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Gelling behavior of different grades of chitosan: Comparative study

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Abstract
Chitosan is versatile excipient, aid for controlled release and bioadhesive polymer, depending on the route of delivery. It is also use as an absorption enhancer promoting drug uptake across the mucosal barrier. Chitosan are widely used as gelling agent in *in situ* gel preparation. There are various grades of chitosan are available in the market. But all the grades are not suitable for the formulation of the *in situ* gel formulation. Some of the grades are showing good viscosity, some having good mucoadhesive properties. This experiment gives idea about the gelling properties and mucoadhesive properties of the different grades of chitosan. The different grades of chitosan which are used for the study are *viz*. High density chitosan, Low density chitosan, Chitosan from shrimp shell, Chitosan deacetylated (75%), Chitosan deacetylated (90%). The low density chitosan found to be good gelling agent for the formulation of *in situ* gel formulations. It having good viscosity and mucoadhesive property. In conclusion we can said that low density chitosan can serve as a good polymer for the formulation of *in situ* gel preparation.

Keywords: Chitosan, chitin, gelling behavior, gelling agent, *In situ* gel

1. Introduction
Chitin and chitosan (CS) polymers are natural aminopolysaccharides having distinctive structures, multidimensional characteristics and a broad range of applications in pharmaceutical and other industrial areas [1-3]. CS is manufactured by partial deacetylation of naturally insoluble chitin extracted from exoskeletons of crustaceans, fungi and insects (Fig. 1). The application of CS are reduced due to the acetylated groups, rigid framework as well as low aqueous solubility. When chitin is partly deacetylated and transformed to CS, the number of amino groups and its aqueous solubility are increased. There is a proportionate rise in CS deacetylation and an improved performance in case of biocompatibility and biodegradability [4, 5].

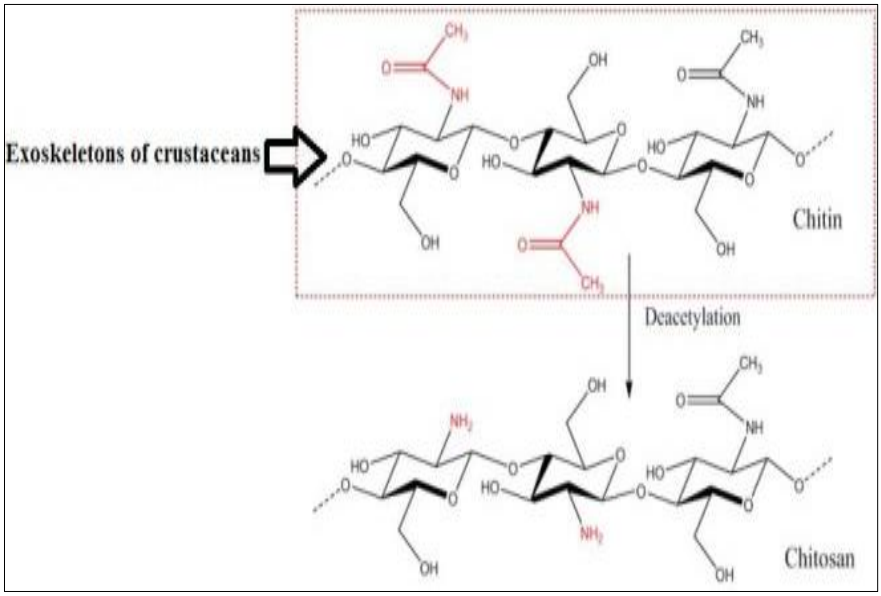

Fig 1: N- Deacetylated chitosan

CS having the excellent futuristic ability with immense possibilities for structural modification to impart the required characteristics and functions, research and development work on chitin and CS have reached the stage of intense operations in many parts of the World. CS having beneficial characteristics like better biocompatibility and biodegradability with ecological safety and low toxicity with flexible biological activities such as antimicrobial activity and poor immunogenicity have offered abundant possibilities for further development [6].

CS is a pH-dependent cationic polymer that is becoming prominent in the biomedical era. CS is a weak base with pka 6.5 that can be dissolved in dilute acetic acid. Due to the presence of amines and hydroxyl groups, CS molecules can create hydrogen bonds that lead to the crystalline structure of the polymer. CS remain in form of liquid up to pH 6.2 once dissolved in acidic medium. Neutralization of CS aqueous solutions with a pH exceeding 6.2 results to hydrated gel precipitate [7, 8].

CS occurs in distinct molecular weights and degrees of acetylation. The molecular weight of CS is found in the range of between 50-2000 KD. Hydrophilic polymers, (CS) may undergo systemic absorption in the human body; therefore the polymer should have defined molecular weight to eliminate it by renal filtration. *In vitro* research have shown that CS can be degraded by a number of enzymes such as β-N-acetyl hexosaminidase, chitosanase, chitinase and chitin deacetylase. CS can be biodegraded by lysozyme, acid, gastrointestinal enzymes and colon bacteria in the human body [9, 10].

The primary parameters affecting the properties of CS are its molecular weight (MW) and its degree of deacetylation (DD) [11]. There are various of sources of CS and the fact that it is commercially accessible with a broad spectrum of DD and MW. In order to optimize the required application, it is essential to take into account the impacts of these parameters on biomedical operation. The molecular weight of CS is probable to be the most significant property, as a minimum molecular weight is often required to attain the desired functions [12, 13].

CS have various application in different fields including pharmaceutical, medical, cosmetics, agricultural and food industries. CS is biocompatible and biodegradable and it is non toxic and non-immunogenic degrading product. CS is bioadhesive and bacteriostatic, acts as chelating agent, hemostatic agent and antioxidant. These polymer is also used to control bleeding via incorporating a procoagulant which helps in clotting. The pharmaceutical applications of chitosan include drug and gene delivery, wound dressing, tissue repair, and tissue engineering [14-20].

There are various grades of chitosan are available in the market. But all the grades are not suitable for the formulation of the *in situ* gel formulation. Some of the grades are showing good viscosity, some having good mucoadhesive properties. This experiment gives idea about the gelling properties and mucoadhesive properties of the different grades of chitosan. The different grades of chitosan which are used for the study are *viz*. High density chitosan, Low density chitosan, Chitosan from shrimp shell, Chitosan deacetylated (75%), Chitosan deacetylated (90%).

2. Material and Methods

2.1 Material

Different grades of CS were purchased from different vender. High and low density CS were purchased from the Marine Chemical (Meron), Cochin. CS from Shrimp Shell was procured from Himedia. CS deacetylated (75%), CS deacetylated (90%) and The ß-glycero phosphate disodium salt hydrate were purchased from Sigma-Aldrich. Preservative methyl paraben, other chemicals and solvents were procured from licensed vendors of pharmaceutical grades.

2.2 Preparation of CS gel

1. High density CS, Low density CS, CS from shrimp shell, CS deacetylated (75%), CS deacetylated (90%) were used for the study, these different grades were dissolved in 1% acetic acid to get 0.5, 1, 1.5, 2, 2.5% concentration.
2. ß-glycero phosphate disodium salt hydrate (ß-GP) was added into the above mixture as buffering agent to adjust the pH of the mixture.
3. Resulting mixture was evaluated for the gelling behavior by using 1% NaOH solution [21-23].

2.3 Physical characterization

Prepared solution comprising distinct grades of CS was noted for its color, odor, clarity and appearance. The transparency was examined by visual inspection on a black and white backdrop.

2.4 Gelling capacity

Different concentrations of CS and β-GP were prepared and evaluated for the gelling capacity in order to prepare *in situ* gel. The gelling ability was determined by putting 100 µl of formulated liquid in a bottle comprising 2 ml of 0.1% NaOH solution and visually evaluating the development of gel and check the time for gelation (24). From the observations we have classified as (+) Gels after few minutes, dissolves rapidly, (++) immediate gelation and remains for few hours, (+++) immediate gelation and remains for extended periods [25].

2.5 pH

The formulation pH was recorded using a pH meter (Mettler Instruments, Germany). Triplicate experiments were conducted [26].

2.6 Spredability

A sample of 0.5 g of each formulation was placed between two slides (separated into 5 mm squares) and left for about 5 min where no further spread was expected. Spread circle diameters were measured in cm and taken for spreadability as comparison values. The findings acquired are average of three determinations [27].

2.7 Viscosity

The viscosity of the formulations has been determined using the programmable Viscometer (Brookfield, RVDV pro II, USA). To determine the viscosity of the solution, 5 ml of the formulation was transmitted to the sample cell which was placed carefully in a small volume sample adapter. The guard leg was put around the adaptor by constantly stirring the sample. The sample viscosity at various rotations per min (RPM) ranging from 0.5 to 100 RPM was evaluated. The motion of the helipad was regulated to prevent the spindle from touching any portion of the sample holder, particularly the bottom. A typical run was involved change in the angular velocity at a controlled speed from 0.5 to 100 RPM after every 10 sec. Viscosity values were observed at each RPM. The test was repeated three times for the same gel sample, and

the average reading was observed [7, 28-31].

2.8 Syringability
The syringeability was determined using two techniques. Qualitative evaluation of syringeability in which the solution was evaluated by passing through a 24-gauge needle to check for ease of administration. The second technique was Syringability assessment using the Brookfield Texture Analyzer [17].

2.9 Mucoadhesive study
A tensile test was used to evaluate the mucoadhesive properties of the various formulations, where the measurement of maximum force, mucoadhesion as well as work of adhesion required to detach the formulations from a mucosal tissue was evaluated. Mucosal tissue was collected from freshly sacrificed animals in the slaughter house and separated from the underlying tissues, washed, cut into smaller parts and thoroughly rinsed. Using the Brookfield texture analyzer fitted with a 5 kg load cell, the mucoadhesive characteristics of formulations were assessed. Cyanoacrylate glue was used to attach sections (> 2 mm in thickness) taken from the inner part of the mucosal membrane surface to the reduced end of the texture analyser sample (10 mm in diameter). The gels have been packed into a holder and kept at 37 °C. The probe holding the mucosa was lowered to the gel surface at a steady velocity of 0.1 mm/s until a contact force of 0.05 N was applied for 2 min in contact with the gel surface. The probe was then shifted vertically up at a constant velocity of 0.1 mm/s and the resulting force distance graph determined the maximum detachment force (F) and the area under the curve (AUC). The work of mucoadhesion (Work, mJ cm^{-2}) was calculated from the following equation:

$$\text{Work} = \frac{\text{AUC}}{\pi r^2}$$

where, πr^2 = the gel-contacted mucosal surface.
All analysis have been repeated at least three times [32-35].

3. Results and Discussion
3.1 Appearance
The appearance of the gels has been examined for transparency. The transparency of the formulations was assessed by visual inspection under the black and white background. The batches were found clear against both black and white backgrounds.

3.2 Gelling capacity
Gelling capability of the formulated solution was assessed for gelling behavior using 1% NaOH solution. Different CS demonstrates varying gelling behavior at distinct concentrations. (Table 1) demonstrates the gelling capacity of the different grades of CS.

Table 1: Gelling capacity of different grades of CS

Batches (diff. Conc. of CS)	0.5%	1%	1.5%	2%	2.5%
High Density CS	-	-	-	-	+
Low Density CS	-	-	+	++	++
CS From Shrimp Shell	-	-	+	++	Very thick solution
CS deacetylated (75%)	-	-	-	+	Very thick solution
CS deacetylated (90%)	-	-	-	+	Very thick solution

*+ good, ++better

3.3 pH
pH of all the formulation were tested with the use of digital pH meter. The pH of the formulations was in the range of 6.3-6.8.

3.4 Spreadability
The spreadability is the distance travelled by the formulations before the transition to gel. The Spredability of formulation was found in the range of 14-20 mm.

3.5 Viscosity
Viscosity was measured at 100 rpm and room temperature using spindle number 3. The viscosity of all the batches was shown in (Table 2). The viscosity of the *in situ* gel increased as there is increase in the concentration of CS. The viscosity of the formulation directly influenced the Syringability.

Table 2: Viscosity of Formulation

Batches (diff. Conc. of CS)	Viscosity (CPS)				
	0.5%	1%	1.5%	2%	2.5%
High Density CS	1100	1250	1275	1330	1400
Low Density CS	1200	1360	1450	1600	1800
CS From Shrimp Shell	1300	1500	1650	1790	2000
CS deacetylated (75%)	1360	1400	1500	1700	2050
CS deacetylated (90%)	1450	1500	1620	1750	2090

3.6 Syringability
Syringability was measured by passing through a 24-gauge needle to test the ease of administration. The 2% Low density CS was shown good Syringability properties than other grades of CS.

3.7 Mucoadhesive Properties
Some of the optimized batches were selected for the evaluation for mucoadhesive property based on the above parameters. Fig. 2 indicates that the CS from shrimp shell shows the better mucoadhesive properties than the other formulation. Low density Chitosan are also having good mucoadhesive property. Mucoadhesive property of the different formulation were found in range of 10.5-18.3 (Fig. 2).

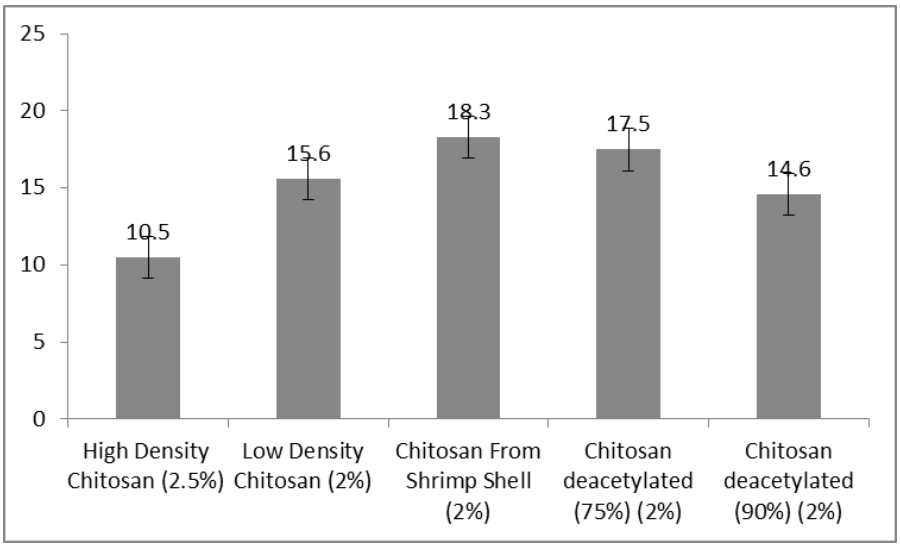

Fig 2: Mucoadhesive properties of formulation

4. Discussion

All the batches of in situ gel of chitosan are clear and transparent they do not show any precipitation. Therefore the gel is stable and not show any precipitate. CS is a pH-dependent cationic polymer therefore liquid form of gel is convert into the gel form when it expose it to alkaline pH. All formulation lie in pH range which is near to neutral i.e.6.3-6.8. At particular concentration the CS convert into gel when it expose to alkaline medium. Low density CS (2%) and shrimp shell CS (2%) have excellent gelling properties compared to other CS.

The spreadability plays a significant role in accordance with patients and helps to uniform application of gel to the skin. High spreadability of gel requires less time to spread and it will easily spread on mucosal membrane. The rise in the concentration of polymer i.e. CS reduced the spreadability. Higher concentration having low spredability than the lower concentration of CS.

The viscosity of the formulation directly influenced the Syringability. Lower the viscosity, higher the syringability.

Quantification of mucoadhesion is essential in order to guarantee that the adhesion provided by formulations is adequate to assure prolonged retention at the application site, but not excessively, because may result in harm to the mucous membrane. Mucoadhesion study gives the thorough assessment of the detachment phenomenon of various formulations under examination. Though the Chitosan from shrimp shell shows highest mucoadhesive property but it do not have good spredability as well as syringability.

5. Conclusion

CS is versatile excipient, aid for controlled release and bioadhesive polymer, depending on the route of delivery. It is also use as an absorption enhancer promoting drug uptake across the mucosal barrier. There are various grades of CS are available in the market. But all the grades are not suitable for the formulation of the *in situ* gel formulation. Some of the grades are showing good viscosity, some having good mucoadhesive properties.

This experiment gives idea about the gelling properties and mucoadhesive properties of the different grades of CS. At 2% of concentration CS showed good gelling behavior in the alkaline medium. From the various evaluation parameter the low density CS and high density CS showed the good syringability as well as spreadability. The CS from shrimp shell showed the good mucoadhesive property but it form very thick solution so it did not pass the syringability test.

The low density CS (2%) showed the good gelling behavior, spreadability. It also passes the syringability parameter. Though it having low mucoadhesive property than the CS from shrimp shell but it showed consistently good result than other grades of CS. Therefore low density CS can be used as a gelling agent for the formulation of *in situ* gel formulations.

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