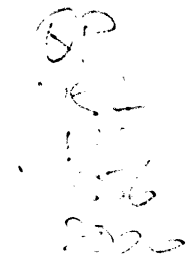

RHEOLOGICAL AND STRUCTURAL PROPERTIES OF LECITHIN-BASED ORGANOGELS

by

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Toronto, Ontario, Canada, 2003

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presented to Ryerson University

in partial fulfillment of the
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in the Program of
Chemical Engineering

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ABSTRACT

Rheological and Structural Properties of Lecithin-based Organogels

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The objectives of this study were to develop lecithin-based organogels made of a biocompatible organic phase and to study the structural and rheological properties of organogels made with different organic phases. The materials used were soybean lecithin of >90% purity (phosphatidylcholine), distilled water and the following organic phases: isooctane (ISO), mineral oil (MO) and isopropylpalmitate (IPP). Phase diagrams for the PC/Water/ISO, PC/Water/ISO/MO (20:80 vol/vol), PC/Water/IPP, PC/Water/IPP/MO (50:50 vol/vol and 30:70 vol/vol) and PC/Water/MO systems were analysed using polarised light microscopy. A narrow region of organogel formation was observed in all systems at molar ratios of water to lecithin between values of 0.4 and 4, depending on the organic phase used. Small-angle X-ray diffraction studies for the PC/Water/ISO system revealed the existence of a highly ordered two-dimensional crystal lattice likely formed by the bundling of the cylindrical reverse micellar tubes. The results for the PC/Water/IPP and PC/Water/MO systems indicated that there was not enough structural material in the organogels to observe any level of molecular organisation. In summary, this study demonstrated that it was possible to develop biocompatible lecithin-based organogels.

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I would like to express my deep gratitude for the support and direction, as well as for the numerous opportunities to advance in my studies, provided by Dr. Dérick Rousseau. His enthusiasm for learning is inspiring. I would also like to warmly thank Shane Hodge for his valuable input in the early stages of this research, as well as Dr. Srebri Petrov of the Chemistry Department, University of Toronto, for his willingness to share much of his knowledge.

The generous financial assistance provided by the National Science and Engineering Research Council and Ryerson University for the Graduate Student Award are also gratefully acknowledged.

This would not be complete without thanking my family members, relatives, church family and close friends for their prayerful support and continual interest for the length of my studies. To my loving and patient husband, Russel Singh; my parents, Keith and Annette Mootoosingh; my brothers and sisters, Hayden & Kathy-Ann, Andy & Wendy, Kirby; my nephew, Christopher and my niece, Arianna - you all make my life complete.

Finally, all glory goes to my Heavenly Father, with whom are all things made possible.

"*And Jesus looking upon them saith, With men it is impossible, but not with God: for with God all things are possible.*" Mark 10:27 KJV.

This is dedicated to
my friend and sister-in-Christ

Ann Persad

February 9, 1980 – November 24, 2005

for whom work of this nature
is made worthwhile.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

Symbols

β_s	Full-width-at-half-maximum (Radians)
$\overline{d}$	Characteristic size of molecular structure (Å)
$\frac{\Delta}{\overline{d}}$	Degree of disorder
I(q)	Scattering intensity (Relative Units)
G	Elastic modulus (Pa)
G'	Storage modulus (Pa)
G"	Loss modulus (Pa)
λ	Wavelength of X-rays (1.54056 Å)
L	Mean long-range order dimension (Å)
η_a	Apparent viscosity (Pa·s)
η_s	Zero-shear viscosity (Pa·s)
η^*	Complex viscosity (Pa·s)
q	Scattering vector ($Å^{-1}$)
r_m	Radius of interaction (Å)
R_g	Radius of gyration (Å)
R	Radius (Å)
2θ	Scattering angle (Radians)
σ	Shear stress (Pa)
w_O	Ratio of molarities of water and lecithin
$w_{O,(gel)}$	Critical w_O for gel formation
$\dot{\gamma}$	Shear rate (s^{-1})

Acronyms

AOT	Bis-2-ethylhexyl Sodium Sulfosuccinate
CCGM	Cross-Coupled Goebel-Mirrors
EO	Ethyl Oleate
FWHM	Full-Width-At-Half-Maximum
GADDS	General Area Diffraction Detector System
IFT	Indirect Fourier Transform
IPP	Isopropylpalmitate
ISO	Isooctane
KF	Karl Fischer
MO	Mineral Oil
NMR	Nuclear Magnetic Resonance
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PGSE	Pulsed Gradient Spin-Echo
PI	Phosphatidylinositol
PS	Phosphatidylserine
PLM	Polarised Light Microscopy
QLS	Quasielastic Light Scattering
SANS	Small-angle Neutron Scattering
SAXD	Small-angle X-ray Diffraction
SAXS	Small-angle X-ray Scattering
SQDEC	Square-Root Deconvolution

CHAPTER 1 - INTRODUCTION

With the discovery of lecithin organogels in the 1980s, came interest in their possible applications in the food, cosmetic and pharmaceutical industries. Since then, there has been great interest in organogels, particularly in the pharmaceutical industry, due to their potential as transdermal drug delivery systems, in lieu of more conventional delivery routes (intramuscular, intravenous, oral). It is thought that the drug release behaviour of organogel matrices depend on how the organogel components self-assemble to form a structural network [Kumar and Katare, 2005; Schurtenberger *et al.*, 1990; Scartazzini and Luisi, 1988]. However, to this day, there remains a thorough lack of understanding of how organogels are organized at a molecular level. It is believed that a better understanding of the structural network in lecithin organogels would enhance our understanding of where guest molecules may be located and how they might interact with organogel components, thus impacting their usage as controlled delivery and release vehicles. As has been shown in the past, an organogel's continuous phase will have a substantial impact on its rheological properties. However, most continuous phases used have not been food-grade, typically consisting of organic solvents. In developing organogels for human use, it is important to understand the influence that a biocompatible continuous phase will have on molecular ordering and rheological behaviour, as this would aid in the design of organogels suitable for topical applications, as well as in their future industrial production.

The purpose of the following sections is to introduce lecithin organogels, by presenting background information on lecithin and the theory behind the formation of organogels, their properties and their potential applications. This section concludes with the main objectives and hypothesis of this thesis.

1.1 Lecithin

Lecithin is the common name for 1,2-diacyl-sn-glycero-3-phosphocholine. It is a naturally occurring, fully biocompatible surfactant that is used extensively in many common applications, such as in animal and human foods, medicine and cosmetics [Shchipunov, 2001; Gunstone and Padley, 1997; Zhang and Proctor, 1997]. Lecithin belongs to substances called phosphoglycerides or phospholipids, which make up more than fifty percent of the lipid matrix of biological membranes of most living organisms, and which are essential to cellular metabolism [Angelico *et al.*, 2003]. The polymorphic behaviour of lecithin in solvents has been recognized for many years [Luzzati *et al.*, 1968].

Lecithin is made up of several different phosphatides, mainly phosphatidylcholine (PC) and phosphatidylethanolamine (PE) [Szuhaj and List, 1985]. Others include phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglycerol (PG). The composition of lecithin depends greatly on its degree of purification. From inception, organogel formation has been observed only when using lecithin containing a high percentage of phosphatidylcholine (>90%). Hence, in the following, the word 'lecithin' will be used to indicate phosphatidylcholine, unless otherwise specified. It has been shown, however, that lecithin with a high percentage of phosphatidylethanolamine does not inhibit gel formation, but rather shifts the region of their formation to that of a higher water concentration than that of phosphatidylcholine [Shumilina *et al.*, 1997].

The molecular structure of lecithin is illustrated in Figure 1. It consists of residues of phosphoric acid, choline, glycerol, and two fatty acids. The lecithin molecule is dipolar, where the two hydrocarbon chains of the fatty acid esters are non-polar and the remainder of the molecule is polar. The lecithin molecule is also zwitterionic since the polar portion contains the choline residue, which is positively charged, and a phosphate group, which is negatively charged. In comparison to the other phosphatides listed above, at a pH of 7 only the head-group of PC and PE possess a positive charge while the others, PS, PI and PG, are negatively charged [Walde *et al.*, 1990]. It is this property of the lecithin

(phosphatidylcholine) molecule that facilitates the formation of the lecithin organogel, as is discussed in Section 1.2.

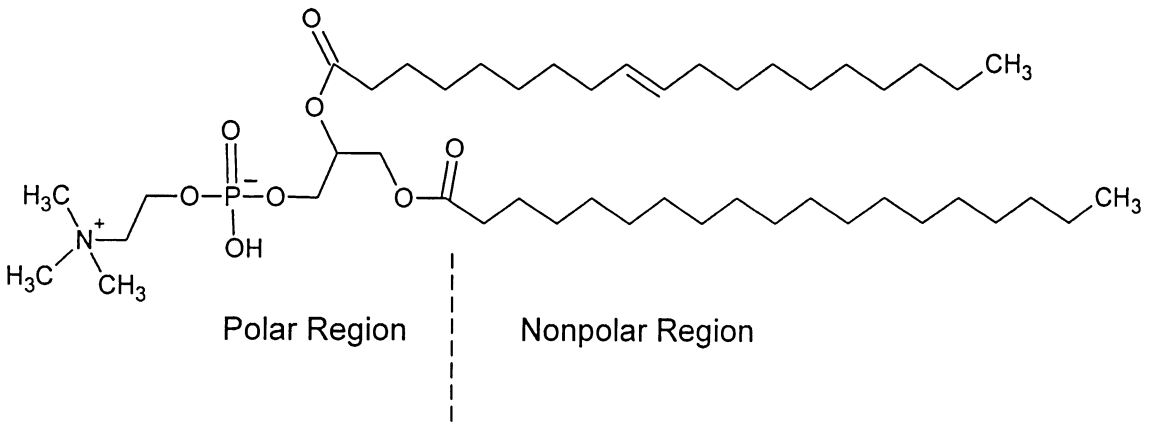

Figure 1. The molecular structure of a molecule of lecithin (phosphatidylcholine) (after Shchipunov, 2001).

1.2 Formation of Lecithin Organogels

Surface-active compounds, or surfactants, typically contain two distinct regions in their chemical structure. The hydrophilic head (water-liking) and hydrophobic tail (water-hating) regions are responsible for the aggregation of these molecules, known as micellisation. In an aqueous solvent, the hydrophobic part of the surfactant molecules are protected from the aqueous phase by the hydrophilic groups (Figure 2(a)) whereas in the case of surfactant molecules in an organic solvent, the structure of the micelles is 'inverted' or 'reversed', with the hydrophilic heads being shielded from the organic phase by the hydrophobic chains (Figure 2(b)). The primary reason for micelle formation is to attain a state of minimum free energy (Florence and Attwood, 1988) in which the interfacial tension between the oil and water phases is reduced and the system is at or near thermodynamic equilibrium.

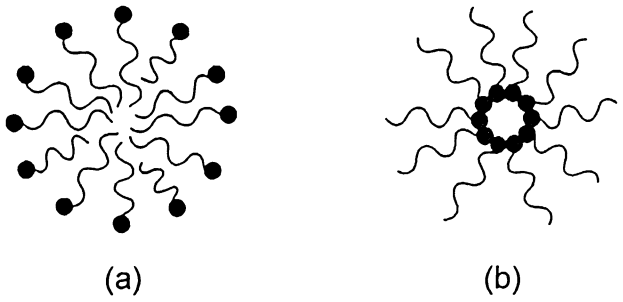

Figure 2. Cross-sections of (a) spherical micelle and (b) spherical reverse micelle [after Norde, 2003].

When water is added to an organic solution containing lecithin, the lecithin molecules naturally self-assemble into reverse micelles [Walde *et al.*, 1990]. At a molecular level, the added water molecules interact with part of the lecithin head group. Figure 3 illustrates the bonding thought to occur between water molecules and phosphatidylcholine molecules when in an organic solvent. With the continual addition of water, the micelles begin growing in length [Florence and Attwood, 1988]. Eventually these elongate enough to change from a spheroid to a cylindrical shape. With further water addition, these cylinders continue to elongate until they start to bend or become tubular. At a certain critical water concentration, these tubular reverse micelles start to overlap and become entangled. The entangled network of tubular reverse micelles then entraps the solvent molecules, thus forming a rigid network known as the lecithin organogel. At this point, known as the gelation point, the mixture experiences a very sharp rise in viscosity ($>10^4$ times) [Shchipunov and Shumilina, 1995]. It generally occurs within a very narrow range of water content, usually <5 wt% for most organic phases.

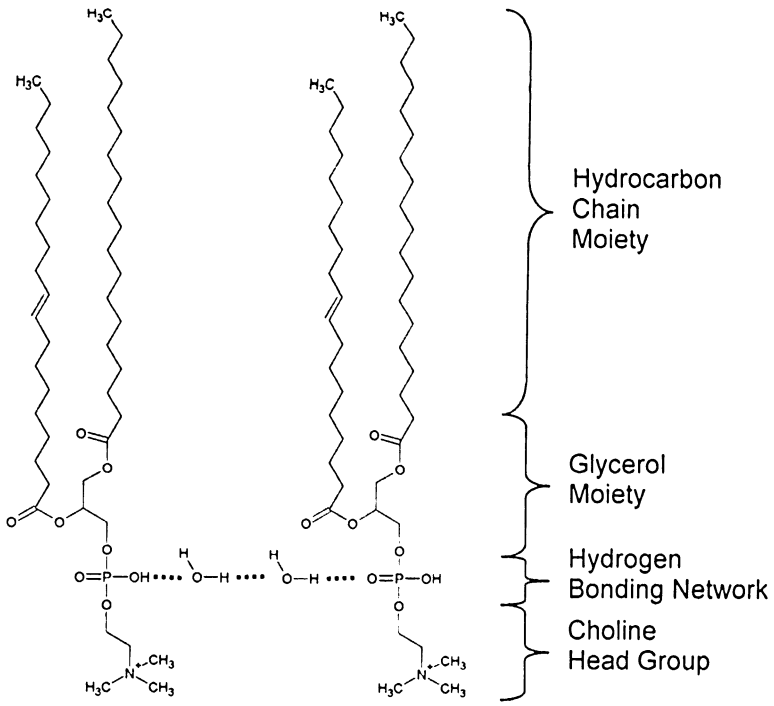

Figure 3. The bonding between water molecules and lecithin (phosphatidylcholine) molecules [after Kumar and Katare, 2005; Shchipunov and Shumilina, 1995].

When more water is added and the gel point is passed, the viscosity of the mixture then decreases, until enough water is added to induce separation of the system into two or three phases [Luisi *et al.*, 1990; Schurtenberger *et al.*, 1990]. For the present research, a gel is defined as a viscous mass that exhibits little or no flow on a short time scale (on the order of seconds) when inclined or inverted [Ferry, 1970].

The three-dimensional network of tubular reverse micelles is held together by hydrogen bonds between the molecules of lecithin and water, and hydrophobic interactions between the organic and aqueous phases. Figure 4 provides a schematic representation of the development of the tubular reverse micelles that make up the rigid network of the lecithin organogel.

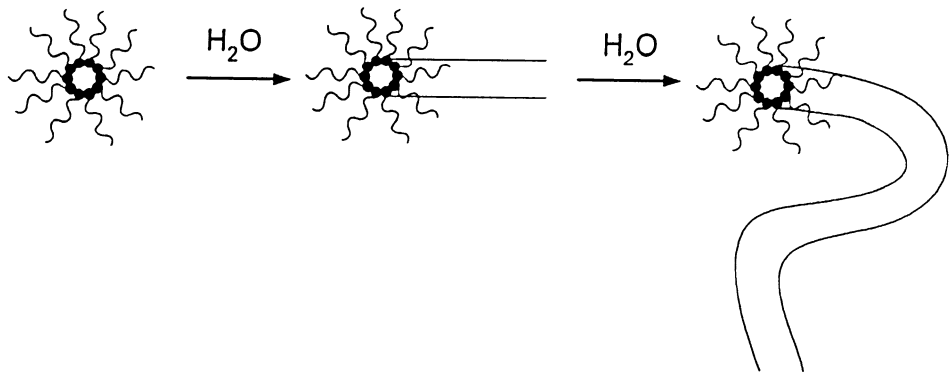

Figure 4. A schematic representation of the development of a tubular reverse micelle [after Luisi *et al.*, 1990].

The water concentration in the organogels is defined as the parameter, w_O, which is the ratio of the molar concentration of water to the molar concentration of lecithin, given in Equation [1]:

$$w_O = \frac{[H_2O]}{[Lecithin]} \qquad [1]$$

The parameter $w_{O(gel)}$ represents the critical value of w_O at which an organogel is initially formed, that is, where sufficient molecular ordering is present to induce an increase in viscosity greater than 10^4 times that of the lecithin solution without water [Shchipunov and Shumilina, 1995]. However, based on previous research, this value is somewhat independent of lecithin concentration but strongly dependent on the organic phase used to prepare the gel [Kumar *et al.*, 2005; Luisi *et al.*, 1990; Schurtenberger *et al.*, 1989; Scartazzini and Luisi, 1988]. Typical values of w_O have been found to be in the range of 1 to 12 for many organic materials [Scartazzini and Luisi, 1988]. The organic phase must facilitate the formation of the lecithin reverse micelles by providing a suitable environment for intermolecular and intramolecular interactions in the lecithin and organic phase molecules [Kumar and Katare, 2005]. Thus many, but not all, solvents may be used to form organogels.

The organic phases used in this study were isooctane (ISO), mineral oil (MO) and isopropylpalmitate (IPP), the molecular structures of which are illustrated in Figure 5. Of the three materials, isooctane is the smallest in size, containing only eight carbons, and also contains the most branching. Mineral oil is a complex mixture of liquid hydrocarbons (≥15 carbons) of primarily straight-chained molecules and some branched-chain paraffins, naphthenes and aromatics [Niraula *et al.*, 2004; Yin Wang *et al.*, 2004]. It was selected for its biocompatibility as it has been widely used for numerous years in cosmetic and medical applications [Yin Wang *et al.*, 2005; Margaroni, 1999; Nash *et al.*, 1996]. Isopropylpalmitate is produced by the esterification reaction between isopropyl alcohol and palmitic acid [Estrin *et al.*, 1982]. It too is known for its biocompatibility and its potential for use in drug delivery applications [Willimann and Luisi, 1991]. Isopropylpalmitate contains an ester linkage that may also influence the formation of lecithin reverse micelles. The ability of this portion of the molecule to hydrogen bond may cause it to compete with lecithin for water, thus potentially inhibiting the initial formation of spherical reverse micelles and their growth into flexible cylinders.

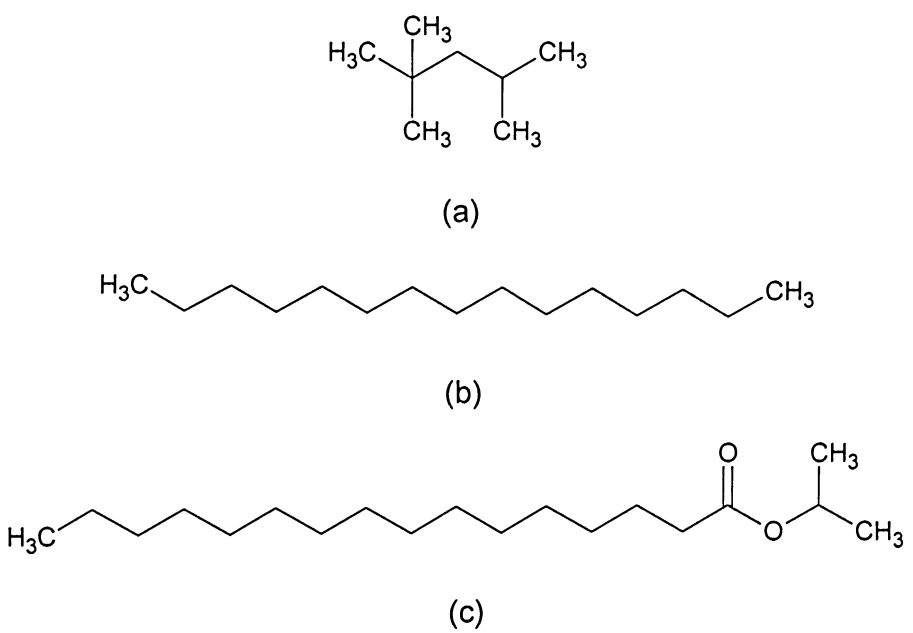

Figure 5. The molecular structures of (a) isooctane (b) a representative component of mineral oil and (c) isopropylpalmitate.

1.3 General Properties of Lecithin Organogels

Gels are an intermediate state of matter, that contain regions of both solid and liquid components [Murdan *et al.*, 1999]. The interconnected molecules that form the three-dimensional network of the gel are considered the solid component that entraps or immobilises the continuous liquid phase [van Esch and Feringa, 2000]. In the case of hydrogels, the continuous phase is aqueous, whereas the continuous phase of organogels is organic in nature.

There are two classifications of gels that are based on the nature of the bonds between the molecules of the solid matrix: chemical and physical. In chemical gels, the molecules form a permanent network that is held together by covalent bonds. In physical gels (such as lecithin organogels), the three-dimensional network of the molecules is held together by weak interactions, namely hydrogen bonds, and electrostatic and van der Waals interactions [Murdan *et al.*, 1999].

Organogels are gels that have been described as 'living or equilibrium polymers' [Angelico *et al.*, 2005; Drye and Cates, 1992] and 'asymmetric bicontinuous microemulsions' [Tlusty and Safran, 2000]. The first of these names has been given since organogels, like polymers, are formed by a rigid crosswork of tubes, but unlike polymers, these tubes have the ability to dissociate and recombine [Jerke *et al.*, 1997; Schurtenberger, 1994]. This dynamic network is therefore one that is described as transient or temporary, though the organogels themselves possess long-term stability on the order of months. The length of the tubular reverse micelles is dependent on parameters such as lecithin concentration, water content, temperature and shear, also in agreement with theories used in classical polymer science [Palazzo *et al.*, 2003; Cates and Candau, 1990].

Organogels are characterised by the following physico-chemical properties:

a) *Very high viscosity* – Organogels are characterised as being highly viscous materials, which is due to the formation of the very rigid structure of the organogel network. The actual viscosity of the organogels formed varies considerably depending on the nature of the organic phase used.

b) *Viscoelasticity* – Organogels exhibit viscoelastic behaviour as is predicted by theory for entanglement networks of semi-dilute polymer solutions [Ferry, 1970]. This means that organogels have properties of both viscosity and elasticity, the latter indicating that the gels change shape with an applied load, but regain their shape once the load is removed.

c) *Non-birefringency* – Organogels are isotropic, and thus are completely non-birefringent. They appear perfectly dark when viewed under polarised light.

d) *Thermoreversibility* – Upon heating, the structure of an organogel is destroyed, as the hydrogen bonds are broken due to the excess energy acquired by the lecithin and water molecules with the addition of heat. Upon subsequent cooling and the loss of this extra energy, organogels regain their original structure and revert back to being a rigid network by the reformation of these bonds.

e) *Thermodynamic stability* – Organogels are thermodynamically stable systems and therefore possess long-term stability. This makes them suitable for drug and cosmetic applications for which a long shelf-life is desirable.

f) *Optical clarity* – Organogels are perfectly transparent. Although solutions of lecithin in an organic phase may be opaque, the addition of water generally causes them to become optically clear [Scartazzini *et al.*, 1988].

1.4 Potential Applications

One of the greatest potential applications of lecithin organogels is for the transdermal delivery of drugs and bioactive ingredients, such as nutraceuticals. Conventional methods of administering drugs include intramuscular, intravenous and oral route. Oral ingestion has the disadvantage of being affected by many factors such as the pH of the intestinal tract, the intestinal transit time and metabolism by the liver. These factors substantially affect the level of the drug within an individual. Although injections and infusions help to avoid some of these problems, they still do not provide a means of controlled release, nor do they result in sustained levels of the drug or bioactive ingredient within the body.

Intact skin provides another possibility as a route of entry, although it has not been generally regarded as suitable, due to its impenetrability to most chemicals. In fact, very few drugs are able to penetrate the skin in order to enter the body in sufficient amounts for therapeutic use. Nevertheless, the advantages of using this approach, however, would include the elimination of the aforementioned influences of systemic circulation, as well as provide controlled release of a drug treatment that could be simply applied and also rapidly terminated. Systems that would enhance the skin's permeability to facilitate drug delivery are, therefore, of immense interest.

Lecithin organogels may be used as a topical ointment for controlled drug/bioactive ingredient release due to their inherent stability [Mackeben *et al.*, 2001], which would give prolonged and continual drug/bioactive supply directly into the circulatory system. Other advantages may include a very low risk of skin irritation and a reduction in either the possibility of patient non-compliance or overdose [Dreher *et al.*, 1997]. For lecithin organogels to be made appropriate for use in the pharmaceutical or cosmetic industries, it is essential that the organic phase be made of a suitable biocompatible material [Muller-Goymann and Hamann, 1993; Mackeben *et al.*, 2001]. Long-chain fatty-acid esters, such as isopropylpalmitate [Dreher *et al.*, 1997; Angelico *et al.*,

2005], as well as hydrocarbons such as squalene, have been suggested as suitable materials [Luisi *et al.*, 1990].

It is possible to incorporate guest molecules with various physico-chemical properties [Dreher *et al.*, 1997], such as vitamins, proteins, etc., within organogels. Guest molecules may be located in either the bulk organic phase (e.g. perlyn), at the micellar interphase (e.g. erythrocin) or in the hydrophilic core of the tubular reverse micelles (e.g. ascorbic acid) [Luisi *et al.*, 1990]. With regards to the organogel's ability to enhance the permeability of the skin to chemicals, it is believed that the combination of lecithin and isopropylpalmitate may work together to possibly enhance skin penetration [Dreher *et al.*, 1997; Gupta and Garg, 2002].

Willimann and Luisi [1991] successfully used lecithin organogels as a matrix for the delivery of scopolamine (to control motion sickness) and broxaterol, a bronchodilatory agent, using isopropylpalmitate as the organic phase. Since then, numerous therapeutic compounds have been incorporated into lecithin organogels, including muscle relaxants, hormones, antithyroid drugs, steroids and non-steroidal anti-inflammatory drugs (NSAIDS) [Kumar and Katare, 2005]. Studies have also been performed to evaluate the potential of organogels as carriers of anti-tumour drugs, such as polyamidine compounds [Nastruzzi and Gambari, 1994]. These authors developed formulations based on several organic solvents, including isopropyl palmitate and myristate. It was found that the application of these organogels resulted in the reduced cell growth of cancerous tumours.

1.5 Objectives

The two key objectives of this study were to:

1. Develop a lecithin organogel made of a biocompatible organic phase that could potentially be used for food, pharmaceutical, medical or cosmetic applications.

2. Investigate the structural and rheological properties of lecithin organogels made with various organic phases.

1.6 Hypothesis

The rheological and structural properties of organogels can be modulated with the type of organic phase used in their formation.

CHAPTER 2 – LITERATURE REVIEW

2.1 The Discovery of the Lecithin Organogel

The first organogels were prepared by solubilising gelatin in the water phase of the AOT (bis-2-ethylhexyl sodium sulfosuccinate)/water/isooctane system. The resulting product, termed a 'microemulsion gel', was characterised by having a very high viscosity, up to 1000 Pa·s [Scartazzini and Luisi, 1988]. Scartazzini and Luisi [1988] began investigating the possibility of producing organogels made with biocompatible materials. Soybean lecithin, a widely used biocompatible surfactant, was selected for this purpose.

The results of their study showed that organogels could be made by using purified surfactants (i.e. lecithin containing >90% phosphatidylcholine) dissolved in an appropriate solvent to which small amounts of water were added. These gels were highly viscous, completely transparent and not birefringent. This phenomenon was observed with approximately fifty organic solvents, which included linear, branched and cyclic alkanes, ethers and esters, fatty acids and amines. Table 1 lists some of these organic phases and the corresponding water concentration needed for organogel production.

Although the molecular structure of the organogels was not determined in this study, some generalised conclusions were made. Since the organogels were formed with a stoichiometric amount of water to lecithin, it was suggested that the water molecules were tightly bound to the polar head of the lecithin molecule. It was also suggested that slight differences in the molecular structure of the organic phases had an effect organogel viscosity.

Finally, it was concluded that lecithin organogels would be useful in the preparation of pharmaceutical formulations, mainly because guest molecules could be entrapped in either the organic or aqueous phases.

Table 1. Some organic materials found to produce lecithin organogels, with the corresponding water concentration required for gel formation [after Scartazzini, and Luisi, 1988]. w_o is defined as per Equation [1].

ORGANIC MATERIAL	WATER CONCENTRATION, w_o
Ethyl laurate	4
Butyl laurate	7
Ethyl myristate	5
Isopropyl palmitate	3
Isooctane	2
Cyclopentane	8
Cyclohexane	6
Cycloheptane	7
Cyclooctane	7
Cyclodecane	12
Methyl cyclohexane	7
Tert-butylcyclohexane	4
Phenylcyclohexane	12
Bicyclohexyl	4
1,3,5-triisopropylbenzene	3
Octylbenzene	6
Trans-decalin	5
(1R)-(+)-trans-pinane	6
(1R)-(+)-cis-pinane	10
n-pentane	3
n-hexane	3
n-heptane	2
n-octane	2
n-nonane	2
n-decane	2
n-undecane	2
n-dodecane	1
n-tridecane	1
n-tetradecane	2
n-pentadecane	1
n-hexadecane	1
n-heptadecane	1
2,3-dimethylbutene	4
1-hexene	6
1,7-octadiene	7
Tripropylamine	4
Tributylamine	2
Triisobutylamine	3
Trioctylamine	2

2.2 Elucidation of the Lecithin Organogel Structure

Directly following the discovery of lecithin organogels, Schurtenberger *et al.* [1990] delved into the elucidation of their molecular structure. The phase behaviour and properties of the lecithin/water/isooctane system was investigated using polarised light microscopy (PLM), ^{2}H and ^{31}P nuclear magnetic resonance (NMR) and small-angle neutron scattering (SANS). Dynamic shear viscosity investigations were also made using a rheometer with disk and plate geometry.

Once the region of organogel formation was determined, careful examination by polarised light microscopy (PLM) showed no indication of the presence of any birefringent material. Nuclear magnetic resonance, both ^{2}H and ^{31}P NMR, experiments done on the samples revealed no presence of liquid crystalline order within the samples. Therefore, the formation of a hexagonal or lamellar liquid crystalline phase was eliminated as a possible explanation for the high viscosity of these samples.

Another possibility still remained of the samples being a cubic liquid crystalline phase, since it was possible for this phase to exhibit isotropic behaviour as well. This option was also ruled out based on the results of small-angle X-ray scattering (SAXS) and SANS experiments, which were in clear disagreement with typical results produced by cubic liquid crystal structures. The resulting scattering curves obtained from these highly viscous samples did not support the presence of a cubic liquid crystal phase.

It was then postulated that the addition of water to lecithin/isooctane solutions induced aggregation of the lecithin molecules into long and flexible cylindrical reverse micelles. At a high enough lecithin concentration, these micelles would entangle to form a transient network, similar to semi-dilute polymer solutions. Using SANS and quasi-elastic light scattering (QLS), together with a study of their rheological properties, it was determined that the molecular structure of lecithin organogels did indeed consist of long, cylindrical reverse micelles that

aggregated to form a transient entangled network [Schurtenberger *et al.*, 1990]. Using SANS measurements of the reverse micelles under shear in another study, direct results were further obtained supporting the theory of the lengthening of the micelles with the addition of water, independent of lecithin concentration [Schurtenberger *et al.*, 1990a].

More detailed investigations into the growth of the tubular micelles were performed using the system of lecithin/water/cyclohexane [Schurtenberger *et al.*, 1991; Schurtenberger *et al.*, 1992]. Cyclohexane was used instead of isooctane used in previous studies because it had a much higher w_o value than that of isooctane (6 and 2 respectively). This meant that properties of the individual particles could be determined in the dilute region where the micellar size was still very large even at low lecithin concentrations. By analysis of small-angle neutron scattering, the radius of gyration of the cylindrical micelles, R_g, was determined using a Guinier plot of the scattering data to be 18.3 ± 0.8 Å. This gave a value of 25.9 ± 1.1 Å of the cross-sectional radius of the cylinders, R. The value of the persistence length (degree of flexibility), l_p, was determined to be 110 ± 30 Å, which was in good agreement with the theoretical value of approximately 125 ± 30 Å. In another study, the water-induced lengthening of the tubular reverse micelles in the same ternary system was clearly demonstrated by small-angle neutron scattering by applying the methods of the indirect Fourier transform (IFT) and square-root deconvolution (SQDEC) using cyclohexane as the organic phase [Schurtenberger *et al.*, 1996].

Rheological experiments on organogels were done to compare their viscoelastic behaviour with that of entanglement networks in polymer solutions [Schurtenberger *et al.*, 1989]. Using the system of lecithin/water/isooctane, it was observed that the zero-shear viscosity and the elastic shear modulus of the gels increased with increasing volume fraction of lecithin, but both decreased with increasing temperature. It was also deduced that the zero-shear viscosity of the gels increased by several orders of magnitude with as little as three molecules of water per molecule of lecithin.

Further research [Shchipunov and Hoffman, 2000; Luisi *et al.*, 1990] showed that organogels exhibit the characteristic features of entanglement networks of giant cylindrical micelles analogous to semi-dilute polymer solutions. In studying the viscoelastic behaviour of lecithin organogels made of different organic solvents (tributylamine, hexadecane, isopropylmyristate), it was found that the values of their storage modulus (G'), their loss modulus (G'') and their complex viscosities (η^*) showed dependency on frequency. At low frequencies, η^* became independent of frequency. At high frequencies, G' approached a plateau region, providing the value of the elastic shear modulus, G.

The research group of Angelico *et al.* [2005] investigated the microstructure of organogels formed by biocompatible organic phases. Oils, such as the fatty-acid esters of isopropylpalmitate (IPP) and ethyloleate (EO), were used for organogel preparation. An investigation of the phase behaviour of the lecithin/water/IPP and lecithin/water/EO was made. The microstructure of the organogels was principally determined based on analysis by pulsed gradient spin-echo nuclear magnetic resonance (PGSE-NMR), used to observe the molecular motion of lecithin and water in the samples. They found that the lecithin self-diffusion coefficients were low and actually decreased significantly with increasing water content [Angelico *et al.*, 2005]. This is in contrast to the results obtained previously [Ambrosone *et al.*, 2001] for the lecithin/water/isooctane system, which showed an increase in the lecithin self-diffusion coefficients with increasing water loading, indicating an increase in the branch density of the system. It was therefore maintained that organogels made of fatty acid esters were disconnected and rod-like in shape, that is, there was no bending of the cylinders to form long flexible tubes.

In comparing the results of organogels made from IPP and EO, it was found that the size of the reverse micelles in the gels made from IPP were larger than that of the gels made from EO. It was also noted that there was a difference in the viscosities of the organogels produced in the two systems, with the IPP gels having a higher viscosity than the EO gels. It was therefore inferred that the longer cylindrical micelles of the IPP gels resulted in the higher viscosity.

In summary, it has been found that lecithin organogels produced with a hydrocarbon solvent (e.g. hexane, isooctane, decane, etc.) as the organic phase have a molecular structure that consists of long and flexible reverse micelles. Lecithin organogels produced with a fatty-acid ester (e.g. isopropyl palmitate, ethyl laurate, etc.) as the organic phase likely consist of rod-like reverse micelles, with no evidence of curvature.

CHAPTER 3 - MATERIALS AND METHODS

3.1 Materials

Soybean lecithin (Fisher Scientific, Napean, ON, Canada), used as the surfactant in organogel preparation, consisted of soybean phosphatidylcholine (PC) with a purity of at least 90% and with an average molecular weight of 773 g/mol [Angelico *et al.*, 2005]. Soybean lecithin generally contains ~0.7 – 2.3 wt% moisture (0.3 to 1 molecule of water to 1 molecule of lecithin) [Angelico *et al.*, 2005]. The lecithin used in this study was found to contain 0.03 wt% moisture as determined by Karl Fisher Analysis (Section 3.2.5). In this study, water content is expressed in terms of water added to the samples. The soybean lecithin was kept refrigerated (4 °C) at all times.

Three organic solvents, isooctane (ISO), mineral oil (MO) and isopropylpalmitate (IPP) were used as the continuous phase in this study. The solvent isooctane (2,2,4-trimethylpentane) (density = 0.686 g/ml) of HPLC grade was obtained from Fisher Scientific (Napean, ON, Canada) together with mineral oil (density = 0.849 g/ml). Isopropylpalmitate (90% pure) (density = 0.853 g/ml) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All experimental samples were prepared using distilled water. All chemicals were used without further purification.

3.2 Methods

3.2.1 Ternary Phase Diagram Development

Ternary phase diagrams were developed for the following systems:

a) lecithin-water-isooctane
b) lecithin-water-isooctane-mineral oil (20:80 vol/vol)*
c) lecithin-water-isopropylpalmitate
d) lecithin-water-isopropylpalmitate-mineral oil (50:50 vol/vol)*
e) lecithin-water-isopropylpalmitate-mineral oil (30:70 vol/vol)*
f) lecithin-water-mineral oil

*The ratios in brackets beside the systems represent the volume percent of each organic phase present in the system in the order listed.

The method of preparation of these samples is described as follows. For systems (a) to (e) above, stock solutions were prepared by weighing appropriate amounts of lecithin and organic phase(s) into 250 mL glass flasks, which were stoppered. The contents were allowed to mix for at least 24 hrs with a magnetic stirrer, until all the lecithin was dissolved and a visually homogeneous solution was produced. These stock solutions were then divided into 5 mL portions, to which increasing volumes of distilled water were added up the point of gelation and beyond the region of gel formation. Once the water was added, the samples were vortexed (~5 mins, 25 °C). In preparing samples for system (f) above, the same procedure was applied, except that the stock solution of lecithin and mineral oil was heated to approximately 85 °C (~2-3 hours) to aid dissolution of lecithin in the mineral oil. The stock solution was then kept heated to approximately 60 °C while preparing the 5 mL samples since it became very viscous upon cooling. Samples were observed visually immediately after mixing and after approximately 24 hrs to confirm the phases present.

For each system, a large number of samples, concentrated in the very water-poor region, were prepared for the determination of the phase boundaries. Since the region of organogel formation was of interest, only the boundaries of this region were determined for most of the systems. For the systems (a) to (f)

above, the phase diagrams were developed based on the analysis of ~125, ~25, ~130, ~110, ~30 and ~50 samples, respectively.

3.2.2 Polarised Light Microscopy (PLM)

A Zeiss Axioplan-2 light microscope (Zeiss Instruments, Toronto, ON, Canada) with a 20x objective was used to aid basic visual inspection in order to identify the different phases produced. Organogels and liquid crystals were sampled using a Pasteur pipette and placed on viewing slides (Fisher Scientific, Napean, ON, Canada), upon which a cover slip (Fisher Scientific, Napean, ON, Canada) was gently placed. Samples were viewed within 24 hrs after preparation. A Q-Imaging CCD camera was used to capture the images, which were analysed by Northern Eclipse Software (version 6.0, Empix Imaging, Mississauga, ON, Canada).

3.2.3 Rheometry

Rheological measurements were carried out within 48 hrs of sample preparation, on samples in each system with lecithin concentrations of 0.1 mol/L (77.3 g/L) with increasing water contents, using an AR 2000 Rheometer (TA Instruments, New Castle, DE, USA) with a cone-and-plate geometry (40 mm, 2°) (Figure 6). Samples (0.59 ml) were placed on a Peltier plate that was set and held at approximately 25°C throughout the experimental run. Care was taken to obtain as pure a sample as possible, from regions where two or more phases existed using 5 ml syringes (Fisher Scientific, Napean, ON, Canada). In experimental runs with samples containing isooctane, a solvent trap was used to prevent evaporation. This was achieved by filling the well with isooctane so that the air within the solvent trap would be saturated with the isooctane vapour.

Each experimental test was performed in duplicate, using a fresh sample each time, under controlled shear stress of 0.001-1000 Pa. The zero-shear viscosity, which is the apparent viscosity at low shear-rates, is used in characterising shear-thinning fluids such as gels [Rao, 1999]. The zero-shear viscosity was

evaluated from the plateau of viscosity on a plot of viscosity versus shear rate [Shchipunov and Hoffman, 2000; Schurtenberger, 1994; Luisi *et al.*, 1990] by applying a linear least-squares fit to the portion of the graph that was approximately linear. This characteristic parameter was selected for plotting in order to identify the w_0 value in each system that corresponded to an organogel with maximum measured viscosity.

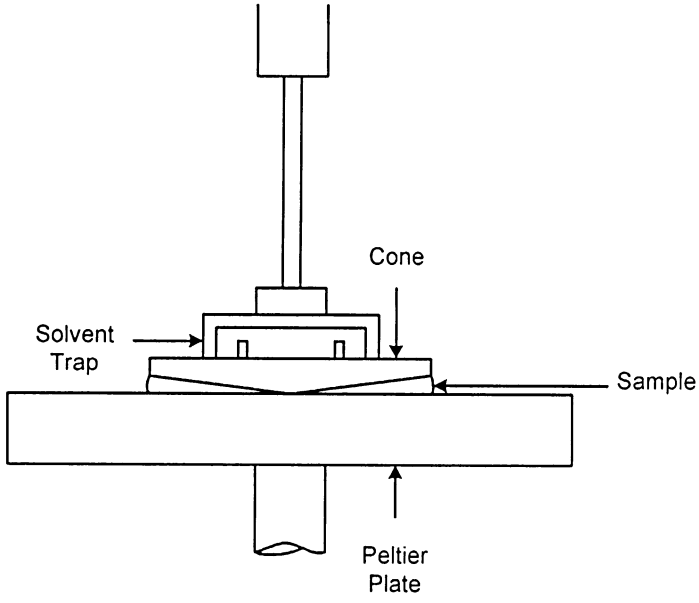

Figure 6. A schematic diagram of the cone and plate geometry used for rheological measurements.

3.2.4 Small-angle X-ray Diffraction (SAXD)

Small-angle X-ray diffraction measurements were performed on a NANOSTAR (Bruker AXS, Madison, WI, USA) at wavelength λ = 1.54056 Å (Figure 7). The radiation source was a point-focus Cu X-ray tube, with the primary optical system being CCGM (Cross-coupled Goebel-Mirrors) used for precise beam focusing. The sample-to-detector distance was 64.65 cm. The type of detector was a high-resolution proportional (wire-type) 2D area detector (General Area Diffraction Detector System, GADDS). The beam size had a pinhole diameter of 300 μm. The volume between the sample and the detector was kept under vacuum during the measurements to minimise scattering from air. All SAXD

measurements were performed on samples in each system with lecithin concentrations of 0.1 mol/L (77.3 g/L), with increasing water contents. Experiments were performed at 25°C, ~24 hrs after the samples were prepared. The samples were loaded onto the sample holder and held in place by scotch tape, whose scattering was subtracted from the raw spectra of the samples. The software used for data collection and processing was the SAXA/WNT v. 4.1.13 (2003). Crystal lattices were analysed using the program INDX (Beta Version, June 1999).

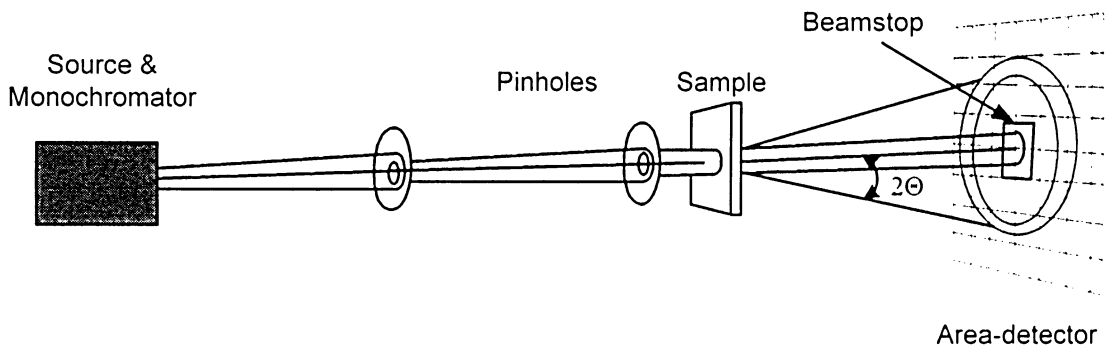

Figure 7. A schematic diagram of the apparatus used in small-angle X-ray diffraction analysis [after Erlacher, 2004].

A range of the scattering vector, q, of approximately 0.007 to 0.25 Å $^{-1}$, was covered where:

$$q = \frac{4\pi \sin\theta}{\lambda} \qquad [2]$$

and 2θ is the scattering angle in radians. From the Bragg peaks of the scattering curves of intensity, $I(q)$ versus q, structural parameters characterising the degree of internal order of the samples were obtained. The mean long-range order dimension, L, estimates the size of the areas or clusters where periodic spatial order occurs in the samples. This value was determined using the Scherrer equation, as follows [Svergun, 2000; Alexander, 1969; Vainshtein, 1966]:

$$L = \frac{\lambda}{\beta_s \cos\theta} \qquad [3]$$

where β_s is the full-width-at-half-maximum (FWHM) intensity of the peak (in radians) at a scattering angle 2θ. The value of β_s was determined using the profile fitting software TOPAS™ (© 2000 Bruker AXS).

The value of the radius of interaction, r_m, gives the maximum separation between spatially correlated ordered regions and is described by Equation [4] below [Svergun *et al.*, 2000; Vainshtein, 1966]:

$$r_m = \left(\frac{\pi}{2.5}\right)^2 \frac{\lambda}{\beta_s} \qquad [4]$$

Finally, the relative mean square deviation of the distance between the ordered regions, that is, the degree of disorder, $\frac{\Delta}{\bar{d}}$, in the systems was calculated using Equations [5] and [6] below [Svergun *et al.*, 2000; Vainshtein, 1966]:

$$\frac{\Delta}{\bar{d}} = \frac{1}{\pi}\sqrt{\frac{\beta_s \bar{d}}{\lambda}} \qquad [5]$$

where

$$\bar{d} = \frac{2\pi}{q_{max}} \qquad [6]$$

In Equation [5], λ is defined as in Equation [3] and β_s as in Equation [4]. Also in Equation [5], $\bar{d}$ displays the most frequently distributed distance between identical material formations in the structure. It provides the characteristic size of these formations.

According to theory, the values of L and r_m should increase with increasing order in the system. On the other hand, $\frac{\Delta}{d}$ should decrease if the system becomes more ordered [Svergun et al., 2000].

3.2.5 Karl Fischer Analysis

A Karl Fischer Coulometer (Metrohm Limited, Herisau, Switzerland) was used to determine the water content of the pure solid lecithin used in sample preparation.

CHAPTER 4 - EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Lecithin-Water-Isooctane-Mineral Oil

4.1.1 Ternary Phase Behaviour

A partial ternary diagram for the lecithin/water/isooctane (PC/Water/ISO) system at 25°C is presented in weight fractions in Figure 8. It is a partial ternary phase diagram since the weight fractions of the components do not cover the full range of values from 0 to 100 wt%. This diagram describes the short-term phase behaviour of the system, as the information was collected 24 hours after sample preparation.

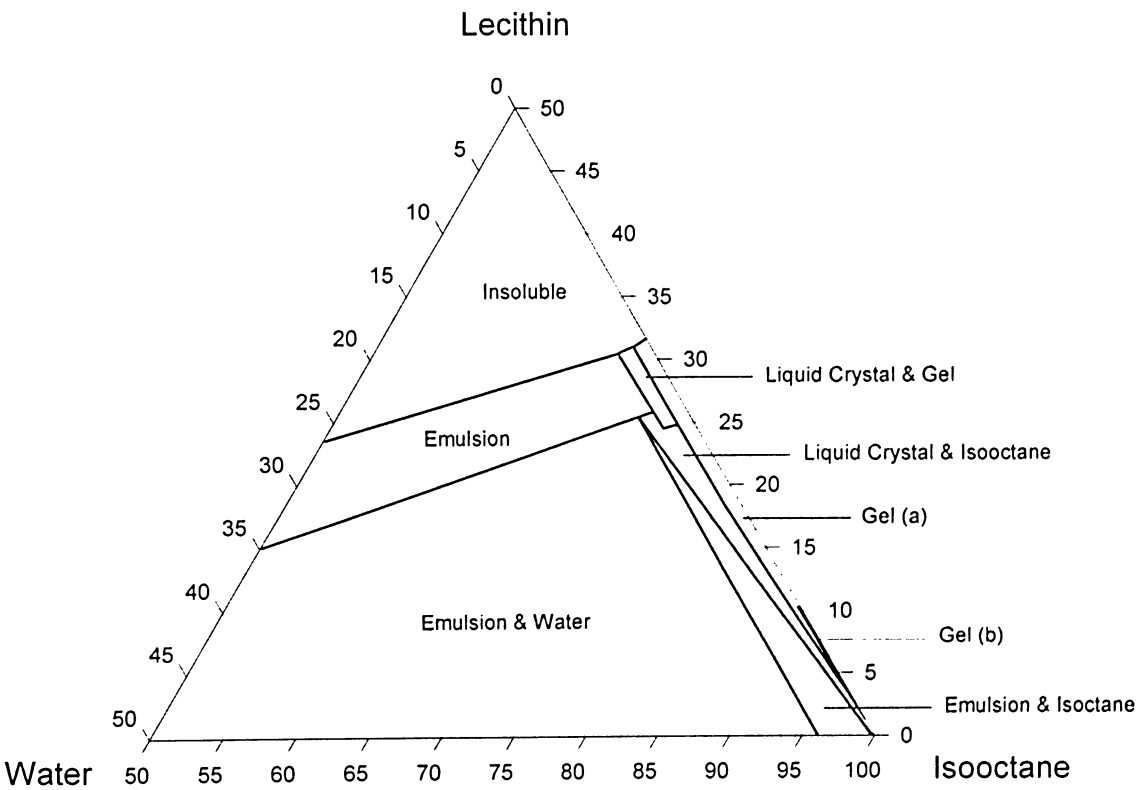

Figure 8. Partial ternary phase diagram of the (a) PC/Water/ISO (solid lines) and (b) PC/Water/ISO/MO (20:80 vol/vol) (shaded) systems.

Above ~33 wt% lecithin, it was found that the lecithin was insoluble in the organic phase for the system containing pure isooctane. The largest region obtained is occupied by two-phase region of an emulsion and water that is bordered by the phase boundaries of a maximum lecithin of concentration of ~27 wt% and a minimum of ~2.5-4.0 wt% water. Above this, at lecithin concentrations up to ~33 wt%, a region of emulsion formation was found. In moving towards areas of lower water concentrations, a two-phase region of an emulsion and isooctane is encountered. This area is bordered by a maximum lecithin concentration of ~27 wt%, and has a minimum water wt% of ~2.5 that extends up to ~5 wt%. With a further decrease in water concentration, one of two things can be found. Up to a maximum lecithin concentration of ~25 wt%, a two-phase region of liquid crystal and isooctane is found. In a small region above this, another two-phase region of a liquid crystal and an organogel is found.

The region of major interest and focus was one in which organogels were formed. This was found to be a narrow strip along the 'water-lean' region of the diagram, as seen in Figure 8. The organogels produced were highly viscous, optically transparent and completely non-birefringent [Figure 9]. An increase in water concentration up to ~2 wt% resulted in an increase in the viscosity of the samples, until gelation occurred. At the gel point, the viscosities of the samples were at their highest values, and corresponded to a range of values of the ratio of the molar concentrations of water to lecithin, w_0, of ~2.5-3.0. This range is in agreement with that found in the literature [Luisi *et al.*, 1990, Angelico *et al.*, 2003]. It was also found that with increasing lecithin concentration, more water was required for organogel formation. This is also in agreement with other research [Schurtenberger *et al.*, 1990; Angelico *et al.*, 2003].

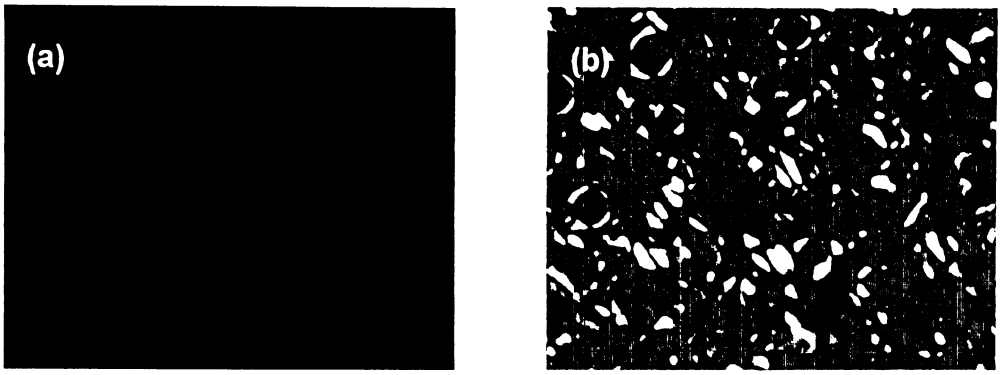

Figure 9. Polarised light microscopy of samples in the PC/Water/ISO system for (a) an organogel ($w_O \approx 2.8$) and (b) a liquid crystal ($w_O \approx 4.0$).

For the lecithin/water/isooctane/mineral oil (PC/Water/ISO/MO) system, only the region of organogel formation was determined. Organogels only existed the in the very small shaded region as seen in Figure 8. Gels formed in this region followed the same trend as with the pure isooctane gels. With increasing lecithin weight fraction, water concentration up to a maximum of ~0.6 wt% was required for gel formation. Once passed the gel region with increasing water, a liquid crystal phase was produced, which was confirmed by the use of polarised light microscopy (Figure 10). Anisotropic phases can be recognized by their optical birefringent nature. Organogels are isotropic and thus optically non-birefringent. They therefore show only a dark background when studied using microscopy with polarised optics. Anisotropic liquid crystalline phases, however, have their own characteristic textures that can be easily observed [Angelico *et al.*, 2005].

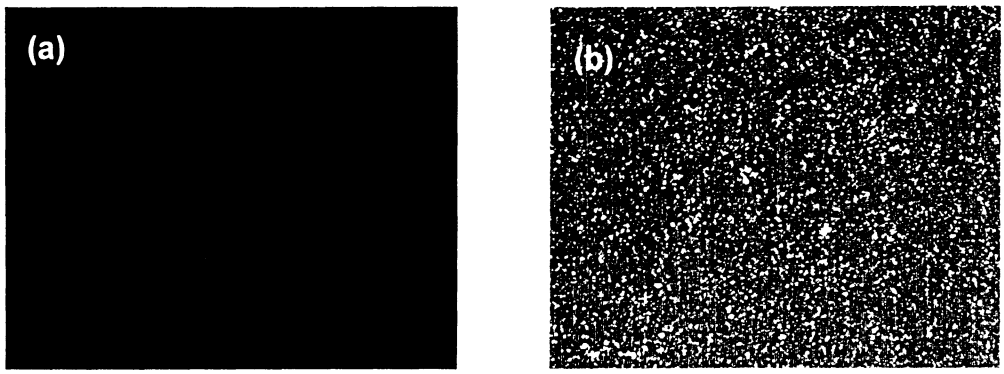

Figure 10. Polarised light microscopy of samples in the PC/Water/ISO/MO (20:80 vol/vol) oil for (a) an organogel ($w_O \approx 0.8$) and (b) a liquid crystal ($w_O \approx 2.0$).

4.1.2 Rheological Behaviour

In this study, the rheological behaviour of samples made using isooctane (ISO) and a mixture of isooctane and mineral oil (MO) in the ratio of 20:80 vol/vol as the organic phase, respectively, is examined. Figure 11 illustrates the dependence of apparent viscosity (Pa·s) on shear rate (s^{-1}) of samples prior to gelation, at the gel point and after gelation (a liquid crystal) for the PC/Water/ISO system. The main feature of this graph is that the apparent viscosity of the organogel is constant with increasing shear rate. It therefore exhibits Newtonian behaviour, very similar to the low viscosity reverse micellar solution. This behaviour shows that the organogels have reached structural equilibrium, as studies have shown that the relationship between shear stress and shear strain is directly proportional at low shear stresses for gels at equilibrium [Rudraraju *et al.* 2005]. From the graph, the organogel phase exhibited Newtonian behaviour between shear rate values of ~0.0001 and 0.1 s^{-1}. Results for the flow behaviour of the liquid crystal structure demonstrated non-Newtonian behaviour.

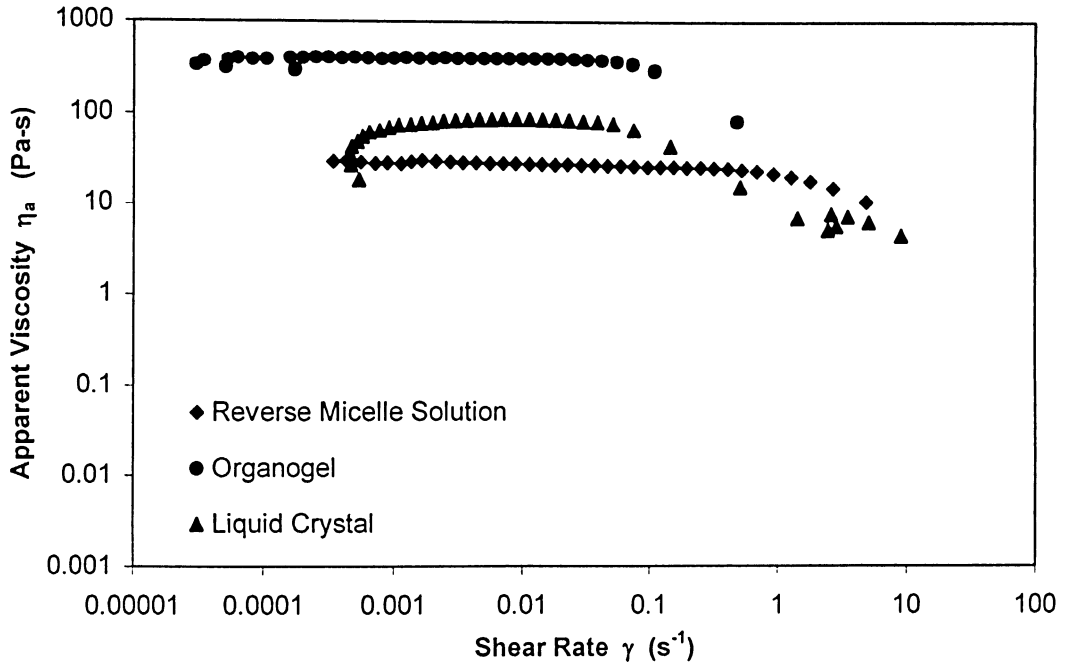

Figure 11. Apparent viscosity η_a (Pa·s) versus shear rate γ (s^{-1}) for samples in the PC/Water/ISO system.

Another feature of the organogel is that at a critical shear rate, the viscosity begins to abruptly decrease, which is likely due to the breakdown of the structural network within the organogels [Rao, 1999; Mewis, 1979]. The abrupt change of viscosity of the organogel occurred at a shear rate value of ~0.1 s^{-1}.

Figure 12 illustrates the dependence of apparent viscosity (Pa·s) on shear rate (s^{-1}) for an organogel and for a liquid crystal in the PC/Water/ISO/MO (20:80 vol/vol) system. The results for samples in this mixed system follow the same general trend as that of the pure isooctane system. Again, the main feature of this graph is that the apparent viscosity of the organogel is relatively constant with increasing shear rate, although for a much narrower region than that of the pure isooctane system. The viscosity of the organogel decreases at a shear rate of ~0.001 s^{-1}, which is lower than in the pure isooctane system. This may indicate that its structure is less organised than that of the isooctane gels, and hence breaks down at a lower value.

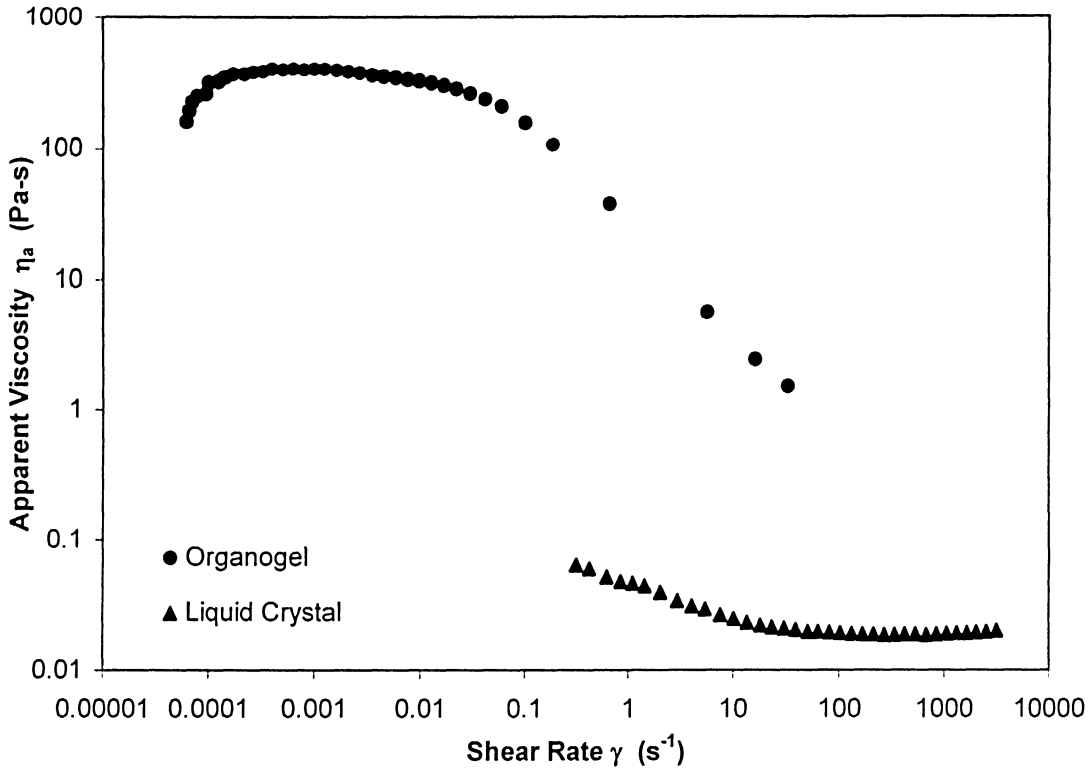

Figure 12. Apparent viscosity η_a (Pa·s) versus shear rate $\dot{\gamma}$ (s^{-1}) for samples in the PC/Water/ISO/MO (20:80 vol/vol) system.

Using plots of apparent viscosity versus shear rate, the zero-shear viscosity of the organogels was determined for both systems from the plateau region. Figure 13 illustrates the relationship between the zero-shear viscosity, η_s, of the samples for both the PC/Water/ISO and the PC/Water/ISO/MO (20:80 vol/vol) systems with water content, w_o. The plot of the PC/Water/ISO system shows that the water content modulates the viscosity of the samples. The point of maximum viscosity corresponded to the organogel with highest measured viscosity, which was ~507 Pa at w_o = 2.78. Before this maximum, the samples were clear liquids until a w_o value of ~2.00 was reached. These liquids represent reverse micelle solutions with low viscosities.

Following the point of maximum viscosity, there is a decrease in gel viscosity. This decrease in viscosity has been attributed to the formation of transient branch points that can break and reform during shearing [Cirkel *et al.*, 2001; Cirkel *et al.*, 1998]. With continued addition of water, the viscosity continues to

drop significantly, which corresponds to the regions of the phase diagram where two phases are found (the portion of the plots after the dashed line). Values of w_O between ~3.7 and 5 represent the region where both a gel and an isooctane solution are found. This is followed by another two-phase region where a liquid crystal and isooctane is found. These results are comparable to other studies that have found an increase in viscosity of the samples with an increase in water content, followed by a decrease [Schurtenberger *et al.*, 1994; Schurtenberger *et al.*, 1992; Luisi *et al.*, 1990].

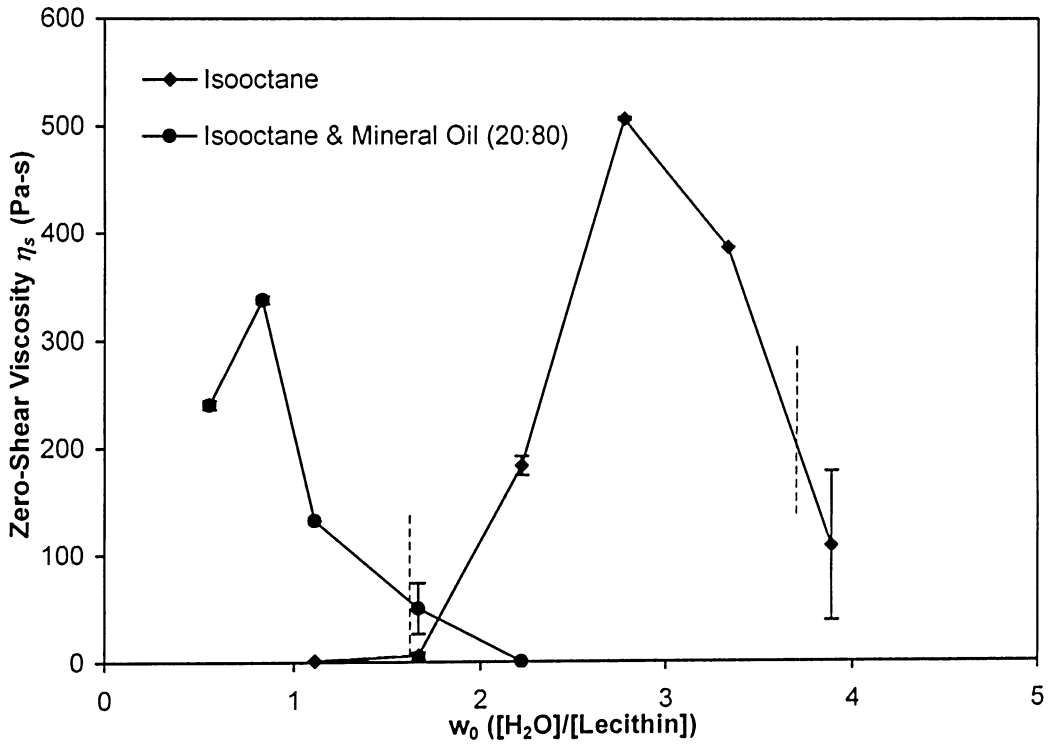

Figure 13. Zero-shear viscosity of samples in the PC/Water/ISO and PC/Water/ISO/MO (20:80 vol/vol) systems as a function of water content, w_O. Values right of the dashed lines represent the w_O values where two phases are formed (see text for details).

The plot of the PC/Water/ISO/MO system (Figure 13) follows the same general trend as the PC/Water/ISO system. The organogel with the highest measured viscosity (~337 Pa·s) occurred at w_O = 0.83. Above w_O = 1.67, the two-phase region begins, which contains a liquid crystal with the organic phase. Again,

the liquid crystal was chosen as the representative sample for this two-phase region whose viscosity is contained in the graph.

The key difference between the two systems is the larger w_O for the isooctane system to attain gelation (w_O = 2.00 - 3.50) versus the mixed system (w_O = 0.56 - 1.67). This difference in w_O values may be due to the fact that the viscosity of the mixed system, even without the addition of water, is higher than that of the isooctane system without added water. It may be that less growth of the cylindrical reverse micelles is required to produce a rigid network in the mixed system than in the pure system. Another difference is the lower viscosity of organogels in the mixed system (up to 338 Pa·s) versus the pure system (up to 507 Pa·s). The higher viscosity of the pure isooctane gels suggests that its structure may be more organised than in the mixed system.

4.1.3 Small-angle X-ray Diffraction (SAXD) Results

The SAXD curves obtained for samples within the PC/Water/ISO system for increasing values of water content (w_O = 0.56 to 3.47) are illustrated in Figures 14, 15 and 16. The SAXD curves for solutions at w_O = 0.56, 1.33 and 1.50 are shown in Figure 14. Of the three samples, there is evidence of some degree of internal order in the sample containing the least amount of water (w_O = 0.56), as small Bragg peaks appear at q values of approximately 0.11 and 0.15 Å. At this low water concentration, the sample is still a low viscosity liquid likely containing spherical reverse micelles (Schurtenberger *et al.*, 1992; Florence and Attwood, 1988). With increasing water, the peaks disappear with w_O values of 1.33 and 1.50. There is, however, a slight increase in the intensity over a wide range of q values. This is possibly representative of the beginning of a change in the structure of the spherical reverse micelles within the solution, as it is has been established that in the process of organogel formation, spherical reverse micelles elongate with the addition of water (Scartazzini e*t al.*, 1988].

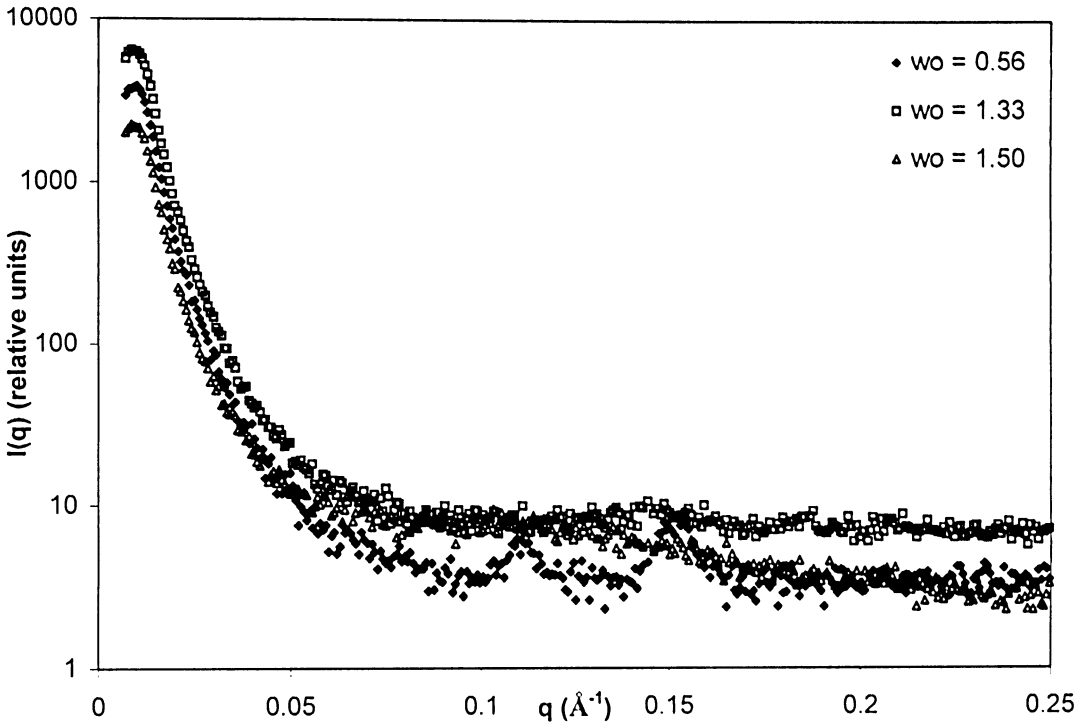

Figure 14. SAXD curves of samples in the PC/Water/ISO system, with different w_O values.

Figure 15 contains the diffraction patterns for samples at higher w_O values. Clear Bragg peaks appear in samples at w_O = 2.00 to 2.78 that have higher viscosities than the samples mentioned above. The Bragg peaks of these diffraction curves illustrate that the internal structure of the samples possess some degree of order. At a w_O value of 2.00, the first Bragg peak appears at a q value of ~0.10 Å. This peak is stronger at w_O = 2.50. With an increased water content, more individual structural units are likely formed. The sample with a w_O value of 2.78 was the most viscous organogel in this system. Its diffraction pattern clearly shows the appearance of second and third Bragg peaks that overlap, at q values of approximately 0.14 and 0.15 Å.

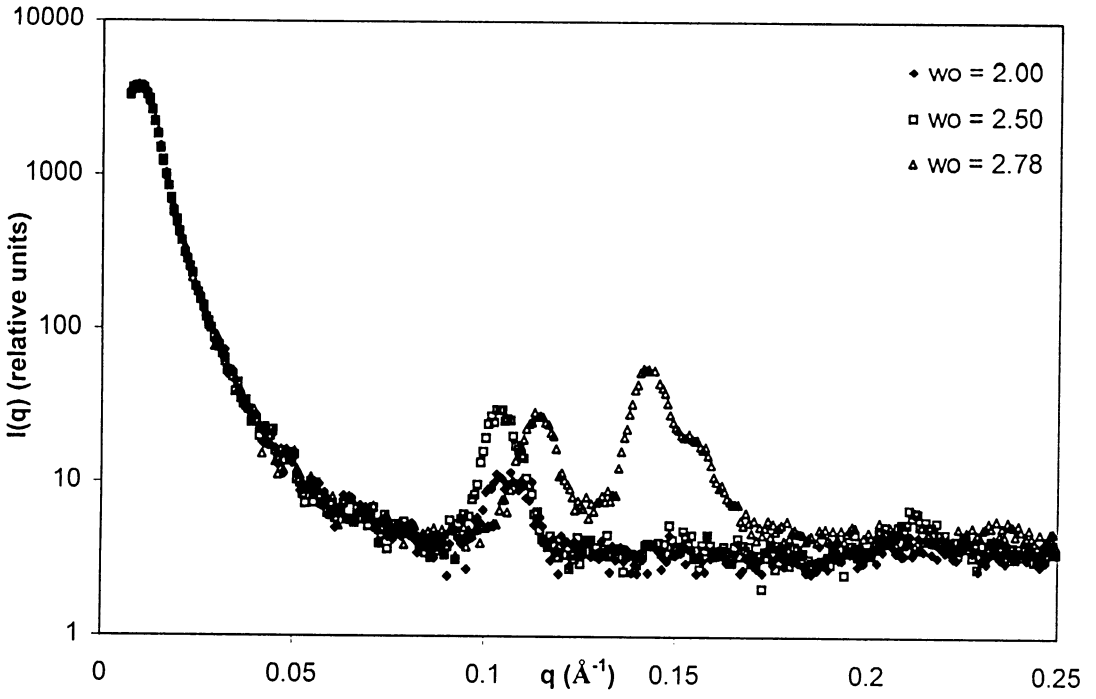

Figure 15. SAXD curves of samples in the PC/Water/ISO system, with different w_O values.

Figure 16 contains the SAXD curves for samples with w_O values greater than 2.78. At w_O = 3.00, three peaks at q values of 0.11, 0.13 and 0.15 Å still appear, though these are visibly diminished. At w_O values of 3.33 and 3.47, there is the presence of peaks at q values of 0.11, 0.15 and 0.23 Å.

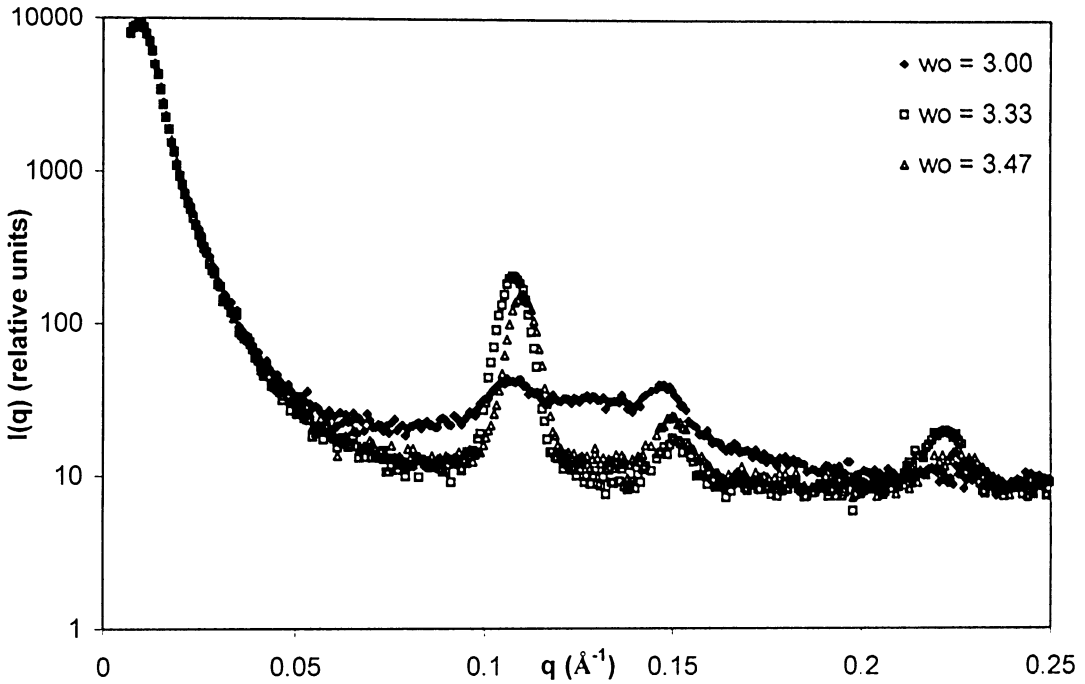

Figure 16. SAXD curves of samples in the PC/Water/ISO system, with different w_O values.

Table 2 presents the structural parameters that characterise the internal order of the samples in the PC/Water/ISO system with varying water content for the peak at q = 0.11 Å. This peak was selected as it offers information about the characteristic size of the molecular structure of the organogel, as will be discussed later. The structural parameters were calculated from the Bragg peaks seen in the diffraction patterns using Equations [2] to [6].

From these results, it can be seen that for samples with w_O = 2.00 to 3.47, the values of the mean long-range order, L, and the radius of interaction, r_m, both experience an increase with increasing water content in the samples, from 618 to 884 Å and 977 to 1396 Å, respectively. In contrast, the values of the degree of disorder decrease from 0.099 to 0.081. The notable exception to these trends is found in the organogel at w_O = 3.00, which may be due to an inexact determination of the full-width-at-half-maximum values used in the calculations caused by the ill-definition of the peaks. The overall trends are, however, in agreement with theory, and clearly illustrate that the degree of

order within the molecular structure of the PC/Water/ISO system is water concentration-dependent. It may be postulated that the reduced value of the $\bar{d}$ of ~55 Å at w_O = 2.78 as opposed to ~60 Å for all the other samples may be attributed to the very close packing of the cylindrical reverse micelles that may occur in the organogel of maximum viscosity. The physical meaning of the calculated values of the mean long-range order and the radius of interaction as related to the molecular structure of the organogels will be discussed later on.

Table 2. Structural characteristics of organogel molecular structure in the PC/Water/ISO system obtained from SAXD data.

w_O	q_{max} (Å)	$\bar{d}$ (Å)	L (Å)	r_m (Å)	$\frac{\Delta}{\bar{d}}$
2.00	0.11	59.8	618	977	0.099
2.50	0.10	60.8	721	1137	0.092
2.78	0.11	54.9	742	1170	0.087
3.00	0.11	60.0	475	749	0.112
3.33	0.11	58.2	841	1328	0.084
3.47	0.11	57.1	884	1396	0.081

These peaks represent interplanar distances or d-spaces (Å), which provide evidence for the existence of long-range order that defines a crystal lattice. The values of the d-spacings were calculated using Equation (6). An attempt was therefore made to find the lattice that was formed by indexing the observed reflections. In doing so, it was found that one main lattice existed within the range of samples that were tested.

The basic lattice was found for samples corresponding to the regions both before (w_O = 0.56, 2.00, 2.50) and after (w_O = 3.33, 3.47) the region of the most viscous gels (w_O = 2.78, 3.00). Based on the peak analysis, this lattice was determined to have two-dimensional ordering with a unit cell in the shape of a square. The lack of evidence of ordering along the c-axis was based on the absence of any peaks corresponding to the (111) position, clearly indicated that

the ordering was two-dimensional. The square-shaped unit cell forms the lattice given by Space Group #11, p4mm (Appendix: Figure A1). The SAXD curves are illustrated in Figure 17 and the peak analysis is listed in Table 3, which contains the d-spaces and the corresponding indices for increasing water contents of the samples both before and after the region of maximum organogel viscosity.

Table 3. SAXD analysis for samples in the PC/Water/ISO system (2D lattice).

w_O ([H_2O]/[PC])	Indices (hk)	2-theta (degrees)	d-spacings (Å)
0.56	(11)	1.55	56.78
	(20)	2.13	41.47
2.00	(11)	1.47	59.90
2.50	(11)	1.45	60.76
	(22)	3.00	29.44
3.33	(11)	1.52	58.19
	(20)	2.13	41.46
	(22)	3.13	28.18
3.47	(11)	1.55	57.06
	(20)	2.11	41.93
	(22)	3.17	27.87

In view of the above data, the following can be deduced. The largest d-spacing of ~60 Å, obtained from the first reflection on the SAXD curves, provides the length of the side of the unit cell making up the p4mm lattice. The characteristic size, or lattice parameter, of the ordered structure is thus 85 Å, given by the index (10). The Bragg reflection corresponding to a d-spacing of 85 Å does not appear in the SAXD curves for any of the samples since this reflection is not allowed based on lattice conditions (Appendix: Figure A1).

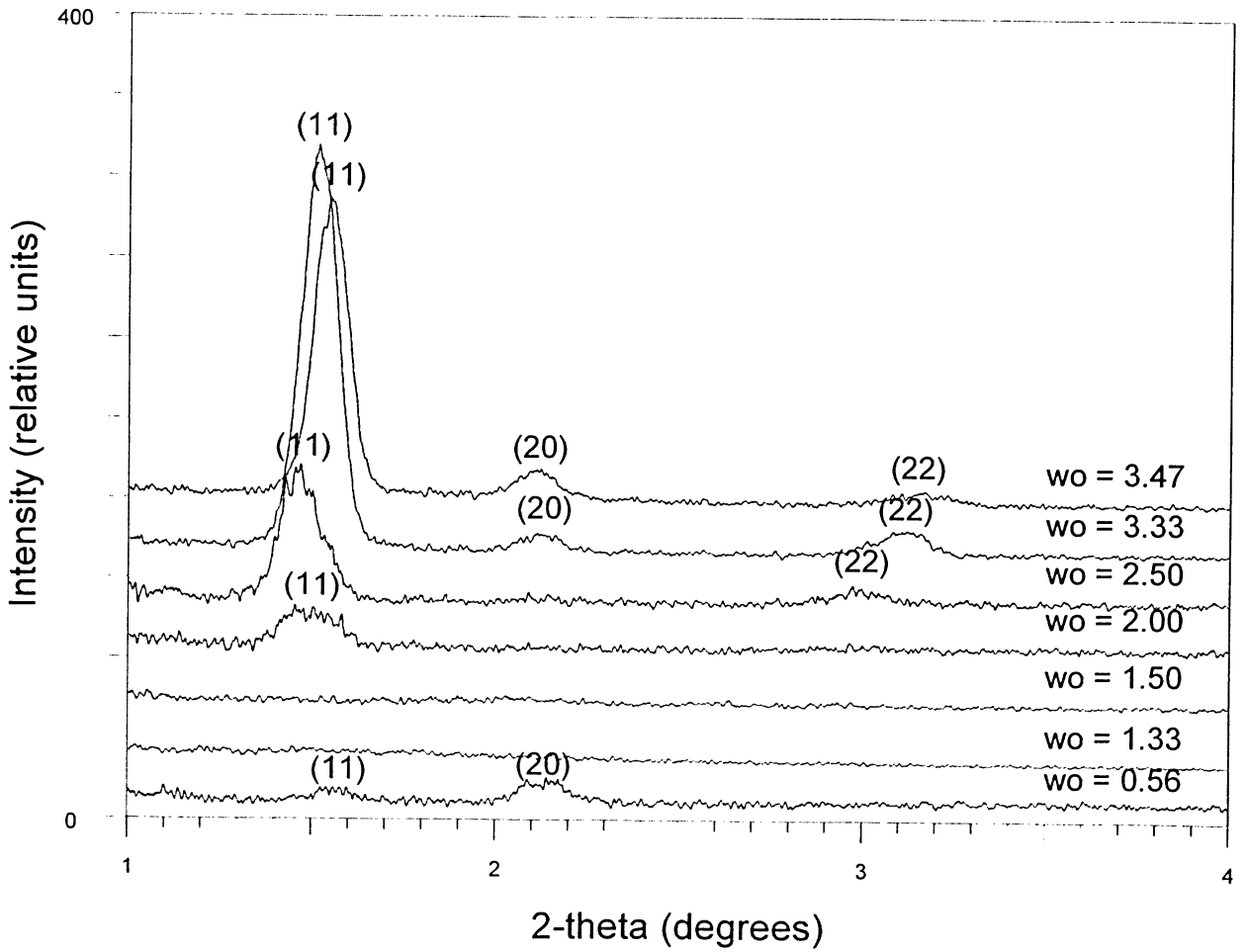

Figure 17. SAXD curves for samples from the PC/Water/ISO system before and after the region of the most viscous gels. The 2D square lattice indices and the corresponding w_o values are shown. Plots have been shifted upward for ease of viewing.

The evaluated lattice appeared to have no significant change with increase of water, as the largest d-spacing provided by the peaks of the SAXD curves remained within the same range for most of the analysed gels (~60 Å). In such a lattice, the value of 60 Å may be related to the mean diameter of the structural units, which in this system is most likely a cylindrical reverse micelle. This postulate is supported by the results of other studies that have found the cross-sectional radius of the reverse micelles to be approximately 30 Å [Schurtenberger *et al.*, 1996; Schurtenberger *et al.*, 1991]. Such an arrangement can be achieved by close packing or bundling of cylindrical shaped reverse micelles, as shown in Figure 18 (a) and (b). The minimum number of these structural units forming the bundle would therefore have to be nine, in order to achieve the crystal structure of the p4mm lattice.

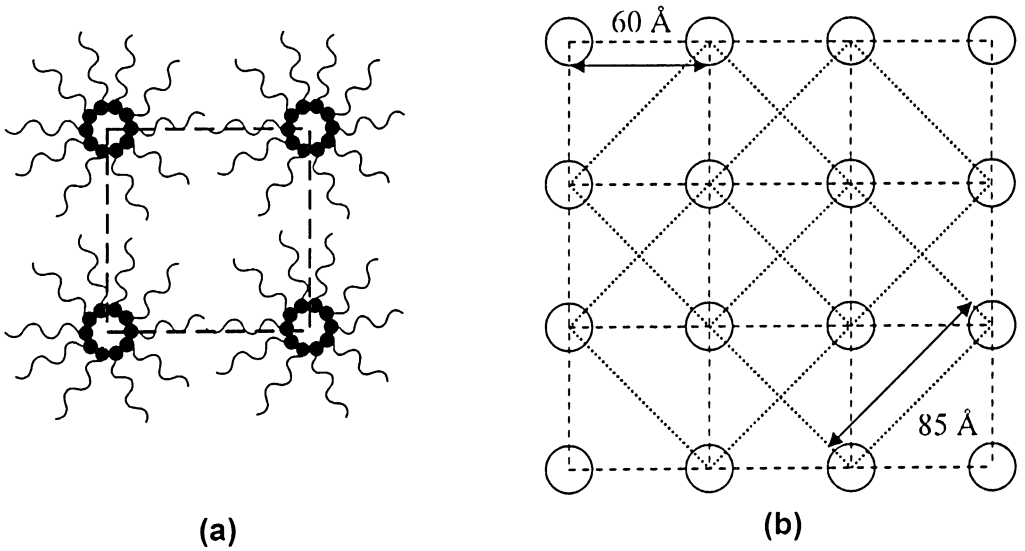

Figure 18. (a) [100] projection of the 2D unit cell (length of side = 60 Å) formed by the cylindrical bundles (dashed lines) in organogels in the PC/Water/ISO system and (b) [100] projection of the arrangement of the unit cells to form the structure of the cylindrical bundles. The circles represent the cross-section of cylindrical reverse micelles as shown in (a).

The SAXD results for samples with w_o = 2.78 and 3.00 are illustrated in Figure 19 and their peak analysis, in Table 4, contains the d-spaces and the corresponding plane indices. These results indicate that, with an increase in water content from just 2.50 to 2.78, a structural transformation takes place, based on the appearance of reflections corresponding to d-spacings of ~44 - 48

Å. This d-spacing corresponds to the plane index (111), which indicates that the formation of a three-dimensional cubic crystal lattice occurs in the organogels of highest measured viscosity. Based on the crystal lattice determined for samples with w_O values of less than 2.78 and greater than 3.00, however, it is suggested that this three-dimensional structure is related to the p4mm arrangement of the bundles of cylindrical reverse micelles. It is possible that their dynamic nature may allow them to be oriented in different directions, such that they form the faces of the three-dimensional lattice. This may be possible with the very tight packing of a large number of ordered molecular structures. This postulate is supported by the observed reduction in size of the largest d-spacing for the organogel with the maximum measured viscosity (at w_O = 2.78), as discussed previously.

Table 4. SAXD analysis for samples in the PC/Water/ISO system (3D lattice).

w_O ([H_2O]/[PC])	Plane Indices (hkl)	2-theta (degrees)	d-spacings (Å)
2.78	(110)	1.60	54.90
	(111)	2.00	44.12
	(200)	2.17	40.60
3.00	(110)	1.50	58.96
	(111)	1.81	48.67
	(200)	2.08	42.44

The non-birefringency of the organogels studied in the present work as well as in other work [Luisi *et al.*, 1990; Schurtenberger *et al.*, 1990] may be explained by the fact that the ordered regions are made up of unit cells of a square lattice. Materials are optically active if, when plane-polarised light is passed through them, the plane of polarisation is rotated. This phenomenon is not observed in materials that possess a centre of symmetry [Woolfson, 1979]. The perfect planar symmetry of squares result in them being isotropic in nature. They are therefore optically inactive when viewed through polarised light and so are non-birefringent [Schurtenberger *et al.*, 1990]. Another possibility for the samples to show no optical activity is if the size of the ordered regions is well below the limit of detection by the instrument [Woolfson, 1979].

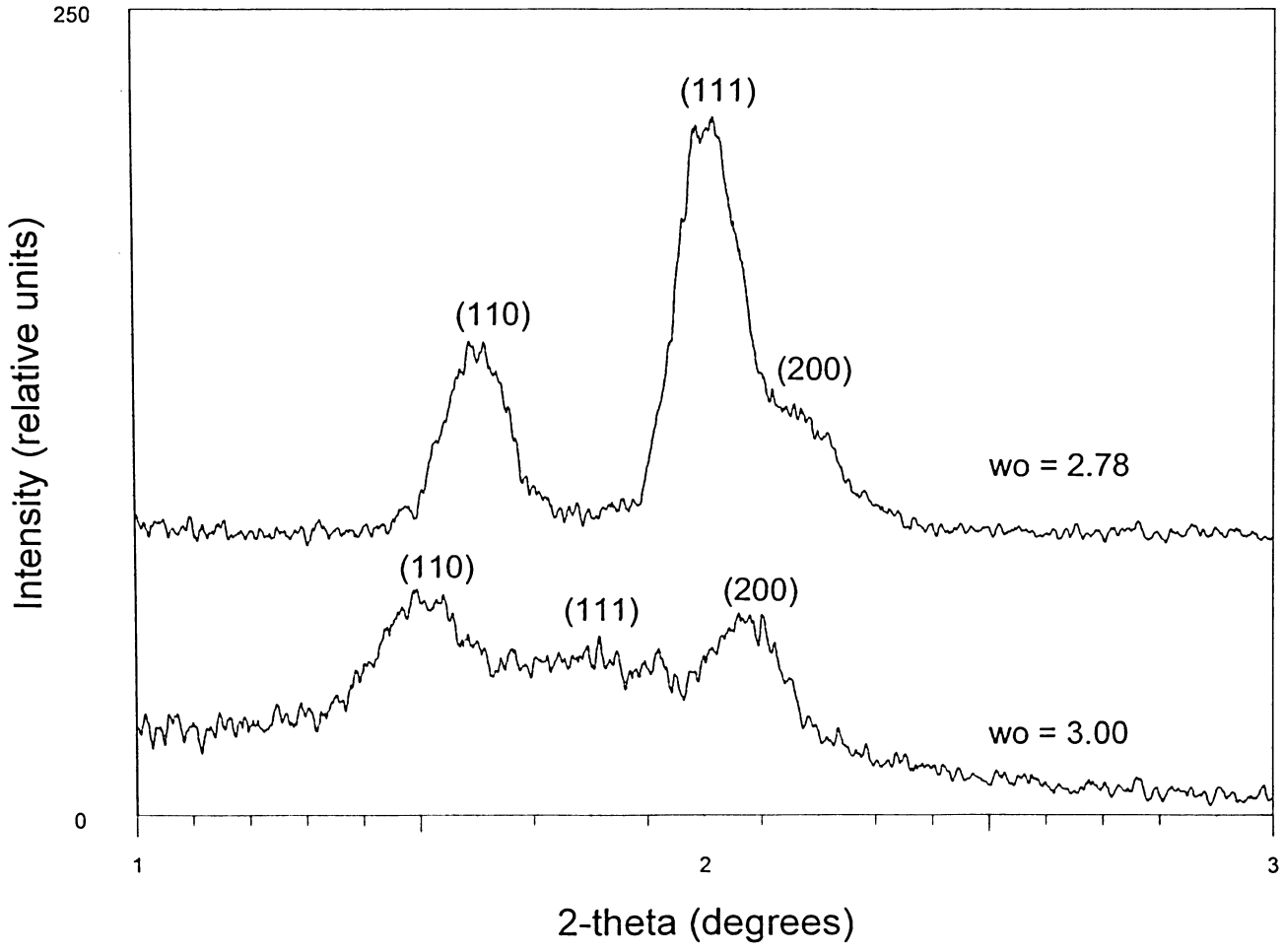

Figure 19. SAXD curves for samples from the PC/Water/ISO system in the region of the most viscous gels. The 3D cubic lattice indices and the corresponding w_o values are shown. Plots have been shifted upward for ease of viewing.

From the values of the structural characteristics shown in Table 2, the values listed of the mean long-range order, *L*, gives the approximate size of the bundles present in the network and the values of the radius of interaction gives the maximum distance between these ordered regions. The parameter, *L*, may correspond to either the thickness or length of these bundled cylinders. The overall molecular structure of the organogels produced in this study, therefore, likely consists of a crosswork of highly ordered elongated reverse micelles that entrap solvent within the pockets of its network.

In summary, organogels made using isooctane are formed by the bundling or close packing of the cylindrical reverse micelles into a square-shaped lattice. This lattice forms part of the entangled network that immobilises the solvent molecules. The diameter of these structures has been found to be approximately 60 Å. The phenomenon of the highly organised packing of the cylindrical reverse micelles has also been observed in studies of other organogels made with various organogelators [Jeong *et al.*, 2005; Sakurai *et al.*, 2003; Gronwald *et al.*, 2002; Terech *et al.*, 1994].

4.2 Lecithin-Water-Isopropylpalmitate-Mineral Oil

4.2.1 Ternary Phase Behaviour

A partial ternary phase diagram for the lecithin/water/isopropylpalmitate (PC/Water/IPP) system at 25°C is presented in weight fractions in Figure 20. This diagram describes the short-term phase behaviour of the system, as the information was collected 24 hours after sample preparation.

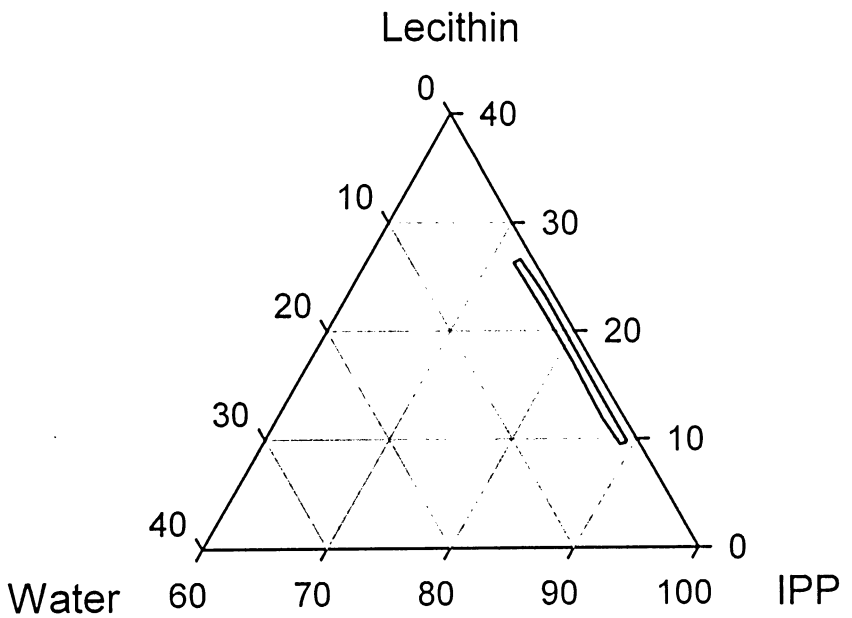

Figure 20. Partial ternary phase diagram of the PC/Water/IPP system.

The region of major interest and focus was the organogels region, found as a narrow strip along the 'water-lean' region of the diagram. An increase in water concentration up to ~1.5 wt%, resulted in an increase in the viscosity of the samples, until gelation occurred. This region is bordered by lecithin weight percents of ~10 to 26 wt%. At the gel point, the viscosity of the sample is at its highest value, and corresponds to a range of w_o values of ~2.0 to 4.0. This range is in agreement with other research [Angelico *et al.*, 2005]. With increasing water content, the formation of three phases was observed. This consisted of the organic phase on top, an organogel in the middle and a liquid

crystal at the very bottom. The liquid crystal was identified by its birefringency under polarised light (Figure 21).

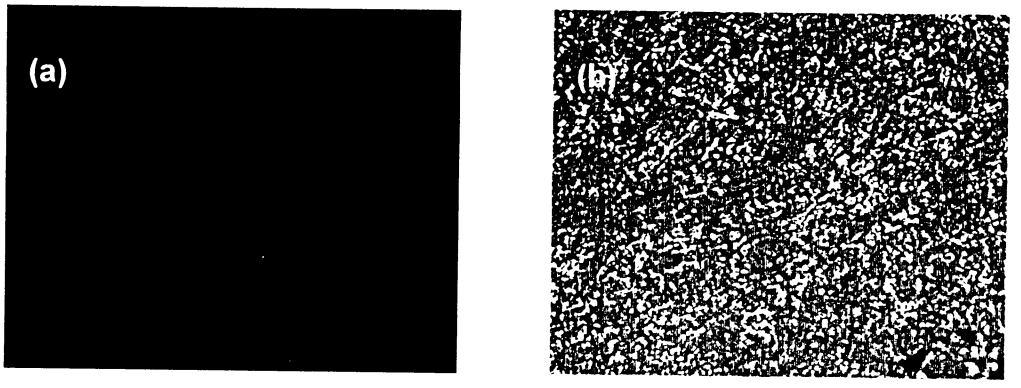

Figure 21. Polarised light microscopy of samples in the PC/Water/IPP system for (a) an organogel ($w_O \approx 3.5$) and (b) a liquid crystal ($w_O \approx 5.6$).

The organogel region for the lecithin-water-isopropylpalmitate-mineral oil (PC/Water/IPP/MO) system with ratios of isopropylpalmitate to mineral oil of 50:50 vol/vol and 30:70 vol/vol were developed (Figure 22).

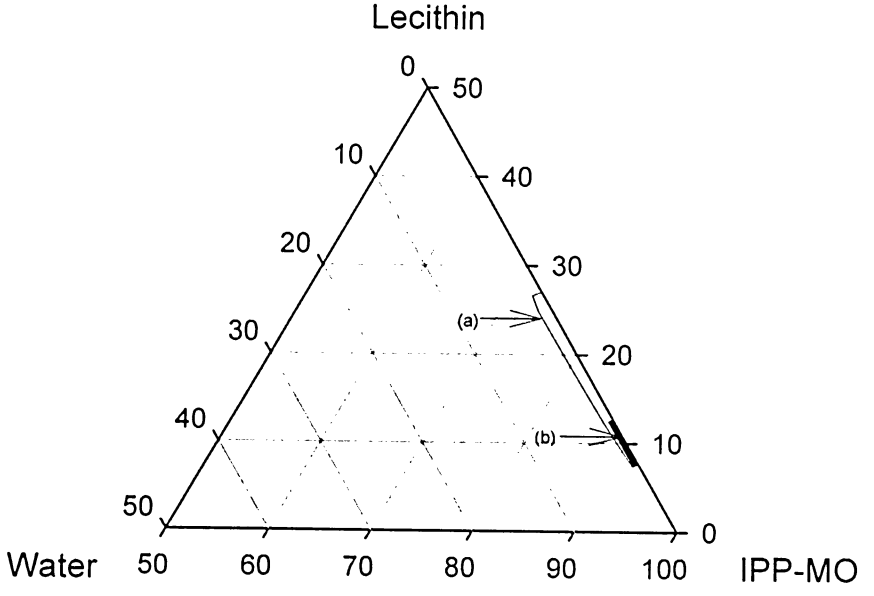

Figure 22. Partial ternary phase diagram of the PC/Water/IPP/MO (50:50) system (solid line) and (b) PC/Water/IPP/MO (30:70) system (shaded).

For the PC/Water/IPP/MO (50:50 vol/vol) system (Figure 22 (a) bound by solid line), the organogel region is bounded by a maximum concentration of ~1 wt% water and 8 - 26 wt% lecithin. For the PC/Water/IPP/MO (30:70 vol/vol) system (Figure 22 (b) shaded area), the organogel region is bounded by a maximum water content of ~0.3 wt% and a range of lecithin content of ~8 to 12 wt%. The difference in the size of the organogel region for these two systems may be due to the low solubility of lecithin in mineral oil, thus causing the systems with more isopropylpalmitate to be larger. Briefly, once passed the region of organogel formation for both systems, it was noted that three-phases formed, consisting of organic phase on top, an organogel in the middle and a liquid crystal at the very bottom. The liquid crystals were identified by their birefringency under polarised light (Figure 23 and Figure 24).

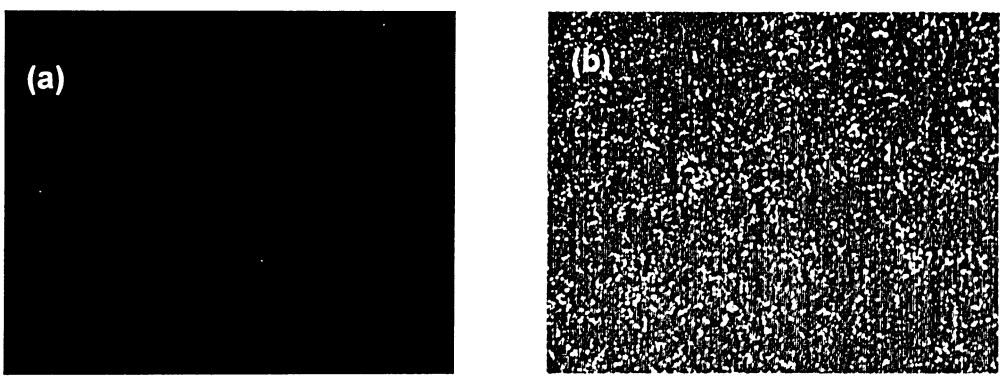

Figure 23. Polarised light microscopy of samples in the PC/Water/IPP/MO (50:50 vol/vol) for (a) an organogel ($w_O \approx 2.0$) and (b) a liquid crystal ($w_O \approx 3.5$).

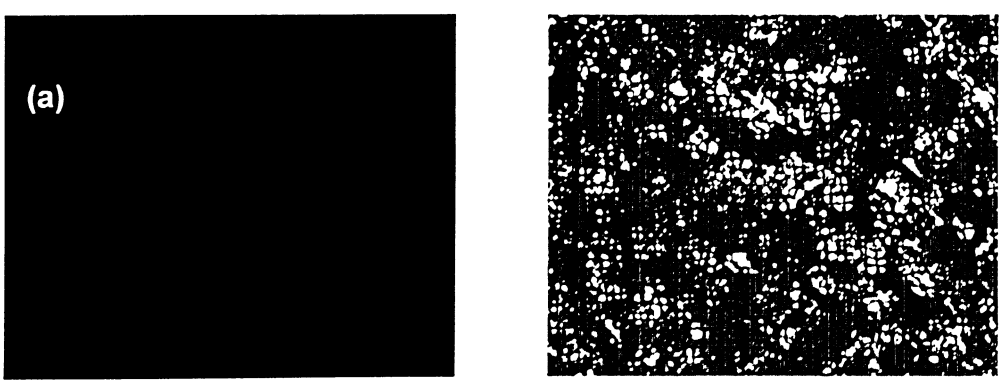

Figure 24. Polarised light microscopy of samples in the PC/Water/IPP/MO (30:70 vol/vol) for (a) an organogel ($w_O \approx 1.0$) and (b) a liquid crystal ($w_O \approx 2.0$).

Finally, the partial ternary phase diagram for the lecithin-water-mineral oil (PC/Water/MO) system was developed. Again, only the region of organogel formation was established (Figure 25) and it should be noted that the preparation of organogels in this system was possible only by heating the mixtures up to ~85 °C. It was found that pure lecithin organogels were formed with water and lecithin contents up to ~0.3 wt% and ~26 wt%, respectively. Above this lecithin concentration, organogel preparation was very difficult, due to the highly viscous nature of the samples. With increased water content, it was found that a two-phased region of an organogel and a liquid crystal existed. The liquid crystal was identified by its birefringency under polarised light (Figure 26).

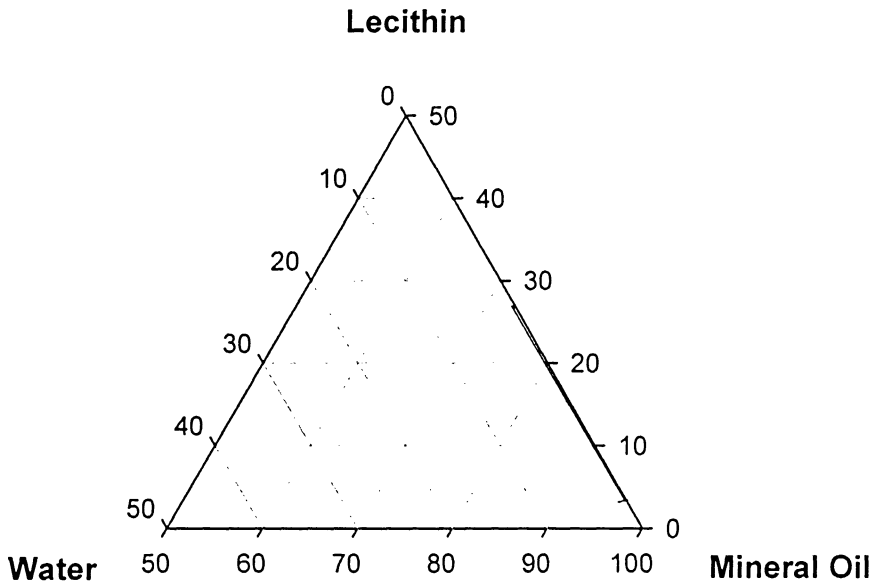

Figure 25. Partial ternary phase diagram of the PC/Water/MO system.

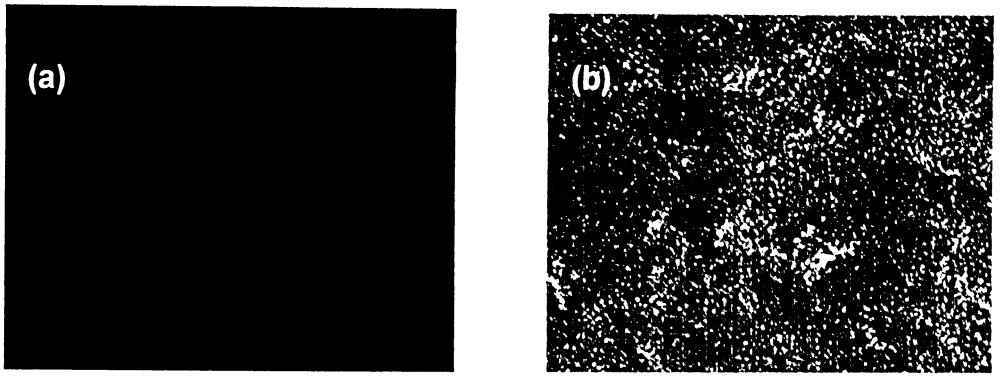

Figure 26. Polarised light microscopy of samples in the PC/Water/MO system for (a) an organogel ($w_O \approx 0.56$) and (b) a liquid crystal ($w_O \approx 1.5$).

4.2.2 Rheological Behaviour

The rheological behaviour of lecithin organogels containing biocompatible materials, such as isopropylpalmitate, as the organic phase has been recently investigated [Angelico *et al.*, 2005]. In the present study, the rheological behaviour of samples made using isopropylpalmitate (IPP), isopropylpalmitate and mineral oil (30:70 vol/vol and 50:50 vol/vol), and mineral oil (MO) as the organic phase was examined.

Figures 27 to 30 illustrate the dependence of apparent viscosity η_a (Pa·s) on shear stress (Pa) of the PC/Water/IPP, PC/Water/IPP/MO (50:50 vol/vol), PC/Water/IPP/MO (30:70 vol/vol) and the PC/Water/MO systems, respectively. The main feature of these graphs is that the apparent viscosity of the organogels in each system is relatively constant with increasing shear rate for a measured range of shear rate values, thereby exhibiting Newtonian behaviour. It should be pointed out however that regions of Newtonian behavior were very narrow for certain samples. At a critical shear rate, the viscosity begins to abruptly decrease, which is likely due to the breakdown of the structural network within the organogels. Such shear-thinning may be due to the breakdown of the structural network within the organogels [Rao, 1999]. The rheological behaviour of the organogels in the three-phased systems was

inconclusive, as it was possible for the gels tested to contain trace amounts of the other phases.

The viscosities of the organogels decrease at shear rate values of ~2 s^{-1}, ~0.4 s^{-1}, ~0.2 s^{-1} and ~0.03 s^{-1} for the systems of PC/Water/IPP, PC/Water/IPP/MO (50:50 vol/vol), PC/Water/IPP/MO (30:70 vol/vol) and PC/Water/MO, respectively. The yield point evolution as a function of shear rate directly relates to an increase in mineral oil content in the samples. It is therefore possible that this decrease in yield point is also directly related to the molecular structures of the organogels, as they may become more disordered with increasing mineral oil content.

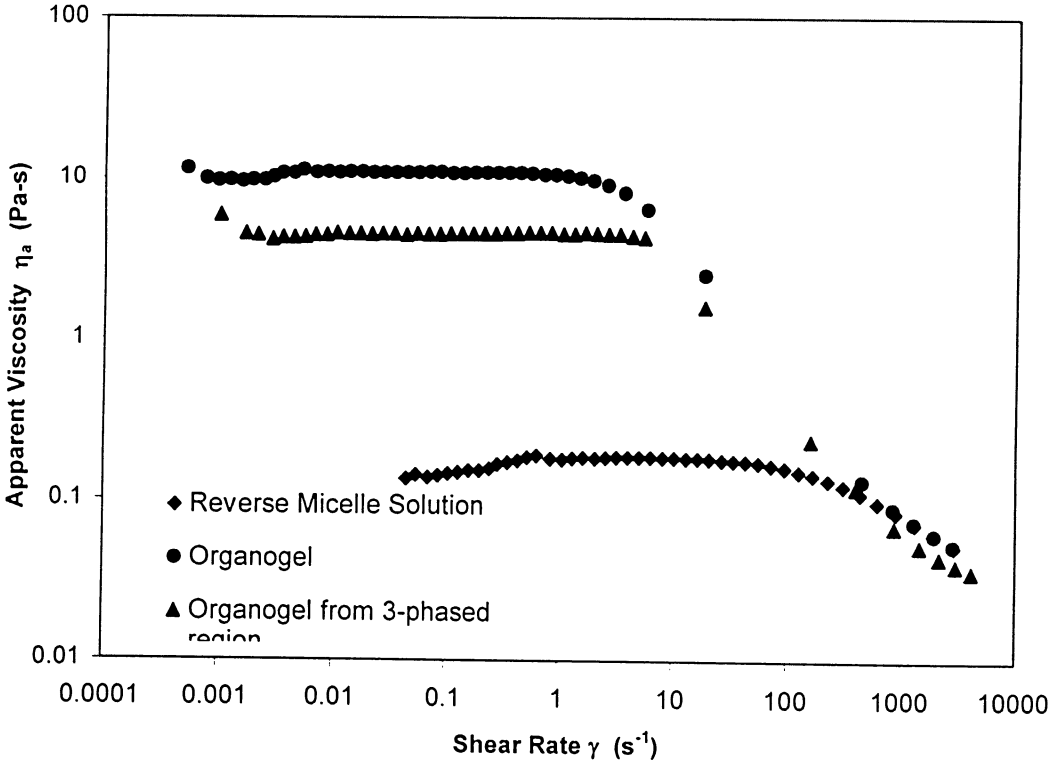

Figure 27. Apparent viscosity (Pa·s) versus shear rate (s^{-1}) for samples in the PC/Water/IPP system.

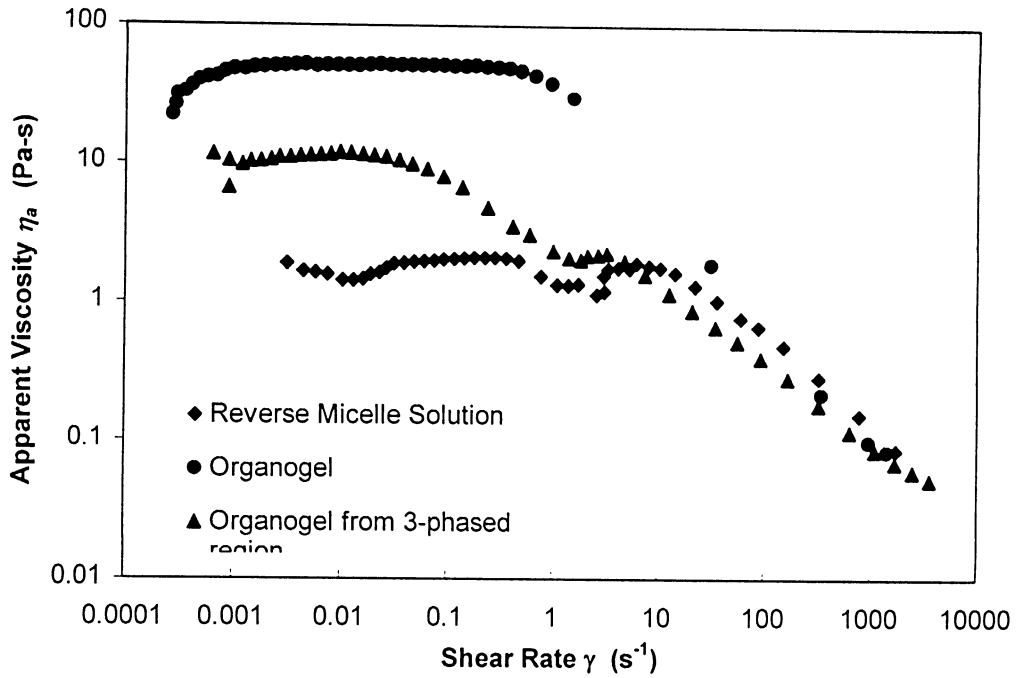

Figure 28. Apparent viscosity (Pa·s) versus shear rate (s^{-1}) for samples in the PC/Water/IPP/MO (50:50 vol/vol) system.

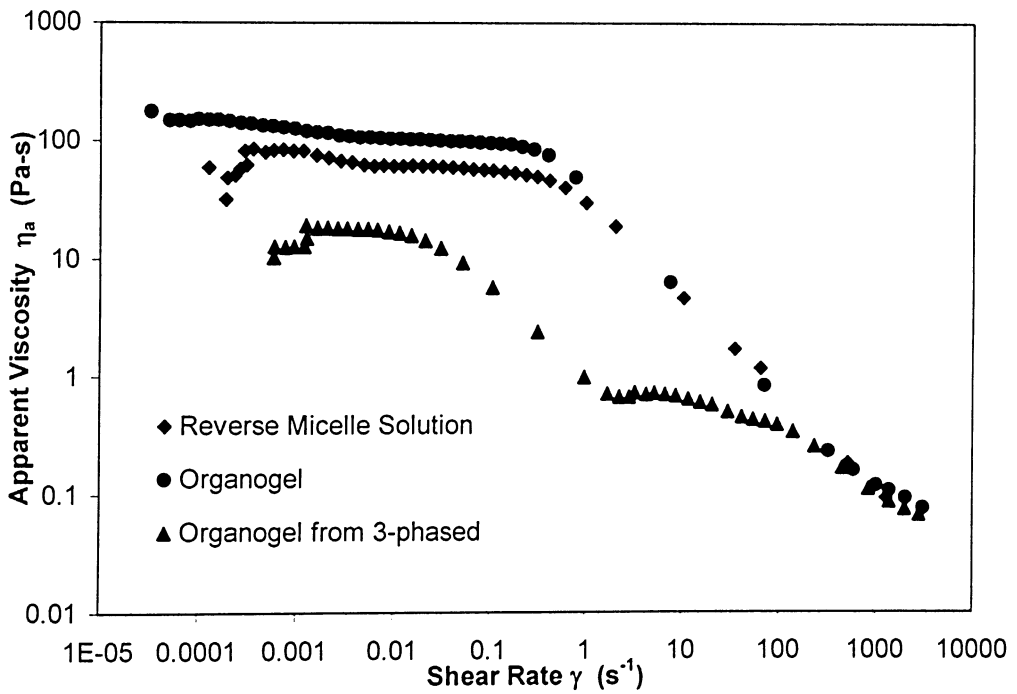

Figure 29. Apparent viscosity (Pa·s) versus shear rate (s^{-1}) for samples in the PC/Water/IPP/MO (30:70 vol/vol) system.

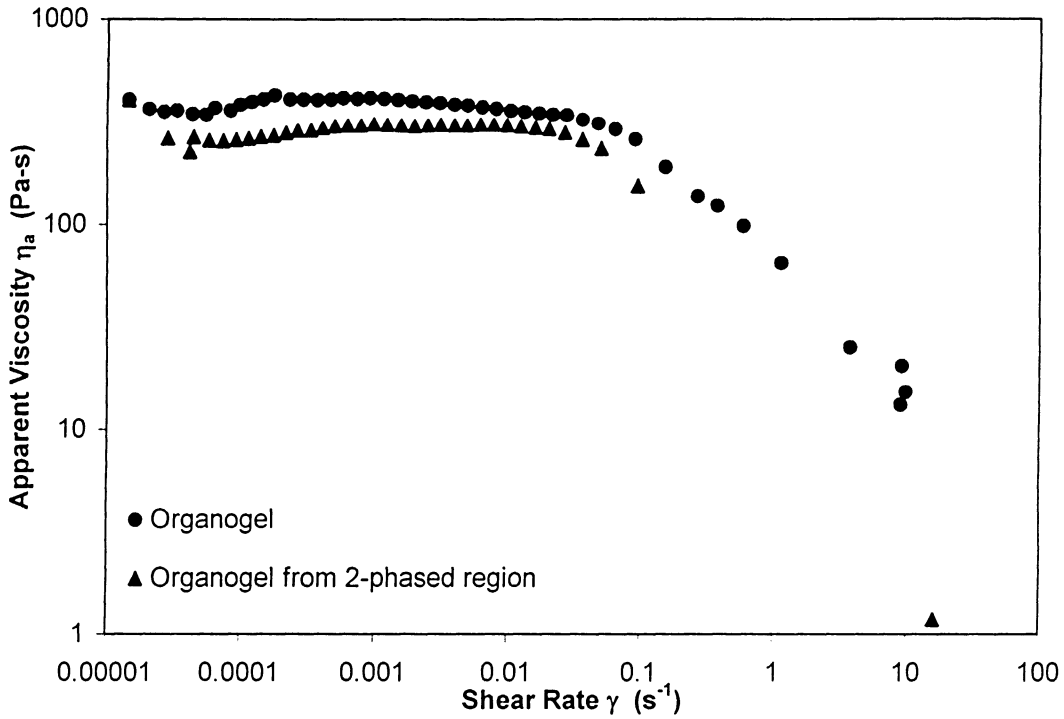

Figure 30. Apparent viscosity (Pa·s) versus shear rate (s^{-1}) for samples in the PC/Water/MO system.

Using plots of apparent viscosity versus shear rate, the zero-shear viscosity of the organogels was determined from the plateau region. Figure 31 illustrates the relationship between the zero-shear viscosity of the samples for the PC/Water/IPP, PC/Water/IPP/MO (50:50 vol/vol), PC/Water/IPP/MO (30:70 vol/vol), and the PC/Water/MO systems with water content, w_0. These graphs helped establish the value of w_0 for the most viscous organogels in each system.

One of the main differences between the plots for the four systems is the amount of water required to produce the organogel of maximum viscosity. The IPP system requires a large amount of water, corresponding to $w_0 \approx 3.5$ to produce an organogel with the highest viscosity. The systems containing a mixed organic phase (the 50:50 vol/vol and 30:70 vol/vol IPP/MO systems) require lower w_0 values than that of the pure IPP, of 2 and 1 respectively, for the production of the organogel with highest viscosity. Finally, the pure MO system requires the least amount of water to attain the organogel with highest

viscosity, corresponding to w_o = 0.50. The reduced values of w_o required for gelation for systems with increasing mineral oil content may be due to the viscous nature of the solutions even before the addition of water. It may also be possible that the isopropylpalmitate system requires more water for the growth of the cylindrical reverse micelles since it may hinder lecithin-water interactions, as discussed in Section 1.2.

The other key characteristic in Figure 31 is the difference in viscosity in relation to mineral oil content. Maximum viscosities for the PC/Water/IPP, PC/Water/IPP/MO (50:50 vol/vol), PC/Water/IPP/MO (30:70 vol/vol) and PC/Water/MO systems are 11 Pa.s, 44 Pa.s, 111 Pa.s, and 399 Pa.s, respectively. Figure 32 gives provides a graphical representation of this evolution.

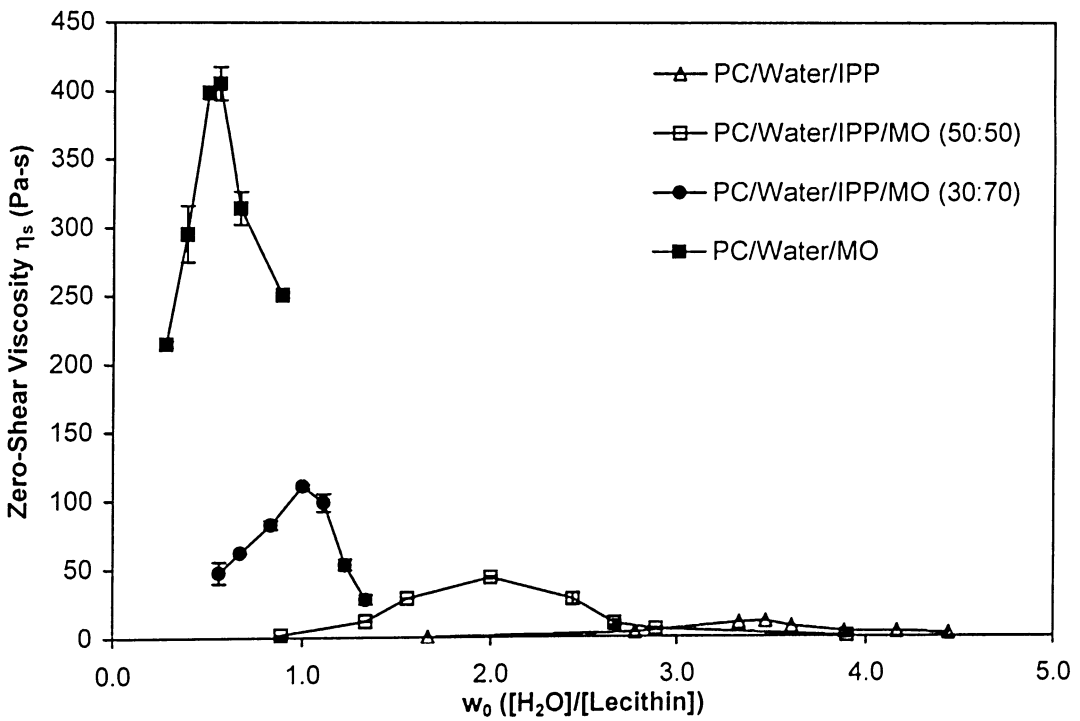

Figure 31. Relationship between zero-shear viscosity and water content for organogels in the PC/Water/IPP, PC/Water/IPP/MO (50:50 vol/vol), PC/Water/IPP/MO (30:70 vol/vol) and PC/Water/MO systems.

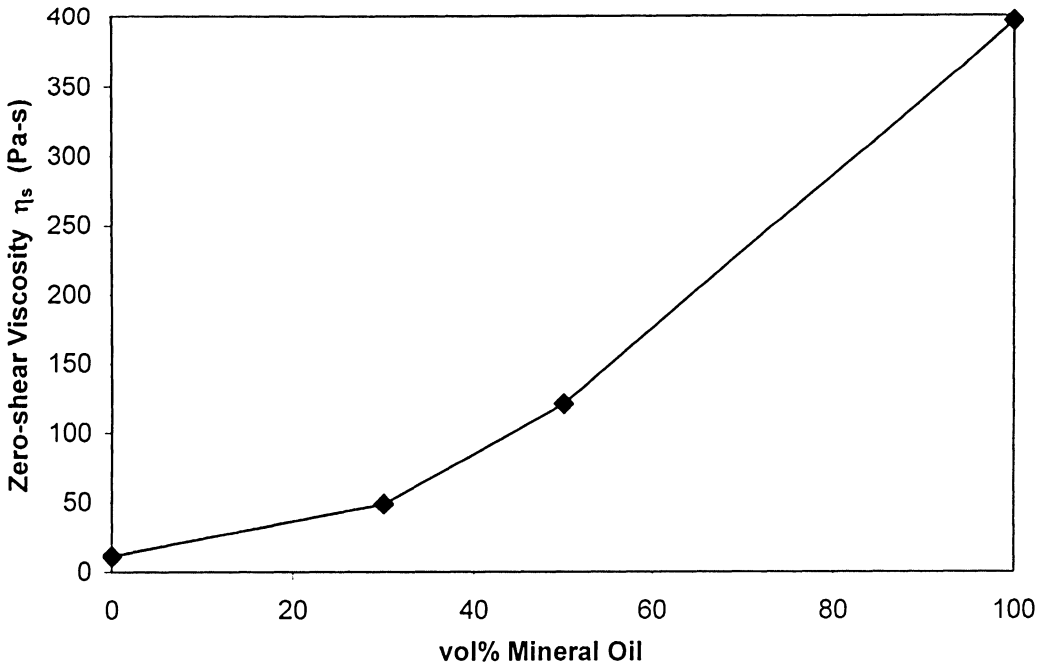

Figure 32. Relationship between zero-shear viscosity and volume percent of mineral oil in organogels.

4.2.3 Small-angle X-ray Diffraction (SAXD) Results

SAXD curves for the PC/Water/IPP and PC/Water/MO systems are shown in Figures 33-36. SAXD curves for the (PC/Water/IPP) system with w_O = 0.56 to 3.47 are shown in Figures 33-35. The sample with w_O = 3.47 corresponds to the organogel of maximum viscosity. For all of these samples, regardless of w_O value there were no Bragg peaks in the scattering curves. The absence of these peaks implies a lack of structural order or too low a concentration of structural units, below the detection limit of the instrument.

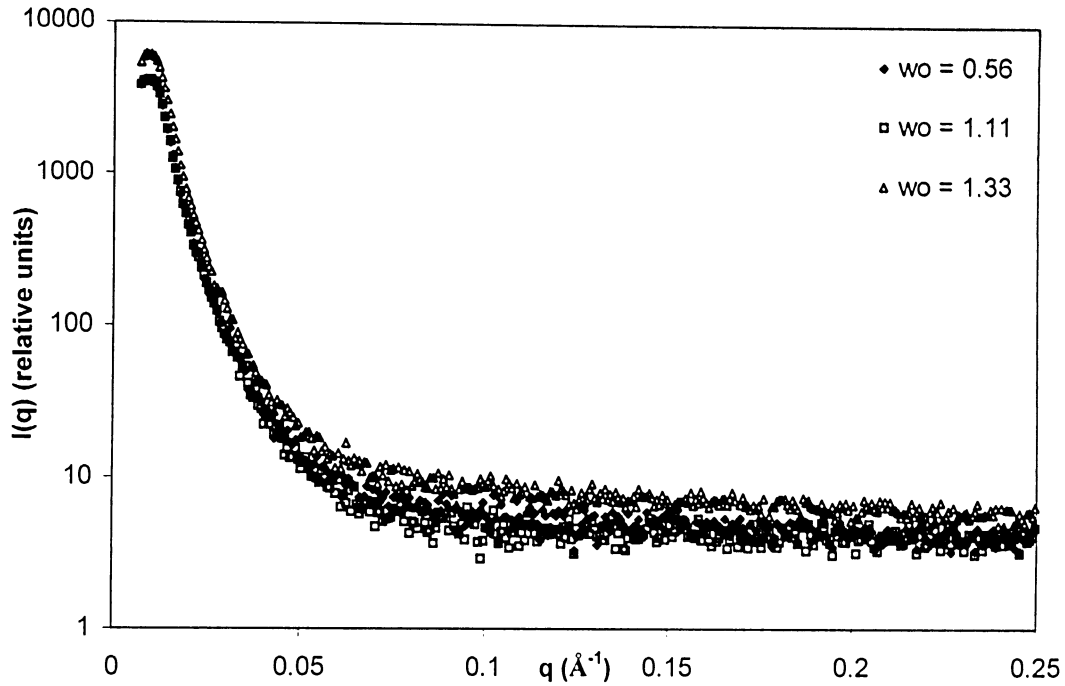

Figure 33. SAXD curves of samples in the PC/Water/IPP system for low w_O values.

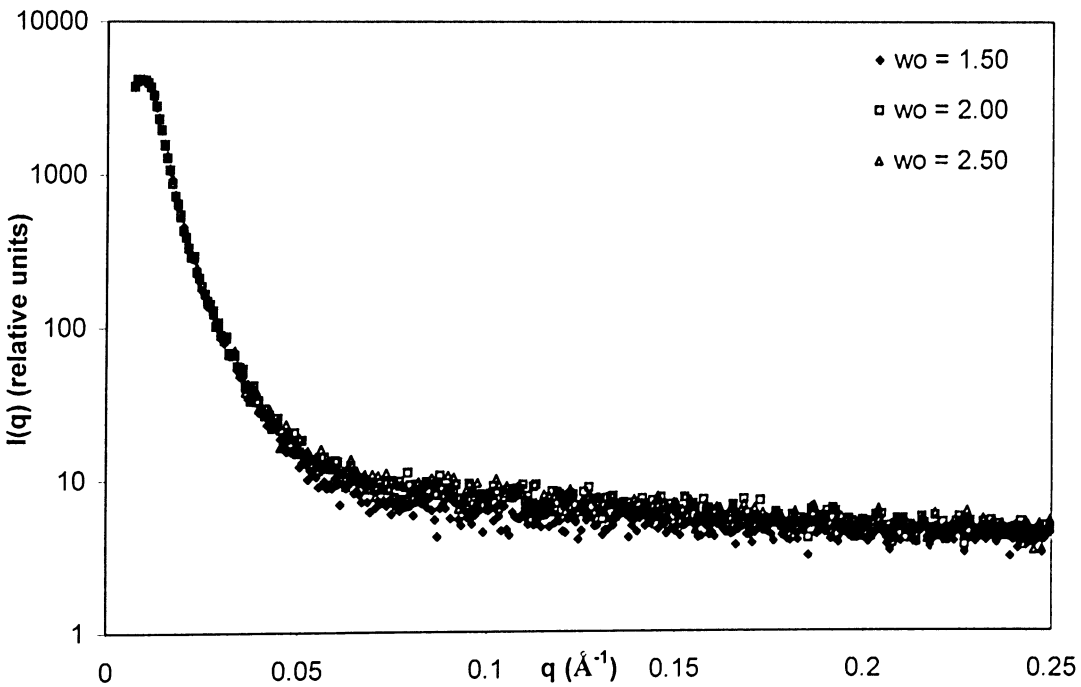

Figure 34. SAXD curves of samples in the PC/Water/IPP system for mid-range w_O values.

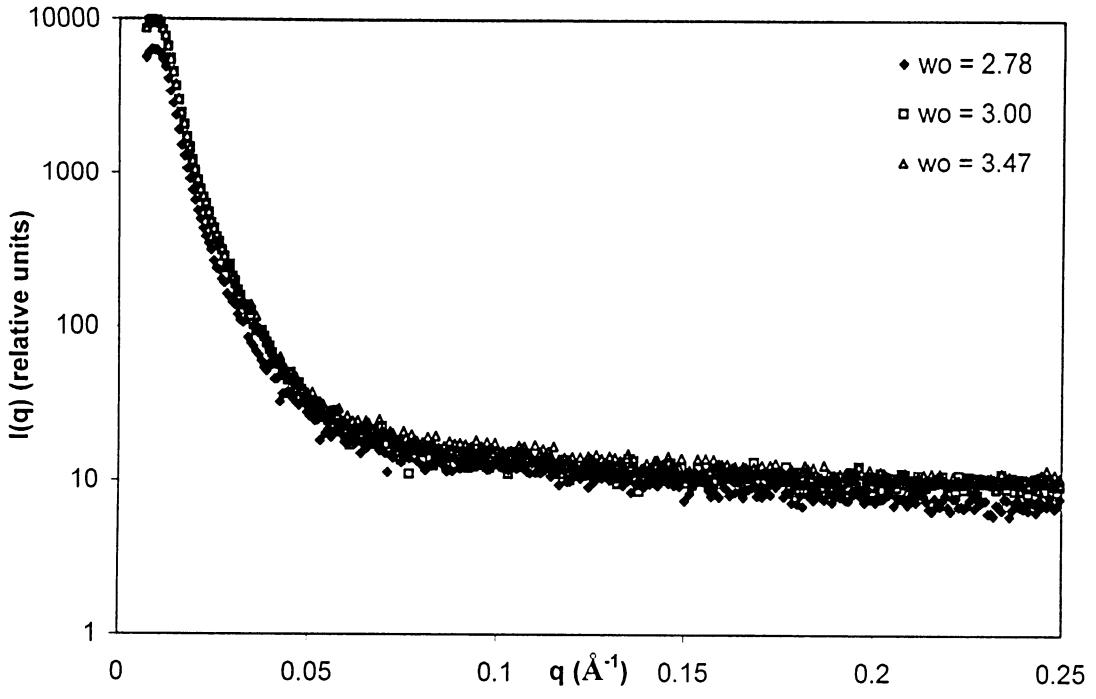

Figure 35. SAXD curves of samples in the PC/Water/IPP system for high w_o values.

Figure 36 shows the PC/Water/MO at w_o = 0.33, 0.44 and 0.56. All three of these samples were organogels as it was not possible to prepare samples of lower w_o values. The sample with a water content of 0.56 corresponds to the organogel of maximum viscosity. It is notable that although these samples are gels, there is no indication of any degree of order as noted by the absence of Bragg peaks. Since these are gels, however, they must possess a molecular structure that is responsible for the high viscosities encountered. The absence of any diffraction peaks implies molecular disorder within the sample or that the concentration of the structural units is too low to be detected by the instrument.

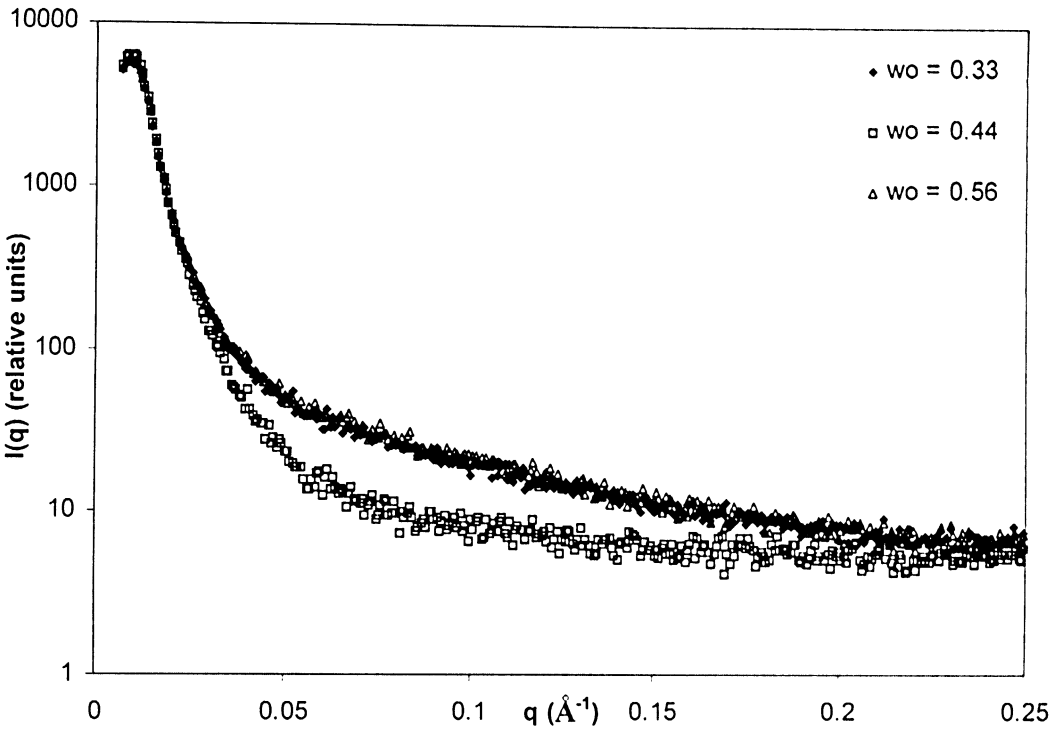

Figure 36. SAXD curves of samples in the PC/Water/MO system, with different w_O values.

These results provide vital information concerning the effect of the organic phase on the nature of the molecular structure they promote. When the results of the PC/Water/MO and PC/Water/IPP systems are compared to the results of the PC/Water/ISO system, it is shown that for the same concentration of lecithin, the formation of tubular reverse micelles is promoted in the latter system but not in the isopropylpalmitate and mineral oil systems. These results clearly demonstrate that the organic phase and w_O values may be used to modulate the viscosity of biocompatible organogels.

CHAPTER 5 – CONCLUSIONS

Lecithin organogels were formed with pure isooctane and with a combination of isooctane and mineral oil. The organogels produced by both systems were characterised by their high viscosity, Newtonian behaviour and non-birefringent nature. Small-angle X-ray diffraction studies of the isooctane system revealed their high degree of molecular organisation, based on the existence of a two-dimensional crystal lattice formed by the bundling of the cylindrical reverse micelles. Organogels based on isooctane and to a lesser extent on isooctane and mineral oil, were highly ordered systems.

Fully biocompatible lecithin organogels formed with isopropylpalmitate and mineral oil or combinations thereof were produced. The viscosity of these systems was strongly modulated by mineral oil content and w_o values. Contrary to the isooctane-based organogels, the small-angle X-ray diffraction results indicated that there was not enough structural material in the organogels to observe any level of molecular organisation.

In summary, the results of this study show that it is possible to prepare biocompatible lecithin-based organogels with both isopropylpalmitate and mineral oil. It also shows that both the rheological and structural properties of the organogels formed depend on the nature of the organic phase used to prepare them.

CHAPTER 6 – RECOMMENDATIONS FOR FUTURE STUDIES

The recommendations to continue work in this area of study are as follows:

(i) Continue to investigate the crystalline structure of the organogels formed in the lecithin-water-isopropylpalmitate and lecithin water-mineral oil systems with a similar approach to this study but at higher lecithin concentrations (i.e. obtain zero-shear viscosity versus water content curves, followed by diffraction data, for samples containing increasing amounts of water).

(ii) Investigate the effect of adding a well-known and well-studied guest molecule such as bovine serum albumin to the organogel made of a biocompatible material of choice, on its structural and rheological properties. This would be followed by incorporating bioactive molecules into these organogels.

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8 – APPENDIX

System: Square

Space Group: #11, p4mm

Bravais Lattice Conditions: h + k = 2n

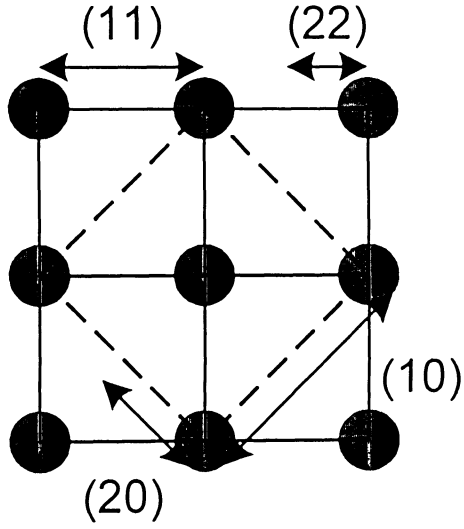

Figure A-1. Space Group #11, p4mm [Hahn, 1983]