1 INTRODUCTION

Polysaccharide-based gels play an important role in modern cosmetic and pharmaceutical formulations. This is mainly due to the characteristics of the polysaccharides, which are, biocompatible, biodegradable, edible, and are available from natural sources such as seaweeds [1]. The polysaccharide-based gels are commonly used as a matrix to disperse medicinal or cosmetic-active ingredients. The fluid-filling interstitial space within the gel network provides a continuous moisturizing effect to the skin [2 – 4]. Carboxymethyl cellulose (CMC) and i-carrageenan (i-C) are frequently used polysaccharides in food and pharmaceutical industries. CMC is a derivative of cellulose, with the carboxymethyl group bound to some of the hydroxyl groups of the glucopyranose monomer backbone [5]. In general, CMC is commonly used as a binding, thickening, and stabilizing agent of various products in cosmetic and pharmaceutical industries [6, 7]. i-C is a linear sulfated polysaccharide with alternating three-linked β-D-galactopyranose and four-linked 3,6-anhydro-α-pyranose residues [8]. i-C has been used in the preparation of foodstuff such as dairy products and jellies, due to its typical gelling strength [9, 10]. Besides the food industry, i-C has also been used in the preparation of soft gel for the oral drug delivery system [11, 12].

The liposome drug carrier has been widely investigated since it was discovered by Bangham and co-workers (Figure 1a) [13]. However, single type, non-surface modified liposomes have been found to hardly survive in the blood stream, due to fast elimination by the mononuclear phagocyte system [14, 15]. The recognition and removal of the liposomes from the blood stream, as foreign particles, has been promoted by the adsorption of proteins that circulate from the blood stream onto the surface of the liposome [14]. This disadvantage inhibits the liposome’s function as a drug carrier and reduces its circulation half-life [15]. In order to overcome this disadvantage, surface-modified lipo-
Liposomes have been developed [16–18]. Studies have revealed that the presence of chitosan and acylated chitosan on the surface of the oleic acid liposomes can enhance the stability and bioavailability of the liposome [19].

The great popularity of liposomes as drug carriers has been attributed to their versatile nature and ability to entrap hydrophilic and hydrophobic drugs (Figure 1b). This could protect the drugs from degradation and increase the therapeutic efficacy of the drugs by reducing their toxicity and side effects [20, 21]. For this reason, the liposome has been widely used in pharmaceutical and medical applications. Incorporation of the liposome into biopolymer gel is one of the approaches for the preparation of pharmaceutical formulation for topical application. Liposomes have the ability to improve the drug deposition and permeation rate through the skin especially for hydrophilic drugs [22–24]. In addition, the long retention time of the gel on the skin can prolong the release rate of the liposomes from the gel matrix, as well as reduce the dosing frequency of the therapeutic drugs that are encapsulated in the liposomes [25, 26].

Rheological behavior is an important characteristic of the gel, as it can reveal the information on the gel strength, rearrangement of the internal gel network structure, spreading ability, and stability of the gel during storage and transportation [27, 28]. The rheological behavior of the gels that correspond to spreadability [29] can directly influence the treatment consistency at the targeted site for topical application gels, such as, liposomal gels. Poor spreading of the gel can reduce the homogeneity of the liposome dispersion at the skin [30, 31]. As a result, the liposome’s diffusion rate from the gel through the skin is affected and it may cause adverse effects due to the incorrect dosage of drugs that have been transferred [32, 33]. In this study, the liposomal gels have been prepared by loading three different types of liposomes: oleic acid liposome, chitosan-modified oleic acid liposome, and acylated chitosan-modified oleic acid liposome. The objective of the present work is to prepare polysaccharide-based liposomal gel and to study the rheological behavior of the liposomal gel.

2 MATERIALS AND METHODS

2.1 MATERIALS

All solutions and samples were prepared by using deionized water with a resistivity of 18.2 Ω·cm (Barnstead Diamond Nanopure Water Purification, Barnstead International, Iowa USA). i-C was obtained from Dai-Ichikogyo Seiyaku Co. Ltd. (Japan). Boric acid and hydrochloric acid (HCl) were obtained from Merck (Germany). Sodium hydroxide (NaOH) was purchased from Fluka (Switzerland). GC grade oleic acid (99 %) was purchased from Sigma (USA). Potassium chloride was purchased from R&M (UK). Palmitoyl chloride was purchased from Aldrich (Switzerland), and used as received. Acridine orange hydrochloride hydrate was purchased from Aldrich (USA). Chitosan, with an average molecular weight of 10,000 Da (low molecular weight chitosan) and 25,000 Da (high molecular weight chitosan) were prepared by depolymerizing the 150,000 Da chitosan which was obtained from Acros Organics (USA).

2.2 PREPARATION OF ACYLATED CHITOSAN

Acylated chitosan, with a 18 % degree of acylation, was prepared from high molecular weight chitosan. First, 1 %(w/v) of high molecular weight chitosan solution was prepared by dissolving an appropriate amount of high molecular weight chitosan in 1 % of acetic acid solution. The pH of the high molecular weight chitosan solution was adjusted to 7, following which, 27 μL of palmitoyl chloride was added. The mixture was stirred under magnetic stirring at room temperature to yield acylated chitosan. After five hours, the mixture was neutralized and acylated chitosan was precipitated using acetone. The acylated chitosan was collected by the centrifugation method at 5000 rpm for two minutes at 25°C. The collected acylated chitosan was washed several times with chloroform to eliminate the free fatty acid, followed by drying under a vacuum oven at 30°C, overnight [34].

2.3 PREPARATION OF OLEIC ACID, CHITOSAN-COATED, AND ACYLATED CHITOSAN-COATED LIPOSOMES

The preparation of oleic acid, chitosan-coated, and acylated chitosan-coated liposomes was reported in a previous study [35, 36]. Briefly, 30 mM of oleic acid liposome solution was prepared by dissolving an appropriate amount of oleic acid in 50 mM of borate buffer (pH 8.8). The low molecular weight chitosan and high molecular weight chitosan solutions, with a concentration of 0.15 % (w/w), were prepared by dissolving an
appropriate amount of these chitosans in 50 mM of borate buffer (pH 8.8). Next, the pH of the oleic acid liposome and chitosan solutions was adjusted to 7 using HCl (1 M). The liposome solution was added dropwise into the chitosan solution under magnetic stirring. These mixtures were stirred for 24 hours at room temperature. Subsequently, the pH of the mixtures was adjusted to a pH of 8.8 for the formation of a chitosan-coated liposome. The above procedure was repeated for the preparation of an acylated chitosan-coated liposome. These liposome solutions were incubated at 25°C for 24 hours, before being incorporated into a gel.

2.4 PREPARATION OF GEL

The concentration of pure \( i-C \) and CMC was fixed at 1 and 2 \%(w/w), respectively. They were prepared individually by dispersing an appropriate amount of \( i-C \) and CMC into 50 mM of a borate buffer (pH 8.8) that contained 0.2 \%(w/w) potassium chloride. These solutions were then heated to 80°C in sealed vials, until a clear gel was obtained, and were kept at room temperature for 24 hours before proceeding to the next preparation step. Then, a series of mixtures with different amounts of \( i-C \) and CMC were prepared, using the above-mentioned 1 \%(w/w) of \( i-C \) and 2 \%(w/w) of CMC (Table 1). These mixtures were further diluted with 50 mM borate buffer (pH 8.8) in a ratio of 9:1. The mixtures were then kept at room temperature for another 24 hours before the rheological study. The mixture with the greatest rigidity and elasticity was selected for the preparation of liposomal gel.

2.5 PREPARATION OF LIPOSOMAL GEL

Liposomal gels were prepared by dispersing the prepared liposomes (10 \%(w/w)) into the selected gel mixture described previously, using a Vortex mixer (Uzusio VTX-3000L, Japan). The liposomal gels were vortexed for three minutes and stored at room temperature for 24 hours before the rheological study.

2.6 MORPHOLOGICAL STUDY OF LIPOSOMAL GELS

The distribution of liposomes in the selected gel system was observed using the confocal laser scanning microscope, the Leica TCS SPS confocal setup, mounted on a Leica DMI 6000 CFS inverted microscope (Leica Microsystems GmbH, Germany), and was operated under the Leica Application Suite Advanced Fluorescence (LAS AF) Program. The liposomal gels were stained with acridine orange. The sample was at equilibrium at room temperature for five minutes before further analysis.

2.7 RHEOLOGICAL MEASUREMENT

A stress/rate controlled Bohlin Gemini CVO-R Rheometer (Malvern, UK) with a temperature controller, was used to measure the rheological properties of the gel. The measurements were performed at 25.0 ± 0.1°C, with a 4°/40 mm cone and plate geometry, and a 0.100 mm gap. In order to obtain the proper parameter and reliable data from the frequency sweep, an amplitude sweep was performed first in a controlled strain mode,
with the applied strain ranging from 0.01 to 10 units, with the frequency fixed at 0.5 Hz; followed by a frequency sweep, which was performed in a controlled strain mode (a very low deformation strain was chosen from the linear viscoelastic profile from the amplitude sweep) by varying the frequency from 0.01 to 5 Hz.

2.8 STATISTIC

The statistical analyses were performed by using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA). The data were analyzed with one-way analysis of variance [37]. The differences in means and pairwise comparisons were considered to be statistically significant when \( p < 0.05 \) [38].

3 RESULT AND DISCUSSION

3.1 RHEOLOGICAL BEHAVIOR OF PURE 1 % (W/W) I-C AND 2 % (W/W) CMC

The rheological properties of 1 % (w/w) i-C and 2 % (w/w) CMC were evaluated in order to study their feasibility for the preparation of liposomal gels. The rheological behavior was first examined in the amplitude sweep mode. In the amplitude sweep test, the linear viscoelastic region LVR, critical strain \( \gamma_r \), elastic modulus \( G' \), and cohesive energy \( CE \) values were obtained. The LVR and \( \gamma_r \) were used to characterize the flexibility of 1 % (w/w) i-C and 2 % (w/w) CMC, while \( G' \) was used to evaluate the elasticity of the gel and CE described the cohesiveness of the gel’s network structure (Table 2 and Figure 2a) [39]. CE was determined using the following equation:

\[
CE = \frac{i}{2} G' \gamma_r^2
\]

Table 2: The critical strain \( \gamma_r \), elastic modulus \( G' \), cohesive energy \( CE \), slope of \( G' \) from frequency sweep, and Power Law Index PLI of the gel mixture and liposomal gels.

<table>
<thead>
<tr>
<th>Name</th>
<th>Critical strain ( \gamma_r )</th>
<th>Elastic modulus ( G' ) (Pa)</th>
<th>Cohesive energy ( CE ) (Pa)</th>
<th>Slope of ( G' ) from frequency sweep</th>
<th>Power Law Index PLI (PLI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 % (w/w) i-C</td>
<td>0.65</td>
<td>39.41</td>
<td>6.1e+02</td>
<td>0.166e+0005</td>
<td>N/A</td>
</tr>
<tr>
<td>2 % (w/w) CMC</td>
<td>0.36</td>
<td>0.70</td>
<td>0.0464e+006</td>
<td>1.25e+0008</td>
<td>N/A</td>
</tr>
<tr>
<td>8.2G</td>
<td>0.412</td>
<td>23.84</td>
<td>2.023e+004</td>
<td>1.023e+0006</td>
<td>0.136e+005</td>
</tr>
<tr>
<td>7.3G</td>
<td>0.477</td>
<td>18.89</td>
<td>2.149e+007</td>
<td>0.153e+0006</td>
<td>0.163e+004</td>
</tr>
<tr>
<td>5.5G</td>
<td>0.576</td>
<td>11.99</td>
<td>1.989e+004</td>
<td>0.191e+0009</td>
<td>0.234e+004</td>
</tr>
<tr>
<td>3.7G</td>
<td>0.341</td>
<td>9.64</td>
<td>0.502e+002</td>
<td>0.213e+001</td>
<td>0.284e+003</td>
</tr>
<tr>
<td>2.8G</td>
<td>0.289</td>
<td>5.98</td>
<td>0.250e+001</td>
<td>0.327e+003</td>
<td>0.362e+004</td>
</tr>
<tr>
<td>LG-OA</td>
<td>0.41</td>
<td>12.31</td>
<td>1.04e+01</td>
<td>0.178e+001</td>
<td>0.192e+001</td>
</tr>
<tr>
<td>LG-OACHe</td>
<td>0.43</td>
<td>13.38</td>
<td>1.24e+01</td>
<td>0.167e+003</td>
<td>0.185e+001</td>
</tr>
<tr>
<td>LG-OACHeP</td>
<td>0.44</td>
<td>13.45</td>
<td>1.28e+01</td>
<td>0.163e+002</td>
<td>0.183e+001</td>
</tr>
</tbody>
</table>

It was found that the \( G' \) and \( \gamma_r \) of 1 % (w/w) i-C were higher than 2 % (w/w) CMC, which indicated the higher rigidity of 1 % (w/w) i-C (Table 2 and Figure 2). The high CE value of the 1 % (w/w) i-C indicated high cohesiveness within its network structure. These results were mainly attributed to the strong internal gel network structure of i-C. From the frequency sweep test, the \( G' \) of 1 % (w/w) i-C was found to be greater than its \( G'' \) over the selected frequency range and this result indicated the solid-like behavior of 1 % (w/w) i-C (Figure 2b). Besides the viscoelastic properties, the flow behavior of 1 % (w/w) i-C was evaluated by measuring its complex viscosity as a function of frequency. It was found that 1 % (w/w) i-C exhibited high complex viscosity. This result was mainly attributed to the strong internal gel network structure of 1 % (w/w) i-C (Table 2 and Figure 2c). Complex viscosity of 1 % (w/w) i-C was found to decrease with the increasing frequency, and this result showed the shear thinning effect. The shear thinning behavior either originated from the disentanglement of the polymer coil or the increased orientation of the polymer chains in the flow direction.

The 2 % (w/w) CMC exhibited different viscoelastic behavior as compared to 1 % (w/w) i-C. Based on the amplitude and frequency sweep profiles of the 2 % (w/w) CMC, the \( G'' \) curve was always dominant against the \( G' \) over the selected strain and frequency range. These results indicated the liquid-like behavior of the 2 % (w/w) CMC (Figures 2a and 2b). The \( G' \) and CE values of the 2 % (w/w) CMC were also relatively lower than those for the 1 % (w/w) i-C, and a similar result was also reported by the previous studies [40, 41]. It was also found that the complex viscosity of the 2 % (w/w) CMC was 100 times lower when compared with 1 % (w/w) i-C, which indicated that the strength of the network structure in 2 % (w/w) CMC was relatively weak when compared to 1 % (w/w) i-C.

As discussed earlier, it can be concluded that the 1 % (w/w) i-C is a rigid gel, as it exhibits high \( G' \) and \( CE \). The high \( G' \) and \( CE \) can limit the dispersion and reduce the homogeneity of the liposomes within the 1 % (w/w) i-C [42]. The high complex viscosity of 1 % (w/w) i-C will also result in poor spreadability of the dispersed liposomes on the skin [30, 31]. As a result, the diffusion rate of the liposome from 1 % (w/w) i-C into the skin may be lower and this may also cause adverse effects due to the incorrect dosage of drugs that have been transferred [32]. On the other hand, the weak internal structure (low \( G' \), \( CE \), and shear viscosity) of 2 % (w/w) CMC may not be strong enough to hold and stabilize the dispersed liposomes. For these reasons, a series of mixed gel systems have been prepared by mixing 1 % (w/w) i-C and 2 % (w/w) CMC in different ratios (Table 1). In order to identify the optimum composition of the mixed gel...
system that is suitable for the preparation of liposomal gel system, the rheological properties of these mixed gels have been evaluated.

3.2 RHEOLOGICAL BEHAVIOR OF A MIXTURE OF THE i-C AND CMC GEL SYSTEM

3.2.1 Viscoelastic properties

The amplitude sweep profiles of all mixed gels showed that \( G' \) was predominant over \( G'' \). Also, the \( G' \) obtained from the amplitude sweep was found to increase from 2.8G to 8.2G (Table 2). These results indicated that the strength of the internal gel network structure could be improved by increasing the i-C concentration (Figure 3 and Table 2). The changes in CE of the gel mixtures were only 7% when the concentration of CMC in the gel mixture increased to 50% (8:2G to 7:3G) and was considered as negligible (\( p > 0.05 \)). The CE of the gel mixture decreased dramatically from 1.33 Pa to 0.40 Pa (\( p < 0.05 \)) when the concentration of CMC exceeded 50% (3:7G and 2:8G). This result indicated that the elastic strength of the gel network structure for 3:7G and 2:8G was relatively weak compared to 8:2G, 7:3G, and 5:5G.

The \( \gamma_c \) is the critical strain that represents the end of the LVR. The higher the \( \gamma_c \), the longer the LVR is. In other words, gels with a higher \( \gamma_c \) can withstand greater deformability. In this study, the \( \gamma_c \) of all gels was interpreted using a shear stress vs shear strain plot as demonstrated in Figure 3b. The \( \gamma_c \) of the gel mixtures is found to increase from 2.8G to 5.5G. This result indicates that the gel mixture with a higher i-C concentration exhibits greater resistivity against deformation. This is mainly due to the ability of i-C, which can exert more influence on the elasticity and flexibility of the gel mixture. The \( \gamma_c \) value of 5:5G is the highest among the prepared gel mixtures. This result indicates that the 5:5G gel has a greater elastic network structure and can withstand larger deformation. The \( \gamma_c \) values start to decrease (\( p < 0.05 \)) when the concentration of i-C exceeds 0.63% (w/w) (7:3G and 8:2G). Therefore, 7:3G and 8:2G are more sensitive against the applied strain and less flexible. This result is mainly due to the great physical entanglements between the polymer chains. According to the previous studies [43, 44] it is suggested that the i-C chains adopt a random-coil conformation and entangle with each other to form a gel (Figure 4a). This physical entanglement will become more pronounced with increasing i-C concentration, and it will eventually limit the mobility of the polymer chains, leading to the formation of a less flexible and stronger gel.

Increase in the elasticity of the gel with an increasing concentration of i-C was also shown in the frequency profile, where the \( G' \) of all gel mixtures was found to be larger than \( G'' \), over the selected frequen-
where the consistency, $\eta^*$ is the complex viscosity of the mixed gel, $k$ is the consistency, $\omega$ is the frequency, and $n$ is the Power Law Index $PLI$ [47]. The $PLI$ of the gel mixtures was increased with decreasing $i$-C and this result indicated that the gel mixtures had become less shear thinning ($p < 0.05$). This result also implied a decrease in the spreadability of the gel mixtures (Table 2). The complex viscosity of the gel mixtures was found to increase with increasing $i$-C composition (Figure 3d). This might be attributed to the newly formed entanglements between the $i$-C polymer chains, which retarded the flow of the gel mixtures, as discussed earlier in the article.

In order to produce good dispersion of the liposome in the gel system, the $CE$ of the gel must be high enough to suspend the liposome, as $CE$ is related to the work required to overcome the cohesiveness within the gel network structure [39]. Another factor to be considered is the flow property of the gel. The flow property of the gel can influence the stability of the dispersed liposome and also the texture of the gel. For example, high complex viscosity and shear stress will decrease the homogeneity of the liposome dispersion, whereas, low viscosity will lead to phase separation and consequently affect the stability of the liposome-gel mixture. After considering these two factors, 5:5G has been selected for the preparation of the liposomal gel. In this study, three types of different liposomal gels have been prepared, namely oleic acid liposome-in-gel (LG-OA), Chitosan-coated oleic acid liposome-in-gel (LG-OACH), and Acylated chitosan-coated oleic acid-in-gel (LG-OACHP). The morphology of the oleic acid liposome-in-gel is shown in Figure 4. This micrograph shows that the liposomes are distributed and accommodated in the gel network structure.

3.3 EFFECT OF THE LIPOSOME ON THE RHEOLOGICAL PROPERTIES OF 5:5G

3.3.1 Dynamic behavior

The $G'$ value of the liposomal gels was slightly increased from 12 Pa to 13.7 Pa after the liposomes were added to the 5:5G, and this result indicated an increase in the elasticity of the liposomal gels (Figure 5a). This result was mainly due to the rigidity of the liposomes [48]. Under applied shear stress, the liposomes could effectively store the applied stress in their elastic components and thus increase the $G'$ of the liposomal gels (Table 2). The $\gamma_c$ and $CE$ for all liposomal gels were lower when compared to 5:5G ($p < 0.05$). This result indicated that the liposomal gels could be deformed easily when compared to 5:5G. The decrease of $CE$ indicated a decrease in cohesiveness of the gel structure. This result was favorable, as the cohesiveness within the liposomal gels was directly proportional to the flowability and spreadability of the liposomal gels. High $CE$ retarded the flow of the liposomal gels, limited the spreadability of the gel, and thus, influenced the homogeneity and consistency of the gel, upon application [30].

The contribution of liposomes to the elastic component of 5:5G was also shown in the frequency sweep data. According to the frequency sweep profile, all liposomal gels exhibited solid-like behavior with $G'$ greater than $G''$ over the selected frequency range (Figure 5c). The $G'$ profile of all liposomal gels was shifted to a high-

Figure 4: Fluorescence micrographs of oleic acid liposome-in-gel. The liposomes are indicated with arrows.
er value as compared to 5:5G ($p < 0.05$). This result showed the increase in elasticity of the liposomal gels. The slope of $G'$ of the liposomal gels was also found to be slightly lower than 5:5G. The decrease in the $G'$ slope implied that the liposomal gels were more solid-like than 5:5G. These results were in agreement with the results obtained from the amplitude sweep, as discussed earlier, wherein the liposomes could store some of the applied stress in their elastic components. The increase in the elastic behavior of the liposomal gels might be attributed to the presence of liposomes that added an extra “bridging” effect, which linked the adjacent polymer chains in the gel [49]. It was suggested that this bridging effect originated from the hydrogen bonding between the O-H at the backbone of the polymer chains in the gel matrix and the oxygen atom in the fatty acid or the oxygen atom or nitrogen atom of the chitosan molecules at the surface of the liposomes, as the molecules were rich in the -OH groups. On the other hand, the changes in the $G''$ of the liposomal gels were negligible when compared to 5:5G, which implied that the changes in the dissipation energy of the liposomal gels were negligible. This result showed that the presence of liposomes did not affect the physical entanglements of the 5:5G and were just accommodated within the voids of the gel network [26, 45].

3.3.2 Complex Viscosity

The complex viscosity of the liposomal gels was found to be slightly higher than the 5:5G (Figure 5d). This result might be attributed to the presence of the liposomes that added an extra “bridging” effect, which linked the adjacent polymer chains in the gel, as discussed earlier [49]. The liposomal gels also exhibited shear thinning behavior. However, the PLI of the liposomal gels was relatively lower than that of the 5:5G. This result showed that liposomal gels had more shear thinning behavior, with better spreadability, as compared to the 5:5G.

4 CONCLUSION

In this study, liposomal gels were prepared by dispersing oleic acid, chitosan-coated oleic acid liposome, and acylated chitosan-coated oleic acid liposomes into a gel mixture consisting of i-C and CMC. According to the rheological data, a gel mixture with 0.63 % (w/w) of i-C and 0.54 % (w/w) CMC (5:5G) was used to prepare the liposomal gels, due to their optimum viscoelastic properties and moderate flow behavior. The $\gamma$, of 5:5G was the highest among all prepared gels, which indicated the flexibility of the gel. The $CE$ of the 5:5G was also found to be an optimum value among the gel mixtures. Lipo-
somal gels were prepared by mixing the oleic acid, chitosan-coated oleic acid liposome, and acylated chitosan-coated oleic acid liposome into the 5:5G. The result demonstrated that the presence of liposomes in the gel matrix had increased the elastic property of the gel. This was explained by the rigidity of the loaded liposomes. The low CE value of liposomal gels is favorable because high CE will limit the transfer of liposomes from the gel matrix to the targeted site. Besides elastic properties, the higher complex viscosity of the liposomal gels when compared with 5:5G will enhanced the stability of the dispersed liposomes during storage. The presence of liposomes was also found to exhibit greater shear thinning behavior indicated higher spreadability of the liposomal gel when compared with 5:5G.

**ACKNOWLEDGEMENTS**

We would like to extend our gratitude to University of Malaya and the Ministry of Higher Education Malaysia for providing us financial support (PV004/2012A and FRGS, No. FP017/2010A).

**REFERENCES**


