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Research article

Design and preparation of a novel pullulan hard capsule formulation: A promising green candidate and study of crucial capsule features

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ABSTRACT

Plant-based hard capsules have gained considerable attention because of their great properties. Accordingly, designing and developing of these kinds of capsules will be a difficult task. Herein, an innovative pullulan-based hard capsule formulation was prepared for the first time. A series of characterization approaches, including Fourier transform infrared, field emission scanning electron microscope, and rheology analysis, were utilized to figure out the straightforward preparation of a designed hard capsule. Many tests and experiments were performed to achieve the optimum capsule formulation. Based on the obtained results, specifications such as uniform downfall and non-desirable adhesion, and other ideal characteristics of the capsule display the critical function. The gelling promoter of divalent cationic salts is more beneficial than its single-valent counterparts. With respect to the key role of gelling promoter, the presence of chosen $MgSO_4.7H_2O$ salt and the source of selected carrageenan are important parameters to achieve optimal formulation. Moreover, field emission scanning electron microscope images illustrate that the weight ratio of 3.5 (gelling agent to salt) displays uniform surface morphology without any impurities or other foreign materials. Likewise, the outcomes of the rheology test also illustrated that the weight ratio of 3.5 is preferable. Considering the different weight ratios, the benefits of a weight ratio of 3.5 outweigh the other investigated ratios. Overall, the current research addresses substantial information about developing pullulan-based hard capsules for target usage.

1. Introduction

Nowadays, the drawbacks of gelatin-based capsules are recognizable. Indeed, regardless of its beneficial attributes, such as mechanical strength, facile processing, providing essential amino acids, and easy digestion feature, it is substantial that gelatin-based hard capsules illustrate a series of disadvantages, including the activity of amine-functionalized groups and corresponding interaction with

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the active groups of drugs, frangibility at low humidity, transferable spongiform encephalopathy threat, softening at high temperatures, religious opinions and biosafety [1–3]. Yet, many endeavors have been made to fabricate non-gelatin capsules with new ingredients. Pullulan is considered to be a linear polysaccharide generated by aureobasidium pullulans. In more detail, pullulan possesses the chemical structure of maltotriose constituents interconnected by α-(1 → 6) glycosidic connections, and in every maltotriose, the three glucose are linked by an α-(1 → 4) glycosidic linkages. Pullulan biopolymer is used in different fields, such as food and pharmaceuticals [4–7].

Pullulan is an appropriate candidate as an alternate material for hard capsules due to its superior properties, including great film-forming, non-toxicity, tastelessness, biodegradability, solubility, transparency, and non-mutagenic. Moreover, it is well-established that pullulan films demonstrate potential features in various applications. Much research has been devoted to manufacturing pullulan capsules, with many advantages and disadvantages [8,9]. Plant-caps™ is one of the commercial pullulan-based hard capsules, which is declared to possess superior characteristics. One of the most influential parameters in pullulan capsules is polymer molecular weight (M_w). In other words, pullulan with high M_w exhibits high viscosity, strength, and stability. It is preferred that the high Mw of pullulan is a suitable form in the design and development of favorable hard capsules [10,11].

Corresponding to HPMC and starch, pullulan does not configure a gel in its net form; consequently, extra gelling agents need to be mixed with pullulan to achieve hard capsules [12]. Owing to the weak mechanical features of pullulan capsules, use of other practical biopolymers such as cellulose derivatives, gellan gum, and carrageenan is an essential task [13]. Carrageenan has diverse forms like kappa, lambda, and iota, which differ in the number of sulfated ester groups [14]. It is worth mentioning that the Kappa form illustrates the convenient features for use in the formulation of pullulan hard capsules [15]. κ-carrageenan is a natural sulfated polysaccharide extracted from marine red algae. κ-carrageenan is extensively utilized in food, cosmetic and pharmaceutical industries because of its substantial attributes, including gelling, thickening, stabilizing, and textural increasing capacity. It is well-known that κ-carrageenan can form reversible gels with the assistance of gel promoting agents, like metal cations. In gelling process, the composition change of κ-carrageenan from a random coil to a helix form is a main transformation. Subsequently, the aggregation of helices leads to gelation. It is noteworthy that the addition of cations can promote the sequence of conformation and association of helices for the final stability of κ-carrageenan gel. It is well-established that the rheological features of the carrageenan solution display a close relationship with the type of metallic salt and its charge density. It is substantial that introducing of cations can increase the sequencing degree of conformation and aggregation of helixes. Specific cations like k^+ and Ca^{2+} influence the conformational conversion of the coil to the helix in carrageenan. It is also noteworthy that during the gelling process, the percentage of helixes enhances with the alleviating in temperature [16–19].

Further, noteworthy to mention that the mechanical features of hard gelatin capsules are depend on the relative humidity of capsules. One particularly salinity instance of this is the research investigation by Yukoh et al., they investigated the correlation between the water activity and mechanical potential of pullulan hard capsules filled with starch. Owing to the water activity the mechanical strength of capsules dropped. In another words, they argued that the molecular features of pullulan films have a strong correlation with their water content [20].

Today, there is a tremendous attention toward using biodegradable and biocompatible polymer materials in different fields of research. For instance, bioflavonoids, polyurea, PVPVA, PCL, polydimethylsiloxane, PANI and their derivatives have been extensively used in the field of medicine for their unique biological features. Further and even more importantly, though, there are a number of drawbacks associated with this finding but all of them addresses important and unique features [21–30].

To the best of our knowledge, there are negligible reports on the development of pullulan hard capsules with practical applications. Herein, we have carried out a research study about the influence of important parameters on the formulation of pullulan hard capsules. To be more specific, we assessed the kind of gelling promoter or salt, gelling agent, gelling agent to salt ratio, plasticizer, pH and drying time on capsule properties. One of the most important objectives of the current research work is its industrial usages. Accordingly, the current study was carried out in the pilot industrial phase using a production machine with valuable outputs. It is substantial that the outputs of the current research study are in well accordance with the practical utilization features. Importantly, this finding will open a new window and provides key information for industrial scale production of pullulan hard capsules.

2. Materials and methods

2.1. Materials

All of the employed materials were used as received from reliable companies without further purification. Pullulan was obtained from MICROLEX. Magnesium sulfate ($MgSO_4.7H_2O$) was acquired from Merck Company. κ-Carrageenan was received from Selt Marine Group, Sodium lauryl sulfate (SLS) was purchased from Godrej Industry (India). Propylene glycol (PG) was achieved from Kimyagaran Emrooz Chemical Industries Co. (Iran).

2.2. Preparation procedure

166 g of deionized water (DI) was heated to 50 °C to prepare the pullulan capsule solution. 0.48 g $MgSO_4.7H_2O$ was added to the solution. After complete dissolution, 1.2 g κ-carrageenan as a gelling agent was added to the solution, and the temperature was raised to 70 °C for 5 min to activate κ-carrageenan. Then, the temperature was down to 50 °C, and 40 g of pullulan was slowly added to the solution and stirred until it dissolved. Moreover, 0.14 g SLS and 1.0 g PG were added to the solution. The prepared solution was transferred to a bain-marie at 55 °C to remove bubbles. The obtained viscosity was found to be 1100 cp. The obtained solution was

Scheme 1. A schematic representation of the sequence in the preparation of a hard pullulan capsule.

Table 1
The details of various investigated pullulan hard capsule formulations with gellan gum as gelling agent.

Materials (g)	A	B	C	D	E
Pullulan	40.0	40.0	40.0	40.0	40.0
Gellan Gum	1.2	1.2	1.2	1.2	1.2
Salt	CH_3COOK (0.48)	NaCl (0.48)	KCl (0.48)	$MgSO_4$. $7H_2O$ (0.48)	Na_3PO_4 (0.48)
PG	0.5	0.5	0.5	0.5	0.5
SLS	0.21	0.21	0.21	0.21	0.21
Water	206.0	206.0	206.0	206.0	206.0

Table 2
The evaluation of different pullulan hard capsules formulation over κ-carrageenan gelling agent.

Materials (g)	F	G	H	I	J
Pullulan	40.0	40.0	40.0	40.0	40.0
κ-Carrageenan	1.2	1.2	1.2	1.2	1.2
Salt	CH_3COOK (0.48)	NaCl (0.48)	KCl (0.48)	$MgSO_4$. $7H_2O$ (0.48)	Na_3PO_4 (0.48)
PG	0.5	0.5	0.5	0.5	0.5
SLS	0.21	0.21	0.21	0.21	0.21
Water	206.0	206.0	206.0	206.0	206.0

conveyed into a steel dish with dimensions of 10×5×7 cm^3. After the dipping procedure and complete drying, the capsules were stripped off the pin and cut to the appropriate size. Subsequently, the two halves (body and cap) were joined. To achieve the best formulation of the pullulan capsule, the addition sequence of materials is very influential. For further details, in Scheme 1, the sequences and schematic representation of the pullulan hard capsule preparation procedure have been illustrated (Scheme 1).

2.3. Equipment's

The techniques and devices utilized in this investigation consisted of Brookfield Viscose (VTE model, USA), a manufacturing machine (Technophar, Canada), quality control gauges (VSX 136, Mitutoyo, Japan), and a fan dryer (GB 121-3, Greenheck Technology, Canada). The field emission scanning electron microscope (FESEM) was used to explore the morphology and size of the as-prepared hard capsules. The FESEM images were recorded on a Mira3 Tescan. Rheological features of the prepared hydrogel solutions were investigated to obtain the storage modulus (G′) and loss modulus (G″) by employing an oscillatory time sweep trial at 0.4% amplitude and frequency of 1 Hz for 15 min utilizing a rheometer (Physica MCR 300, Anton Paar Ltd., Austria). Therefore, the examinations were carried out at various temperatures for different pullulan hard capsule formulations, including weight ratios of 2.5, 3, and 3.5, respectively. The characteristic of loss modulus (G″) (liquid form) and storage modulus (G′) (gel form) was assessed by a temperature sweep in the range of 25–90 °C at a speed of −2 °C/min.

3. Results and discussion

3.1. Choosing the appropriate gelling agent

Despite the desirable features of pullulan biopolymer, it has several disadvantages. Substantially, pullulan polymer does not illustrate the desirable adhesive feature for the preparation of hard capsules. Accordingly, to enhance the adhesion feature of the polymeric network, the use of gelling agents, including carrageenan and gellan gum, is a necessary task in the formulation. To understand and address the suitable gelling agent for pullulan capsule formulation, carrageenan and gellan gum gelling agents were chosen in the formulation. It is necessary to explain that among the different forms of carrageenan, such as kappa, lambda, and iota, only the kappa form is beneficial for pullulan hard capsule formulation. Based on the performed tests and optimizations, κ-carrageenan was selected for further investigations owing to its compatibility, transparency, and suitable activity. It is essential to explain that the use of gellan gum leads to severe turbidity, non-Newtonian trend, and shedding of the formulation. Hence, it was removed from further

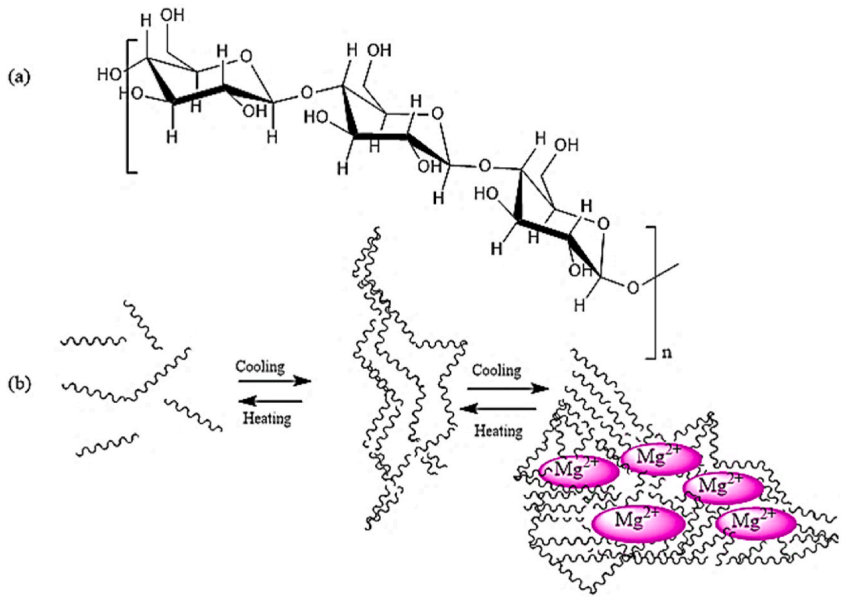

Fig. 1. (a) Chemical structure of the repeating unit of pullulan biopolymer and (b) thermally reversible gelation mechanism of κ-carrageenan in the presence of metal cations.

Fig. 2. A schematic images of hard pullulan capsules.

investigations. Considering the presence of active functional groups on the surface of carrageenan, it is crucial that the κ-carrageenan activates at a temperature of 70 °C. Precisely, providing this temperature is an essential task for the activation of this gelling agent. Therefore, in all experiments, the reaction temperature was set at 70 °C (Table 1 and Table 2). A schematic representation proposed in Fig. 1 to understand and address the interactions of cations and the mechanism of gelation. As demonstrated, cations lead to conformational changes in the carrageenan with the primary coil to helix transition, which may be accompanied by aggregation of these helices resulting in gelation. As a consequence, the existence of cations or gelling promoters displays a critical role in this formulation Fig. 1 (a,b).

3.2. Selecting convenient gelling promoter

The interaction of gelling promoter and gelling agent displays a substantial effect on the feature of the final pullulan solution in terms of appearance and rheology. Likewise, salts with different capacities can demonstrate different interactions with the gelling agent. Accordingly, monovalent and divalent salts such as NaCl, Na_3PO_4, KCl, CH_3COOK, and $MgSO_4.7H_2O$ were examined. Using of KCl causes the solution to become opaque during preparation. However, the prepared solution does not lead to any substantial problems in the final solution and gives a clear solution. Surprisingly, KCl results in a non-Newtonian characteristic in the final solution that this attribute makes the pinning impossible. NaCl also illustrates non-Newtonian features in the final solution. The drying time highly increases in the presence of NaCl salt. Na_3PO_4 salt causes a considerable increase in the final pH of the solution to 12. CH_3COOK in different concentrations and ratios generates a non-Newtonian solution, which makes the pining infeasible. According to the tests, it was found that the $MgSO_4.7H_2O$ results in a clear solution. Additionally, it does not bring a non-Newtonian solution and illustrates excellent rheology. The existence of surfactants in the formulation of hard capsules and any other formulations is considered one of the crucial ingredients. SLS is often used as a surfactant in the formulation of hard capsules. The presence of SLS in formulation acts as a lubricant and causes the solution to have homogenous rheology. In general, utilizing 0.03 percent of SLS is required for the entire formulation. Nonetheless, using more amounts will increase falling. A schematic image of prepared jointed hard capsules (size 1) has been demonstrated in Fig. 2.

Table 3
Inspection of gelling agent/salt ratio and amount in the pullulan capsule formulation.

Materials (g)	K	L	M	N	O	P	Q
Pullulan	40.0	40.0	40.0	40.0	40.0	40.0	40.0
κ-carrageenan	1.2	1.2	1.2	1.2	1.2	1.2	1.2
$MgSO_4.7H_2O$	0.8	0.6	0.48	0.48	0.34	0.30	0.24
PG	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SLS	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Water	206.0	206.0	206.0	206.0	206.0	206.0	206.0

Table 4
Observations on various gel-to-salt ratios and amounts in formulations.

k-carrageenan/Salt (ratio)		k-carrageenan/Salt (ratio)	
		2.3 2.4	2.5 2.6 2.7 2.8
	0.9X 0.95X X	Dome too high Downfall very little Medium wall	Downfall high Wall very weak
	1+0.7X 1+0.1X	Medium dome Downfall very little	Good dome Downfall low

3.3. *Effect of gelling agent/salt ratio on pullulan formulation*

One of the most crucial features in the design and fabrication of the Pullulan hard capsule formulation is the gelling agent/salt ratio. Accordingly, in the primary tests, different ratios of gelling agent to salt, such as 2.5, 3.75, and 5, were evaluated.

Ratios of 5 and 3.5 with 15% pullulan led to undesirable forms during pinning, being not favorable for industrial production. In other words, this phenomenon can be attributed to the presence of high amount of gelling agent in formulation. κ-carrageenan must reach a temperature of 70 °C for activation, and providing this temperature is one of the requirements of this research survey. According to the experiments, the most appropriate ratio of κ-carrageenan to salt was 2.5, with the optimal value of 1.2 and 0.48. It should be explained that less than these values cause the downfall of solution on the pin or undesirable forms, and more than this value cause the problem of unwanted forms. Moreover, being lower than this value gives a very low viscosity and, as a result, a low capsule wall because it cannot activate and gel all the amount of pullulan biopolymer in the solution. The problem encountered after obtaining this ratio during pinning was the fast closing of the dome and the lack of uniform downfall of the solution from the capsule dome during pinning. Based on designed tests and experiments, the non-falling of solutions from the dome remained a challenge. According to observations and experiences, we decided to change the amount of carrageenan to the salt ratio in different ratios to achieve the desired results. By changing the source of carrageenan, we decided to evaluate the relationship between the ratio and amount of carrageenan through several experiments. Hence, a summary of the observations and the correlation between the experimentally obtained parameters is given in Table 3. Various weight percent of gelling agent/salt, including 1.5, 2, 2.5, 3, 3.5, 4, and 5, have been performed in the tests of K to Q.

To assess and address the appropriate ratio of gelling agent and the salt relationship between k-carrageenan/salt (ratio) and κ-carrageenan/salt (amount), we have performed a series of tests in different ratios, and amounts of κ-carrageenan/salt. As can be observed from Table 4, from a weight ratio of 2.5–2.8 and a higher amount of κ-carrageenan/salt X (1 + 0.7X) and (1 + 0.1X) a reasonable trend can be identified.

Table 5 depicts the association between influential parameters (wall, Puncturing (fast dome loading), and downfall) and effective parameters such as viscosity, temperature, amount, and the ratio of κ-carrageenan to salt. As viscosity increases, puncturing is enhanced, and downfall decreases, which is favorable for producing hard capsules from pullulan. In addition, as the temperature rises,

Table 5
Study of various factors and parameters on the properties of pullulan hard capsules.

Effective parameter	Influential parameter		
	Wall	Puncturing (fast dome loading)	Downfall
Increase in viscosity	↑	↑	↓
Rise in temperature	↓	↓	↑
Increasing the ratio of carrageenan to salt	↓	↓	↑
Increasing the amount of carrageenan and salt	↑	↑	↓

Table 6
The examination of guar gum in the formulation of pullulan hard capsule.

Materials	Pullulan	$MgSO_4.7H_2O$	κ-Carrageenan	SLS	Water	PG	Guar gum	Viscosity (cp)
Amount (g)	40	0.34	1.2	0.21	203	16 (D)	0.2	1260

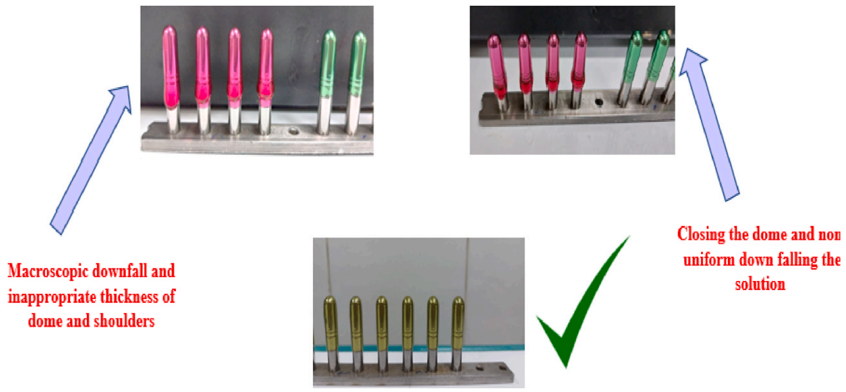

Fig. 3. Images that represent the pinning situation of various pullulan capsule solutions.

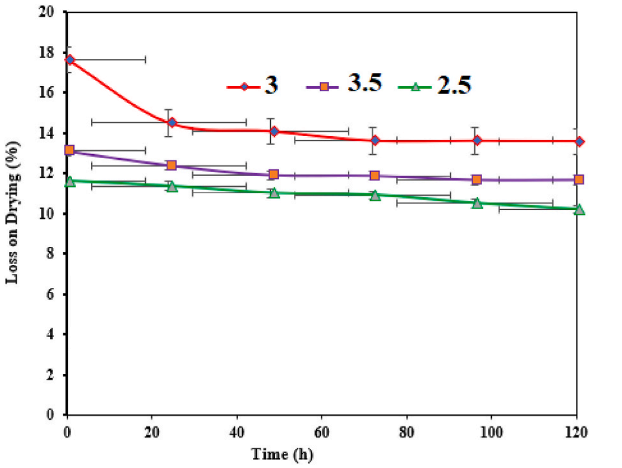

Fig. 4. Loss on drying (%) of prepared pullulan capsules in various intervals including 2.5, 3 and 3.5.

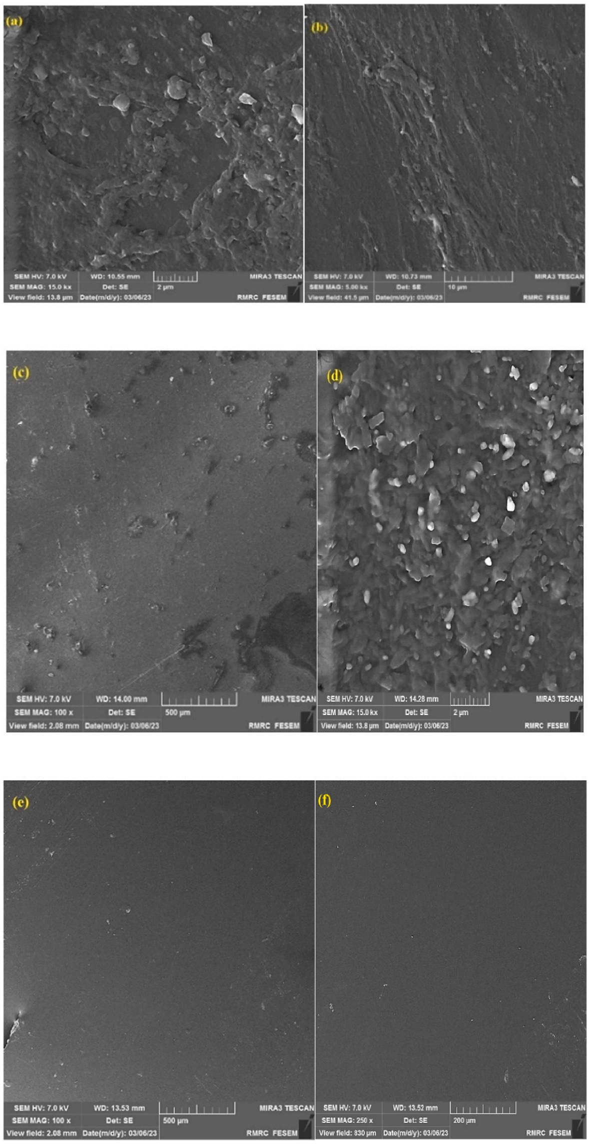

Fig. 5. FESEM images of pullulan at different weight ratio including (a–b) 2.5, (c–d) 3 and (e–f) 3.5.

fast dome loading decrease, which is desirable in practical production. Increasing the ratio and amount of carrageenan to salt leads to reduced puncturing and an enhancement in the wall, both of which are advantageous for pullulan hard capsule production.

3.4. The efficacy of plasticizer, surfactant, and pH on pullulan hard capsule formulation

Employing and examination of a convenient softener or plasticizer agent are considered crucial factor for the formulation of hard capsules. Accordingly, optimal percent and the correct choice of plasticizer in the formulation is an essential task for practical applications. The plasticizers employed in the formulation of pullulan capsules include PG and PEG400. Based on the obtained results, the main influence of plasticizers is on the mechanical properties of the capsules, and changing plasticizer type and percent can somehow affect the degree of capsule downfall. It is worth mentioning that increasing the amount of plasticizer leads to an increase in plasticization and drying of the capsule. Furthermore, to figure out and address the effect of pH on the capsule properties, a series of experiments have been performed on the pH of the pullulan solution in each step. As a result, the appropriate pH for pullulan hard capsule preparation was mentioned between 6 and 7. It is well understood that changing pH to an acidic or basic environment will not be favorable for target applications. As mentioned previously, the uniform downfall was one of the main challenges in capsule formulation. As a consequence, we decided to utilize guar gum in the capsule formulation. The use of guar gum leads to a uniform downfall and achieving an optimal formulation (Table 6). As it illustrated in Fig. 3, a number of phenomena including downfall, inappropriate thickness, shoulders, closing the dome and non-uniform down falling of solution were represented.

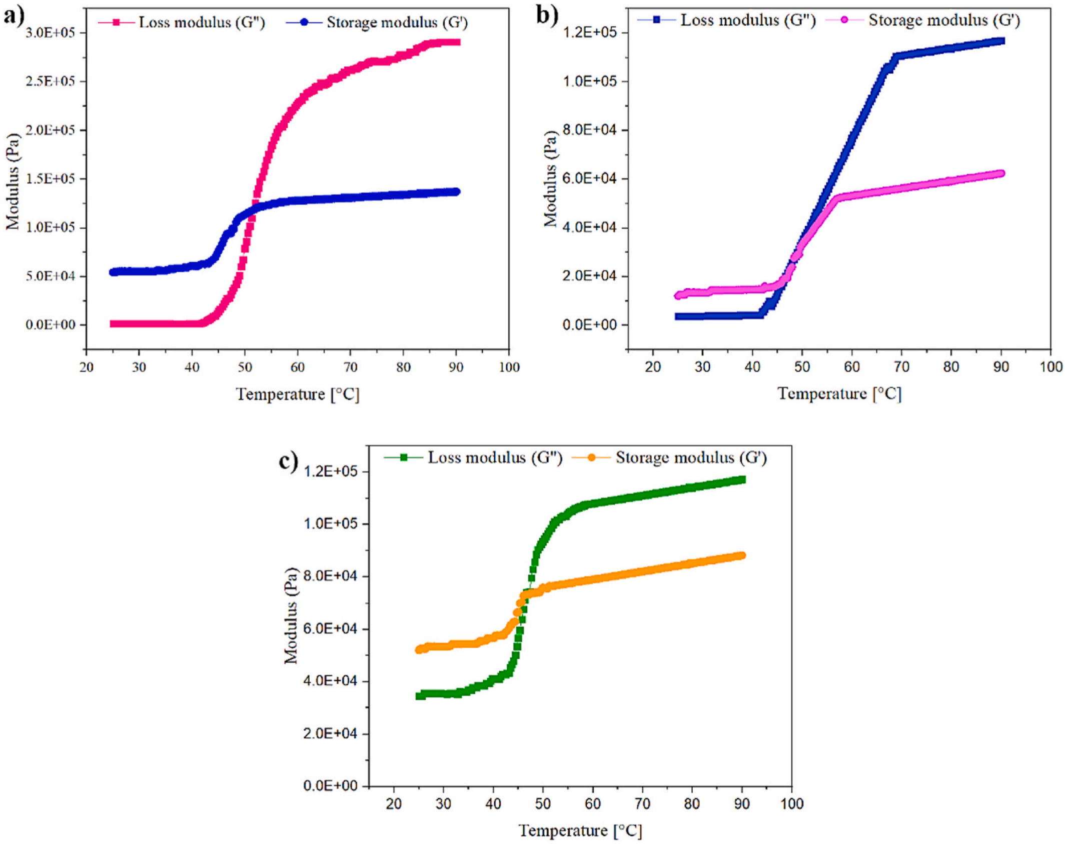

Fig. 6. Temperature sweeps of different gelling agent to salt ratios, including (a) 2.5 (b) 3 and (c) 3.5 for pullulan solutions from 20 to 90 °*C*

3.5. LOD test

Significantly, loss on drying plays a primary role in appraising the physical parameters of capsules. The moisture capacity of a hard capsule should be in an appropriate range for functional applications. In other words, the LOD of a suitable capsule should be in a standard range. For instance, the lower LOD leads to a product with brittle features. Despite considering this key element, the interaction between active pharmaceutical ingredient (API) and capsule is the lowest. Herein, the LOD experiment of 3 different gelling agents to salt ratios such as 2, 3, and 3.5, have been performed. As seen in Fig. 4, the primary moisture content of as-prepared capsules is high. Surprisingly, all graphs illustrate a gradual decrease in the trend of moisture loss. The rate of moisture loss is stable without any remarkable change after 48 h. It should be noted that the conventional hard gelatin capsule displays a moisture content of 13–16%. Moreover, the plant-based HPMC capsules contain a moisture amount of 6–7%. With regard to the practical applications, this characteristic is critical from the initial step. From the moisture content aspect of view, 3.5 fulfills the capsule moisture standards. As a consequence, this weight ratio of gelling agent to salt is convenient for target applications.

3.6. Morphology investigations

The FESEM technique was utilized to realize and address the morphology and surface features of the as-prepared hard pullulan capsules. Hence, the FESEM images of the different prepared pullulan formulations are illustrated in Fig. 5. In more detail, three weight ratios of gelling agent to salt, including 2.5, 3, and 3.5 were chosen for FESEM investigations. As can be observed, the FESEM images of pullulan hard capsules with a weight ratio of 2.5 demonstrate the non-uniform surface properties and morphology. Likewise, the weight ratio of 3 represents an identical feature. In stark contrast, the aforementioned attribute cannot be identified for the weight ratio of 3.5 in the pullulan formulation. Indeed, the surface morphology characteristic is uniform without the existence of any foreign material. As a consequence, due to the appropriate properties of the gelling agent/salt of 3.5, it was chosen as a desirable ratio for further investigations Fig. 5(a–e).

3.7. Rheology study

The rheological test has been performed in detail to perceive the rheological features of prepared pullulan solutions at various

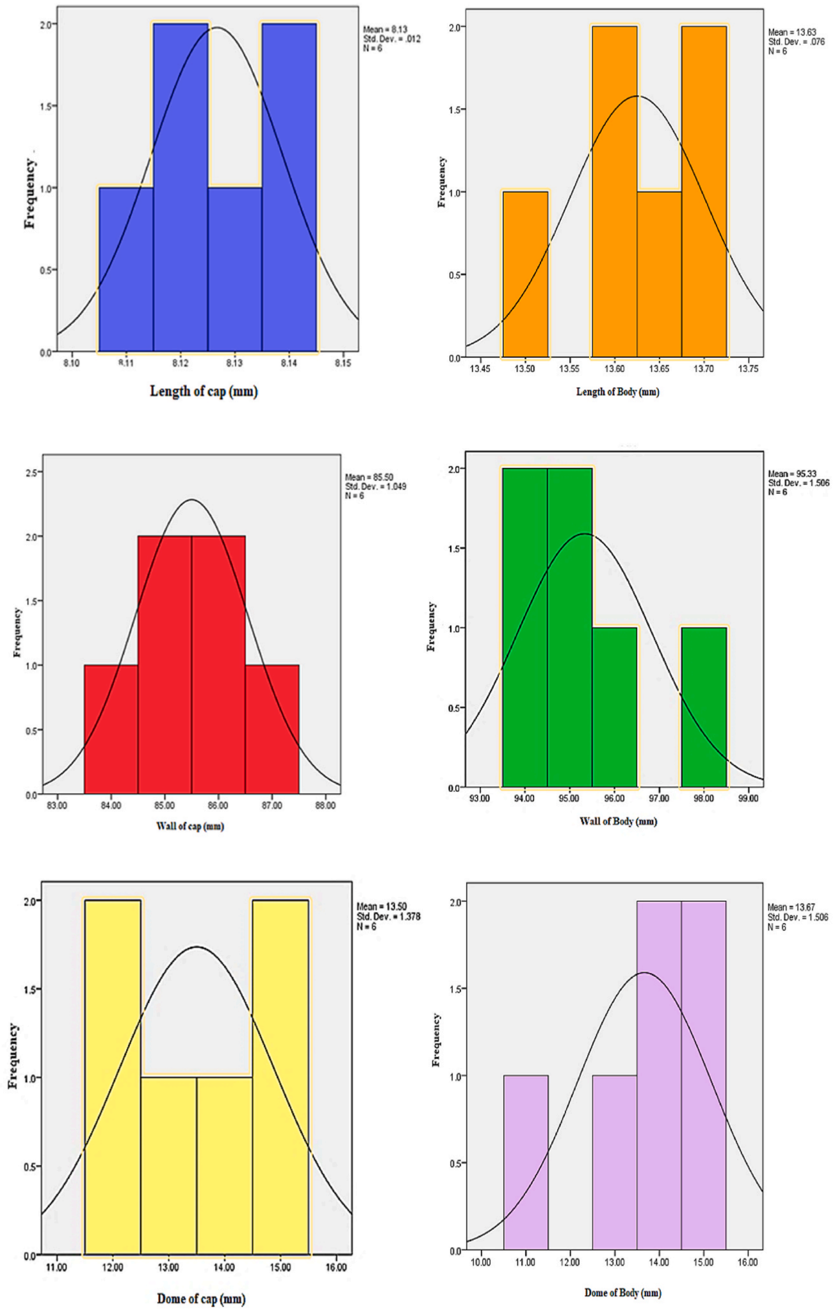

Fig. 7. Statistical evaluation of various parameters of prepared pullulan hard capsule shells (Size 3).

gelling agent to salt ratios. It is well-established that the storage modulus (G′) and loss modulus (G″), which demonstrate the elasticity and viscosity of the material, are very crucial. In the pullulan hard capsule solution, a gelation behavior appears with the enhancement of G'. In more detail, the temperature of liquid transformation to gel is because of the identical value of G' and G". Fig. 6 provides information regarding G′ and G″ as a function of temperature, representing the viscoelastic trend of prepared samples. It is substantial that with the decreasing of temperature, a gelation temperature where G′ equals G″ takes place for three measured solutions. The gelation temperature indicates hard capsules thermosensitivity. In high temperatures, G″ is remarkably greater than G'. According to Fig. 6, the gelation temperatures of examined samples, including gelling agent/salt of 2.5, 3, and 3.5, are 51.2, 49, and 45 °C, respectively. As a consequence, the weight ratio of 3.5 is more favorable for practical use because providing higher temperatures, such as 51.2 °C is not feasible in the production process Fig. 6(a–c).

3.8. Statistical investigations

In order to understand and address the statistical results of examined tests, a serious of quality control (QC) appraisements was carried out. Hence, the length, wall as well as dome for separate cap was performed. As it can be observed in Fig. 7, the statistical measurements for different dimensions have been performed and addressed utilizing the SPSS software results. According to the obtained results all of the obtained data are in well agreement with the standard capsule features without substantial deviations. Regardless of standard dimensions, all of the obtained capsules are in accordance with the GMP conditions. Indeed, these results illustrate a better identification and complementary aspects of the designed pullulan hard capsules [31].

4. Conclusions

An innovative pullulan hard capsule shell was prepared using a facile approach. A detailed investigation of the effect of different preparation conditions on the production of optimum capsules was conducted, including the type of gelling agents, the kind of salts, the amount and ratio of gelling agents to salts, and the pH of the solution. Based on the results, the stretching phenomenon, and non-ideal rheology lead to the unfavorable and inappropriate formulation of hard capsules. It is interesting to note that gelling agent to salt displays a crucial part un the pullulan hard capsule formulation. Indeed, this factor is a major contributing factor to adjusting the pullulan hard capsule physical features. With regard to achieve and address straightforward ratio of this factor, numerous studies at different ratios with various gelling agent and salts were carried out. Consequently, the gelling agent to the salt ratio of 3.5 was chosen as the optimal ratio. It is substantial that this ratio illustrated the superior hard capsule characteristics with respect to industrialization. FESEM results revealed that utilizing the gelling agent to salt ratio of 3.5 manifests favorable characteristics. Besides, rheological tests showed identical outcomes that the weight ratio of 3.5 is a suitable value for further investigations. Regarding the moisture content of prepared hard pullulan capsules, it was found that the prepared samples possess a moisture content of 13.04%. To achieve the desirable hard capsule with respect to industrialization or target usage it is important to consider a wide range of features not a single or individual involving parameter. All in all, the prepared hard pullulan capsules seem to have the convenient potential for practical use.

Data availability statement

The authors do not have permission to share data.

CRediT authorship contribution statement

Ramin Ramezani Kalmer: Funding acquisition, Formal analysis, Data curation, Conceptualization. **Afzal Karimi:** Supervision. **Hamed Ramezanalizadeh:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Data curation. **Mojgan Ghanbari:** Writing – review & editing, Validation. **Dariush Samandarian:** Writing – review & editing, Investigation. **Atefeh Sadjadinia:** Writing – review & editing, Project administration, Investigation. **Samira Gholizadeh dogaheh:** Writing – review & editing, Validation, Supervision. **Seyedehmaryam Moosavi:** Writing – review & editing, Validation, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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