Oily in situ gels as an alternative floating platform for ketoconazole release

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ABSTRACT
Sodium alginate, calcium carbonate, and guar gum were mixed with oils such as olive oil (OO), sesame oil (SO), and medium chain triglyceride (MCT). The oily formulations were found to simplify the preparation of in situ floating gel. This was the aim of this study using ketoconazole (keto) as a model drug. The investigations for the floating property were established by In vitro gelling capacity study and In vitro floating study. Additionally, in vitro release study was applied to find the best formulations to delay the release of keto. Then, selected formulations were studied by FTIR and SEM. Lastly, in vivo gelation was performed to examine the gelation in the rat’s stomach. The results showed all formulations were floating after successful gelation as the least amount of sodium alginate to gel oils was 20% w/w. The gels in SO and OO were better than MCT in delaying keto release, and 30% w/w sodium alginate in SO was the best to delay the release of keto within 8 hours of the release study. Selected gels showed interactions between the keto molecules and the molecules of the gel contents by FTIR study, and SEM showed a difference in the internal structure of selected formulations. Lastly, the 30% w/w sodium alginate in SO proved to gel and remain in the rat’s stomach in the following periods: 30 min, 1 hour, 2 hours, and after 8 hours. Oily suspension formulations showed floating properties in the stomach and slowed the release of keto and specifically 30% w/w of sodium alginate in SO.

INTRODUCTION
There is significant awareness among researchers for in situ floating gels in the stomach as a drug delivery system. This delivery system has good advantages, such as the delayed release of drugs that are absorbed well in the stomach environment and the simplicity of the administration via the oral route. Most studies on in situ floating gels used sodium alginate and calcium salts as the primary polymers and gelling agents, respectively (Stockwell et al., 1986; Shilpa et al., 2003; Tadros, 2010). However, liquid or suspension formulation for in situ gels is not easy to prepare and needs several steps for preparation. This limitation was obvious in methods of preparation such as that by Youssef et al. (2015), where alginate needed to be mixed with water heated to 90 °C to obtain a clear sodium alginate liquid and then cooled to 40°C to add calcium carbonate (Youssef et al., 2015). Additionally, Foster et al. used this desirable mixture by using a high-shear mixer at 12,000 rpm and then cast this mixture on liquid (Foster, 2012). Farrés increased the temperature to 90 °C during preparation to develop a homogenous sodium alginate mix-
ture before adding calcium carbonate (Farrés and Norton, 2014). Because these studies showed the difficulties and complexity of the method of preparation, hence, we tried to find a simple and novel procedure to prepare in situ gels by mixing the desired combination in oil, not in water. Indeed, the main property of oily formulations is the ease of preparation (Kollipara and Gandhi, 2014). Thus, the aim of this study was to prepare a desired mixture of sodium alginate-calcium carbonate with oils using an effortless method. This mixture was achieved by preparing a single dose of the solid content in one sachet and mixing it with a specific volume of oil.

Then, the single dose will be ready to swallow after simple mixing. This single dose will help to overcome issues of content uniformity and loading capacity of drugs, specifically for large-scale production of gels and suspended drugs in gel (Garg, 2010). Therefore, in this current study, oils that are ingested daily by humans and available at the local market were selected: olive oil (OO), sesame oil (SO), and medium chain triglyceride oil (MCT). These oils were previously formulated in the pharmaceutical industry for oral dosage forms of cyclosporine, alfacalcidol, and valproic acid (Müllertz et al., 2010; Pouton, 2006; Colin, 2000).

Strickley (2004) To achieve the aim of this work, ketoconazole (keto) was chosen to formulate with oils in the desired combination because keto is a weakly basic drug keto solubility increases as pH decreases (Adachi, 2015). This property guarantees, in addition to the delayed release of in situ gel formulation, the high availability of keto to be absorbed from the stomach within a narrow window.

The following studies were essential to investigate the possible formation of an oily floating in situ gel starting with the physical properties of in situ gel formulations, such as screening the prepared formulation for their physical appearance, in vitro gelling capacity, in vitro floating ability and viscosity. After conducting those physical studies, an in vitro release study was carried out to investigate the keto release pattern from the prepared formulations. On a molecular level, an FTIR study was performed for the selected formulations to explore the possible bond formation between keto and the molecules of the formulation contents. Additionally, SEM was used to probe the changes in the scaffold morphology due to using different concentrations of sodium alginate. Last, an in vivo study was performed to investigate the gel residence in a rat stomach.

**MATERIALS AND METHODS**

**Materials**

Keto was purchased from Shanghai Macklin Biochemicals Co., Ltd., China, and both CaCO₃ and Na alginate were obtained from Sinopharm Chemical Reagent Co., Ltd., China. Guar gum was a kind gift from Samara Drug Industry, Iraq. Additionally, Carbopol 940 was purchased from Aladdin Industrial Corporation. However, the OO, MCTand SO were purchased from the local market.

**Methods**

**Preparation of in situ oily gel**

There were several trials to determine the least amount of sodium alginate-calcium salt as a suitable combination to solidify in oils after pouring in the release media using either guar gum or carbopol 940, as shown in Table 1 in the supplementary information. All of the solid contents representing a single dose were weighed accurately and were mixed with the required amount of oils. This formulation was prepared by simply mixing using a spatula to obtain a homogenous suspension within seconds. These formulations were kept in a suitable container for further investigations.

**Physical appearance**

All formulations after mixing were screened for homogeneity and lumpy presence.

**In Vitro gelling capacity**

The transfer from an oily suspension formulation to a solid gel was determined by adding a single dose of each formulation in a container of 500 ml 0.1N hydrochloride acid (pH 1.2). Gelation was visualized and described qualitatively, as shown below:

+ = gelation within a few minutes, but the formulation spread rapidly.

++ = gelation occurred immediately but persisted for 12 hours.

+++ = gelation took place and lasted for long periods after 24 hours.

**In vitro floating study**

The jars of the USP dissolution apparatus were filled to 500 ml with 0.1HCl (pH 1.2), set at 37°C and used to determine the floatation time of formulations. After pouring the oily suspension formulations in the jars of the dissolution vessel, the time to achieve the full buoyancy of formulations to the surface of the media was recorded as the floating lag time where the duration of floating was reported as gelled formulations that stay floating persistently.
Table 1: The detailed content of all formulations

<table>
<thead>
<tr>
<th>No. of formulations</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keto</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
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</tr>
<tr>
<td>Sod. alginate</td>
<td>20%</td>
<td>20%</td>
<td>20%</td>
<td>25%</td>
<td>30%</td>
<td>25%</td>
<td>30%</td>
<td>25%</td>
<td>30%</td>
</tr>
<tr>
<td>Guar gum</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
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<td>CaCO3</td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
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<td>7%</td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
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<tr>
<td>OO Up to 100%</td>
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<td>100%</td>
<td>100%</td>
<td>100%</td>
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<td>100%</td>
<td>100%</td>
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<tr>
<td>MCT Up to 100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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<tr>
<td>SO Up to 100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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Table 2: Floating time

<table>
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<tr>
<th>No. of formulations</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
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<tr>
<td>Floating time</td>
<td>05:21</td>
<td>02:46</td>
<td>06:46</td>
<td>03:57</td>
<td>02:25</td>
<td>04:46</td>
<td>03:13</td>
<td>03:29</td>
<td>02:18</td>
</tr>
<tr>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>0.026</td>
<td>0.026</td>
<td>0.022</td>
<td>0.0091</td>
<td>0.03147</td>
<td>0.01819</td>
<td>0.0049</td>
<td>0.00398</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The viscosity of suspension and solid formulations

<table>
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<th>No. of formulations</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspension formulation in Pa (SD)</td>
<td>137 (17)</td>
<td>65(7)</td>
<td>115(12)</td>
<td>245(13)</td>
<td>450(18)</td>
<td>215(23)</td>
<td>408(19)</td>
<td>425(13)</td>
<td>751(32)</td>
</tr>
</tbody>
</table>

Figure 1: A: Release profiles of keto from in situ gels in OO, MCT, and SO oils in 0.1 N HCl, pH 1.2, with 20% w/w sodium alginate in all formulations; the data represent the mean ± SD. B: Release profiles of keto from the physical mixture of keto in OO, MCT, and SO in 0.1N HCl, pH 1.2; the result represents the mean ± SD.
Viscosity measurement

Viscosity was measured for the oily suspension formulations at room temperature using a Brookfield digital viscometer (Model no. LVDV 2P230), and the spindle was S6. This test was performed in triplicate, and each run lasted 60 seconds.

In vitro drug release study of the in situ gel formulation

The release study of keto was performed by stirring with the paddles of the USP dissolution apparatus (Type II), and the stirring speed and temperature were set at 50 rpm and 37 °C ± 0.5 °C, respectively.

After good mixing of a freshly prepared single dose of oily formulation, it was poured in 900 ml of 0.1 N HCl, pH 1.2, 3ml of the release fluid were drawn according to the selected time frame, and replaced with the same volume of freshly prepared media.

Then all collected samples were analyzed at 270 nm. This study was performed in triplicate, and the mean of 3 samples at each time point was calculated after converting to a concentration using the following equation: \( y=2.43x+0.0341 \).

This equation represents the equation of the calibration curve, which was constructed of several dilutions of keto in the form of 0.1N HCl, pH 1.2.

FTIR

The FTIR test was performed using a Shimadzu FTIR-8400S instrument as the scans were run from 400 to 4000 cm\(^{-1}\). The cell plates were 201-77160-20 and KRS-5 for KBr to sample the suspension formulation with and without keto and for the keto powder, respectively.

SEM

The selected in situ formulations were poured into the release media and then collected after some time of gelation to ensure that the oils were leached from the formulation. This process was followed by a slow drying process by placing the gels on filter paper, which was changed periodically to guarantee dried gels. The surfaces and cross-sections of these gels were the targets of the investigation. This test was run using TESCAN, VEGA 3-Czech Republic, and the prepared samples were placed on adhesive tape that was already fixed to an aluminum stub. A gold coating process was applied to the selected samples under an argon atmosphere.

In vivo study of in situ gelling

An in vivo study was applied according to the
Figure 4: A and B: Spectrograms showing peaks associated with the carbonyl groups in the SO and OO formulations with and without keto against spectrograms of keto. C and D: Spectrograms showing peaks related to C-Cl groups in the SO and OO formulations with and without keto against spectrograms of keto. E and F: Spectrograms showing peaks associated with the hydroxyl groups in the SO and OO formulations with and without keto against spectrograms of keto.

guidelines and approval of the ethical committee of research for animal studies at the Pharmacy College, Mustansiriyah University, using eight healthy adult female Wistar rats weighing 250-300 gm. The rats were kept in plastic cages with dimensions of 20 x 25 x 35 cm. Before starting this study, those rats were housed for 10 days under specific conditions of room temperature (21 ± 1°C) and light stream cycles of 12 hours of light and 12 hours of darkness. Pellets and water were easily accessible as soon as this study started, and the study was finished on the 20th of March, 2019. Methylene blue (0.1% w/w) was added as a dye to the selected formulation to distinguish the oily gels from the stomach tissue clearly, and one milliliter of the stained suspension formulations was given to the rats with the aid of an oral gavage tube. The rats were prohibited from food for 24 hours before running the experiment with water-free access because these animals were anesthetized by intramuscular administration of 50 mg/kg ketamine and 5 mg/kg xylazine. The rats’ stomachs after abdominal dissection were taken at
Figure 5: A and B: SEM images of the surfaces of in situ gels of 20% w/w and 30% w/w sodium alginate; these images were scaled against 500 μm. C and D: SEM images of the inside of in situ gels of 20% w/w and 30% w/w sodium alginate; these images were scaled against 500 μm. E and F: Magnified images at 10 μm of the inside of insitu gels of 20% w/w and 30% w/w sodium alginate. All images are of gels prepared using 5% w/w guar gum in SO.
0 min before administration of the selected formulations as control, then at 30 min, 1 hour, 2 hours, and 8 hours after giving the formulation. After that, photographs were taken of the stomach to verify that the gel formed and stayed in the stomach.

RESULTS AND DISCUSSION

Preparation of oily in situ gel

Indeed, many trials were performed to prepare oily suspensions of sodium alginate with calcium carbonate alone but were not successful as they disseminated quickly after gelation, and this result was also observed by Karemore and Avari (Karemore and Avari, 2019).

Thus, many studies combined different polymers with sodium alginate and calcium carbonate, such as by adding 0.8% w/w hydroxyl propyl methyl cellulose to 2.5% w/w sodium alginate to prepare an in situ spiramycin gel (Sharma et al., 2014).

As clarified in Table 1 in the supplementary information, two polymers were included in our work: guar gum and carbopol 940. The least amount of sodium alginate with guar gum and carbopol 940 in different oils that gelled after pouring in 0.1 NHCl was 20% w/w in the presence of calcium carbonate.

The proportion of sodium alginate to calcium carbonate was 3:1, and this proportion was kept constant for all formulations in this study.

Physical appearance

All in situ suspension formulations of keto were screened for their appearance, homogeneity, and color. The prepared formulations from SO and OO were faint yellow to yellow in appearance, while the formulations of MCT were faint white in appearance. All preparations were homogenous suspensions and showed no lumps.

In Vitro gelling capacity

The in vitro gelling capacity test was performed to investigate the ability of oily in situ suspension formulations to gel upon contact with the release fluid, and all oily formulations of the in situ gel presented instant gelation (+++) after being poured in 0.1N HCl, pH 1.2, and stayed intact for 24 hours. The gelation characteristics (+++) of all oily formulations were similar to gels made by Makwana et al., which consisted of 1.5 w/v sodium alginate in combination with hydroxypropyl methyl cellulose (Makwana et al., 2016).

In Vitro floating study

First, the in vitro floating test was performed to check the lag time that represents the time needed for the gels to float, and the lag time of all formulations was within the range of 2:18 min to 6:46 min. The detailed lag time of each formulation is shown in Table 2 in the supplementary information. Second, the duration of floatation was monitored, and all formulations showed more than 24 hours.

Both the lag time and the duration of floatation results were similar to those obtained by Youssef et al. (2015) for formulations of sodium alginate, gellan gum, and calcium carbonate, as the lag time was between 1 min and 4 min, and the formulations showed a duration of floatation of more than 24 hours (Youssef et al., 2015).

As shown above, all physical studies, including the physical appearance, in vitro gelling capacity, and in vitro floating studies, presented promising in situ gels.
Viscosity measurement of the in situ gelling solutions

Measuring the viscosity of suspension formulations is essential because these formulations are intended to be taken orally, and the viscosity should not be very low for pouring control; thus, the obtained suspension formulation should show a suitable range of viscosity (Garcia et al., 2015). The viscosity of suspension formulations of 20% w/w sodium alginate in the presence of guar gum with OO, MCT, and SO was 137 Pa, 65 Pa, and 115 Pa, respectively (the detailed data in the supplementary information are shown in Table 3). Additionally, as sodium alginate's content increased to 25% w/w and 30% w/w in the suspension formulations with OO, the viscosity increased to 245 Pa and 450 Pa, respectively. This result was similar to that for the suspension of sodium alginate in the presence of guar gum with SO, which showed an increase in viscosity to 215 Pa and 408 Pa, respectively. In the last two formulations, guar gum was replaced with carbolip 940 and showed the same changes, namely, an increase in the viscosity of the suspension formulations (425 Pa and 751 Pa). The viscosity of 30% w/w sodium alginate formulated with carbolip 940 in SO was not close to the range of suspension formulations in this work (65 Pa to 450 Pa) but was close to the viscosity range (74 Pa -694 Pa) of agellan gum in situ gel in a different work (Rajinikanth and Mishra, 2008). In summary, the viscosity of the in situ formulations was acceptable, and all suspension formulations showed an increase in viscosity as the concentration of sodium alginate increased.

In vitro drug release study of the in situ gel formulation

The release study was performed to examine the effects of different formulations and additives on the release of keto. This study started with the model drug with the least concentration, 20% w/w sodium alginate, in the presence of 5% w/w guar gum that gelled OO, MCT, and SO upon pouring in the release medium. The results are shown in Figure 1A, which exhibits that approximately 60% w/w of keto was released from the MCT formulation within the first 20 min, less than 60% w/w was released from the OO formulation, and 50% w/w was released from the SO formulations. Gradually, the release of keto from the MCT formulation increased and reached 100% w/w within 3 hours of the experiment; in contrast, the OO and SO formulations released approximately 90% w/w keto within the experimental time. The release study shown in Figure 1A shows that the in situ gels formulated with OO and SO slowed the release of keto compared with the in situ gels formulated with MCT. Notably, within the initial minutes of the release study, all oils were captured within the formulations once the suspension was transferred to gels in the release media however, over time, oils leached out from the formulations, especially the MCT oil, which completely vanished within the release media. This phenomenon might cause the keto molecules that escaped from the in situ gel formulated with MCT oil to be solubilized by the acidic media via great contact with the release media’s acidic fluid due to the high miscibility of MCT in 0.1N HCl, pH 1.2. In summary, the 20% w/w sodium alginate in SO and OO slowed the release of keto better than 20% w/w sodium alginate in MCT. Oily formulations slow the release of drugs (Weiniger et al., 2012) hence, the physical mixture of oils and keto was investigated to explore if the slow release of keto was due to the oils or oily in situ gels. This question was clarified in Figure 1B, which shows that the three physical mixtures of OO, MCT, and SO released more than 90% w/w within the first 20 min; then, until the end of the three hours of the release study, all formulations released the whole kept content and showed constant values of keto. Figure 1B shows that the physical mixtures of oils and keto did not slow the release of keto compared with the oily in situ gels, as shown in Figure 1A. As can be seen, the oily in situ gels showed a capacity to slowing the release of keto.

After the release study demonstrated the best results of the formulations in SO and OO, another factor was added to investigate the effect of SO and OO formulations on keto release by increasing the percentage of sodium alginate to 25% w/w and 30% w/w and keeping the guar gum concentration constant, as shown in Figure 2. The keto release pattern of 25% w/w sodium alginate was identical to the release pattern of 20% w/w sodium alginate in OO formulations, as shown in Figure 2A however, the 30% w/w sodium alginate gel slowed the release of keto and released approximately 58% w/w, 90% w/w and 100% w/w of keto after 180 min, 7 hours and 8 hours respectively. Figure 2B presents the effect of different concentrations of sodium alginate on the release of keto from SO formulations, and the release of keto compared with that from 25% w/w OO formulations and 30% w/w sodium alginate formulations was slow throughout the 6 hours of the release study, with keto release rates of approximately 62% w/wand 56% w/w, respectively within the first 3 hours of the release study. Then after 7 hours and 8 hours of the release experiment, the 25% w/w sodium alginate formulations released 91%w/w and 100%w/w of keto, respectively. The 30% w/w sodium alginate formulations...
released less keto and were 88% w/w after 7 hours and 100% after 8 hours. The 30% w/w sodium alginate formulations released percentage was the same as anthocyanin that released within 3 hours of the release study from 3.5% w/v sodium alginate and 1.5% w/v calcium carbonate (Celli et al., 2016). Moreover, an equal proportion of sodium alginate and calcium carbonate (1% w/v) released approximately 55% w/w amoxicillin within 3 hours of the release study (Rajinikanth et al., 2007). In short, the results showed a decrease in the percent-age released of keto as the percentage of sodium alginate increase in the oily formulations and especially the 30% w/w sodium alginate in SO.

Additionally, the effect of replacing guar gum with carbopol 940 using the same percentage, 5% w/w, was studied in SO. As shown in Figure 3, an increase in the sodium alginate concentration did not retard the release of keto, and the 25% w/w and 30% w/w sodium alginate in OO, as shown in Figure 2A, gave the same release pattern. In a word, the addition of carbopol 940 in the presence of sodium alginate did not show changes in the release of keto, as shown by the addition of guar gum.

FTIR

The 30% w/w keto formulations in OO and SO in the presence of guar gum were examined by FTIR because their keto release was much slower than that of MCT formulations. The FTIR study was run to understand the possibility of interactions between the keto molecules and other molecules in the sus-pension formulations that could persist after gelation, such as sodium alginate and guar gum (the structures of keto, guar gum, and sodium alginate are shown in the supplementary information). Thus, as shown in Figure 4, the FTIR data for the keto sus-pension formulation were compared against those of both the keto powder alone and the blank sus-pension formulation to exclude and observe the com-mon functional groups. As clarified previously in dif-ferent studies, keto has carbonyl functional groups, and C-Cl might have a high tendency to form hydro-gen bonds with the hydroxyl groups of sodium alginate and guar gum (Kakkar, 2015; Mistry et al., 2015; Tiwari et al., 2018).

Karolewicz et al. (2014) As shown in Figure 4A and Figure 4B, the spectrogram of keto alone shows a peak related to the carbonyl of keto at 1643 cm$^{-1}$ and either disappeared or shifted to 1746 cm$^{-1}$, as shown in the spectrograms of SO and OO formulations with and without keto. However, the carbonyl (of sodium alginate) is already available in these spectrograms. In a different work, a peak shift of 22 cm$^{-1}$ associated with the carbonyl group of keto indicated the formation of hydrogen bonds between keto and poly(2-hydroxyethyl-methacrylate) (Mistry et al., 2015), or the disappearance of the peak in the infrared spectrogram might be due to the hydro-gen bond formation (Spoto, 1994). Additionally, the peak associated with the C-Cl group in the keto spectrogram was at 825 cm$^{-1}$. It completely vanished from the spectrograms of the SO and OO for-mulations with and without keto, as shown in Figure 4C and Figure 4D. This suppression of the peaks associated with the C-Cl group for keto might be due to the interactions between keto and polymers. Additionally, as shown in Figure 4E and Figure 4F, the peaks disappeared from the spectrograms of the OO, and SO formulations with or without keto, and the peaks associated with the hydroxyl groups at approximately 3300 cm$^{-1}$ to 3500 cm$^{-1}$ could be due to the interactions between guar gum and sodium alginate molecules in addition to a possible extended interaction with the keto molecules. The interactions between the functional groups of keto with the functional groups of sodium alginate and guar gum might help to capture the keto molecules within the in situ gel scaffold and slow the release of keto. The carbopol addition in the SO-keto formula-tion was studied, as shown in Figure 1 (supplemen-tary information), and the spectrogram of the blank formulation showed a low-intensity peak associated with the carbonyl group. This finding could be justi-fied by the carbonyl group in both molecules of carbopol 940 and sodium alginate, which might highly interact with the hydroxyl group of sodium alginate. In turn, the interactions between the scaffold and keto molecules decreased, which was dissimilar to keto in SO and OO with guar gum formulations, lead-ing to the rapid release of keto from the carbopol SO formulation. After all, the FTIR study showed possible interactions of hydrogen bonds between func-tional groups of keto molecules and the functional groups of the molecules of the polymers in the for-mulations.

SEM

SEM was run to probe the surface and the cross-section of the network of the sodium alginate gel upon gelation. As expected, the network density of gels affects drug release thus, this study was run to test different concentrations of sodium alginate 20% w/w and 30% w/w in SO, as SO formulations showed the best slow release. Figure 5A, Figure 5B, Figure 5C, and Figure 5D represent images taken at the 500 µm scale, whereas Figure 5E and Figure 5F represent images taken at 10 µm, as shown by the fibrillar network of gels. Figure 5A and Figure 5B signify images of the surfaces of 20% w/w and 30% w/w gels of sodium alginate, respectively, as both
were rough and porous and did not show different structures. However, the cross-section of both concentrations of gels exhibited different structures. Figure 5C shows circular cavities of the inside morphology of the 20% w/w sodium alginate gel, and the inside of the 30% w/w sodium alginate gel was a network of connecting strip-like structure. The morphology of sodium alginate gels prepared in aqueous media was studied by Liu et al. (2016) by field emission scanning electron microscopy. The result showed that as the concentration of sodium alginate increased from 2% w/w to 4% w/w, the thickness of the fibers increased however, the increase in concentration did not change the fibrillar network morphology (Liu et al., 2016). The diameter of the cavities of the networks in Figure 5C and Figure 5D were measured using ImageJ software and are 293.61 μm ± 67 and 92.95 μm ± 67.36 for the 20% w/w and 30% w/w sodium alginate gels, respectively. The small diameter of the 30% w/w sodium alginate scaffold supports the slow release of keto compared with the low concentration of the sodium alginate scaffold, as keto could be captured within the narrow cavities of the scaffold. In brief, the cross-section morphology showed changes as the concentration of sodium alginate increased from 20% w/w to 30% w/w, whereas the surface morphology of both concentrations showed the same construction.

**In Vivo gelation study**

This study was prepared to show that the extent of gel formation within a rat stomach was the same as that in vitro. The 30% w/w SO with 5% w/w guar gum formulation was the candidate for the in vivo gelation study because its slow release was the best. Figure 6 represents six images, with panels Figure 6B to Figure 6E representing a sectioned stomach to show the in situ gelation and the residence of the gel in the stomach, whereas Figure 6A shows an empty sectioned stomach as a control. Furthermore, Figure 6F exhibits an intact stomach containing the gel before sectioning. Additionally, the gels were dyed with methylene blue to be easily distinguished from normal tissue. Figure 6B, Figure 6C, Figure 6D and Figure 6E show the blue colored gel in the sectioned stomach 30 min, 1 hour, 2 hours, and 8 hours, respectively, after administering the selected in situ suspension to the rats. Bubbles are clearly shown in Figure 6B, indicating the release and effervescence of calcium carbonate in the acidic stomach media within 30 min of gelation (Rajinikanth et al., 2007). To sum up, Figure 6 shows that the suspension formulation of 30% w/w sodium alginate in SO in the presence of 5% w/w guar gum gelled and remained for long periods in the stomach of rats could be correlated with the in vitro gelation.

**CONCLUSION**

This research was designed to simplify the method of preparation of in situ gels composed mainly of sodium alginate-calcium carbonate and oils. Our method of in situ gel preparation was simple and easy and needed gentle mixing by a spatula. The selected oils in this work were MCT, SO and OO as the least amount of sodium alginate needed to solidify the oils in the presence of calcium carbonate and 5% w/w guar gum was 20% w/w. The 30% w/w sodium alginate in SO formulation was the best at slowing the release of keto compared with other formulations.

This slow release was primarily due to the hydrogen bonds between the keto molecules and the content of the formulation, as demonstrated by the FTIR study, which helped to connect the keto molecules within the formulations. Second, the keto molecules could be captured within the small orifices of the connecting strips, such as the structure of the gel scaffold, as shown by the SEM images of the scaffold of 30% w/w sodium alginate.

Last, the in vivo study supported the in vitro gelation data by showing that the selected formulation gelled and remained in the sectioned stomach after 30 min, 1 hour, 2 hours, and 8 hours of administration.

**Compliance with ethical standards**

The animal study was conducted according to the guidelines and approval of the ethical committee of research for animal studies at the Pharmacy College, Mustansiriyah University, Baghdad –Iraq.

**Conflict of interest**

The authors stated that they have no conflicts of interest.

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None

**Informed consent**

The consent was obtained from the participants in the current study.

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