Sys Rev Pharm 2020; 11(5):850-861
A multifaceted review journal in the field of pharmacy

To Consider The Organogel Of Span 40 And Span 60 In Sesame Oil As A New Member In The Gastro Retentive Drug Delivery Systems

Zainab Saad Kaddoori [1] , Masar Basim Mohsin Mohamed [2] , Wesam R. Kadhum [3] , Nawfal Am. Numan [4]

[1] AL-Mustansiriyah University, Pharmacy college, Pharmaceutics Department, Baghdad, Iraq,

[2] AL-Mustansiriyah University, Pharmacy college, Pharmaceutics Department, Baghdad, Iraq,

[3] Department of Pharmacy, Al-Kut University College, Wasit 52001, Iraq,

[4] Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan: 19328,

Corresonding author: Masar Basim Mohsin Mohamed**,** masarmohamed328@gmail.com

ABSTRACT

To explore the ability of organogel for floating in acidic media to obtain a novel member gastroretentive dosage form testing span 40 and span 60 as a gelators, sesame oil (SO) as an apolar phase and cinnarizine (CN) as a model drug to produce an organogel as a gastroretentive drug delivery system. Floating properties were studied like floating lag time and floating duration for the organogels in both solid and liquid status. Characterizations of organogels were studied by assessment of thermal behavior by tabletop rheology and probing the organogels morphology with an optical microscope, the assembly of gelators was investigated by Fourier-transform infrared spectroscopy (FTIR). Oscillatory rheology studies, the amplitude sweep and frequency sweep were conducted. These were followed by in- vitro release and in-vivo floating studies. The results showed that all span organogels floated instantly and for around 24 hours in both statuses. Also, all span organogels showed reversible transition temperature and higher than 37 ºC and the optical images showed fibrillar scaffold where the fibers length of span 40 organogel were significantly shortening with increasing concentration of span 40 and the non significant change was with span 60.The hydrogen bonds were responsible on gelator-gelator interactions by a shift in the peaks that associated with the carbonyl and hydroxyl groups. Moreover, organogels showed the viscoelastic properties as the 20% w/w of span 40 and span 60 organogels were frequency independent. Last, organogels slowed the release of CN and persisted in rat's stomach for 12 hours.

Keywords: span 40- span 60- low molecular weight organogelator- organogel- floating system-gastroretentive drug delivery system.

Correspondence:
Masar Basim Mohsin Mohamed
AL-Mustansiriyah University, Pharmacy college, Pharmaceutics Department, Baghdad, Iraq,
*Corresponding author: Masar Basim Mohsin Mohamed
email-address: masarmohamed328@gmail.com

INTRODUCTION

The oral route is a major route in drug delivery as it is easier to manufacture and more convenient to individuals; although, some problems in this route are needed to be resolved concerning erratic absorption, the low solubility of weakly basic drugs and short gastric residence time. Gastro retentive drug delivery systems (GRDDS) provide new and essential therapeutic options to solve these problems by prolonging the gastric residence time and improve the bioavailability of weakly basic drugs. GRDDS includes: swelling and expanding system, bio/mucoadhesive system, high-density system, magnetic system and low-density system (floating system)[1] as the floating system has been studied extensively. Side by side, the capability of the low molecular weight organogelator (LMOG) capturing oil and because they are easy to formulate as this agrees with the sustained release requirements that improve the bioavailability of the drug; these led in this work, an investigation for floating properties to the organogel and seeking to designate a new member for a floating system to be delivered in soft gelatin capsules. This approach and according to our knowledge hadn't been studied till now. In parallel, a full characterization for organogels and their depot properties were assessed as a proven feature[2]. The range of LMOGs for organogels preparation was sorbitan monopalmitate (span 40) and sorbitan monostearate (span 60) as they represented the solid phase and the liquid phase was represented by the sesame oil (SO). Cinnarizine (CN) was used as a model drug which is an H1-receptor antagonist and it was chosen because of its lipophilicity that makes CN a good candidate to be formulated in oil. Also, CN nature as a weakly basic molecule encouraged the scientists to formulate CN in many GRDDS [3].Both spans as lipophilic nonionic surfactants mostly were formulated as vesicles and emulsion for oral route of administration [4-9]while as a LMOG, spans were used as a gelator in organogel for topical preparations such as the organogels of 20% w/w span 40 in groundnut oil and mustard oil where those organogels were stable and prolonged the release of metronidazole[10]. Besides, span 60 in sunflower oil organogel was used to deliver salicylic acid for transdermal application and showed prolonged shelf-life. For the oral rout, spanorganogels were utilized as in span 40/ medium-chain triglyceride organogel that improved the solubility of curcuminoids and span 60 in sorbitanmonooleate with polysorbate 80 organogel to deliver cyclosporine [11-12].The SO in addition to its nutritional benefits, was used as a liquid in organogel oral route [13-14]. To achieve our aim, the floating properties such as floating lag time and floating duration of organogels as a solid and as a liquid were investigated. Besides, organogels were explored for the rheological properties, morphological network and Fourier transform infrared (FTIR). Furthermore, the depot property of the selected organogels was tested by the release study of CN in the gastric media at pH 1.2 as well as in vivo floating was executed to the selected organogels.

Results and Discussion

Preparation of organogel

The assessment of the gel formation was visually examined as this helped to determine the minimum gel concentration (MGC), which is the minimum concentration of gelator required for gelation at 25 °C as shown in figure 1. The vials of the prepared concentrations 1% , 3% , 5%, 7% , 10%, 13%, 15%, 18% and 20% w/w of the span 40 and span 60 in SO were inverted to check the organogel formation where the span 40 in SO showed gelation at 13%, 15%, 18% and 20% w/w whilst the lower concentrations were not gelled. Also, the concentrations of organgels of span 60 started at 10%, 13%, 15%, 18% and 20% w/w. Those results are similar to a different study formulated organogels of span 40 and span 60 in soybean oil while the MGC for span 60 was found to be 16% w/v and 18%w/v for span 40 as the lower concentrations than their MGC were not gelled in soybean oil [15].The differences in the MGC of different gelators could be attributed to the solubility differences of the two gelators, those gelators were soluble in oil at high temperature (65°C) during preparation but when they cooled down; they exposed different gelation. This was due to gelators molecules formed aggregates upon cooling but these self-aggregations of gelators were not sufficient to form a 3D network at low concentration of gelators. The assembly of the aggregates is important and required to build up the 3D network structure for the gel formation. For the next studies, the selecting of the organogel concentrations was by picking up 3 organogels that showed gelation upon inverting such as the concentrations that represented the lower, the middle and the higher. For span 40 and span 60 in SO were (13%, 15%, 20%) (w/w) and (10%, 15%, 20%) (w/w), respectively.

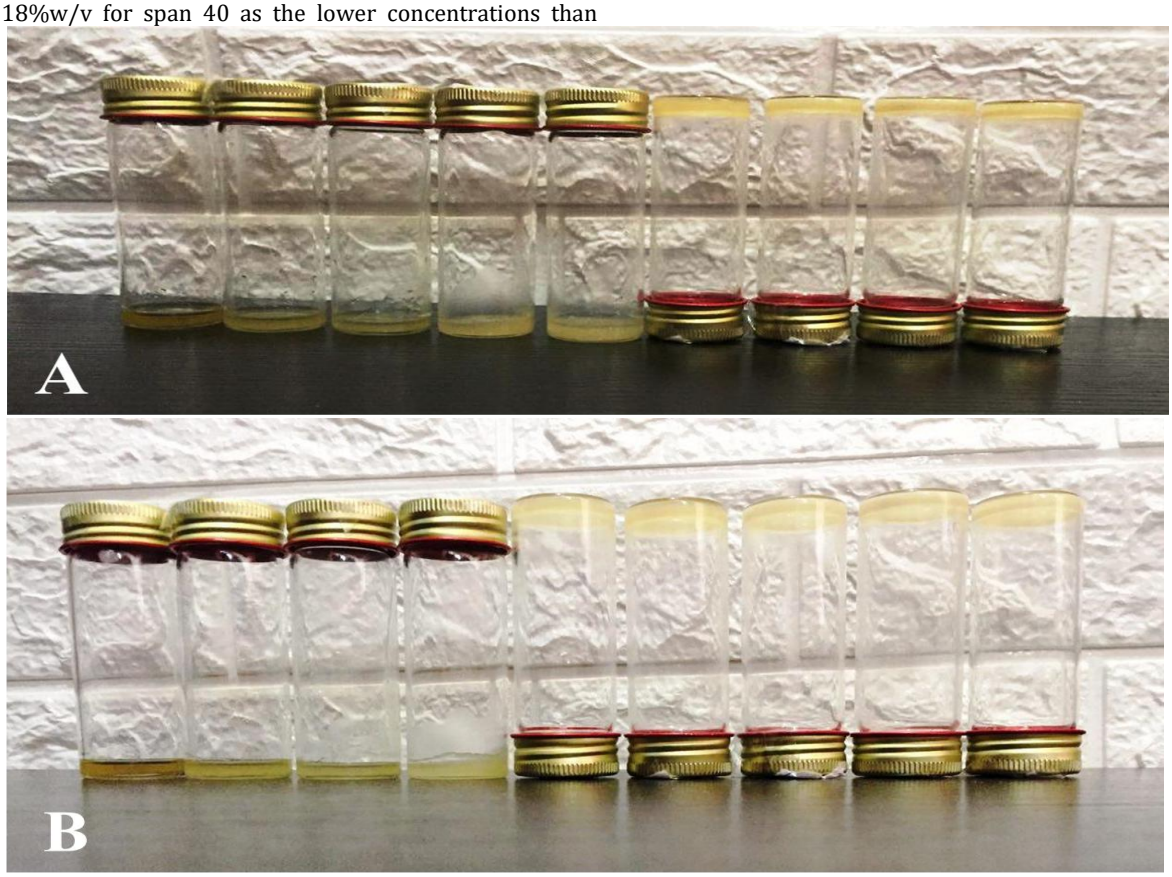


Figure (1): Organogels of span 40 and span 60 in SO as in A and B respectively. Since, all organogels concentrations were from left to right 1%,3%,5%,7%,10%13%,15%,18%,20% w/w and the solid organogel represented as inverted vial.

Investigation of the floating properties

The general floating properties are floating lag time and floating duration which are important to study to guarantee that our organogel meet the aim of our work[16-17].These investigations started with organogel as a solid loaded in the capsule. Lag time parameter: The capsules of all formulations were instantly buoyant and within 5 minutes of the experiment, all capsules shells were melting in 0.1N HCL leaving the solid organogel floated. After that, all formulations showed a continuous floating; this could be justified to the organogel properties and also due to the low density of oils that was prepared with. This outcome was reported in our work as zero lag time. The duration of floating: This was clarified in Table 1 for all organogels where 13% w/w Span 40/SO organogels showed durations of floating 17 hours and it was 24 hours for both of 15% w/w and 20% w/w span 40/SO. These durations were acceptable and could be scored +++. For the selected concentrations of span 60 in SO, the floating duration of 10% w/w and 15% w/w was 20 hours while the 20% w/w floating duration was 24 hours. Those organogels could be also scored as +++ and this result was similar to the duration of oily in situ floating gels of sodium alginate that formulated with guar gum[18].Clearly from the above results, increasing the concentration of LMOGs prolonged the floating duration. As this augmentation could be justified to a denser scaffold of organogels which in turn decrease the liquid penetration; hence, as a result the mass of organogel kept

floating. To conclude, the selected organogels of span 40 and span 60 in SO were floating instantly and showed different floating durations as the highest concentrations showed the longest durations. Table 2 showed floating parameters of organogel as a liquid besides to the gelation time. This study was done to confirm the floating properties of the selected organogels without the aid of the capsule as the last one is well known to float that might help in floating our selected organogels. Markedly as shown in Table 2, all the selected organogels from the lower to the higher concentrations of span 40 and span 60 in SO showed the same gelation times 15 min, 12 min and 7 min after using the same volumes of ethanol 100 µl, 150 µl and 200 µl respectively. It is obvious the volume of ethanol was increased as the concentration of LMOGs in organogel increased and this might be due to the self-assembly of LMOGs increased as the LMOGs concentration increased. This in turn increased the volume of ethanol to disassembly the LMOGs. This method was implemented in different work by using specific amounts of ethanol to liquefy N-lauroyl-L-alanine methyl ester/soybean organogel and an opaque gel was formed in the aqueous media within 2 min after pouring[19].In nutshell, all organogels were liquefied successfully by ethanol and the ethanol volume was increased as the LMOGs concentration increased as this manifest an inverse relationship with the gelation time. Moreover, all liquefied organogels were gelled and showed the same floating parameters when they were solid in capsules. Those floating results were matching our aim of work as the organogels showed appropriate duration floating time in addition to the lag time whether the organogels were solid loaded in a capsule or liquid. Thus the next steps were investigating the organogel for their properties.

Table (1):the floating duration for solid organogels

Organogels	LMOGs /Oil% (w/w)	Floating duration in hour
Span40/SO	13	17
	15	24
	20	24
Span60/SO	10	20
	15	20
	20	24

*All formulations had floating lag time = zero because they are instantly floated.
**All organogels scored +++ due to instant gelation and its duration around 24 hours.

Table (2): The gelation time and the floating parameters for liquid organogels

Organogels	LMOGs /Oil% (w/w)	Ethanol volume µl	Gelation time in minutes	Floating duration in hour
span 40 /SO	13	100	15	17
	15	150	12	24
	20	200	7	24
span 60 /SO	10	100	15	20
	15	150	12	20
	20	200	7	24

*All formulations had floating lag time = zero because they are instantly floated.
** All organogels scored +++ due to instant gelation and its duration around 24 hours.

Table to rheology

Tabletop rheology is a simple and convenient method for characterization of phase transitions from gel to solution (sol) and vice versa, and especially for our thermo sensitive organogels. Phase transition temperature results from sol to gel and gel to sol were illustrated in Table 3 for all selected organogels. Firstly, sol to gel transition temperature (Tsol-gel) for the 13% w/w, 15% w/w and 20% w/w span 40/SO were 38.33ºC, 40.33ºC and 41.66ºC whilst the transition temperature of 10% w/w, 15% w/w and 20% w/w span 60/SO organogels were 40.33ºC, 42.33ºC and 48.33ºC sequentially. The reverse phase of transition temperature of gel to solution for the 13%w/w, 15% w/w and 20% w/w of span 40 organogels which were liquid at 40.33ºC , 42.33ºC and 44.33ºC, respectively. In case of span 60/SO organogels, Tgel-sol were 40.66ºC, 41ºC and 51.66ºC for the concentrations 10% w/w, 15% w/w and 20% w/w correspondingly. The above data obviously indicated a trend of direct relationship between the Tsol-gel / Tgel-sol and the concentrations of gelators which this could be justified to a denser 3D network of organogel was as the concentration of gelators increased [20].Also, the results showed that the Tgel-sol is always higher than Tsol-gel and this pattern was similar to the organogels of 12-hydroxy octadecanoic acid/n-decane[21].To conclude, phase transition for the selected organogels was reversible and higher than 37 ºC which indicated the stability of organogels in the body temperature 37 ºC.

Table (3): The transition temperature of organogels

Organogels	LMOGs /Oil% (w/w)	Solution to Gel transition temperature(ºC)	Gel to Solution transition temperature(ºC)
Span 40 /SO	13	38.33±0.57	40.33±0.57
	15	40.33±0.57	42.33±0.57
	20	41.66±0.57	44.33±0.57
Span 60 /SO	10	40.33±0.57	40.66±0.57
	15	42.33±0.57	41.00±1.00
	20	48.33±0.57	51.66±0.57

Optical microscopy

This study was executed to probe the cross section morphology of the organogel scaffold and this is demonstrated in the Figure 2 where the images A, B, and C present the13% w/w, 15% w/w and 20% w/w span 40 organogels in SO. From the first glance to the 3 images, an apparent febrile is shown. This network morphology is similar to a different study which used span 40 organogel

in mustard oil[10].Also, the images of the selected concentrations of span 60 organogels in SO are shown in D, E and F in figure 2 which also displays fibrillar networks but they were shorter and more branching comparing with span 40 organogels. This morphology in our work is likewise of the span 60 organogel/ soybean oil network [22].These images were further studied by ImageJ software to confirm fiber length of each organogel precisely as clarified in Figure 2 G and H. Firstly, the length of fibers were 10 µm, 10 µm and 8 µm for 13% w/w, 15% w/w and 20% w/w span 40/SO organogels, respectively. Differently in span 60 organogels, the fibers were shorter than span 40 organogel and they were 5 µm, 3.66 µm and 3.33µm for 10% w/w, 15% w/ and 20% w/w, respectively. Thus, One way ANOVA was applied using SPSS to find if the difference in the length fiber with concentration of gelator for each gelator was significant or not. The statistical analysis came conformity to the visual finding, where the fiber length of all selected concentrations of span 40 organogels were significantly shortening with concentration ($p < 0.05$) differently in span 60 were the fiber length of all selected concentrations of span 60 organogels were not significantly shortening with concentration ($p > 0.05$).

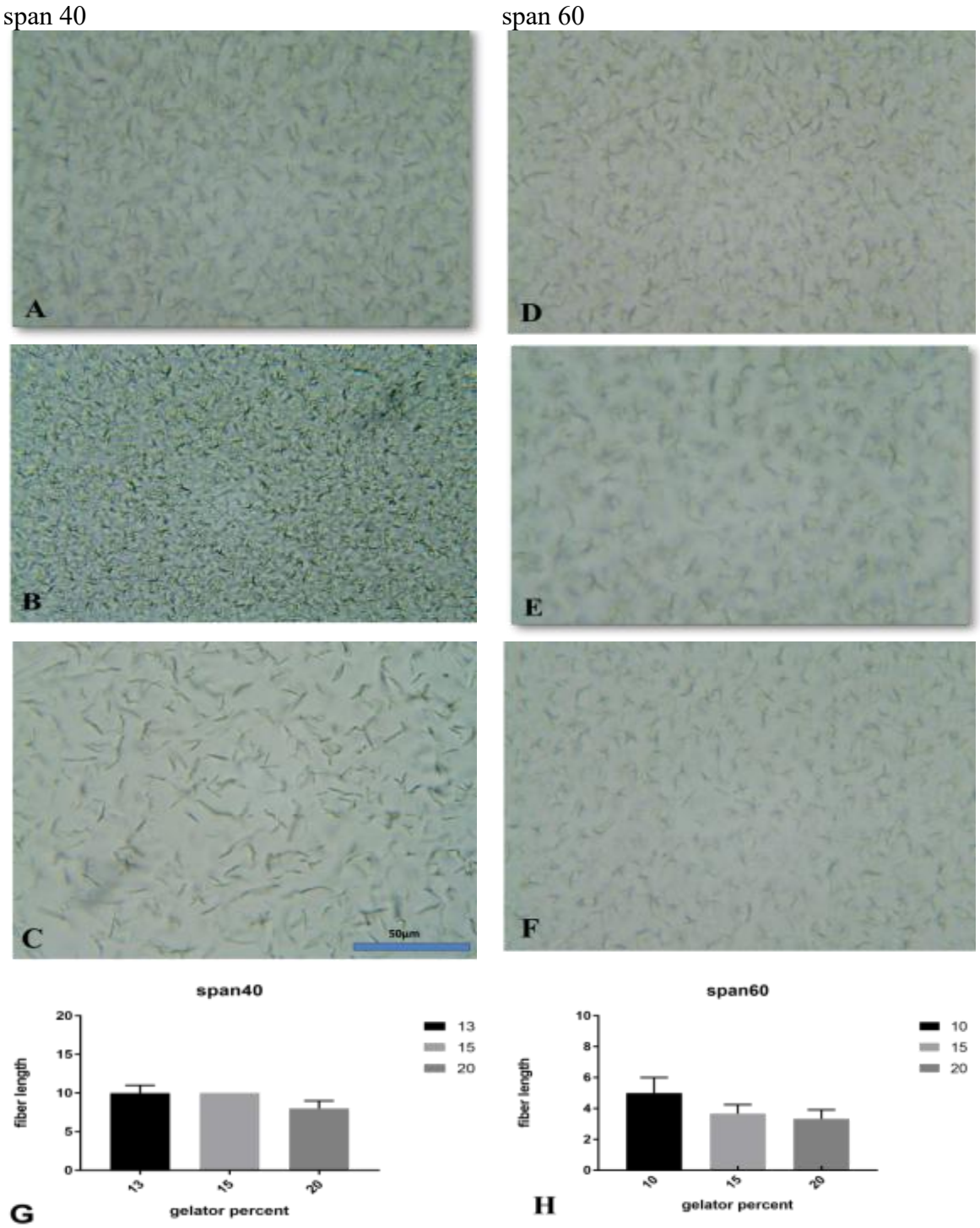


Figure (2): Microscopic images of span 40 and span 60 organogels in which A, B and C representing 13% w/w, 15% w/w and 20% w/w while D, E , and F representing 10% w/w, 15% w/w, 20% w/w in SO. Bar charts show fiber length of organogels where G for span 40 and H for span 60. Error bars represent standard deviation, n =3.

Fourier transform infrared (FTIR)

FTIR spectrum study gives qualitative information concerning physical interactions amongst gelators that are responsible for gelation and the interactions between components of the organogel. The highest concentration of the selected organogels spectrograms was compared against gelators (span 40 and span 60) as well as SO spectrogram where in A and B in Figure 3 focusing on the carbonyl related region for both organogels where a peak at 1745 cm^{-1} in the spectrogram of 20% w/w span 40 organogel which shifted from the peak of span 40 spectrogram at 1734 cm^{-1}, despite the SO spectrogram showed the peak at the same site (1745 cm^{-1}). This trend was similar to the carbonyl associated peak of the span 60 spectrograms as shown in B Figure 3. This outcome indicates possible hydrogen bindings that help in self-assembly of LMOGs rather than referring it to the carbonyl peak of the SO. However, Sat apathy *et al* prepared span 40 organogel in mustard oil and this shift in the carbonyl region were not shown in their FTIR spectrograms[10]. Also, Behera*et al* in a different study formulated organogel of span 60 in sunflower oil and their FTIR study did not show any shift in the carbonyl peaks[23].Additionally, C and D Figure 3 focus on hydroxyl region (from 3300 cm^{-1} to 3700 cm^{-1}) as spectrograms of organogels in SO showed the low intensity of a broad peak compared with the raw span 40 and span 60 spectrograms. This result was similar to Behera*et al* as they attributed this change to the intermolecular hydrogen bonding between span 40 molecules that were responsible for gelation in sunflower oil[24]. To sum up, all FTIR spectrograms showed a shift in the carbonyl associated peaks as well as the low intensity of peaks associated with the hydroxyl group of span 40 and span 60 organogels that all helped in the constitution of the scaffold by hydrogen bindings.

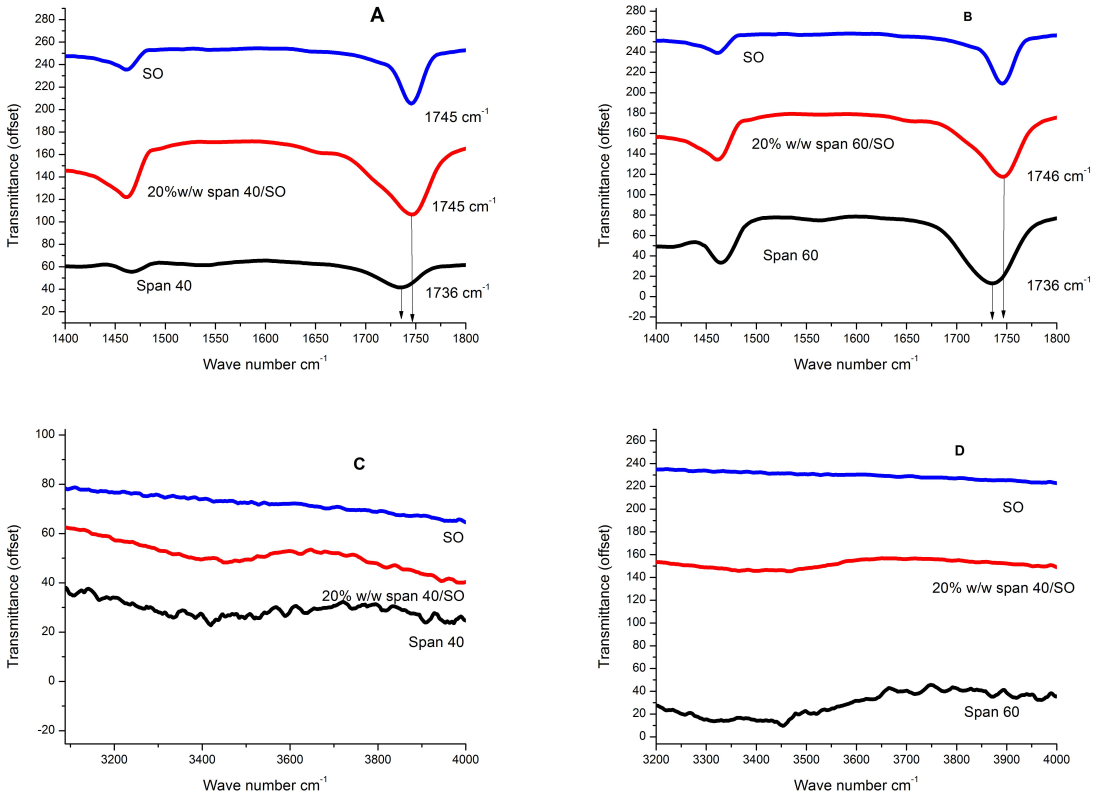


Figure (3): FTIR spectrograms of span40, span 60, SO and 20% w/w span40 organogels and 20% w/w span60 organogels where A and B represent the region of carbonyl in all spectrograms while C and D represent the all spectrograms of hydroxyl region.

Oscillatory rheology studies

Rheological studies were proposed in this work to determine viscoelastic properties of organogels; these tests permit access to the viscoelastic properties of organogels. Oscillatory studies are the amplitude sweep and the frequency sweep as those tests were applied to span 40 and span 60 in SO organogels.

Amplitude sweep

The first part of rheology study was to investigate the amplitude sweep test to identify G' (storage modulus), G'' (loose modulus), LVER (linear viscoelastic region) and the flow point for each selected organogel in SO. G' represents the solid phase that shows the strength or the elasticity of organogel whereas G'' represents the liquid phase of the organogel. Moreover, the LVER represents the continuity of the same values of G' while the flow point means G'=G''. These parameters reflect the strength of any preparation where the strength is important to be determined in this work to guarantee the resistance that can show by organogels toward the stomach contractions and motions. All the amplitude sweep figures are shown in Figure 4 and their parameters

are listed in the Table 4. Starting with G' and G'', all organogels showed increase in those values as the concentration of gelator increased and all G' values are larger than G''. This direct relationship was similar to the organogels of monoglycerides that showed the same relationship between concentration and G' with G'' [25].Secondly, a direct relationship was shown between LVER values and the concentrations of span 40 organogels and those LVER values statistically showed significant difference ($P < 0.01$), whereas this relationship was the opposite for span 60 organogel. Span 60 LVER values were decreased as span 60 concentration increased in the organogel; though, the difference was not significant ($p > 0.05$). The LVER trend of span 40 organogels is the same of the ricinelaidic acid/canola oil organogel as showed an increase in LVER with the increase of ricinelaidic acid concentration[26].Whereas the inverse relationship of LVER that was shown by span 60 organogels is likewise the decrease in LVER values as the concentration of tetradecylamine in ethylene glycol increased [27]. The dissimilarity in the LVER that was shown by span 40 and span 60 organogels could be referred to the assembly of span 40 and span 60 gelators to form the transient junctions that responsible on the 3D structure formation. We think that the increase in the LVER values of span 40 organogels with the increase in the concentration of span 40 leads to the more connecting scaffold as this was not shown in the almost constant values of LVER of span 60 organogels. In the case of span 60 organogels, the increase in the concentration led to more dense scaffold rather than connecting scaffold. The 4th parameter in amplitude sweep is the flow point and all organogels showed the same pattern of increasing the flow point values with the decrease of the organogel concentration. This correlates the impact of the increasing organogel concentration on the formation of less connecting aggregates of assembled gelators that responsible for the formation of the 3D scaffold. Most of the organogels and at the end of the test show that the strain breaks the organogels and leads to a transformation from solid to liquid statues (G'' > G').

To conclude, all organogels showed G' larger than G'' and increase of both values as the concentration increase. An inverse relationship was shown by all organogels between the concentration and the flow point. Moreover, LVER values pattern of span 40 organogels were different from span 60.

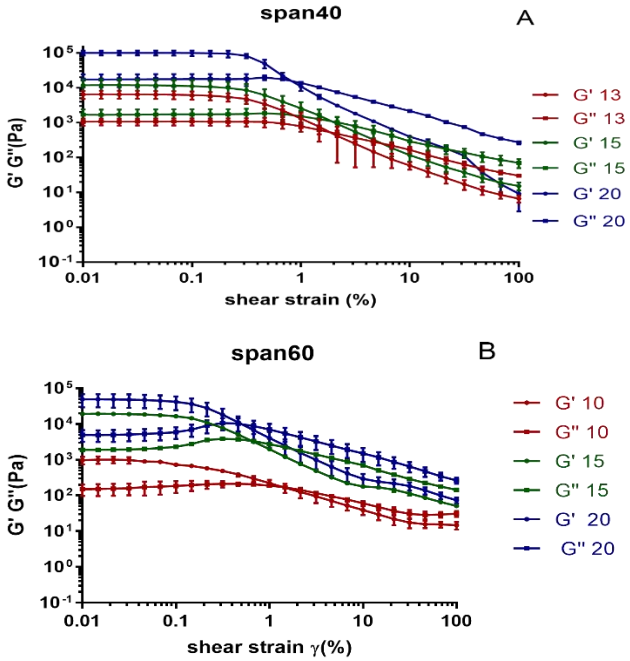


Figure (4): The amplitude sweep test where A and B represent span40 organogels and span60 organogels respectively

Table (4): Amplitude sweep parameters G', G'', LVER and flow point of all selected organogels

Organogels	LMOGs/Oil % (w/w)	G' (pa)	G'' (pa)	LVER (%)	Flow point(%)
span 40/ SO	13	6002±1532	1070±248	0.07±0.01	2±1.3
	15	11003±5412	1718±694	0.06±0.01	1.95±.70
	20	97379±19151	17725±6506	0.12±0.04	0.79±0.18
span 60/ SO	10	873±117	169±63	0.05±0.01	1.83±0.14
	15	17464±2090	2219±274	0.04±0.003	0.65±0.05
	20	42602±3727	5955±2002	0.04±0.004	0.48±0.56

Frequency sweep

This test was executed by applying different angular frequencies with constant strain that is selected from the LVER. The 13% w/w and 15% w/w span 40/ SO organogels as shown in A and B Figure 5 displayed parallel curves that represent G' and G'' values at angular frequency rate starting from 0 to 70 rad s^{-1} and 80 rad s^{-1} respectively whereas those curves of two organogels intersected at high frequencies. Differently, the curves of G' and G'' of 20% w/w span 40/SO organogel did not cross at any angular frequency as shown in C Figure 5. Furthermore, the 10% w/w span 60/SO organogel as in D and E Figure 5 is very weak and show very low values of G' and G'' at low angular frequency after that the two curves of G' and G'' crossed at 30 rad s^{-1}. Also, 15% w/w span 60 /SO was frequency-dependent at 70 rad s^{-1} and higher till 100 rad s^{-1}nevertheless, the 20% w/w span 60/ SO organogel was frequency-independent within the full range of the angular frequency as shown in F Figure 5. Both the 20% w/w of span 40 and span 60 in SO were not affected by any frequency and their figures presented parallel curves of G' and G'' where G' was higher than G''. This means that both organogels were solid at all frequency range, whereas the lower selected concentrations of span 40 and span 60 organogels were frequency dependent at high rates which means they were liquid at high rates as shown in Figures 5 where their G'' values higher than G' values. Those patterns were similar to the frequency sweep test that was applied to the organogels of monoglycerides in cod liver oil where their low concentrations, the 5% w/w and 7% w/w demonstrate a cross curves of G' and G'' at high frequency rate; in the opposite, the 9% w/w organogel showed frequency-independent trend[27].To sum up, frequency sweep test identifies that the 20% w/w of span 40 and span 60 were solid and elastic at different rate of angular frequency while the lower concentrations of span 40 and span 60 organogels were solid at a low rate of angular frequency and liquid at high angular frequency.

span 40 span 60

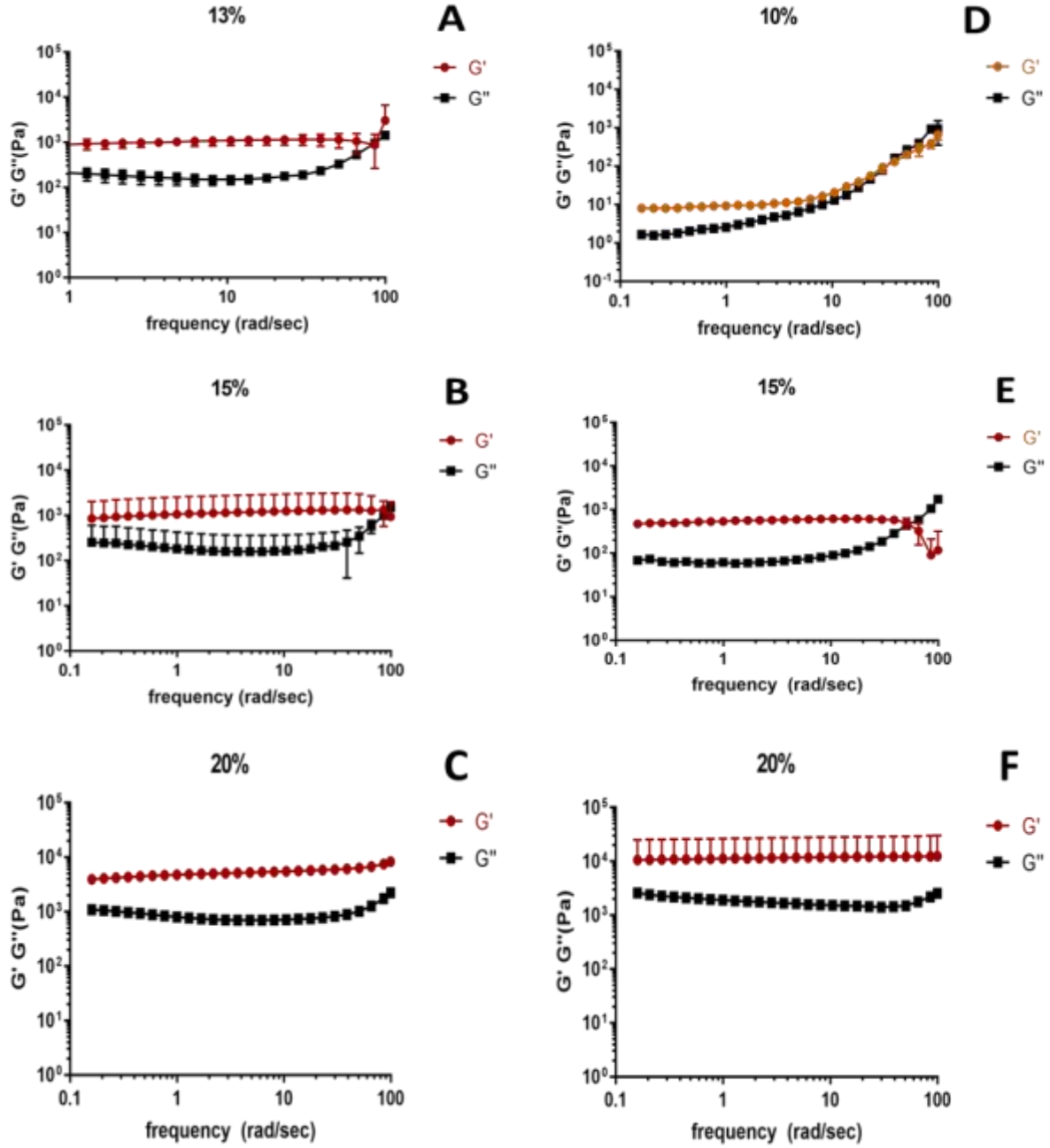


Figure (5): The frequency sweep test where A, B and C represent 13%,15% and 20% w/w span 40/ SO organogel while D, E and F represent 10%, 15% and 20% w/w span 60/SOorganogel, respectively

In-vitro study

The in-vitro CN release study for the two sets of floating organogels was determined in gastric pH 1.2 for 24 hours to investigate the depot property for organogels. A control was run along with all the release investigations which composed of 25 mg CN that was solubilized in SO to compare with the organogels as this is shown in the Figure 6 A and B. Those controls were prepared because of the oils known ability to retard the release of hydrophobic drugs. The SO control showed a release 80% w/w CN after 6 hours. The selected concentrations of span 40 organogels in SO showed a difference in their release pattern and statistically the difference was significant ($p < 0.05$) where 13% w/w, 15% w/w and 20% w/w span 40/SO organogels released 65% w/w, 55% w/w and 48% w/w CN after 6 hours of the study respectively. Then, the same approach as before, those organogels released CN 79% w/w, 66% w/w and 53% w/w after 12hr of the experiment and 90% w/w, 80% w/w and 65 %w/w at the end of the release experiment respectively. However, span 60 organogels delayed the CN release as after 6hr of the release study, 65% w/w, 54% w/w and 48% w/w of CN was released from 10% w/w, 15% w/w and 20% w/w span 60/SO respectively. Also, those organogels after 12hr released 77% w/w, 64% w/w and 54% w/w CN then the same order of organogels after 24hr released 94% w/w, 75% w/w and 63% w/w CN respectively. As shown above, the 20% w/w of both span 40 and span 60 in SO organogels were the best to delay the release were both after 12 hr released around 53% w/w CN and this percentage was lower than those CN tablets that were formulated as floating tablet composed of sodium alginate and they released 60% w/w CN[28]. This outcome estimates that

the 20% w/w span 40 or 60 in SO in addition to the floating property, it has also the depot property.

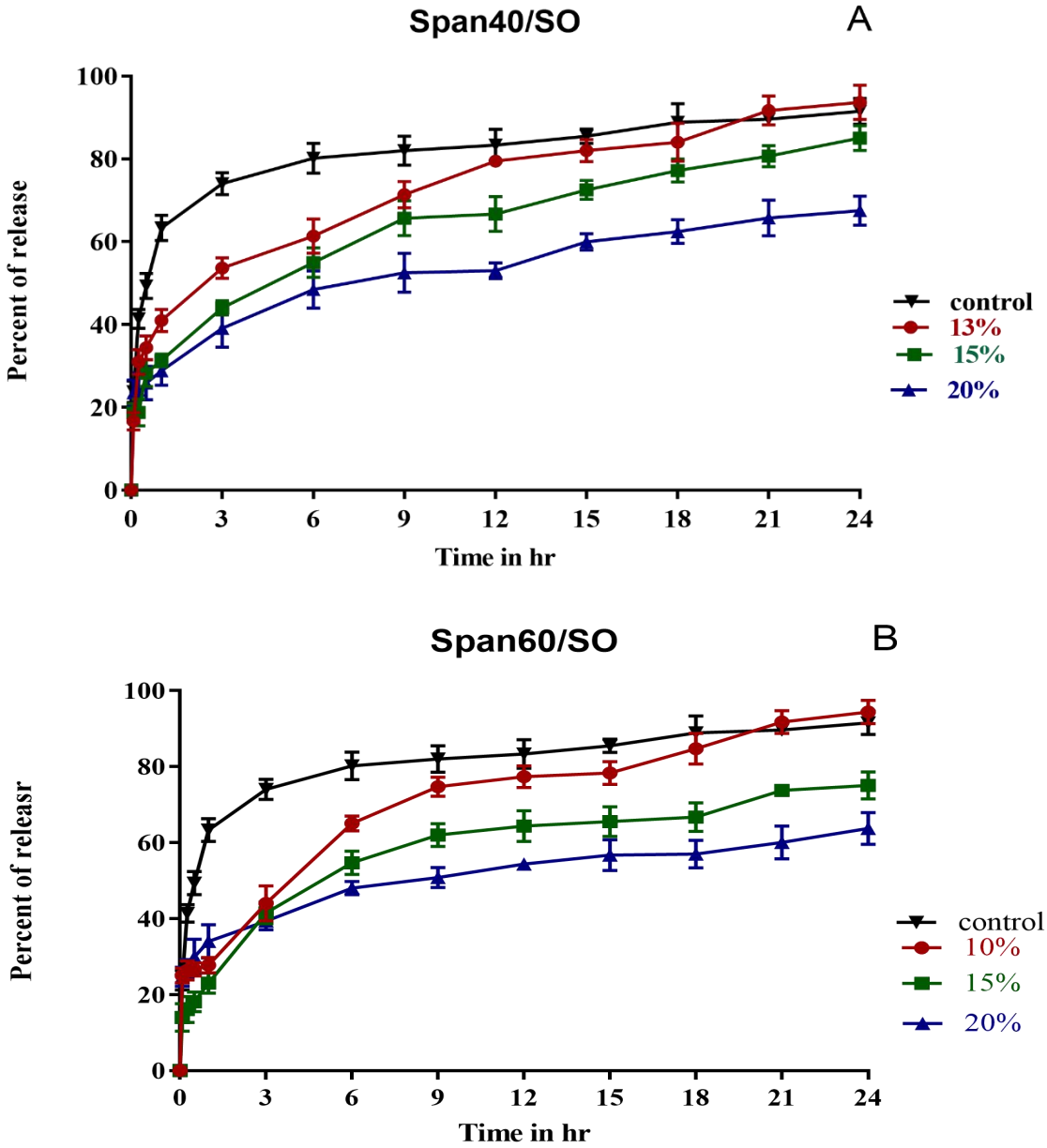
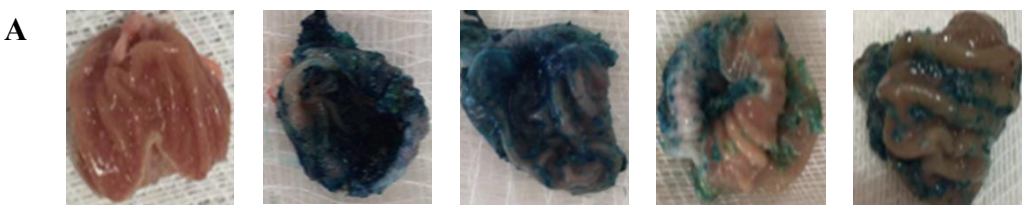


Figure (6): In-vitro CN release, A represents span 40/SO organogel, B represents span 60/SO organogel and control of SO as a black curve

***In-vivo* floating study**

This study was demonstrated to the highest concentrations of spans organogels as shown in Figures7. Photos were taken to prove the gel formation and its floating for extended period of time. The organogels at the four times intervals persist in the stomach as shown in Figure A7 for span 40 organogels and Figure B 7 for span 60 organogels, respectively. The same result achieved by AA Aboelwafaetal for raft liquid GRDDS which persist in rat stomach for 8 hours [29].In conclusion, the selected organogels succeeded to be retained in stomach for 12 hours as a floating delivery system.



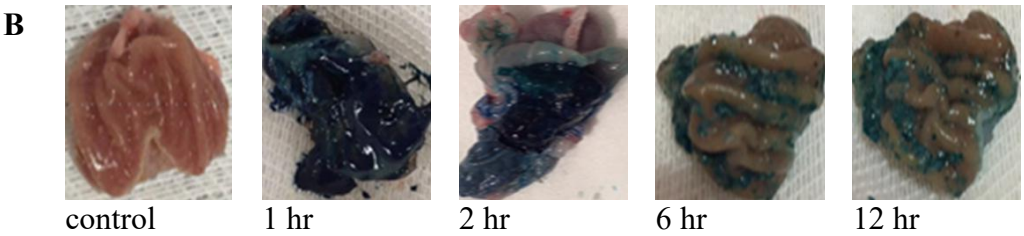


Figure (7): Photos that show the presence of organogels in rat's stomach in different time intervals where A represents span 40/SO organogel and B represents span 60 organogel following oral adminsteration

MATERIALS AND METHODS

Materials

Span 40 and span 60 were purchased from Sinopharm Chemical Reagent Co., Ltd, Sesame oil was purchased from Emad local factory and Cinnarizine was purchased from Baoji Guokang Bio-Technology Co., Ltd–China. Nine healthy adult female Wistar rats weighing 200-225 gm were purchased from the experimental animal center in college of pharmacy, AL-Mustansiriyah University.

Preparation of organogel

Firstly, the organogel was prepared by weighing a specific amount according to the following concentrations of span 40 and span 60 (1%, 3%, 5%, 7%, 10%, 13%, 15%, 18%, 20%) (w/w) in vials then completing the weight to 1 gm with SO, the vials were incubated in a water bath for 40 min at 65°C till gain a clear solution to ensure the solubility of gelators in SO. Then, the vials took out from the water bath and let them cool at room temperature for 45 minutes. These vials were inverted individually to check the organogel formation and the result was recorded either as solid organogel or liquid organogel when there was no flow or flow of organogel preparation, respectively. The last one also pointed as no gelation. Secondly, the CN organogel was prepared as the above method in addition to the 25mg of CN which was weighed with the selected amount of the gelators then both were solubilized in oil.

Investigation of floating properties

The organogels were investigated for the floating parameters in 2 statuses either as a solid organogel loaded in a capsule or as a liquefied organogel. Firstly, for the first status; this procedure started with pouring the hot liquid organogel solution into the body of a hard gelatinous capsule size 00 and then allowed them to cool and solidify gradually. At this point, the capsule was placed in a beaker filled with 200mL of 0.1 N HCl which was already at 37°C and as the capsule within the medium, a continuous stirring was kept by a magnetic stirrer that adjusted at 100 rpm. Secondly, when the status of organogels as a liquid; this was to ensure both the floating of the organogel after gelation, and the intake of organogel by rats via the aid of gavages tube to reach stomach's rat as this was for further study in our work. Liquefaction of organogel was performed by adding a sufficient amount of ethanol to the components of organogel in a glass vial then put the vial in the water bath at 65°C. This disturbed the organogel formation, and then the liquid organogel was taken and then injected into a 200mL of 0.1 N HCl, 37°C that rotated at 100 rpm. At this step and before investigating the floating parameters, an observation to the change in the status of the organogel from liquid to solid as this reported a gelation time. The floating parameters are the lag time and the duration of floating where first the floating lag time which was set by observing the time for organogels to float. Secondly, the duration of floating that represents the duration time of persistence organogels to be floating on the 0.1 N, HCl surface. These parameters were checked visually as well as organogels were classified as followings according to the outcome of the floating parameters:

+ = represents of few minutes of gels to solidify but with rapid gel disseminating.

++ = represents of instant gelation that continued for just 12 hours.

+++ = As ++ but organogels last for 24 hours.

Table top rheology

All the vials of the organogels were incubated in a water bath at 80 ºC then the temperature was going down each 2°C to reach 32 ºC as the average rate was 2°C/ 15 minutes. At the end of the 15 minutes periods, the vials were tilting 45° to inspect organogels status whether solid or liquid. This phase of the procedure showed the transition temperatures from liquid to solid for all organogels. This part of the study was followed by opposite phase through increasing the temperature (2°C/ 15 minutes) as this phase represented the transition temperatures from solid to liquid for all organogels. This study was performed in triplicate for each organogel.

Optical microscopy

Microscopic images were taken to probe the morphology of organogels using optical microscope and slides. These slides were prepared by adding a drop of melted organogel on 25 • 75 • 1 mm glass slide immediately after removing the vial from the water bath using a micropipette. A 22• 22 • 0.15 mm glass cover slip was placed on the top of the gel and compressed gently then left for 15 min. The slide after that was transferred into the microscope stage to inspect and capture images by using the software and the camera. All the captured images were analyzed using Image J to measure the length of fibers by taking the average of the fiber's length from 3 images where at least 15 fibers were scaled per each image.

Fourier transform infrared (FTIR)

FTIR was applied for selected organogels using Shimadzu FTIR-8400S. The spectra recording were from 400 to 4000 cm^{-1} where the cell plate 201-77160-20 was for oils and KRS-5 for KBr to test solid samples and organogels.

Oscillatory rheology studies

Rheological measurements were carried on Anton par mcr302 rheometer using plate-plate configuration (pp25SN61895) for all measurements that were at 25 ºC and the data evaluation was by the aid of the Rheoplus software. A triplicate of each test was applied for the selected organogels. This study was executed at University of Petra/Pharmaceutical Center (UPPC).

Amplitude sweep

The amplitude sweep test was implemented to identify storage modulus (G'), loss modulus (G''), the linear viscoelastic region (LVER) and the flow point for each formulation as the oscillatory strain range was set from 0% to 100% at angular frequency 10HZ.

Frequency sweep

The other oscillatory study was the frequency sweep as the chosen strain was within this range 0.01-0.08 % depending on LVER values that obtained from the amplitude sweep study for each formulation. Also, the angular frequency changed from 0.1 to 100 rad s^{-1}.

In-vitro study

In-vitro release study for CN organogels loaded in capsules was performed using USP type II apparatus (paddle type) and the jars were filled with 900 ml of 0.1N HCl- pH 1.2 solutions that adjusted at 37±0.5°C and100rpm. The capsule was placed into the jars of the apparatus then according to the following time frame (0.083, 0.25, 0.5, 1, 3, 6, 9, 12, 15, 18, 21 and 24) hours; 5 ml was withdrawn from the release media then substituted with equal volumes of fresh medium. Then each sample filtered by a Millipore filter 0.45 µm papers and properly diluted and measured by UV-visible spectrophotometer at 254nm (λ max of CN) and this was performed in triplicate. Each time point was representing an average of 3 triplicates and converted to a concentration using the following equation $y = 0.0642\ x$, this equation symbolizes the equation of the calibration curve, which constructed of several dilutions of CN in 0.1N HCl, pH 1.2

***In-vivo* floating study**

According to the guidelines and approval of the ethical committee of research in the college of pharmacy, Mustansiriyah University for animal studies, the *in-vivo* study was applied using 9 healthy adult female Wistar rats weighing 200-225gm. Prior starting this study, those rats were kept for 10 days in plastic cages under standard situation (12 hours of light and dark cycle, 24°C, 35-60% humidity) with free access to food and water. Then, the rats were forbidden from food for 24 hours before running the experiment with water-free access. Methylene blue (0.1% w/w) was added as a dye to the selected formulation to distinguish the organogels from the stomach tissue in addition to the ethanol to keep the status of organogel as liquid. One milliliter of the stained liquid formulations was given to the rats with the aid of an oral gavages tube. These animals were anaesthetized by intramuscular injection of 50 mg/kg ketamine and 5 mg/kg xylazine then abdomen was dissected to investigate the floating formulation in the rats' stomach. Photos for stomach were taken at 0 min before administration of the selected formulations as a control, then at 1 hour, 2 hours, 6 hours and12 hours after giving the formulation.

Statistical analysis

All measurements were carried out in triplicates as statistically analyzed and presented in Figures and Tables where the mean was statistically followed by the standard deviation (± SD). Analysis of variance (independent sample T-test) and (One way ANOVA) were performed using SPSS version 16.0.

CONCLUSIONS

The selected organogels floated instantly and showed longer floating durations with increasing concentration of gelator, in both statuses of the organogels (solid and liquid) as the20% (w/w) of both spans showed the longest durations. By doing table top rheology, the phase transition for the selected organogels was reversible and higher than 37 ºC which indicated the stability of organogels in the body temperature 37 ºC. Also, all organogels showed fibrillar type of scaffold and a tendency of a decrease in the fiber length as the concentration of gelators in organogels increased (significantly in span 40 and not significantly in span 60). Also, FTIR spectrograms showed a shift in the associated peaks of carbonyl and hydroxyl groups of both span organogels that all contributed in the assembly of the scaffold by gelator-gelator interactions. From amplitude sweep, span 40 showed better viscoelastic properties than span 60 organogel since it showed higher values of G' and LVER than span 60 organogel while frequency sweep test identifies that the 20% w/w of span 40 and span 60 were solid and elastic at different rate of angular frequency (frequency independent) whereas the lower concentrations of span 40 and span 60 organogels were solid at a low rate of angular frequency and liquid at high angular frequency (frequency dependent). From in- vitro study, 20% w/w span 40 and 60 in SO were the best in delaying the release of CN than lower concentration so they gained the depot property. Lastly, *in-vivo*study in rats confirmed that the selected organogels succeeded to be retained in stomach for 12 hours as a floating delivery system.

Acknowledgment

The authors acknowledge the support received from Mustansiriyah University, College of pharmacy, Baghdad, Iraq. The authors also acknowledge the help from University of Petra/Pharmaceutical Center (UPPC) during the oscillatory rheology studies, Amman, Jordan.

REFERENCES

1. Mandal UK, Chatterjee B, Senjoti FG. Gastro-retentive drug delivery systems and their in vivo success: A recent update. Asian Journal of Pharmaceutical Sciences. 2016; 11(5): 575-584, doi.org/10.1016/j.ajps.2016.04.007
2. Mohamed, Masar Basim Mohsin. *Organogels for intratumoural delivery.* PhD thesis. University of Nottingham. 2017.
3. Ghareeb MM, Issa AA, Hussein AA. Preparation and characterization of cinnarizine floating oil entrapped calcium alginate beads. International Journal of Pharmaceutical Sciences and Research. 2012; 3(2): 501.
4. Bayindir ZS, Yuksel N. Characterization of noisomes prepared with various nonionic surfactants for paclitaxel oral delivery. Journal of pharmaceutical sciences. 2010; 99(4): 2049-2060, doi:10.1002/jps.21944.
5. Toshimitsu Y, Florence AT. Vesicle (niosome)-in-water-in-oil (v/w/o) emulsions: an in vitro study. International journal of pharmaceutics, 1994; 108(2): 117-123, doi.org/10.1016/0378-5173(94)90322-0
6. Pratap S. Jadon PS, Gajbhiye V, Jadon RS, Gajbhiye KR et al., Enhanced oral bioavailability of griseofulvin

via niosomes. AAPS pharmscitech, 2009. 10(4): 1186, doi: 10.1208/s12249-009-9325-z
7. Hasan AA, Madkor H, Wageh S. Formulation and evaluation of metformin hydrochloride-loaded niosomes as controlled release drug delivery system. Drug delivery. 2013; 20(3-4): 120-126, doi: 10.3109/10717544.2013.779332
8. Sharma SK, Chauhan M, Anilkumar N. Span-60 Niosomal Oral Suspension of Fluconazole: Formulation and *In Vitro* Evaluation. Asian journal of pharmaceutical research and health care, 2009; 1(2): 142-156.
9. Wang L, Dong J, Chen J, Eastoe J, Li X Design and optimization of a new self-nanoemulsifying drug delivery system. Journal of colloid and interface science. 2009; 330(2): 443-448, doi: 10.1016/j.jcis.2008.10.077.
10. Satapathy D, Sagiri SS, Pramanik K Development of mustard oil-and groundnut oil-based span 40 organogels as matrices for controlled drug delivery. Designed Monomers and Polymers. 2014; 17(6): 545-556, doi.org/10.1080/15685551.2013.869652.
11. Yu HH, Liu KSD, Huang Q. Development of a food-grade organogel with high bioaccessibility and loading of curcuminoids. Food chemistry. 2012; 131(1): 48-54, doi: 10.1016/j.foodchem.2011.08.027
12. Murdan S, Andrýsek T, Son D. Novel gels and their dispersions--oral drug delivery systems for ciclosporin. International journal of pharmaceutics. 2005; 300(1-2): 113-124, doi: 10.1016/j.ijpharm.2005.06.002
13. Li Z, Huang Y, Fan D, Li H, Liu S, Wang L. Core–shell-type organogel–alginate hybrid microparticles: a controlled delivery vehicle. Chemical Engineering Journal. 2015; 264: 134-145.
14. Wright AJ, Marangoni AG. Formation, structure, and rheological properties of ricinelaidic acid-vegetable oil organogels. Journal of the American Oil Chemists' Society. 2006; 83(6): 497-503
15. Mukherjee S, et al., Comparative study of span 40 and span 60 based soy-gels for topical drug delivery. Asian J Pharm Clin Res. 2019; 12(6): 259-265.
16. Getyala A, Gangadharappa HV, Prasad MS, Reddy MPK. Formulation and evaluation of non-effervescent floating tablets of losartan potassium. Current drug delivery. 2013; 10(5): 620-629, doi:10.2174/1567201811310050013
17. Rani BS, Hari BNV, Reddy AB, Punitha S, Devi P, kam VR. The recent developments on gastric floating drug delivery systems: An overview. Int J Pharm Tech Research. 2010; 2: 524-534.
18. Sabar MH, JAAFAR IS, Mohamed MBM. Oily in situ gels as an alternative floating platform for ketoconazole release. International Journal of Research in Pharmaceutical Sciences. 2020; 11(2): 2638-2649.
19. Jadhav NK, Patil KA, Patil JK, Pawar SP. A review on organogels: lipid based carrier systems. Pharma Science Monitor. 2012; 3(4): 3132-3143.
20. Edelsztein VC, Andrea S, Cormack M, Ciarlantini M, Chenna PHD. Self-assembly of 2,3-dihydroxycholestane steroids into supramolecular organogels as a soft template for the in-situ generation of silicate nanomaterials. Beilstein journal of organic chemistry. 2013; 9(1): 1826-1836, doi:10.3762/bjoc.9.213
21. Laupheimer M, Preisig N, Stubenrauch C. The molecular organogel n-decane/12-hydroxyoctadecanoic acid: Sol–gel transition, rheology, and microstructure. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2015; 469: 315-325, https://doi.org/10.1021/la991545d
22. Ibrahim MM, Hafez SA, Mahdy MM. Organogels, hydrogels and bigels as transdermal delivery systems for diltiazem hydrochloride. Asian journal of pharmaceutical sciences. 2013; 8(1): 48-57, https://doi.org/10.1016/j.ajps.2013.07.006
23. Behera B, Sagiri SS, Pal K, Srivastava A. modulating the physical properties of sunflower oil and sorbitan monopalmitate-based organogels. Journal of applied polymer science. 2013; 127(6): 4910-4917, https://doi.org/10.1002/app.37506
24. B. Behera, V. Patil, S. S. Sagiri, K. Pal, S. S. Ray. Span-60-based organogels as probable matrices for transdermal/topical delivery systems. Journal of applied polymer science. 2012; 125(2): 852-863, doi: https://doi.org/10.1002/app.35674
25. Sein A, Verheij JA, Agterof WG. Rheological characterization, crystallization, and gelation behavior of monoglyceride gels. Journal of Colloid and Interface Science. 2002; 249(2): 412-422, doi:10.1006/jcis.2002.8287
26. Marangoni AG, Garti N. Edible oleogels: structure and health implications. 2018; Elsevier, Paperback ISBN: 9780128142707
27. Liu G, Hong X, Tsang SCE. Rheological studies of alkylamine-ethylene glycol organogel: Chain length dependent structural diversity. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2013; 434: 1-8, doi:10.1016/j.colsurfa.2013.05.032
28. Nagarwal RC, Ridhurkar DN, Pandit J. In vitro release kinetics and bioavailability of gastroretentive cinnarizine hydrochloride tablet. AAPS Pharm Sci Tech. 2010; 11(1): 294-303, doi: 10.1208/s12249-010-9380-5
29. Abouelatta SM, Aboelwafa AA, El-Gazayerly ON. Gastroretentive raft liquid delivery system as a new approach to release extension for carrier-mediated drug. Drug delivery. 2018; 25(1): 1161-1174, doi:10.1080/10717544.2018.1474969