (9ml) added organogel having higher activity.

Table 6: Antimicrobial activity of organogel

Samples	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Candida albicans* (mm)
0.5ml	4.00±0.28	1.90±0.13	10.75±0.75
1.5ml	5.25±0.36	3.70±0.25	10.75±0.75
3ml	5.25±0.36	5.60±0.39	11.00±0.77
6ml	5.50±0.38	11.00±0.77	11.50±0.77
9ml	6.00±0.42	13.20±0.92	13.75±0.96
Std.	13.00±0.91	13.50±0.94	14.25±0.99

CONCLUSION

This study reports the successful development of novel Lecithin mixture based organogels for the first time. The gels were developed by fluid-filled mechanism. The developed organogels were found to be stable in nature and were able to sustain heat shocks for prolonged period. Based on the preliminary studies,

it seems that the Lecithin-sunflower oil mixture based organogels may be tried as a drug carrier for transdermal bioactive agent delivery and used as antimicrobial agent.

REFERENCES

Aliotta F., *et al*., (2000) *Evidence of percolative phenomena in a lecithin-based gel.* Physica B: Condensed Matter, 276-278: p. 347-348.

Angelico R., *et al*. (2000) *Biocompatible Lecithin Organogels: Structure and Phase [`]click' chemistry.* Tetrahedron Letters 49 (8): p. 1340-1343.

Awoyinka O, Balogun I. O, Ogunnowo A. A. (2007) Phytochemical screening and *in vitro* bioactivity Of *Cnidoscolus aconitifolius* (Euphorbiaceae). *J Med Plant Res*, 1(3): 63-65.

Brown SB (ed.) (1980) An Introduction to Spectroscopy for Biochemists. London: Academic Press.

Jeong B, Kim S. W and Bae Y. H. (2002) *Thermosensitive sol-gel reversible hydrogels. Interface.* Langmuir, 12(26): p. 6443-6445.

Kokashi C. J, Kokashi R. J and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc*, 47: 715-717.

Murdan S and Gregoriadis G, (1999) Florence AT, Novel sorbitan monostearate organogels. *Journal of pharmaceutical sciences*, 88(6): 608-614.

NCCLS-National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. PA: NCCLS Publications; (1993), p. M2-A5.

Sahoo S, Kumar N, Bhattacharya S, Sagiri S. S, Jain K and Pal K. (2011) Organogels: properties and applications in drug delivery. Des Monomers Polym, 14:95–108.

Scartazzini R and Luisi P. L. (1988) Organogels from Lecithins., *J. Phys. Chem*, 92, 829-833.

Scartazzini R, and Luisi P L. (1955) Organogels from Lecithins., J. Phys. Chem, 92, 938-942.

Shaikh I. M *et al*., (2009) *Aceclofenac Organogels: In Vitro and In Vivo Characterization.* Current Drug Delivery, 6(1): p. 1-7.

Shchipunov Y. A. (2001) Lecithin organogel a micellar system properties. Colloids Surf A; 183–185:541–54.

Shchipunov, Y. A. (1996) *Lecithin organogels: rheological properties of polymer-like micelles formed in the presence of water. Colloid J*, 57: p. 556-560.

Xue-Qing Li A. (2006) Vladimir Stepanenko,a Zhijian Chen,a Paulette Prins,b Laurens D. A. Siebbelesb and Frank Wurthner. Functional organogels from highly efficient organogelator based onperylene bisimide semiconductor. Chem. Commun., 3871–3873.

Source of support: Nil;
Conflict of interest: None declared