

Physico-Chemical Properties of Pseudoceramide in Relation to Bilayer-Forming Ability

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Abstract

The bilayer forming ability of pseudo-ceramide PC104 in octanoic acid/water/n-octyl β -D-glucoside mixtures was investigated through the phase diagram. Because of its low solubility in water and of its crystallization, pseudoceramide PC104 was dissolved in octanoic acid, which is nontoxic additive for foods and cosmetics. The mixtures formed four different phases (L1, L2, LC and two phases). Depending on the concentration of PC104 in octanoic acid, the region of each phase was extended or contracted. On the contrary to the region of L2, regions of lamellar phase and L1 phase were expanded. The bilayer-forming ability of PC104 was explained on the basis of concentration of PC104 at interface and interaction between PC104 and octanoic acid. From FT-IR results, it was found that the interactions of PC104's polar head group with octanoic acid increased as the amount of PC104 in octanoic acid increased. Also emulsion size and size distribution have been studied depending upon the emulsification path. Droplets of emulsion prepared from lamellar phase were smaller and more homogeneous compared to those of emulsions formed from L2 phase.

Keywords: Bilayer-forming ability, Pseudo-ceramide, Phase diagram, Lamellar structure, Interfacial tension, FT-IR

INTRODUCTION

Stratum corneum (SC), the upper layer of skin, is considered as the main barriers to maintain the skin moisture and trans-epidermal water loss [1, 2]. The effective barrier functions of SC have been attributed to a bilayer-forming capacity of SC lipids in the intercellular space of SC. Therefore, the mechanism for the self-assembly of a lipid

bilayer has been a subject of considerable interests. Stratum corneum lipid bilayers consist of ceramides, cholesterol, cholesterol esters and free fatty acids, etc [3].

Elias suggested that the presence of ceramides and sphingolipid is the basis for the structural organization of SCL in bilayers [4, 5]. Wertz et al showed that ceramides could take part in the bilayer formation and that fatty acids were essential for this to occur [6, 7]. Stratum corneum lipids retain water in stratum corneum by forming lamellar structure [8, 9]. Also lamellar structure was considered to play a crucial role in the barrier property of SC [8]. Ceramide is composed of a long-chain alcohol to which long-chain fatty acid is linked by an amide bond. Ceramide has a polar head group formed by two or three hydroxyl groups and the amide group. These free hydroxyl groups and the amide group can form an extensive intermolecular hydrogen bond network [10, 11]. FT-IR and FT Raman spectroscopy were employed to study the molecular structure of the SC [12-15].

Even though ceramides constitute the major fraction of SC, they exist in nature only in a small quantity. To make them commercially available for dermatological applications, recently, Imokawa and co-workers synthesized a pseudoceramide, named sphingolipid E, which had a molecular structure analogous to that of the natural non-hydroxy fatty acid ceramide of type II [8, 16-19]. They examined the dermatological applications of sphingolipid E to the dry skin and scaly skin, and found that the synthetic ceramide exhibited water-retaining properties similar to those of natural lipids. The synthetic pseudo-ceramide has been suggested to be useful as a skin moisturizer and/or a drug carrier. Many other pseudoceramides were also reported for the application in cosmetics and foods.

In the present study, we have investigated the bilayer-forming ability of the mixture of n-octyl β -D-glucoside /water/octanoic acid containing pseudoceramide. We used pseudo-ceramide PC104 synthesized by Pacific Co. (Seoul, Korea)[20]. The oil used was octanoic acid. It is nontoxic in vivo use, so it can commercially be used at the cosmetics and foods. Nonionic surfactants of EO derivatives have a cloud point and it was suspected to crystallize the ceramide in long time storage. As an emulsifier, n-octyl β -D-glucoside (hence force referred to as C8G1) was used. Using C8G1 as a surfactant would prevent crystallization of PC104 in long time storage. Phase diagram of PC104 containing oil with C8G1 were constructed and the effects of concentration of PC104 on the phase behaviors have been investigated.

Since ceramides are used in an emulsified products such as cosmetics, the effects of PC104 on the droplet size and size distribution of emulsion was also studied. Usually, the droplet size and size distribution of emulsion are strongly dependent on the path of emulsification in the phase diagram. Thus, characterization of emulsion was performed at a certain ratio of water concentration.

MATERIALS AND METHODS

Materials

The pseudo-ceramide 1,3bis(N-(2-hydroxyethyl)-palmitoyamino)-2-hydroxypropane (PC104, Figure 1) was obtained from Pacific Co.(Seoul, Korea). Octanoic acid was purchased from Aldrich Chemical Co. and n-octyl β -D-glucoside(C8G1) was from Sigma Chemical Co. and used as received. Octanoic acid was chosen as an oil phase since it can dissolve PC104 more than 50.0 wt.% and lamellar structure formed by pseudoceramide are stabilized by fatty acid in SC. The melting point of octanoic acid has been reported to be 16°C. Thus, it was liquid form in our experimental range of temperature.

Methods

Phase diagram

The phase diagrams were constructed using a traditional titration method. The samples were prepared at twenty different points of different compositions. PC104 was dissolved in octanoic acid at 0%, 10%, 25% and 50% by weight, respectively. Liquid crystalline regions were examined by means of visual inspection through crossing polarizers. Appropriate amounts of C8G1, octanoic acid containing PC104 were weighed into the microtube and water was added. The samples were sealed and vigorously stirred. The sealed tubes were placed in the incubator at 25°C until the equilibrium states were obtained.

FT-IR spectroscopy

Ceramides are amphiphilic molecules and have a polar head group. Thus, IR spectroscopy makes it possible to elucidate the structural information from the polar region of ceramide. The absorption wavelength of C=O stretching vibration associated with a C-N stretching in PC104 were measured. These wavelengths were reflected the

extent of interaction among amido carbonyl groups of PC104. The infrared measurements were carried out with MAGNA-IR 760 SPECTROMETER (Nicolet Inc., USA).

Interfacial tension

The amount of PC104 adsorbed per unit area of oil/water interface can be calculated indirectly from slope in interfacial tension vs. concentration curves near the critical micelle concentration. The interfacial tension method was selected to study surfactant adsorption at oil/water interface. And the area per molecule at the interface provides information on the degree of packing and the orientation of the adsorbed surfactant molecules. The interfacial tension measurements were performed with K12 Mk6 (Krüss

$$\Gamma = -\frac{1}{RT} \frac{\partial \gamma}{\partial \ln C}$$

GmbH, Germany) using Wilhelmy plate method. The Gibbs adsorption equation is

where Γ is concentration of PC104 at oil/water interface in mol/cm², γ is the interfacial tension, C is molar concentration of PC104 in oil phase, $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ and T is the absolute experimental temperature.

Procedure for emulsification

Mixtures of octanoic acid containing PC104 (25 wt.)/C8G1/water were prepared at 52.5:22.5:25, 45:30:25 and 37.5:37.5:25 ratios by weight as shown in figure 2-(c), respectively. Then, the doubly distilled water (electrical resistivity 18.2 M Ω) was added into each sample to form emulsions of compositions marked as (a1), (a2) and (a3). Emulsions of a2 and a3 were formed by adding water into lamellar phase and emulsion of a1 was prepared by adding water into L2 phase. The same procedure was repeated to form emulsions of (b1, b2, b3) and (c1, c2, c3). After adding water, samples were vortexed for 5 minutes. The droplet size and size distribution of resulting emulsions were investigated by optical microscopy Optiphot-2 (Nikon, Japan).

RESULTS AND DISCUSSION

PC104 was soluble in most alkanolic acids. It seemed that the solubility increased as the chain length of alkanolic acid decreased at room temperature. In this study, we dissolved PC104 in octanoic acid, which is liquid form at room temperature and has a high PC104

solubility. It is fortunate that alkanolic acid can dissolve PC104 in respect of application in cosmetics since fatty acid can stabilize the lamellar structure formed by pseudoceramide.

Ternary phase diagrams

The ternary phase diagrams of octanoic acid/water/C8G1 system at 25 °C are presented in figure 2 . The diagrams showed the existence of oil-in-water (L1), water-in-oil (L2) microemulsion regions, lamellar (Liquid Crystal) regions and two phases regions in all systems. L1 phase was observed in the narrow region for all phase diagrams. L2 regions may extend further towards the water corner. But the region of L2 phase decreased as the amount of PC104 in octanoic acid increased. All systems have broad LC regions. Water holding capacity in W/O microemulsion system was maximum 31wt% of water

As increasing PC104 in octanoic acid the phase behavior was differed in some extents. L2 region was contracted and LC and L1 regions were extended. The phase diagrams showed that the more concentration of PC104 in octanoic acid, the easier formation of an isotropic region (L1) at these systems. From phase diagrams we hypothesized that PC104 is adsorptive in the O/W interface and it may lead more wide regions of LC structure. As the content of PC104 in octanoic acid increased, the region of lamellar structure was expanded. Thus, it can be inferred that PC104 fosters the formation of lamellar structure in our system.

To clarify the effects of PC104 in the formation of LC regions, interfacial tension of octanoic acid/water interface and FT-IR absorption have been investigated.

Interfacial tension

Several authors addressed the factors necessary for the formation of LC. LC (lamellar structures) would be formed at a certain range of R values [21]. Packing parameter (R) determines the overall packing shape of the surfactant at interface. We could guess the surface curvature of PC104 molecules. In this study we see that a large increase in viscosity was observed, and PC104's packing parameter was calculated in previous studies that $R = 1.03$ [22]. PC104's packing parameter was preferred to form the lamellar structure.

The interfacial tensions of water/octanoic acid containing PC104 were measured in terms of PC104's concentration in octanoic acid. For a given system, interfacial tension

data were shown in figure 3. The addition of PC104 resulted in lower interfacial tension values than those occurred with octanoic acid/water alone. The amount of PC 104 adsorbed per unit area of water/octanoic acid interface had been calculated by use of Gibbs adsorption equation.

Γ was calculated to be 2.17 mol/cm^2 using Gibbs adsorption equation and the area per molecule at interface was 76.55 \AA^2 . Considering interfacial tension data and packing parameter, PC104 was adsorbed at interface and form condensed interfacial films at oil/water interface.

FT-IR measurements

From the FT-IR study, it was found that the polar head groups of PC104 are sensitive to hydrogen bonding and/or polarity effects. The strong hydrogen bond plays a decisive role in the stabilization of lamellar structure [23, 24]. Thus, we performed infrared (IR) measurements to elucidate the interaction of polar head group of PC104 with octanoic acid. The difference in absorption wavelength of these mixtures reflexes the degree of interaction among the amido carbonyl groups of PC104. We assume that the lower the absorption wavelengths, the stronger the interaction.

An intermolecular hydrogen bond between the carboxyl group of octanoic acid and the amido carbonyl group of PC104 were presented in figure 4. We found that the amide C=O peak occurred at 1607.77 cm^{-1} when pure PC104 was in its most stable state and at 1619.42 cm^{-1} when PC104 is dissolved in CHCl_3 with no interaction. As the concentration of PC104 in octanoic acid increased, C=O stretching peak were shifted to the lower wavelength. A strong hydrogen bond formed between PC104 and octanoic acid was induced by increasing PC104 concentration and influences the magnitude of hydrophilicity of oil.

It was suspected that this hydrophilicity might contribute to the phase behavior of phase diagrams. From the studies of IR and interfacial tension data it is suggested that PC104 has significant effects on the formation of LC phases.

The characteristics of the emulsion

The lamellar structures and L2 phase were converted into emulsion by adding more than 60 wt% of water. Emulsions have been investigated in their size and size distribution. Figure 5 shows emulsion droplet sizes depending on the amount of water added. (a), (b)

and (c) have an oil/C8G1 ratio of 7:3, 6:4, 5:5 respectively. The droplet size of emulsion prepared by adding water into L2 phase was over 3 μ m at given concentrations. But emulsions prepared by adding water into lamellar phase have small and homogeneous droplets because they passed through a liquid crystalline phase during the process of emulsification (24). From the results it has been possible to produce the small and homogeneous emulsion regions at appropriate concentrations. Instead of adding water into L2 phase, the addition of water into lamellar phase is preferable in preparing emulsion containing PC104.

CONCLUSION

The region of lamellar structure in the mixture of octanoic acid containing PC104 /water/C8G1 have been increased as the amount of PC104 in octanoic acid increased. Since the packing parameter of PC104 was found to be 1.03, PC104 might help the formation of lamellar structure in our systems. Interfacial tension and adsorption data indicate that PC104 is adsorbed at the O/W interface to change of interfacial properties. In the IR studies the amido carbonyl group of PC104 interacts strongly through a hydrogen bond with the carbonyl group of octanoic acid. By increasing the concentration of PC104 in octanoic acid, strong hydrogen bond between PC104 and octanoic acid exists and hydrophilicity of oil might be increased. PC104 confers its surface activity to the formation and enlargements of LC region. The size of emulsion droplets was much smaller when the emulsification path crossing the LC region compared to that of emulsion which does not path crossing the LC region.

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Figure captions

Fig 1. Molecular structure of PC104

Fig 2. Ternary phase diagrams for octanoic acid/water/C8G1 system at 25 °C. (a) octanoic acid without PC104, (b) 10wt%, (c) 25wt%, (d) 50wt% of PC104 in octanoic acid.

Fig 3. Interfacial tension at water/octanoic acid containing PC104 interface as a function of PC104 concentration in octanoic acid.

Fig 4. Maximum FT-IR peaks of amido carbonyl group of PC104 in octanoic acid.

Fig 5. Average droplet sizes of emulsions at various compositions shown in figure 2(c); a (a1, a2, a3 from left), b(b1, b2, b3) and c(c1, c2, c3).

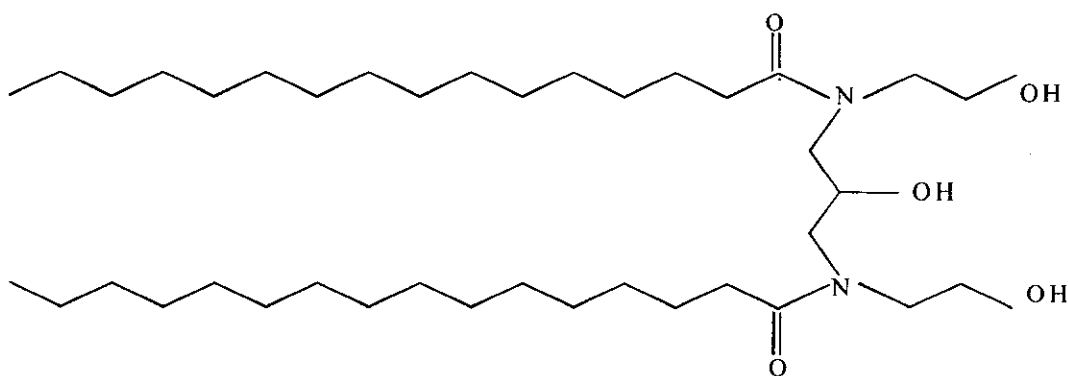


Fig. 1.

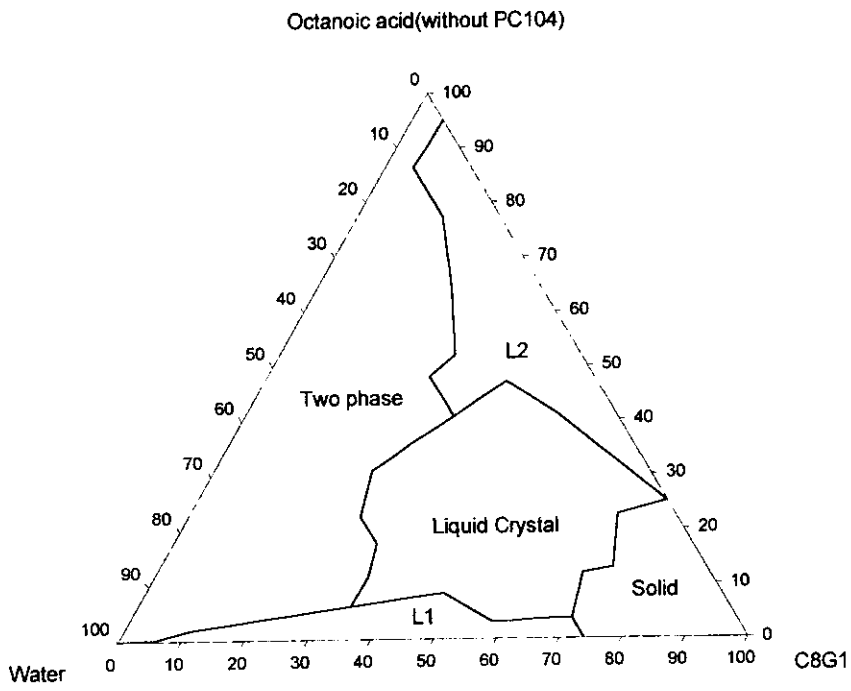


Fig 2.(a)

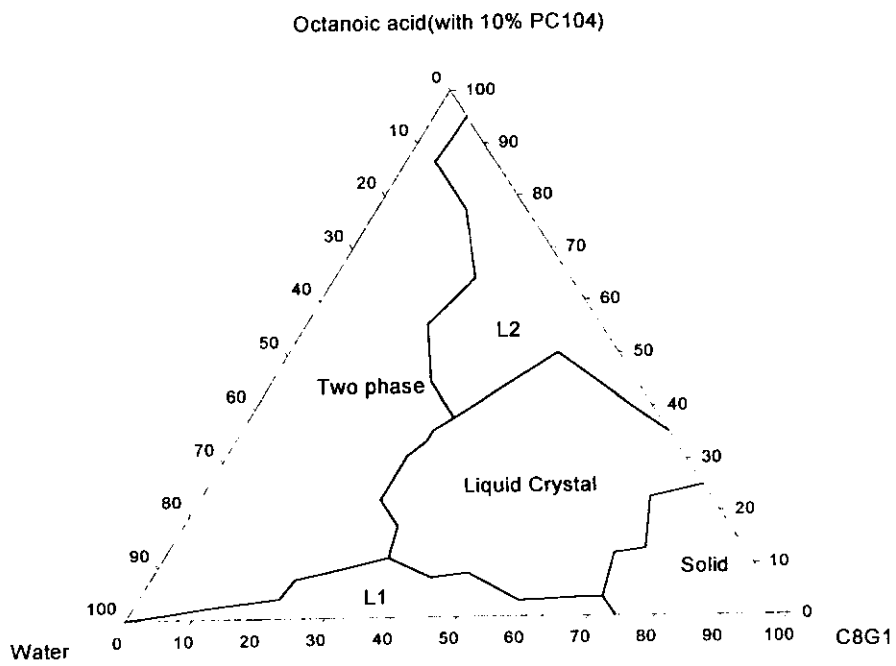


Fig 2. (b)

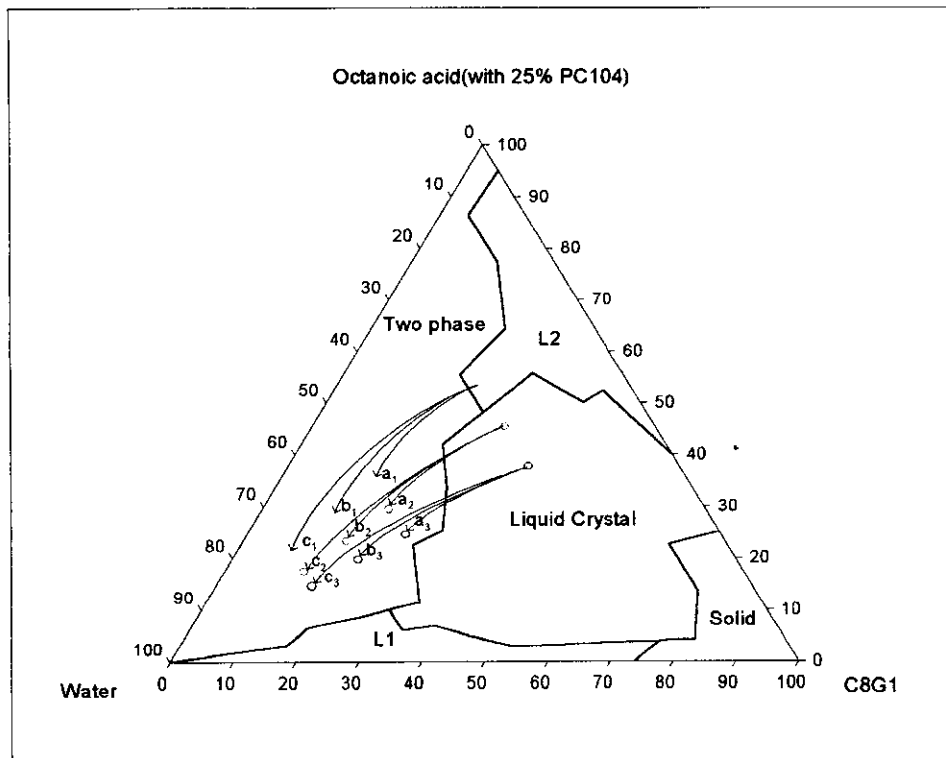


Fig 2. (c)

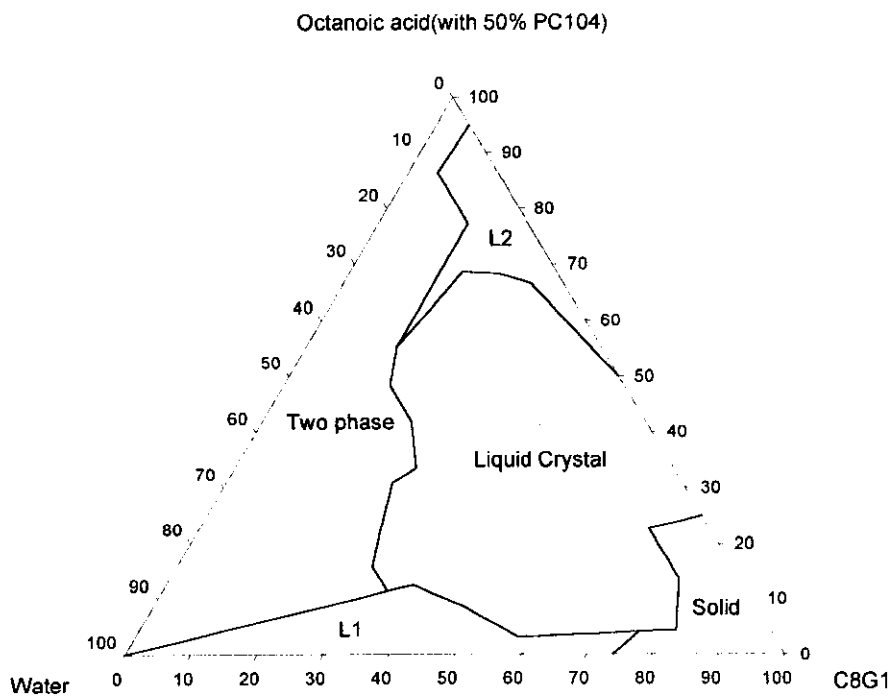


Fig 2. (d)

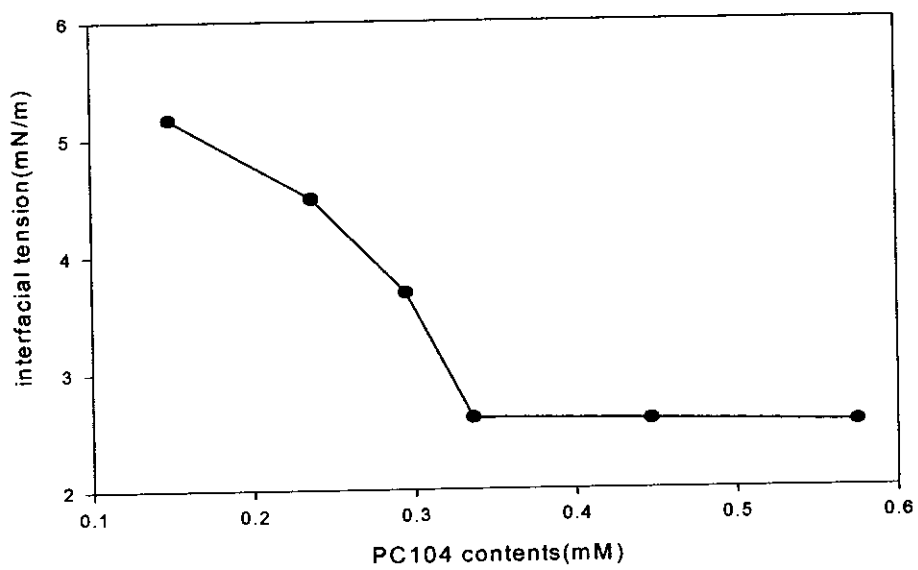


Fig. 3

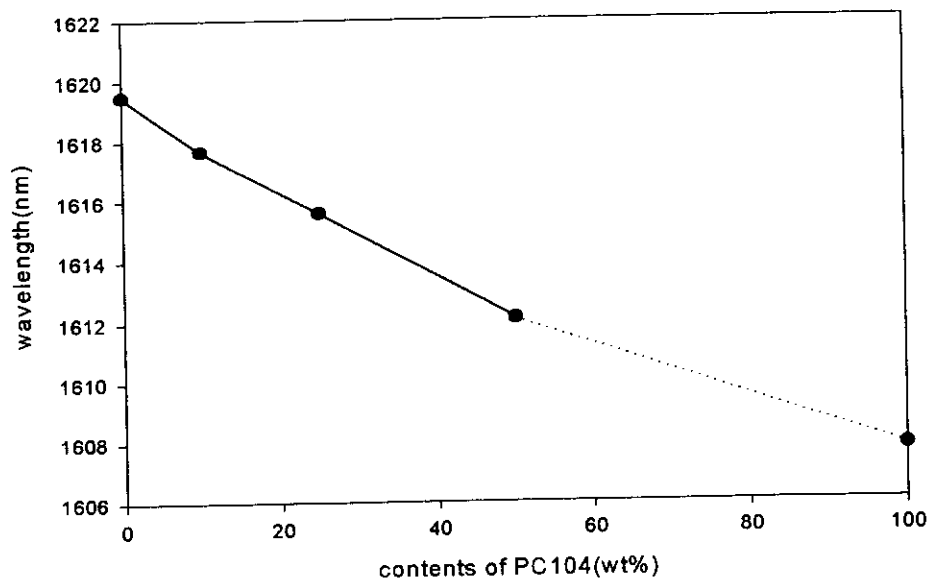


Fig. 4

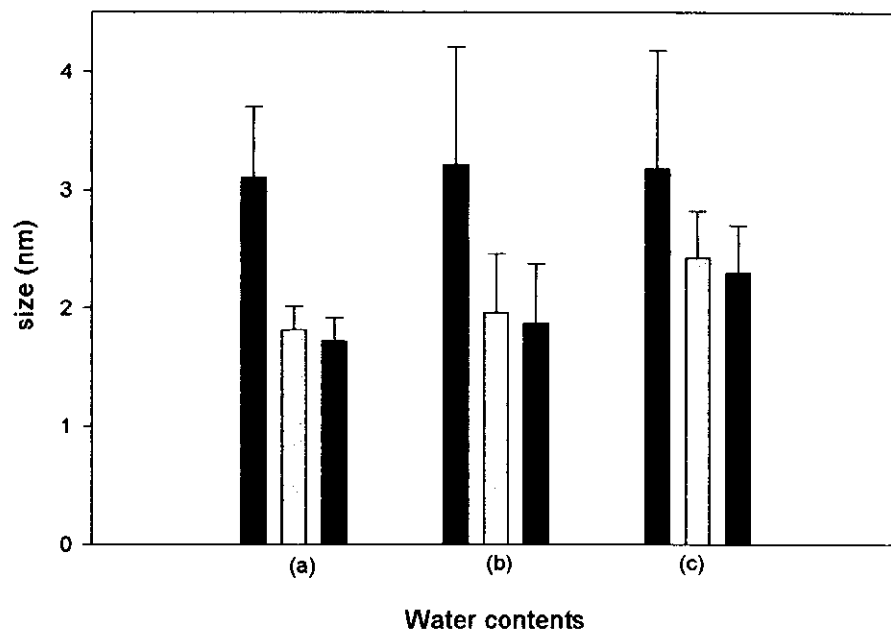


Fig 5.