

## PLASTICIZING EFFECT OF CHOLINE CHLORIDE/UREA EUTECTIC-BASED IONIC LIQUID ON PHYSICO-CHEMICAL PROPERTIES OF AGAROSE FILMS

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Agarose films were formed with the addition of 30 to 70 wt% choline chloride/urea eutectic-based ionic liquid (ChCl/Urea). The ChCl/Urea was prepared through complexation at a 1:2 mole ratio. The films were prepared by dissolving ChCl/Urea in distilled water followed by dispersion of the agarose at 95 °C. The solution was gelled at room temperature, and the formed gel was dried in an oven overnight at 70 °C. Mechanical testing indicated that the agarose film containing 60 wt% ChCl/Urea had higher tensile extension and tensile strain at break compared to the pristine agarose film. The addition of ChCl/Urea also reduced the glass transition temperature ( $T_g$ ) of agarose films. Cross-section SEM images of the agarose films showed that surface roughness disappeared with the incorporation of ChCl/Urea. FTIR spectra confirmed the presence of intermolecular hydrogen bonding between agarose and ChCl/Urea. XRD patterns demonstrated that an amorphous phase was obtained when ChCl/Urea was added. Agarose films containing more ChCl/Urea exhibited higher transparency, as measured by a UV-Vis spectrometer. In summary, the physicochemical properties of agarose films were evidently affected by the incorporation of the ChCl/Urea as a plasticizing agent.

*Keywords:* Agarose film; Eutectic-based ionic liquid; Plasticizer; Choline chloride/urea

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### INTRODUCTION

Ionic liquids consist of cations and anions and are generally regarded as environmentally benign solvents, since they are non-volatile and reusable. They have a relatively low melting point (commonly < 100 °C) and extremely low vapour pressure. Ionic liquids have unusual solvent properties; for example, they are highly stable, highly polar, non-flammable, thermally stable, electrically conductive, miscible with certain organic solvents and/or water, capable of solubilizing most organic and inorganic materials as well as a number of biopolymers, chemically inert, and can be customized (Han *et al.* 2009; Liebert and Heinze 2008; Shamsuri and Abdullah 2010).

Previous studies have indicated that ionic liquids could be used as non-volatile plasticizers for synthetic polymers such as poly(methyl methacrylate) and poly(vinyl chloride). They were found to be compatible with both polymer systems (Rahman and Brazel 2004; Shvedene *et al.* 2006). Moreover, ionic liquids have improved the thermal

stability of these polymers, lowered their glass transition temperature ( $T_g$ ) (Scott *et al.* 2002), improved elastic modulus (Scott *et al.* 2003), increased flexibility (Lunstrook *et al.* 2010), reduced brittleness, showed good thermodynamic compatibility, and also have better leaching and migration resistance compared to the traditional plasticizers (Rahman and Brazel 2006). Plasticization of natural polymers by ionic liquids has also yielded the same improvement in physicochemical properties as described above. It has also been reported that a natural polymer such as thermoplastic starch that has been plasticized by an ionic liquid is less hygroscopic than a corresponding sample plasticized by glycerol. This is due to a rather strong interaction between starch and ionic liquid, which in turn limits the interaction between starch and water molecules (Sankri *et al.* 2010). It has also been shown that ionic liquids are well dispersed and partly miscible in poly(lactic acid), as evidenced by scanning electron microscope images and differential scanning calorimetry results (Park *et al.* 2010).

Nevertheless, imidazolium, pyridinium, phosphonium, and other tetraalkylammonium-based ionic liquids that have been used as plasticizers usually have been prepared through alkylation and/or metathesis reactions. However, these ionic liquids are too expensive (probably due to the high cost of precursors) and the preparation procedures for making them are also very complicated (Klein-Marcuschamer *et al.* 2011). Therefore, the use of these ionic liquids is not economically feasible and may be impractical for large-scale applications. This research is particularly interested in using an inexpensive precursor to produce ionic liquid and is therefore focused on a eutectic-based ionic liquid (also known as a deep eutectic solvent) that was prepared through complexation.

A eutectic-based ionic liquid is composed of a mixture of quaternary ammonium salt and a complexing agent, forming a eutectic mixture with a melting point much lower than either of the individual components (Abbott *et al.* 2001). The main advantage of this ionic liquid compared with common ionic liquids is its ease of preparation; choline chloride only needs to be mixed with metal chloride or a hydrogen bond donor at an elevated temperature by stirring until they melt into liquid form. A high yield (> 99 wt%) could be obtained economically with this approach due to 100% reaction mass efficiency (Hou *et al.* 2008). The physical, as well as chemical properties of these ionic liquids are similar to air- and water-stable ionic liquids and have some superior characteristics with wide liquid-range (Abbott *et al.* 2004). The components of the eutectic-based ionic liquid can be easily found because choline chloride has currently received widespread attention as a precursor for preparation of this ionic liquid (Abbott *et al.* 2003) due to its low cost and its biodegradable and biocompatible properties (it is being widely used as a feed additive for livestock) (Morrow and Maginn 2004). Choline chloride could be complexed with metal chlorides ( $ZnCl_2$ ,  $SnCl_2$ ,  $FeCl_3$ , *etc.*) or hydrogen bond donors (including amides, carboxylic acids, and alcohols) through hydrogen bonding and will produce a homogeneous liquid of ions with a significantly decreased melting point (Abbott *et al.* 2006).

Agarose is a polysaccharide whose natural abundance, non-toxicity, biodegradability, renewability, and low cost make it an interesting resource for producing eco-friendly materials. In addition, agarose has the ability to form thermo-reversible gels

simply by cooling a hot aqueous solution of agarose (Dumitriu 1998). The gels were also found to form freestanding films upon drying.

The films made of agarose are clear, transparent, strong, and heat-sealable (The *et al.* 2009). Their water vapour permeability did not appreciably differ in comparison to the starch films, arabinoxylan films, or cellulose derivative films (Norton *et al.* 1999). However, agarose films are usually very brittle (Nieto 2009), not flexible, and fragile at low moisture contents, thus making its usage restricted. Preventing brittleness and achieving highly flexible films can be accomplished with the addition of a plasticizer. For that reason, the authors have produced some agarose films using a eutectic-based ionic liquid as a plasticizer. The plasticized agarose films that are transparent and flexible have great potential applications in fabrication of safety films for display devices, windows, and so on. Moreover, they also have potential to be applied in production of biodegradable films for wrapping substances, holding things, dividing sections, barring dimensions, and so forth.

A choline chloride/urea eutectic-based ionic liquid was chosen as a potential plasticizer for investigation, as it possesses several useful properties such as a low melting point (12 °C), biodegradability, low toxicity, and a low cost (Abbott *et al.* 2003). The most important feature of choline chloride/urea is that it is non-acidic compared to a wide variety of low melting point eutectic-based ionic liquids, for instance choline chloride/oxalic acid, choline chloride/ZnCl<sub>2</sub>, and choline chloride/phenol that are acidic in nature and consist of dicarboxylic acid, Lewis acid, and carboric acid, respectively. These acids could hydrolyze the agarose, especially at high temperatures used for the preparation of film, as observed in preliminary experiments. Hitherto, the use of this ionic liquid along with hydrocolloid for preparation of agarose films has not yet been reported.

In the present study, choline chloride/urea eutectic-based ionic liquid was added to agarose so as to evaluate its plasticizing effect on mechanical properties, glass transition temperature ( $T_g$ ), and surface morphology of the films. Fourier transform infrared spectroscopy, X-ray diffraction, and ultraviolet-visible spectrometer were also used to characterize the films.

## EXPERIMENTAL

### Materials

Choline chloride, abbreviated ChCl (99% purity) was procured from Acros. Urea (pure grade) was purchased from Sigma Aldrich. Agarose (analytical grade) was obtained from Merck. All chemicals were used as received.

### Preparation of ChCl/Urea Eutectic-Based Ionic Liquid

The preparation of ChCl/Urea eutectic-based ionic liquid through a complexation reaction was carried out according to Abbott *et al.* (2003). The ChCl was mixed with urea at a 1:2 mole ratio in a beaker, heated at 100 °C, and stirred until a homogeneous liquid was obtained. The resultant ionic liquid was then collected and kept in a desiccator containing silica gels prior to use.

## Preparation of Agarose Films Containing ChCl/Urea Ionic Liquid

The ChCl/Urea ionic liquid (0.69 g) was placed in a rotary evaporator flask and allowed to mix slowly with 56 g of distilled water. The mixture was agitated at room temperature (25 °C) until the ChCl/Urea was completely dissolved. Then, 1.6 g of agarose was dispersed into that solution, and it was heated in a water bath at 95 °C. The dispersion was kept agitated until a transparent solution was observed. The agarose solution was immediately poured onto a glass Petri dish and allowed to cool to room temperature (25 °C) for gelation. The gel was rigorously dried overnight at 70 °C to obtain a freestanding film containing 30 wt% of ChCl/Urea. The composition of ChCl/Urea was varied from 30 to 70 wt%. The films were dried in an oven at 70 °C for at least 24 hours prior to characterization.

## Film Characterization

### *Mechanical testing*

Tensile extension, tensile strain, tensile stress, and modulus at break were measured at room temperature using an Instron Universal Testing Instrument (model 5566) equipped with a 1 kN load cell according to ASTM D882-91 (1992). The film samples were cut into a rectangular shape with a length and width of 75 mm and 10 mm, respectively. The initial grid separation was set to 50 mm and the crosshead speed was 5 mm/min (Tang and Teoh 2001). The readings were taken from seven samples for each composition.

### *Differential scanning calorimetry (DSC)*

DSC analyses were conducted on a Mettler Toledo 822<sup>e</sup> instrument under a constant stream of nitrogen at a flow rate of 50 mL/min. All samples were tightly sealed in aluminium pans and were first heated to 100 °C to eliminate the thermal history with an isotherm of 15 min. The experiments were carried out in a temperature range of -50 to 120 °C at a heating rate of 10 °C/min to obtain the glass transition temperature,  $T_g$ .

### *Scanning electron microscope (SEM)*

SEM (Philips, model XL 30 ESEM) was used to examine the morphology of the films. The film samples for SEM micrographs were cross-sectioned by making a tensile fracture at room temperature and being gold coated. The morphologies were observed at magnification of 3000× with accelerating voltages of 10.0 kV and Spot 3.0. Additionally, the Energy Dispersive X-ray (EDX) analysis was implemented using an X-Max 50 mm<sup>2</sup> SDD (Silicon Drift Detector) and the INCA software from Oxford Instruments to examine the elemental compositions of uncoated agarose films. The data presented in this examination corresponds to seven surface areas.

### *Fourier transform infrared spectrometry (FTIR)*

FTIR analyses were carried out by using a Perkin Elmer Spectrum 100 Series to determine the presence of functional groups in the films and to reveal the interaction between agarose and ChCl/Urea. The FTIR spectra were obtained by using a universal attenuated total reflectance (UATR) equipped with a ZnSe-diamond composite crystal

accessory. Each sample was scanned 16 times, in the range of 4000 to 280  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ .

#### *X-ray diffractometer (XRD)*

XRD analysis was carried out using an X-ray Diffractometer (PANalytical Philips, X'Pert PRO MPD PW3040) employing a monochromatized radiation source of  $\text{CuK}\alpha$  of 1.5405 Å. The voltage and current intensities were 40.0 kV and 30.0 mA, respectively. All samples were scanned in the range of 4 to 40°  $2\theta$  with a step size of 0.01° and step time of 0.2 s at room temperature (25 °C) to characterize the crystallographic properties of the agarose films.

#### *Ultraviolet-visible spectrometer (UV-Vis)*

UV-Vis analyses were conducted on a Perkin Elmer Lambda 25 UV/VIS Spectrometer in the wavelength range of 200 to 1000 nm with a scan speed of 960 nm/min. The lamp change wavelength was 355 nm and slit width was 1.00 nm. The samples of films at a constant thickness of 0.13 mm were set up where the beam could directly pass through them to measure the transmittance (%T).

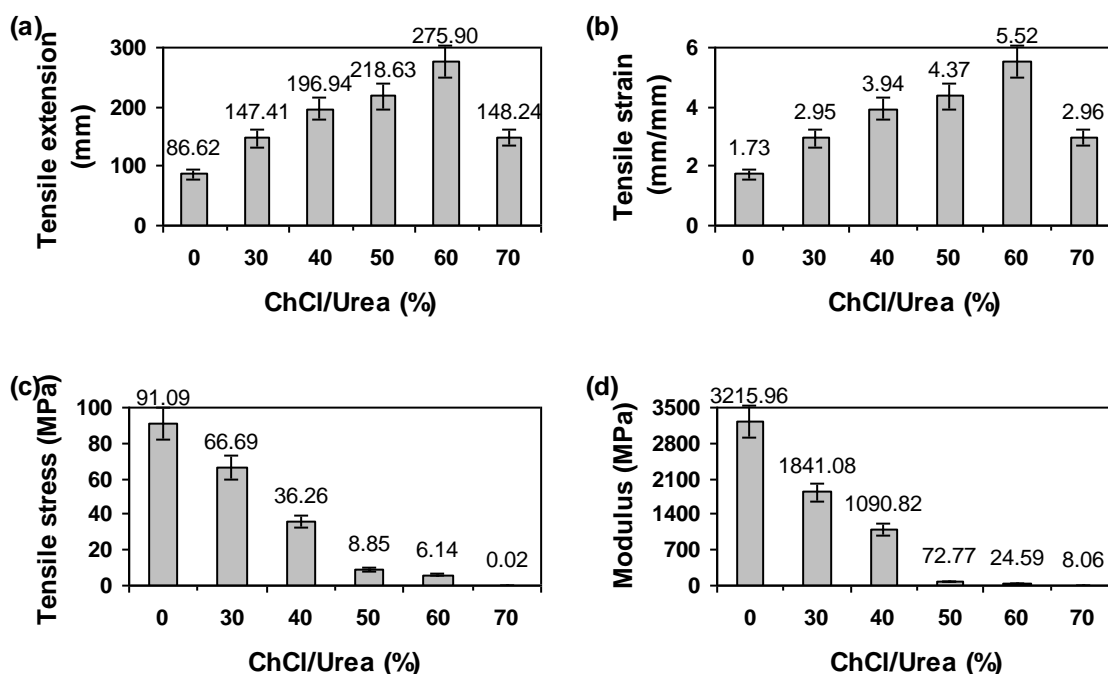
## RESULTS AND DISCUSSION

In the preparation of agarose films with and without ChCl/Urea, air bubbles that commonly emerge within the agarose solutions after the agitating process can affect the appearance of the films and consequently interfere with their final properties. Therefore, the degasification process was carried out to remove the bubbles from the solutions before casting. The process was operated by reducing the pressure inside the flask to a vacuum by means of a water aspirator pump. On top of that, the agarose solutions with or without ChCl/Urea usually have a hysteretic behavior (memory of the past) where they can remain in the liquid state even at elevated temperatures. Hence, the agarose solutions were subjected to the gelation process at room temperature before drying for formation of films.

### **Mechanical Properties**

Figure 1a shows that the tensile extension at break of agarose films increased gradually as the content of ChCl/Urea was increased. A similar trend was observed for the tensile strain at break as the ChCl/Urea content increased (Fig. 1b). The results clearly display the plasticizing effect of ChCl/Urea, which improved the flexibility of the agarose chains. Surprisingly, the addition of 70 wt% ChCl/Urea did not significantly increase the tensile extension and the tensile strain. The maximum increase was observed for the film containing 60 wt% ChCl/Urea. The efficiency of a plasticizer is indeed dependent on its content (Vanin *et al.* 2005). 70 wt% ChCl/Urea is considered less efficient to plasticize the agarose film since it brought about a decrease in tensile extension and tensile strain. It has also been assumed that the decreasing trend will continue when a higher content of ChCl/Urea is embedded in the agarose film. Thus, agarose films containing more than 70 wt% ChCl/Urea were not tested, since they possessed very soft properties.

Meanwhile, the tensile stress at break of agarose films (Fig. 1c) exhibited the opposite trend to the tensile extension and tensile strain, decreasing as the content of ChCl/Urea increased. The modulus at break (Fig. 1d) also showed the same characteristics as the tensile stress results. In the plasticization of a polymer system, the addition of a plasticizer provides larger intermolecular space area between the chains of polymer, thus preventing them from getting close to each other and reducing the energy required for segmental movement (Vieira *et al.* 2011). As a result, the tensile strength and modulus steadily decreases (Sothornvit and Krochta 2005). These reductions are typically revealed by a depression in the second order transition temperature, in particular by lowering the glass transition temperature ( $T_g$ ) (Rosen 1993). In order to further investigate the relationship between the content of ChCl/Urea eutectic-based ionic liquid and the  $T_g$  of the agarose films, DSC analyses were carried out.



**Fig. 1.** Effects of ChCl/Urea content on (a) tensile extension, (b) tensile strain, (c) tensile stress, and (d) modulus of the agarose films

### DSC Characterization

Figure 2 shows the DSC thermograms of the agarose films with different contents of ChCl/Urea, from which the  $T_g$  data were obtained and listed in Table 1. For the case of pristine agarose film, the  $T_g$  was observed at 105.3 °C (midpoint value). This high value underlined high brittleness of pristine agarose film at room temperature. The  $T_g$  value of agarose films containing ChCl/Urea is significantly lower in comparison to the  $T_g$  value of pristine agarose film even at 30 wt% ChCl/Urea (70.2 °C). The depression of the  $T_g$  is probably because of the equivalence of the thermal dilation between the hard matrix (agarose) and the soft phase (ChCl/Urea). The depression in the  $T_g$  also gave indirect evidence that the ChCl/Urea has distributed within the entire agarose film, hence creating

additional free volume and chain mobility (Choi and Park 2004). Therefore, they took less energy to transform from a glassy to a rubbery phase. In addition, it is also clear that  $T_g$  of agarose films decreased with an increasing content of ChCl/Urea. For agarose films containing 40 and 50 wt% ChCl/Urea, the  $T_g$  values found were even lower than that of the pristine agarose film, which were 46.2 °C and 37.5 °C, respectively. The results also showed that the agarose film containing 60 wt% ChCl/Urea had a lower  $T_g$ , leading to a value below room temperature (17.6 °C). Agarose films containing 70 wt% ChCl/Urea reached the lowest  $T_g$  value (6.3 °C). The reason for a low  $T_g$  in this case was attributed to the increased content of ChCl/Urea.

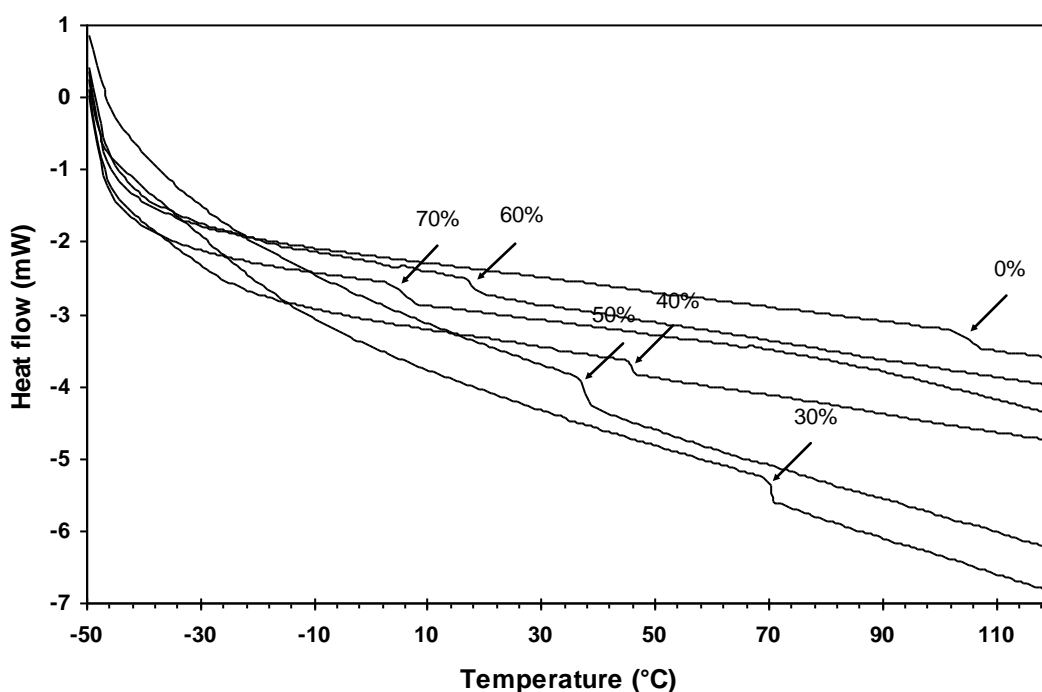


Fig. 2. DSC thermograms of the agarose films

Table 1. Glass Transition Temperature ( $T_g$ ) of the Agarose Films

Agarose (wt%)	ChCl/Urea (wt%)	Onset $T_g$ (°C)	Midpoint $T_g$ (°C)
100	0	104.7	105.3
70	30	69.6	70.2
60	40	45.5	46.2
50	50	36.9	37.5
40	60	16.9	17.6
30	70	5.6	6.3

### Morphology Observation

Figure 3 shows SEM micrographs of the fractured surface (internal section) of the agarose films. The SEM image of pristine agarose film indicated rough surface formation, and it would seem that the brittleness was extremely high (as shown in Fig. 3a). On the contrary, Fig. 3b to 3e clearly indicate that the formation of such a rough surface was no longer discernible, as the content of ChCl/Urea increased from 30 to 60

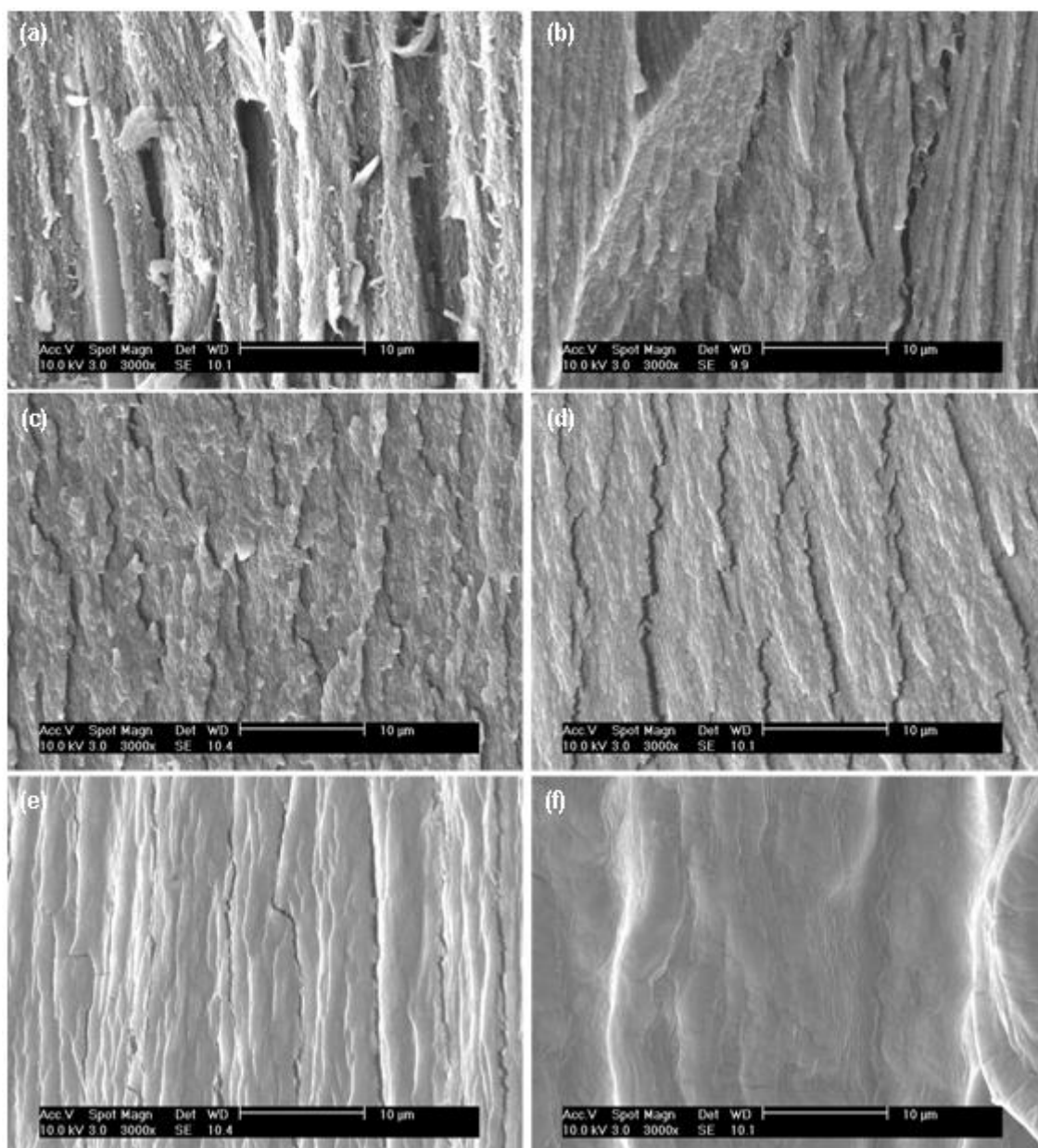
wt%. Furthermore, the agarose film containing 70 wt% ChCl/Urea (Fig. 3f) showed a slightly smoother fractured surface.

Phase separation could not be seen for agarose films containing 30 to 60 wt% ChCl/Urea. Such films exhibited very good surface homogeneity based on EDX area analyses (Table 2) as shown by a low standard deviation (Jembrih *et al.* 2000). This is as expected. However, completely unexpected observations for agarose film containing 70 wt% ChCl/Urea indicated no distinct phase separation; the homogeneity of the surface was good despite its tensile extension, and that tensile strain was less than agarose film containing 60 wt% ChCl/Urea. A loss in extendibility usually takes place when the content of plasticizer goes beyond its compatibility limit in the polymer chain, due to phase separation (Yang and Paulson 2000). Nonetheless, that behaviour does not necessarily imply phase separation between agarose and ChCl/Urea. Scott *et al.* (2003) stated that if the polymer and a plasticizing agent phase separate, the  $T_g$  is only moderately affected by that plasticizing agent. In this case, the  $T_g$  is excessively influenced by ChCl/Urea; thus, it can be concluded that there were no phase separations observed in agarose films. Nevertheless, the absence of phase separations has more to do with the good compatibility between the polymer and plasticizer (Wilson 1995; Godwin and Krauskopf 2007). To verify the existence of compatibility between agarose and ChCl/Urea, FTIR analysis of the agarose films was performed to determine details of specific intermolecular interactions between them.

**Table 2.** Elemental Compositions of the Agarose Films Measured by EDX

ChCl/Urea	Element	Mean (wt%)	Standard Deviation
0%	C	60.58	1.50
	N	-	-
	O	39.42	1.50
	Cl	-	-
30%	C	46.82	1.73
	N	13.82	1.87
	O	36.74	2.30
	Cl	2.62	2.01
40%	C	45.61	2.18
	N	15.62	2.44
	O	34.27	1.67
	Cl	4.50	1.91
50%	C	45.11	2.43
	N	16.98	1.70
	O	32.96	2.53
	Cl	4.95	1.16
60%	C	44.85	2.73
	N	17.29	3.22
	O	28.82	3.31
	Cl	9.04	3.81
70%	C	43.58	2.39
	N	17.96	2.76
	O	29.37	3.15
	Cl	9.09	3.52





**Fig. 3.** SEM micrographs of fractured surface of the agarose films with (a) 0 wt%, (b) 30 wt%, (c) 40 wt%, (d) 50 wt%, (e) 60 wt%, and (f) 70 wt% ChCl/Urea at magnification of 3000 $\times$

### FTIR Characterization

The FTIR spectra of agarose films with different contents of ChCl/Urea are shown in Fig. 4. The bands of FTIR spectra are summarized in Table 3. The FTIR spectra of agarose films exhibited strong intensity with broad bands in the region of 3350 to 3333  $\text{cm}^{-1}$ , which could be assigned to the O–H bond stretching vibration of the alcohol group. The noticeable small bands with medium intensity at approximately 3258 to 3208  $\text{cm}^{-1}$  responsible for the N–H bond stretching of the amide group were only present in the agarose films containing ChCl/Urea. The bands with medium intensity at around 2960 to 2897  $\text{cm}^{-1}$  that were present in every agarose film can be attributed to the C–H bond

stretching of the alkane group. The bands with strong intensity at around 1622 to 1610  $\text{cm}^{-1}$  associated with N–H bond bending of the amide group were only present in the agarose films containing ChCl/Urea. However, the band with medium intensity at 1631  $\text{cm}^{-1}$  was believed to be a feature of tightly bound water present in the polysaccharides (Shamsuri *et al.* 2012). The O–H bond bending vibration of the alcohol group resulted from the strong intensity of the bands at around 1467 to 1451  $\text{cm}^{-1}$ , while the bands with medium intensity at around 1373 to 1369  $\text{cm}^{-1}$  revealed the  $\text{CH}_3$  bond bending of the alkane group. The bands with strong intensity at around 1156 to 1153  $\text{cm}^{-1}$  corresponded to the C–O–C bond stretching of the ether group. The bands that are associated with C–O bond stretching of the alcohol group were found at around 1065 to 1044  $\text{cm}^{-1}$  with strong intensity.

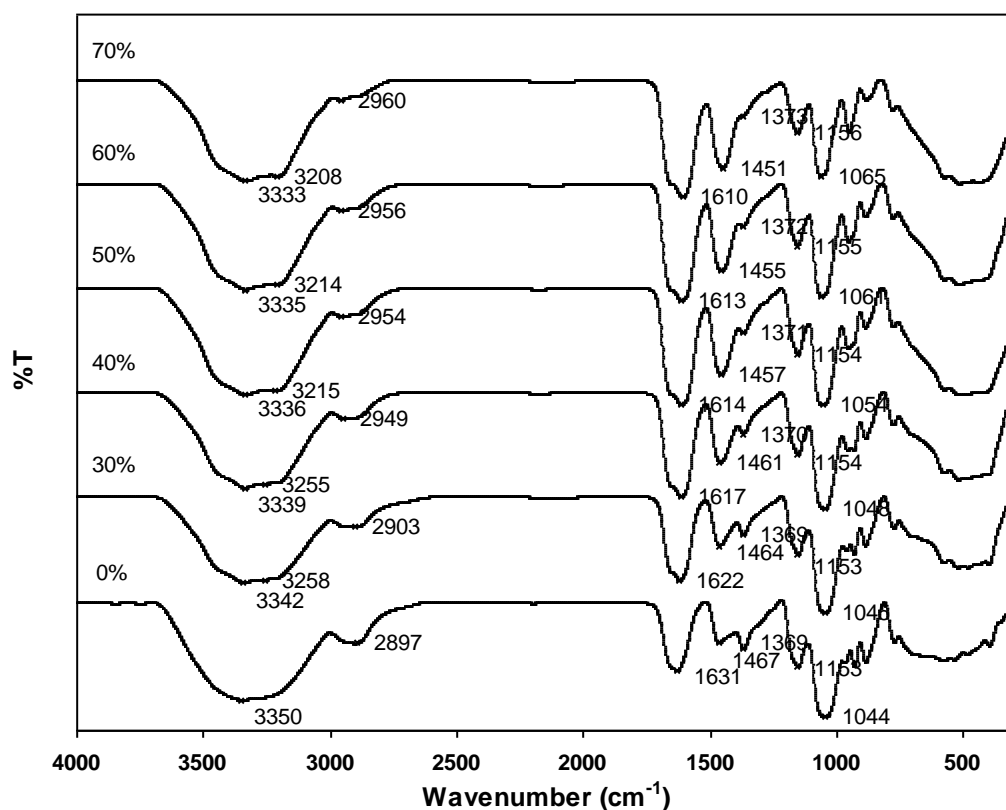


Fig. 4. FTIR spectra of agarose films

Table 3. FTIR Bands of Agarose Films

ChCl/ Urea (%)	Wavenumber ( $\text{cm}^{-1}$ )							
	O–H stretching	N–H stretching	C–H stretching	N–H bending	O–H bending	$\text{CH}_3$ bending	C–O–C stretching	C–O stretching
0	3350	-	2897	-	1467	1369	1153	1044
30	3342	3258	2903	1622	1464	1369	1153	1045
40	3339	3255	2949	1617	1461	1370	1154	1048
50	3336	3215	2954	1614	1457	1371	1154	1054
60	3335	3214	2956	1613	1455	1372	1155	1061
70	3333	3208	2960	1610	1451	1373	1156	1065

Agarose films with different ChCl/Urea contents resulted in a FTIR spectra of a different appearance. This is due to the fact that when agarose and ChCl/Urea are mixed at different compositions, changes in characteristic bands occur as a result of the intermolecular interactions. The bands of both the O–H stretching of the alcohol group and the N–H stretching of the amide group shifted to lower wavenumbers with an increasing content of ChCl/Urea. Similar behaviour was observed at bands of both the N–H bending of the amide group and the O–H bending of the alcohol group. This was due to the increase in the formation of the intermolecular hydrogen bonding between agarose and ChCl/Urea. In contrast, it can also be seen in Table 3 that the bands for C–H stretching of the alkane group and C–O stretching of the alcohol group shifted toward higher wavenumbers as the ChCl/Urea content increased. These changes were caused by the decrease in internal hydrogen bonding. This was supported by the decrease in tensile stress and modulus as previously described. On the basis of this finding, it was considered that the intermolecular hydrogen bonding was formed between the hydroxyl groups of agarose chains with the amide groups of ChCl/Urea. This can be explained by the fact that each urea molecule has two amide groups; therefore they could form stable hydrogen bonding with both the chloride anions in choline chloride and the hydroxyl groups in the polysaccharide molecules (Abbott *et al.* 2003; Ma and Yu 2004). This result led to the deduction that the interaction between agarose and ChCl/Urea existed and the compatibility between them was favoured at a molecular level, explaining the absence of phase separation. Nevertheless, the minor shift in the wavenumbers of both the CH<sub>3</sub> bending of the alkane group and C–O–C stretching of the ether group remained ambiguous. However, this can be considered negligible.

### Crystallographic Properties

Pristine agarose film has broad diffuse peaks around 20° with a small shoulder at 14° (Fig. 5). It can be observed that a peak centered at around 20° showed an orderly structure with a high degree of crystallinity when compared with agarose films with high ChCl/Urea content. The characteristic features of the pristine agarose film peak in this study were similar to previous reports by other researchers (Freile-Pelegrín *et al.* 2007). This is probably due to the slow drying during film casting, providing the opportunity for highly ordered lamellae to be formed. The lamellae have relatively strong intermolecular forces that are also responsible for the increase in the hardness of the film and possess a more brittle behaviour (Carragher and Seymour 2003). This observation was congruent with results obtained from the DSC data (Fig. 2) and SEM images of the fractured surface (Fig. 3).

Nevertheless, the XRD patterns of the agarose films showed a reduction in their relative intensities of the peak at 20° as the content of ChCl/Urea increased. However, the increase of ChCl/Urea content from 30 to 40 wt% had a minimal effect on the peak intensity. In the case of agarose films with 50 and 60 wt% ChCl/Urea, the intensity of the peak at 20° was low, indicating that the degrees of crystallinity of the agarose films were reduced. On the other hand, in the case of agarose film with 70 wt% ChCl/Urea, the peak around 20° completely disappeared, probably as a consequence of the decrease of the crystallinity of agarose film. Compared to pristine agarose film, the addition of ChCl/Urea also enhanced the amorphous character of the films. The broad small peak

around  $14^\circ$  remained almost unchanged, except that its diffraction intensity decreased drastically. Another feature that was accentuated by these results is optical transparency. Therefore, transmittance study of agarose films was further examined by using a UV-Vis spectrometer.

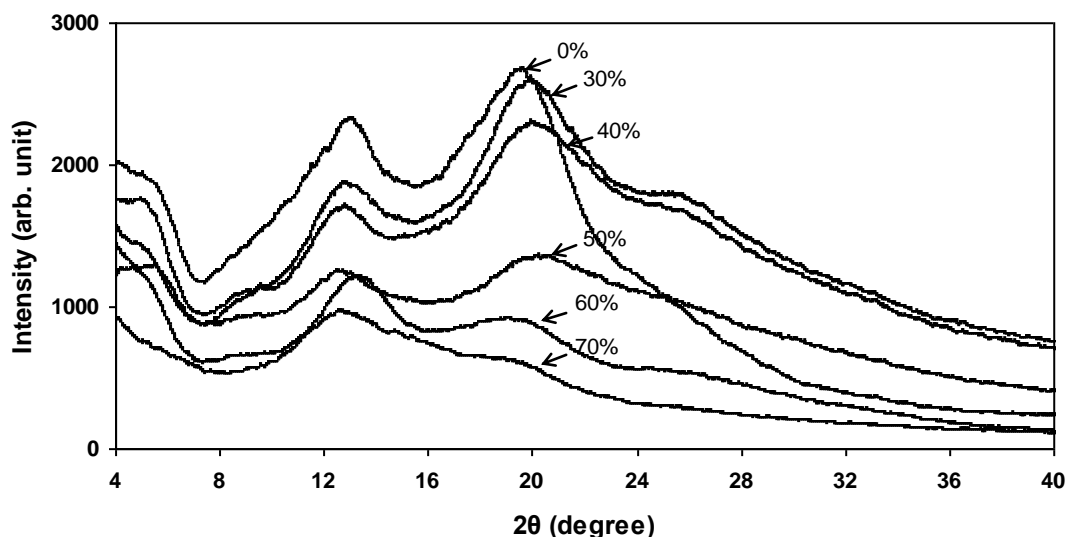


Fig. 5. XRD patterns of agarose films with different contents of ChCl/Urea

### Transmittance Study

All of the film samples considered were transparent and non-colored; however, all samples were subjected to UV-Vis spectroscopy to quantify the transmittance of the films. Figure 6 presents the UV-Vis spectra of the agarose films. The average optical transmittance of the pristine agarose film in the visible range was about 89.41% ( $\pm 0.16$ ), and it was much lower than the transmittance of agarose films containing ChCl/Urea. However, an infinitesimal increase in the transmittance can be observed for agarose films containing 30 and 40 wt% ChCl/Urea with transmittance of 90.53% ( $\pm 0.24$ ) and 90.69% ( $\pm 0.28$ ), respectively. The 50 wt% ChCl/Urea in agarose film showed a higher transparency of 91.23% ( $\pm 0.11$ ), followed by 60 wt%, which was 91.27% ( $\pm 0.12$ ). The highest transparency was obtained for the agarose film with 70 wt% ChCl/Urea, which showed transmittance values reaching up to 91.34% ( $\pm 0.14$ ) in the whole range of the visible spectrum. This is induced by the increase of amorphous phase content in the agarose film (Raphael *et al.* 2010). The orientation structure of the molecules in this phase is random and disordered. Consequently, its molecules are less densely packed than in the crystalline phase of pristine agarose film. This decreased the light scattering intensity, thereby allowing much of the light to pass through them. The overall UV-Vis results were entirely consistent with the results obtained from the XRD patterns, as previously discussed. Therefore, the plasticizing effect of ChCl/Urea on the agarose films provides not only flexibility but also optical transparency. The increase of optical transmittance started to appear at the wavelength 234 nm until 1000 nm, partly because of the absorption properties of the agarose film.

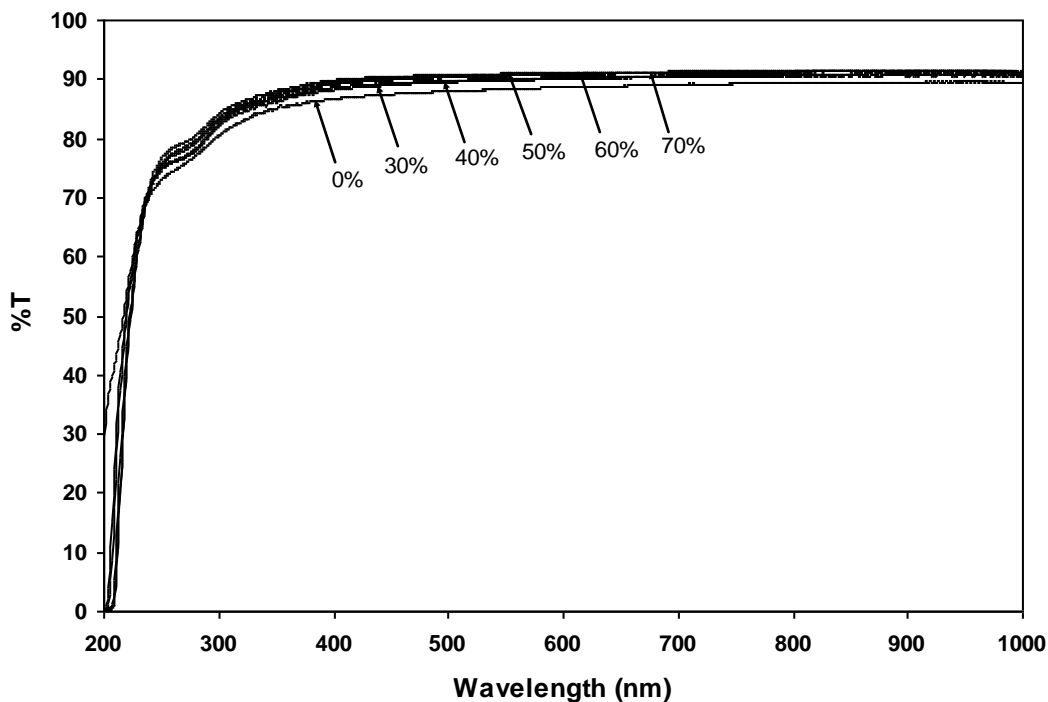


Fig. 6. UV-Vis spectra of agarose films with different contents of ChCl/Urea

## CONCLUSIONS

1. The addition of ChCl/Urea into the agarose films overcame its brittleness. High tensile extension and tensile strain properties was achieved at 60 wt% ChCl/Urea.
2. DSC thermograms indicated a depression in glass transition temperature,  $T_g$ , and SEM images showed that the fracture surface roughness of agarose films disappeared and no phase separation was observed. Thus, it was shown that the use of ChCl/Urea was successful as a plasticizer for agarose films.
3. FTIR spectra confirmed the presence of intermolecular hydrogen bonding between agarose and ChCl/Urea. XRD patterns demonstrated that an amorphous phase was obtained with incorporation of ChCl/Urea.
4. Transmittance study showed that the agarose films containing ChCl/Urea exhibited higher optical transparency compared to pristine agarose. It could be concluded that the presence of ChCl/Urea also affected the intermolecular forces as well as the crystallographic properties and optical transparency of the agarose films.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical support and the facilities provided by the School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, and the Institute of Bioscience, Universiti Putra Malaysia. Research for this paper was completely supported by the Ministry of Science, Technology, and Innovation (MOSTI) under the ScienceFund Grant Scheme (03-01-04-SF1035).

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Article submitted: April 17, 2012; Peer review completed: July 10, 2012; Revised version received: July 26, 2012; Accepted: August 4, 2012; Published: August 13, 2012.