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Influence of hydroxypropyl methylcellulose, methylcellulose, gelatin, poloxamer 407 and poloxamer 188 on the formation and stability of soybean oil-in-water emulsions

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ABSTRACT

Macromolecules of polysaccharides, proteins and poloxamers have a hydrophobic portion and a hydrophilic one that can be used as emulsifiers. Parts of these emulsifiers are safe pharmaceutical excipients, which can replace the irritant low molecular weight surfactants to formulate emulsions for the pharmaceutical field. This project focused on preparing O/W emulsions stabilized with polymers for pharmaceuticals such as polysaccharides, proteins and poloxamers, including hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), gelatin, poloxamer 407 (F127) and poloxamer 188 (F68). Emulsion physical stability was assessed by centrifugation, autoclaving sterilization and droplet size measurements. The stabilization mechanisms of emulsions were determined by interfacial tension and rheological measurements. Results stated that the efficacy of these polymers for pharmaceuticals stabilized emulsions was sorted in the order: F127 > F68 > HPMC > MC > Gelatin.

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1. Introduction

Emulsions are defined as a heterogeneous system consisting of two immiscible phases at least. Emulsifiers are added to the system to lower oil–water interfacial tension and improve the stability of emulsions by increasing repulsion forces between droplets [1,2]. The most common of pharmaceutical emulsifiers are low molecular weight surfactants, although they can cause toxic symptoms in organisms and produce serious environmental pollution [3]. In order to answer the increasing

demand for clean label excipients, natural polymers can replace the potentially irritative low molecular weight surfactants used in pharmaceutical emulsions formulation [4]. Thus, developing much safer molecules for the preparation of emulsions would be a future trend. In natural polymers stabilized emulsions, polysaccharides and proteins generally act as biocompatible emulsifiers [5,6]. Polysaccharides display specific interfacial activity for their amphipathic structure that can be formed by two ways: (i) the protein moiety is linked covalently or physically to the polysaccharide, or (ii) the non-polar chemical groups are attached to the hydrophilic polysaccharide

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backbone [7,8]. Moreover, polysaccharides play an additional role as stabilizer to increase the viscosity of the continuous phase that can slow the movement of the droplets, prevent phase separation and improve the long-term physical stability of emulsions [9]. Proteins contain hydrophilic and hydrophobic residues randomly spread all over the structure that are able to adsorb at the interface and facilitate droplets disruption by lowering the interfacial tension. The protein residues in the aqueous phase also provide steric stabilization against flocculation and coalescence [10]. Actually, polysaccharides and proteins are widely applied into food industry; however, pharmaceutical emulsions are mostly stabilized by low molecular weight surfactants [4,5,8]. It is worth mentioning that poloxamers as synthetic surfactants are widely used for pharmaceutical formulation [11]. Additionally, they are approved for oral or intravenous administration by the FDA by virtue of their high solubilizing capability, low toxicity and prolonged *in vivo* circulation time prior to dissociation [12]. However, poloxamers usually act as cosurfactants and combine with low molecular weight surfactants to prepare emulsions [13]. It is a fact that up to now, pharmaceutical emulsions have not evolved much compared to food emulsions. In the food industry, the better understanding of physicochemistry of natural polymers stabilized emulsions has been a successful approach for the formulation of highly stable emulsions [4]. Hence, systematic learning polymers acting as emulsifiers for pharmaceuticals is necessary.

In the present study, we chose polymers for pharmaceuticals to stabilize O/W emulsions: hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC), the major binder in tablets; gelatin, the main ingredient of hard capsules; and poloxamer 407 (F127) and poloxamer 188 (F68), the principal water-soluble base for many galenic applications (e.g., oral, rectal, topical, ophthalmic, nasal and injectable preparations) [4,14-16]. Indeed, these polymers also possess amphiphilic characteristic according to their structure.

HPMC and MC are water-soluble polysaccharides derived from cellulose, the most abundant polysaccharide in nature. Both HPMC and MC have the same polymeric backbone, a repeating structure of anhydroglucose units [17]. The units endow them the basic hydrophilic that can help themselves to adsorb onto the interface between two immiscible liquids [18]. Due to their hydrophilic character and high molecular weight, the viscosity of aqueous phase based on HPMC or MC system is high, decreasing the flowability and improving the rheological property [19]. HPMC and MC are thus good emulsifiers, enhancing droplet stability against flocculation and creaming [20,21].

Gelatin is a relatively high molecular weight protein obtained by partial hydrolysis of collagen and not by a single chemical substance [22]. The main constituents of gelatin are large and complex polypeptide molecules with the same amino acid composition, which confers interfacial activity to gelatin [23]. Also, it can lower the interfacial tension and adsorb at the oil-water interface preventing droplet from aggregating through the polymeric steric repulsion [22,24].

Poloxamers are block polymers that are synthesized by sequential addition of ethylene oxide (EO) and propylene oxide (PO) monomers in the presence of an alkaline catalyst, forming the basic A-B-A structure: $EO_x-PO_y-EO_x$ [25]. F127 and F68 are produced by altering the number of hydrophilic EO_x and hydrophobic PO_y units [26]. This structure confers an amphiphilic

character to the copolymers and allows them strong adsorption at oil-water interfaces to form stabilizing layers around oil droplets, so they are able to fulfill both the emulsifying and the stabilizing roles [27].

The purpose of this study was to exclusively use HPMC, MC, gelatin, F127 and F68 to prepare stable O/W emulsions, evaluate the physical stability of these emulsions, investigate their stabilization mechanisms and compare the interfacial activity of these emulsifiers. The assessment of emulsion stability was conducted over a 3-month period, combining different methods such as centrifugation, autoclaving sterilization and droplet size measurements. Moreover, the stabilization mechanisms of emulsions were analyzed by interfacial tension and rheology measurements, which favored to get better insight into the stable emulsions and choose the better emulsifiers among these polymers for pharmaceuticals.

2. Materials and methods

2.1. Materials

HPMC (K100M) was a kind gift from Shanghai Colorcon Coating Technology Limited (Shanghai, China). MC (with an average molecular weight of about 20,000 g/mol as reported by the supplier) and Gelatin (Type A gelatin, $pI \sim 7-9$) was purchased from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). F127 (with an average molecular weight of about 12,962 g/mol as reported by the supplier) and F68 (with an average molecular weight of about 8622 g/mol as reported by the supplier) were supplied by BASF Company Ltd. (Shanghai, China). Injectable soybean oil was purchased from Zhonghang Tieling Pharmaceutical Co., Ltd. (Tieling, China). Experiments were carried out with deionized water.

2.2. Methods

2.2.1. The preparation of emulsions

O/W emulsions were prepared by the following steps. Firstly, different mass fractions of emulsifiers and a certain amount of oil were well mixed in a mortar. Deionized water was added to the mortar with constant grinding to prepare mixture. In this system, oil:aqueous phase ratio was 15:85 by weight. Then, the mixture underwent ultrasonic processing by Ultrasonic Cell Crusher (JY92-IIN, Ningbo Xinzhi Biological Co., Ltd., China) for 2 min; this was repeated five times to prepare coarse emulsions. At last, the final emulsions were obtained after high pressure homogenization (Niro Soavi NS10012K homogenization, Via M. da Erba Edoari, Italy) at 700 bar for three, five, eight and fifteen times circulation, respectively. Before homogenization, the pH of the coarse emulsions was adjusted to 8.0 with 0.1 mol/L NaOH.

2.2.2. Droplet size

Creaming would be formed after an increase in droplet size of emulsions [5]. Therefore, it is a convenient way to evaluate emulsion physical stability by determining and comparing droplet size in different time intervals to observe the minimal changes of emulsion droplets.

A dynamic light scattering (Zetasizer Nano-ZS90, Malvern, UK) was used for the droplet size measurement of emulsions, whose characteristic size was always included in the instrument

sensitivity range (0.3–5000 nm). The particle size distribution was characterized in terms of the mean droplet size (Z-average diameter), which was determined by cumulant analysis of the intensity–intensity autocorrelation function $G(q, t)$ and polydispersity index (PDI) [28]. At room temperature (25 °C), the droplet size measurements were carried out. Prior to measurements, the samples were freshly diluted with deionized water to eliminate the influence of multiple scattering and interparticle interaction. It should be noted that all the droplet size measurements were monitored without any visual phase separation. All the samples were carried out at least in triplicate.

2.2.3. Physical stability of emulsions

Physical stability of emulsions was assessed by monitoring the centrifugal stability coefficient, stability after autoclaving sterilization and the evolution of droplet size during short-term storage period.

2.2.3.1. Centrifugal stability coefficient. Centrifugal stability coefficient (K_e) can be taken as a quantitative parameter to estimate emulsion physical stability [2]. In this study, samples were centrifuged (HC-2516, USTC Chuangxin Co., Ltd., China) at two centrifugal conditions: one was at 4000 rpm for 15 min and the other was at 10,000 rpm for 10 min. Then centrifuged and uncentrifuged samples were diluted with the same fold. Finally, the measurements of absorbance were carried out by UV-2000 Spectrophotometry (Shimadzu, Japan) at the wavelength of 500 nm. The value of the centrifugal stability coefficient (K_e) was computed for each emulsion via the equation:

$$K_e = A_0 - A_1/A_0 \times 100\% \quad (1)$$

where A_0 is the absorbance of emulsions before centrifugation and A_1 is the absorbance of emulsions that are drawn from the bottom of centrifuge tube after centrifugation. K_e represents the change in the absorbance before and after centrifugation. The smaller the K_e , the more stable the emulsions [2]. Tests were performed at three times and the mean of the three individual trials was taken for analysis.

2.2.3.2. The physical stability after autoclaving sterilization. Change in the temperature can influence the conformation of polymers for pharmaceuticals that have a negative effect on emulsion physical stability [2]. In order to study emulsion physical stability after autoclaving sterilization, emulsions that went through eight times circulation of homogenization were chosen for autoclave sterilization (LDZX-30FBS, Shanghai Shenan Medical instruments Factory, China) at 121 °C for 15 min. The droplet size of samples was determined before and after sterilization.

2.2.3.3. The physical stability of short-term storage. Centrifugation is a rapid method to evaluate emulsion physical stability, but may not truly reflect the physical stability during the period of storage. To illustrate the physical stability of emulsions under natural conditions, another set of emulsions were used to study the effect of short-term storage on droplet size [5].

Emulsions were placed into vials and stored at room temperature. Droplet size of emulsions was measured after

preparation for 24 h, 7, 30, 60 and 90 d. At the micro level, the physical stability of emulsions was given by comparing the size of emulsions in different time intervals. At the macro level, visible phase separation was an evaluation criterion of instability.

2.2.4. Evaluation of the stabilization mechanisms

2.2.4.1. Interfacial tension. Stable emulsions require the existence of emulsifier, which can adsorb at the oil–water interface and decrease the interfacial tension [29]. Interfacial tension is a useful parameter to characterize and compare the interfacial activity of different emulsifiers [30]. Moreover, the efficiency of emulsification depends on the dosage of emulsifier, and interfacial tension is relevant to the emulsifier concentration [9]. To evaluate the interfacial activity of emulsifiers and search an optimum emulsifier concentration, the measurement of interfacial tension is needed.

The interfacial tension at the oil–water interface was measured by the Wilhelmy plate method using Auto tensiometer (JYW-200b, Chengde Dahua shiyanji Co., Ltd., China). Injectable soybean-oil was selected as oil phase and water phase was composed of polymers solution. It took 30 min to equilibrate after the plate has just touched the water phase by moving the lifting platform. The platinum plate was thoroughly cleaned and dried before each measurement. In all cases, successive measurements were carried out three times, and the standard deviation did not exceed ± 0.2 mN/m.

2.2.4.2. Rheological property. Polysaccharides, proteins and poloxamers have a wide range of application as drug carriers, while their specific applications depend on their structures and rheological properties. Rheology is concerned with the behavior in the transient area between solids and fluids and the flow of substances [31]. The rheological properties of different emulsions may vary considerably and depend on the composition of emulsions. In this experiment, the only difference among emulsions was the composition of aqueous phase, which was varying in the type of emulsifiers. Therefore, it was important to study the influence of continuous phase on the formation of emulsions.

Rheological properties of the polymer solutions were determined using cone and plate geometries (60 mm of diameter; 2° cone angle; 1050 μm gap) on an AR 2000 rheometer (TA, Co., Ltd., New Castle, DE). The temperature was controlled by a circulating water bath and regulated by a Peltier effect at 25 °C. Dynamic frequency sweep was performed in the frequency range from 0.1 Hz to 10 Hz at a linear viscoelastic zone. Flow curves were obtained in the range of shear rate from 0.1 Hz to 120 Hz. Samples were given 5 min to equilibrate after loading into the rheometer.

3. Results and discussion

3.1. Factors influencing the size and the formation of the emulsion droplet

3.1.1. The influence of the emulsifier concentration on the emulsion droplet size

Emulsification involves the sudden creation of a large amount of new liquid interface, and sufficient emulsifiers are needed

Table 1 – Mean droplet size and PDI of emulsions stabilized with HPMC, MC, gelatin, F127 and F68 respectively after eight times homogenization.

Emulsifier	Emulsifier concentration (% w/v)	Mean droplet size (nm)	PDI
HPMC	1	235.13 ± 8.24	0.021 ± 0.019
	2	206.30 ± 5.45	0.255 ± 0.164
MC	1.5	278.70 ± 4.50	0.134 ± 0.098
	2	392.00 ± 8.30	0.088 ± 0.024
Gelatin	5	138.93 ± 5.05	0.076 ± 0.045
	6	148.10 ± 3.29	0.034 ± 0.030
	7	137.37 ± 4.77	0.065 ± 0.060
F127	3	165.50 ± 2.00	0.080 ± 0.027
	5	137.13 ± 1.33	0.101 ± 0.013
	8	130.30 ± 1.10	0.081 ± 0.052
F68	10	136.73 ± 4.22	0.159 ± 0.048
	15	98.24 ± 3.57	0.166 ± 0.027

in this process [5]. Combined with characters of each emulsifier, such as interfacial activity and viscosity, different emulsifier concentrations are required to prepare finer emulsions [28]. In this study, the emulsifier concentration for each kind of emulsion was different and presented in Table 1.

Fig. 1 clearly illustrates that the emulsifier concentration had a strong effect on the droplet size. From Fig. 1A, 1B, 1C and 1D, we observed the tendency that the higher the concentration of emulsifier, the smaller the droplet size of emulsions. Combining the results, in practical applications, we can choose higher emulsifier concentration such as HPMC (2%, w/v), Gelatin (7%, w/v), F127 (8%, w/v) and F68 (15%, w/v) to prepare finer emulsions. This tendency could be attributed to two main factors: (i) a higher emulsifier concentration conducted itself to fast adsorb onto the oil droplet surfaces, providing more effective protection against coalescence; (ii) a higher emulsifier concentration made more available emulsifier molecules to cover the oil droplet surfaces during

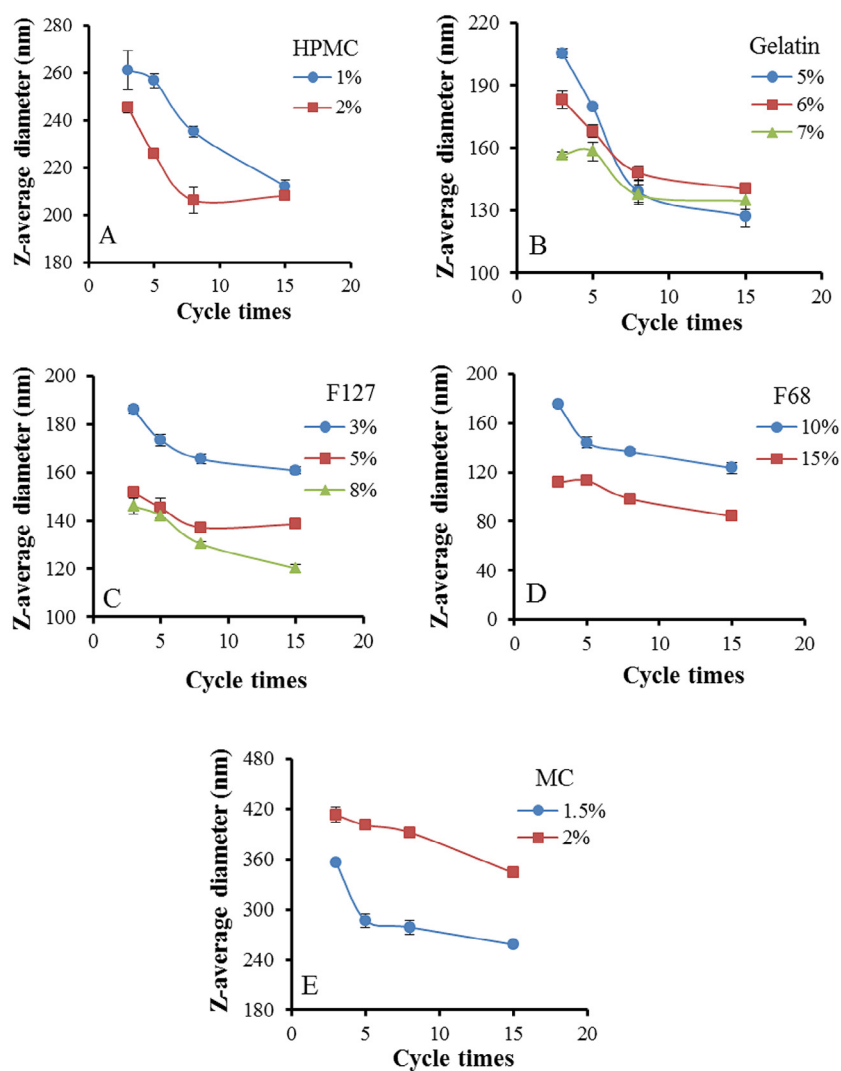


Fig. 1 – Droplet size as a function of cycle times at different emulsifier concentrations. Emulsions stabilized with (A) HPMC, (B) gelatin, (C) F127, (D) F68 and (E) MC.

homogenization [32]. However, as shown in Fig. 1E, there actually existed an increase in the droplet size when the concentration of MC was increased. One possible explanation for this phenomenon was that there were enough emulsifiers to cover all the oil droplet surfaces [32]. Adequate doses of emulsifiers could induce an increase in droplet size through a number of mechanisms: (i) unadsorbed emulsifiers might have formed multilayers around each droplet; (ii) unadsorbed emulsifiers might have promoted droplets flocculation by increasing droplets surface hydrophobicity; (iii) unadsorbed emulsifiers might have formed aggregation in the continuous phase that contributed to the light scattering signal [33]. Especially deserving to be mentioned, MC (1%, w/v) stabilized emulsion appeared phase separation after preparation (results have not been shown). Hence, MC (1.5%, w/v) may be the better emulsifier concentration to prepare stabilized emulsion.

3.1.2. Effect of homogenization on the formation and droplet size of emulsions

In the preparation process of F68 stabilized emulsions, the larger droplets were formed and flocculation was observed after ultrasonication within 2 h (results have not been shown). Fortunately, stable emulsions were produced by homogenization, which generated magnitude of the disruptive forces within the homogenization chamber to overcome the larger droplets together [33]. Meanwhile, homogenization could further increase the contact surface area of the emulsifying molecules and result in the formation of smaller droplets [30]. In addition, under specific homogenization conditions (pressure and number of passes), emulsifiers could quickly adsorb onto the new formed oil-water interfaces against droplets flocculation and coalescence [5]. From the above, the process of homogenization is essential for the formation of stable emulsions.

The mean droplet size of emulsions was decreased with homogenization cycles increased. Nevertheless, fairly small change in droplet size was obtained by increasing homogenization cycles. Taking F127 (8%, w/v) stabilized emulsions for example, cycle times were increased from five to eight, and the size was reduced by 12 nm. By contrast, the size was only reduced by 10 nm when the cycle times were increased from eight to fifteen. The reason for the decrease in droplet size was that more than one pass was enabled to fully use residual emulsifiers that did not yet adsorb at oil-water interfaces and improved the uniformity of emulsions by transferring the already adsorbed emulsifiers from one homogenization to the following homogenization [28]. However, most of the power was dissipated as heat in the homogenization process could not significantly decrease droplet size of emulsions [5]. On the basis of experimental results, eight times circulation could help us obtain stable emulsions, and there is no need to homogenize more times. Thus, we used the emulsions that underwent eight times circulation for further investigations.

3.2. Physical stability of emulsions

3.2.1. Centrifugal stability coefficient

The effect of centrifugal process on emulsion physical stability was investigated at two centrifugal conditions, and results

about centrifugal tests are shown in Fig. 2. From Fig. 2C, 2D and 2E, we conclude that at higher emulsifier concentration, emulsions stabilized with gelatin, F127 or F68 remained relatively stable after centrifugation because the K_e of these emulsions was smaller than those of lower emulsifier concentration stabilized ones. This once again demonstrates that higher emulsifier concentration is beneficial to forming stable emulsions. The higher emulsifier concentration is able to form a thickly protective layer around oil droplets, preventing the oil droplets from moving around and promoting the emulsion physical stability [34,35]. Certainly, all the K_e of these emulsions was increased with centrifugal speed raised, since the accelerated conditions quickened creaming. In Fig. 2A and 2B, we observed that change in emulsifier concentration and centrifugal speeds had no remarkable influence on K_e . It could be explained by the fact that after centrifugal setting, emulsions stabilized with HPMC or MC had an obvious stratification whether the samples remained at high or low emulsifier concentration and centrifugal speed. Meanwhile, this very fact also predicts that HPMC or MC stabilized emulsion would have a poor long-term physical stability, as centrifugation at 4000 rpm for 15 min can produce the same effect on emulsions as keeping them under normal conditions for one year [2]. Based on the theory and comprehensive comparison on every values of K_e , we can speculate that the long-term physical stability of each kind of emulsion can be sorted in the order: F127 (8%, w/v) > F68 (15%, w/v) > Gelatin (7%, w/v) > HPMC and MC. It is worth mentioning that in the actual experimental process, emulsions were drawn from the bottom of centrifuge tube after centrifugation, leading to the large experiment error. To obtain the accurate results about emulsion physical stability, other parameters should be measured.

3.2.2. Effect of autoclaving sterilization on emulsion physical stability

Emulsion physical stability was also assessed after autoclaving sterilization. Sterilization might be a process of re-emulsifying or irreversible redistribution of emulsifier within the oil and aqueous compartments that has influences on emulsion physical stability [36]. In this experiment, not only was the droplet size increased among four kinds of emulsions after autoclaving sterilization, but phase separation appeared in the MC stabilized emulsions. The comparison about droplet size and PDI before and after sterilization is shown in Table 2. During autoclaving sterilization, macromolecule chains of emulsifier aggregated, and the small positive temperature perturbations accelerated the aggregation of chains. The increase of droplet size was attributed to the aggregation between different macromolecule chains [37]. Due to the shrinking of the aqueous phase volume between the droplets and a net force pulling the droplets together, the final results about the aggregation of chains was phase separations [38]. On account of bad physical stability of MC stabilized emulsion after autoclaving sterilization, the application range of MC stabilized emulsion in pharmaceutical fields is limited.

3.2.3. Effect of short-term storage on emulsion physical stability

Emulsions were stored under the natural conditions, and they exhibited good physical stability without phase separation

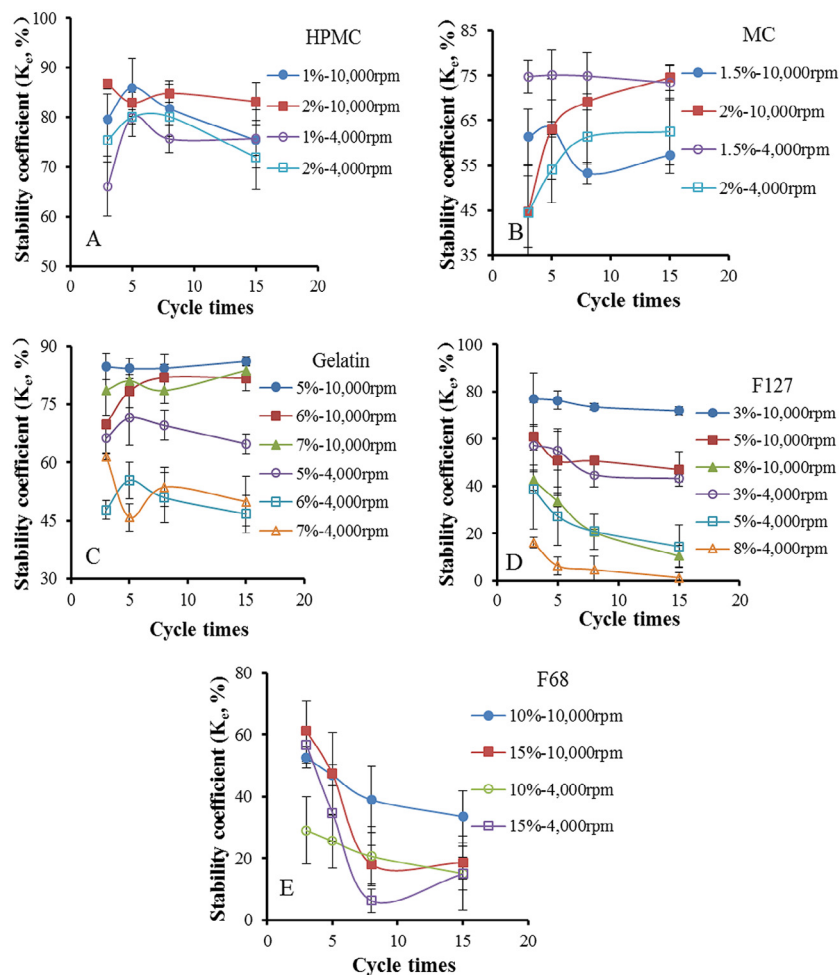


Fig. 2 – Effects of emulsifier concentration and centrifugal speed on K_e . Emulsions stabilized with (A) HPMC, (B) MC, (C) gelatin, (D) F127 and (E) F68.

during the period of 3 months' storage except for gelatin stabilized ones. Emulsion physical stability about short-term storage was also assessed via the variation of mean droplet size as time went on. Results in Fig. 3 highlight that all the droplet size of stable emulsions had the tendency to increase as time went by. The increased droplet size could be assigned to a reduction in the rigidity and an increase in the fluidity

of emulsifier layer, leading to the fusion of emulsifier layers and the formation of larger droplets [39].

As shown in Fig. 4, the droplet size also increased in gelatin stabilized emulsions after storage over 7 d. However, after two weeks of storage, no stable emulsions existed for the visible phase separation. It was likely that the low interfacial activity and viscosity of gelatin solution induced particles to form

Table 2 – Effect of autoclaving sterilization on droplet size.

Emulsifier	Emulsifier concentration (% w/v)	Mean droplet size (nm)		PDI	
		Before sterilization	After sterilization	Before sterilization	After sterilization
HPMC	1	235.13 ± 8.24	263.85 ± 7.42	0.021 ± 0.019	0.095 ± 0.050
	2	206.30 ± 5.45	262.67 ± 6.02	0.255 ± 0.164	0.034 ± 0.016
Gelatin	5	138.93 ± 5.05	165.72 ± 2.17	0.076 ± 0.045	0.093 ± 0.017
	6	148.10 ± 3.29	224.60 ± 5.66	0.034 ± 0.030	0.444 ± 0.049
	7	137.37 ± 4.77	161.27 ± 5.23	0.065 ± 0.060	0.044 ± 0.010
F127	3	165.50 ± 2.00	173.60 ± 5.57	0.080 ± 0.027	0.020 ± 0.097
	5	137.13 ± 1.33	149.23 ± 4.54	0.101 ± 0.013	0.029 ± 0.122
	8	130.30 ± 1.10	143.23 ± 3.01	0.081 ± 0.052	0.049 ± 0.064
F68	10	136.73 ± 4.22	153.08 ± 2.34	0.159 ± 0.048	0.103 ± 0.022
	15	98.24 ± 3.57	112.15 ± 2.38	0.166 ± 0.027	0.118 ± 0.009

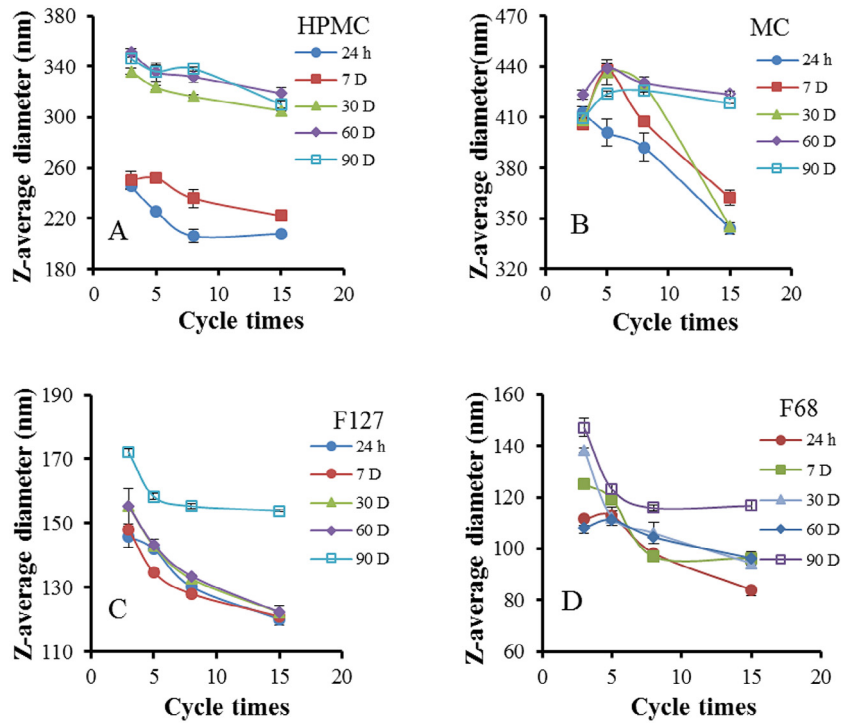


Fig. 3 – Changing process of droplet size after storage over 90 d. Emulsions stabilized with (A) HPMC (2%, w/v), (B) MC (2%, w/v), (C) F127 (8%, w/v) and (D) F68 (15%, w/v).

large aggregation, and the rapid deposition was attributed to hydrogen bonds [1]. Based on experimental results, it is easy to summarize that gelatin may not be an appropriate emulsifier to prepare stable pharmaceutical emulsions.

3.3. Evaluation of the stabilization mechanisms of emulsions

3.3.1. Interfacial tension of emulsifiers

The interfacial activity of emulsifiers was characterized by measuring the interfacial tension at the oil-water interface, in comparison with the interfacial tension of a pure water-soybean oil system. Results are reported in Fig. 5. The adsorption

of macromolecular emulsifiers at the oil-water interface go through three different stages that affect the measurements of interfacial tension: (i) the diffusion from the bulk to the boundary layer at the interface, (ii) the molecular adsorption at the interface and the penetration into the oil phase, and (iii) the molecular reorganization at the interface [40]. The process of dynamic adsorption could last for a long time, particularly in the step of reorganization at the interface [41]. In order to attain an accurate interfacial tension, the setting

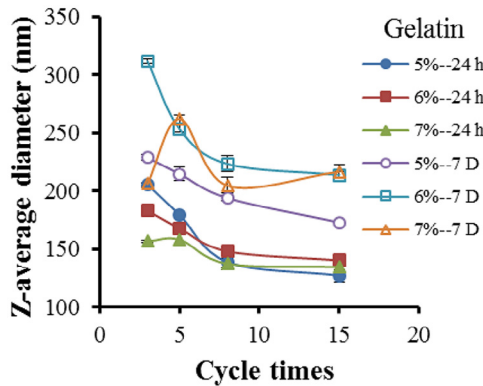


Fig. 4 – Droplet size of gelatin stabilized emulsions after storage over 24 h and 7 d at different emulsifier concentrations.

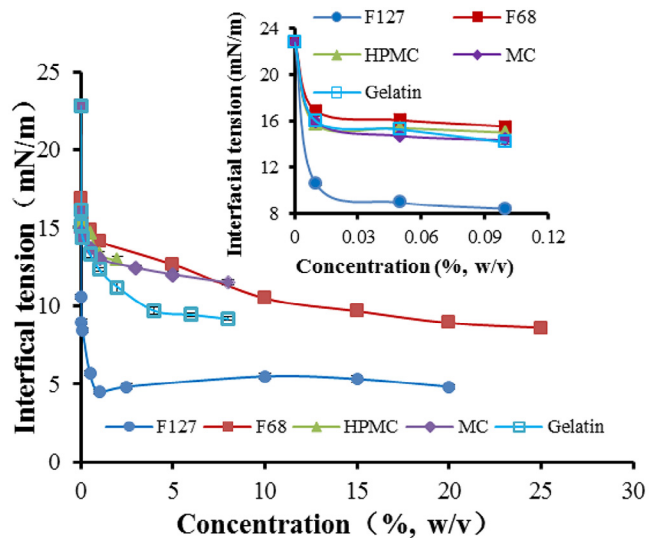


Fig. 5 – Oil-water interfacial tension of HPMC, MC, gelatin, F127 and F68 solution at different concentrations.

about 30 min of equilibration time was necessary. Additionally, upon the platinum plate touching the viscous solution, the resistance among the sticky sample appeared, especially for the HPMC or MC solution at high concentration. Hence interfacial tension was measured at a relatively lower concentration range for each emulsifier.

The interfacial tension of pure water-*soybean oil* system was about 22.83 mN/m, and the value was similar to the previous investigation that was also measured by Wilhelmy plate method [42]. Compared to the interfacial tension of pure water, all the samples were able to significantly reduce the interfacial tension, indicating the adsorption capability of samples at the interface [29]. In the same concentration range of 0 to 0.2% (w/v), the interfacial tension of samples was categorized in the increasing order: F127 < Gelatin < MC < HPMC < F68. This order was also about the comparison of interfacial activity, for the interfacial tension is the quantitative parameter of interfacial activity [28]. With different numbers of interfacial contact groups per molecule, the measurements of interfacial tension for each kind of emulsifier was varied [40]. Evidently, at the same concentration, the more contact polymer groups of emulsifier there were, the higher the interfacial activity.

As expected, with the continuously increased emulsifier concentration in water phase, the interfacial tension was decreased and reached a relatively constant value at high emulsifier concentration. The constant value indicated that the oil-water interfaces was saturated with emulsifiers [32]. In the selected concentration range of each sample, the minimum interfacial tension was sorted in the order: F127 < F68 < Gelatin < MC < HPMC. This order was different from the former one, for the emulsifier of F68 lowered more interfacial tension at the higher concentration. It illustrated that the increased emulsifier concentration in water phase can increase the number of effective contact polymer groups and lower the interfacial tension [40]. Combine with this theory, we are better able to understand why the higher emulsifier concentration of gelatin, F127 and F68 can improve emulsion stability in centrifugation tests and had the smaller droplet size in contrast to HPMC or MC stabilized ones. Hence, we can increase the emulsifier concentration in a suitable range to prepare finer emulsion. In addition, the lowest interfacial tension and highest interfacial activity of F127 solution indicated that F127 was the better emulsifier in comparison with other emulsifiers in this study to prepare finer emulsion.

3.3.2. Rheology analysis on continuous phase

Fig. 6 displays the storage modulus (G') and loss modulus (G'') of sample solutions. Except for F68 solution, within the entire frequency range, all the samples exhibited that G'' was significantly greater than G' . It illustrated that these samples had a highly liquid-like structure with the predominant viscous behavior. F68 solution had a solid-like structure and mainly possessed the elastic behavior for the G' was greater than G'' [43]. Even though phase separation occurred without homogenization, F68 stabilized emulsions possessed the good physical stability after short-term storage and autoclaving sterilization. The stability was primarily attributed to the elastic behavior of F68 solution for the network that was formed by F68 molecules [29,31]. In all the samples, a crossover between the two moduli was not observed in the full frequency range. In other

Table 3 – Linear equation and η_a of flow curves.

Sample solution (w/v)	Linear equation	η_a (Pa·s)	δ_0 (Pa)	R^2
HPMC (1%)	$\delta = 0.1153 \gamma + 0.1167$	0.1153	0.1167	0.9999
MC (1.5%)	$\delta = 0.2108 \gamma + 0.3437$	0.2108	0.3437	0.9998
Gelatin (5%)	$\delta = 0.0310 \gamma + 0.0656$	0.0310	0.0656	0.9970
F127 (3%)	$\delta = 0.0022 \gamma + 0.0687$	0.0022	0.0687	0.9976
F68 (10%)	$\delta = 0.0064 \gamma + 0.0637$	0.0064	0.0637	0.9996

words, polysaccharide, protein and poloxamer solutions in this study did not show any gel behavior.

The dependence of shear rate versus shear stress revealed that all evaluated solutions were categorized as non-Newtonian fluids specific to plastic fluid, which had a constant viscosity after shear stress greater than themselves yield stress [44]. Results are presented in Fig. 7, and the constant viscosity was calculated by Bingham Flow Equation:

$$\delta - \delta_0 = \eta_a \gamma \quad (2)$$

where δ represents shear stress, δ_0 represents yield value, η_a represents apparent viscosity and γ represents shear rate. The apparent viscosity (η_a) of various solutions is shown in Table 3 and classified in the order: MC > HPMC > F68 > Gelatin > F127. Where macromolecular chains are long and heavily twisted, the viscosity of solution is high [39]. Hence, the order was related to the aggregation level of macromolecular chains in water. Apart from F68 solution, viscosity and interfacial activity had the same order, for the relatively unfolded macromolecule chains had more effective groups contacting with the interface [40]. Due to the net structure of F68 molecule, it had the high viscosity and low interfacial tension. The order of viscosity could explain the better physical stability of emulsions containing HPMC or MC compared to gelatin, even if gelatin had advantages in lowering interfacial tension and forming smaller droplets. HPMC and MC clearly enhanced the viscosity of continuous phase and conferred them a better physical stability. Thus, viscosity plays an important role in emulsion physical stability.

4. Conclusions

Emulsions stabilized with HPMC, MC, gelatin, F127 or F68 were systematically investigated, including (i) factors influencing the size and the formation of the emulsion droplet, (ii) emulsion physical stability and (iii) stabilization mechanisms of emulsions. Emulsifier concentration and the process of homogenization were the major factors on the formation of smaller droplet size. Emulsion physical stability was determined by three methods, and the results clearly showed that phase separation of MC or gelatin stabilized emulsions after autoclaving sterilization and storage respectively manifested poor physical stability. Conversely, HPMC, F127 or F68 stabilized ones possessed the good physical stability. Stabilization mechanisms of emulsions were deduced from interfacial tension and rheology measurements: both interfacial tension and viscosity had much different effects on the emulsion physical

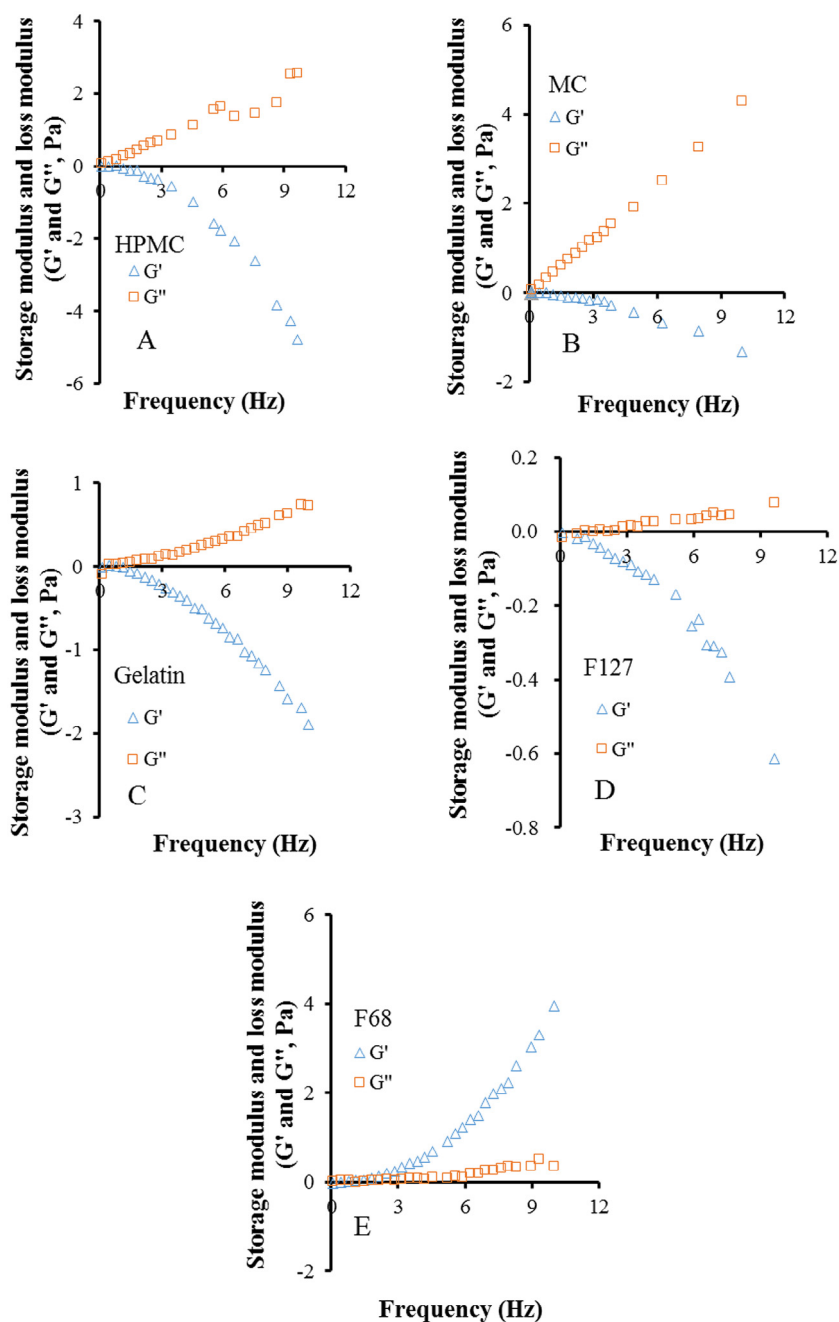


Fig. 6 – Mechanical spectra showing the storage modulus (G') and loss modulus (G'') as a function of frequency at strain = 0.5% for the samples. Spectra recorded (A) HPMC (2%, w/v) solution, (B) MC (2%, w/v) solution, (C) gelatin (6%, w/v) solution, (D) F127 (5%, w/v) solution and (E) F68 (15%, w/v) solution.

stability. During storage period, good physical stability of HPMC or MC stabilized emulsion was mainly due to the high viscosity in solution. Nevertheless, low interfacial activity and viscosity resulting in gelatin stabilized emulsion exhibited poor physical stability. Although F127 had the lowest viscosity in solution, it could efficiently lower oil–water interfacial tension, which contributed to the formation of stable emulsion. F68 showed limited interfacial properties, but forming the network by F68 molecules was the key factor to stabilize emulsions. Compared comprehensively, F127, F68 and HPMC are commendable emulsifiers to form stable O/W pharmaceutical emulsions.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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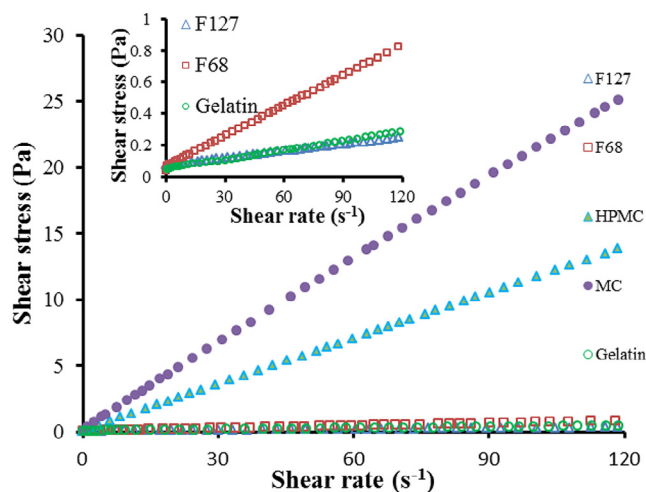


Fig. 7 – Flow curves representing the shear rate dependence of the shear stress for the samples of HPMC (1%, w/v), MC (1.5%, w/v), gelatin (5%, w/v), F127 (3%, w/v) and F68 (10%, w/v).

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