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In-situ gelling characteristics of Gellan gum at various simulated independent physiological conditions

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ABSTRACT

Gellan gum forms strong and clear gels at physiological ion concentration and widely investigated for use as an in-situ gelling agent in various routes of administration viz. nasal, ocular and oral etc. In situ gelling properties of gellan gum are attributed to ionic contents, temperature, pH, non- electrolytes etc. These physiological variables are different for different routes of administration. Therefore it was interesting to observe the gelling performance in vitro at such physiological conditions. The independent variables affect viscosity in the order as ionic strength> pH> non-electrolytes. All the batches showed instantaneous gelation and gel melting temperature >37°C. The effect on gel melting temperature was in the order as, ionic strength> pH> non- electrolytes. The effect on swelling factor of gellan gum in the presence of independent variables was in the order as ionic strength> pH> non-electrolytes. Mucoadhesive strength is neither dependent on concentration of electrolytes nor on concentration of non-electrolytes. But, the order of variables affecting mucoadhesive strength was pH> ionic strength≈ non- electrolytes. Ionic strength was found the variable dominantly affecting viscosity, gel melting, swelling and mucoadhesive strength.

Keywords: Ionic strength, Viscosity, Gel melting temperature, Swelling

INTRODUCTION

In-situ gel is the drug delivery system that is in sol form before administration in the body, however once administered, it undergo gelation at the site to form gel [1]. Polymer gels are produced by two ways, either by the formation of chemical cross linking or by physical cross linking [2].

Now days with increasing global environmental problems, many efforts have been carried out to develop the effective use of traditional natural polymers because these polymers are biodegradable and bio-absorbable. The biocompatibility of hydrocolloids is well known in use of drug delivery system. Therefore the medical applications are rapidly increasing. Many microbial polysaccharides are growing for commercial use and as they are produced from microbes, their production is on a large scale by industrial fermentation. It is possible to prepare microbial polysaccharides with consistent quality as per requirements. Therefore there are no issues of variability. Gellan gum, likewise, is one of widely used fermentation materials. It can form a transparent gel in the presence of multivalent cations, which is resistant to heat and acid.

This research paper focuses on the in-situ gelling properties of gellan gum which undergo gelation in presence of mono, di and polyvalent cations. Gellan gum is available as Gelrite™ or Kelcogel™ in market. It is an anionic deacetylated exocellular polysaccharide obtained by secretions of Pseudomonas elodea [3] with at extra saccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucuronic acid residues [4,5]. Chemical structure of the polysaccharide possess a tetrasaccharide repeat unit consisting of two glucose (Glc) residues, one glucuronic acid (GlcA) residue, and one rhamnose (Rha) residue [6,7]. These are linked together to give a tetrasaccharide repeat unit. Use of gellan gum in drug delivery is widely reported e.g. for oral delivery of theophylline, in situ gelling gellan formulation as vehicle is reported. Gellan gum solution containing calcium chloride (as a source of Ca^{2+}) and sodium citrate, which forms complex with the free Ca^{2+} ions and releases them only in the acidic environment of the stomach. In this way, the formulation remains in liquid form until it reaches the stomach where gelation of gellan gum is instantaneous [8]. Gellan gum is a multi-functional gelling agent can be used alone or in combination with other products to produce a wide variety of interesting textures. Gellan gum acts as a thickening or gelling agent and can produce textures in the final product that vary from hard, non-elastic, brittle gels to fluid gels [9].

EXPERIMENTAL SECTION

Materials used

Deacetylated gellan gum used in this study was gift sample from C P KELCO, Mumbai, India. All salts viz sodium chloride (NaCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), aluminium trichloride ($AlCl_3$) etc used in this study were of analytical grade and were purchased from Loba chemicals, Mumbai, India.

Methods applied

FTIR

The compatibility study was carried out by using FTIR (Jasco M4100). Before carrying out FTIR study, the polymer samples were kept for one month at room temperature for complete interaction. After one month the FTIR study was carried out and scans were obtained at resolution of 2 cm^{-1} from 4000 to 400 cm^{-1}. The polymer samples were dried in hot air oven at 60 ^{0}C for 1 h for removal of moisture. Then dried potassium bromide was placed in sample holder of FTIR and blank sample was run. Then mixtures were prepared in potassium bromide 1:5 w/w ratios of polymer samples. The spectral peaks were observed for any change in peak intensity and wave-number and it was compared with the pure gellan gum (deacetylated) sample [10].

Preparation of gellan gum gel to study the effect of electrolytes

Gellan gum (deacetylated) gel was prepared in presence of different electrolytes vise sodium chloride (monovalent), calcium chloride and magnesium chloride (divalent) and aluminium trichloride (trivalent). To prepare gel, gellan gum powder 1g was dissolved in 100 ml distilled water using magnetic stirrer at 100 rpm. Then six such solutions were prepared in different beakers. In these beakers 0.5 %, 1%, 1.5%, 2%, 2.5%, 3% w/v of respective electrolyte solution (sodium chloride, calcium chloride, magnesium chloride or aluminium trichloride solution) was added. They were stored for 24 h for proper swelling at cool place.

Preparation of gellan gum gel to study the effect of pH

Gellan gum (deacetylated) powder 1g was dissolved in 100 ml distilled water using magnetic stirrer at 100 rpm. Then six different solutions were prepared having pH 2, 4, 6, 7, 8 and 10 in respective beakers and the pH was adjusted by using dilute hydrochloric acid or dilute sodium hydroxide. They were kept for 24 h for proper swelling at cool place.

Preparation of gellan gum gel to study the effect of non- electrolyte

Gellan gum (deacetylated) powder 1g was dissolved in 100 ml distilled water using magnetic stirrer at 100 rpm. Then six such solutions were prepared in different beakers. In these beakers 0.5 %, 1%, 1.5%, 2%, 2.5%, 3% w/v of non-electrolyte (sucrose) solution were added respectively. All containers were stored for 24h for proper swelling at cool place.

Evaluation of in-situ gel: independent effect of physiological variables

Viscosity of formulation

The viscosity measurements were carried out by using Brookfield programmable RVDV-II+ pro model (Brookfield Eng. Lab., Inc.USA). The gel sample was placed in small sample adapter. Temperature was kept 37^{0}C. Viscosity was recorded [11]. Viscosity in centipoises can be calculated by multiplying dial reading into dial factor. All the results were taken in triplicate.

Gel melting temperature

Gel melting temperature of aqueous solution of gellan gum was measured by using procedures reported by Choi et al. [12]. A 1ml volume of gel was transferred to 10 ml transparent vial placed on a magnetic stirrer. The vial was heated at an increasing rate of 1^{0}C/min with constant stirring at 100 rpm. Increase in temperature at which gel liquefy is gel melting temperature. All the results were taken in triplicate.

Swelling factor

Gels of gellan gum dispersions were prepared as per the procedure described above. The samples were then centrifuged for 15 minutes at 2200 rpm by using microcentrifuge. The weight of the sedimented fraction was determined. The swelling factor was calculated as the ratio of the weight of the swollen polymer granules to the weight of the dry polymer. All the results were taken in triplicate.

Mucoadhesive strength

The mucoadhesive force, the detachment stress of the formulation was determined using a modification of the mucoadhesive force measuring device used by Choi et al. A section was cut from the nasal mucosa of sheep and instantly secured with the mucosal side out into each glass vial. The vials were stored at 37±0.5°C for 10 min. One vial connected to the balance and the other fixed with the gellan gum gel (0.5ml) added and the height adjusted so that the gel is placed between the mucosal sides of both vials. Water from a burette was allowed to fall in a beaker drop by drop till the detachment of vials. Increasing weight of water added gradually would detach the two vials [13]. Mucoadhesive force (dyne/cm²) was determined from the minimal weights of water that detached the two vials. All the results were taken in triplicate.

Mucoadhesive Strength (dynes/cm ^{2}) = mg/A

Where, m = weight required for detachment in g, g= acceleration due to gravity (980cm/s^2) and A = area of mucosa exposed (cm$^{2)}$

RESULTS AND DISCUSSION

FTIR

The compatibility study was carried out by FTIR. The sample prepared in 1%w/v proportion of polymer in water and dried at room temperature, kept for one month for complete interaction. The peaks corresponds to the functional groups of pure gellan gum (Figure 1) were compared with gel of gellan gum (Figure 2). There were no any significant changes observed in spectra of polymer. The disappearance of C-O group of carboxylic acid from that of FTIR spectra of gel sample confirms cross linking of cation in gellan gum structure.

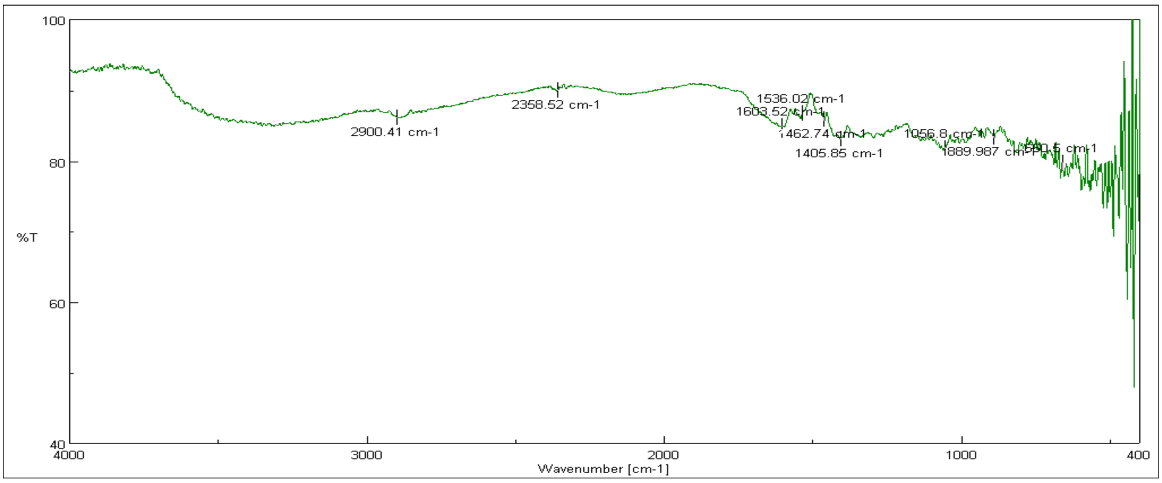

Figure 1: FTIR spectra of gellan gum dry powder

Selection of working range of physiological variables

In this study, electrolytes and non-electrolytes concentration ranged from 0.5- 3% w/v, as the different body fluids contain concentration ranging from 0.5-3% w/v of electrolytes, e.g. nasal fluid contains 0.745% w/v of sodium chloride and ocular fluid contains 0.65% w/v of non- electrolyte [14]. The pH range used was 1-8 to cover the pH range of gastric fluid (1-3) and ocular fluid (7.4). To correlate this study with the physiological conditions, these ranges had been selected.

Evaluation of in-situ gel: Independent effect of physiological variables

VISCOSITY MEASUREMENT

Effect of electrolytes on viscosity measurement of gellan gum

Sodium chloride is monovalent cation containing salt available in the biological fluids so it was selected for this study. Viscosity values were obtained at Spindle no.7 at 20 rpm. Up to certain point, the viscosity increased and then sharp decrease was observed. It may be due to electrostatic repulsion between carboxyl groups present in gellan gum is shielded by increasing concentration of cations and as a result, helix formation and aggregation of molecules get promoted. However in coil formation of polymers, the electrostatic shield leads to reduction of coil dimensions, so there is decrease in viscosity [15]. Calcium chloride is a salt containing divalent cation. The gels prepared in presence of calcium ion were the stronger gel than gel produced in presence of sodium ions. As the concentration of calcium chloride increased, the gel became more uneven and brittle. Viscosity was measured by using spindle no.4 at 30 rpm. The viscosity was dependent on ionic content of formulation. As the concentration of calcium chloride increased, the viscosity of solution also increased. Magnesium chloride is a salt containing divalent cation, so selected for the study. The gels prepared in presence of magnesium ion were the stronger gel than gel produced in presence of sodium ions. As the concentration of magnesium chloride increased, the gel became more uneven. Viscosity values obtained for all batches using Brookfield viscometer at spindle no. 4 at 30 rpm. The viscosity directly depends on ionic content of formulation. As the concentration of magnesium chloride increased the viscosity of solution also increased. It could form more brittle gels than the monovalent cations.

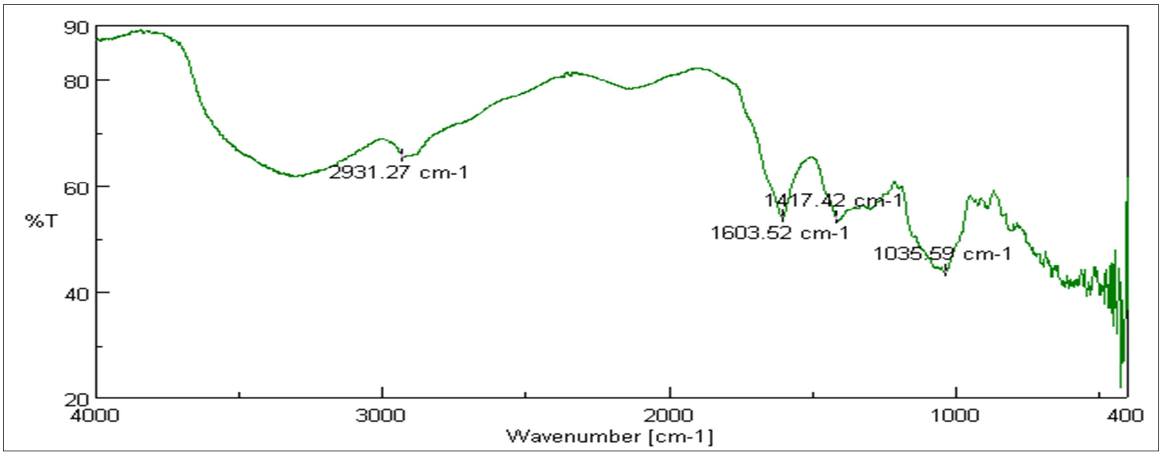

Figure 2: FTIR spectra of gellan gum gel

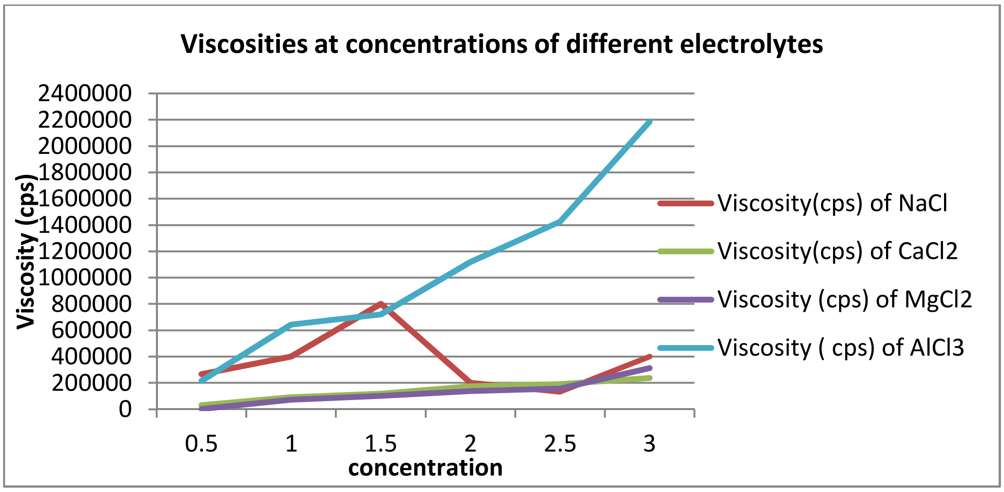

Figure 3: Viscosities in presence of monovalent, divalent and trivalent cations

The effect of monovalent and divalent cations had shown different behavior on the gelling properties of gellan gum, these studies further led to observe the effect of trivalent cation on viscosity of gellan gum. Viscosity values were obtained for all batches by using spindle no 5 at 30 rpm. The viscosity directly depends on ionic content of formulation. As the concentration of aluminium trichloride increased the viscosity of solution was also increased. The heat was generated when aluminium trichloride reacted with gellan gum dispersions. Figure 3 shows that viscosity of gel prepared in presence of trivalent cation is more than gels prepared in presence of monovalent and divalent cations. The gels prepared in presence of magnesium and calcium ions were having almost same viscosity, but viscosity of gel prepared in presence of monovalent sodium was fluctuating.

Effect of pH on viscosity of gellan gum
The viscosity values obtained for all batches using Brookfield viscometer (Spindle no.7 Speed 30 rpm). In the acidic environment the viscosity of the solutions was increased as the pH increased, but when it enters in to the neutral and basic environment, the viscosity remains constant (Figure 4). In presence of acidic pH, the hydrogen might be acting as gel activator; such gels are called as acidic gels [16].

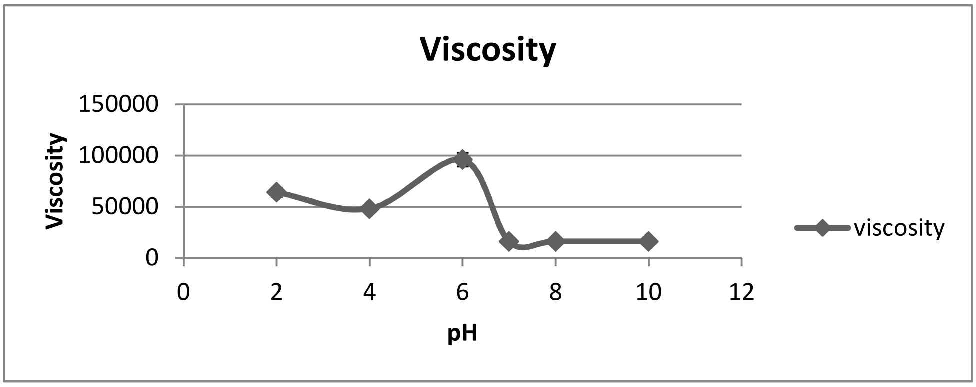

Figure 4: Viscosity at different pH

Effect of non- electrolyte (sucrose) on viscosity of gellan gum
The viscosity values were obtained for all batches using Brookfield viscometer spindle T-D at 10 rpm. As the concentration of sucrose increased the viscosity of solution showed random results as shown in figure 5. It shows that gellan gum is not dependent on non- electrolyte concentration for the gelling ability, but in presence of sucrose, it forms an elastic gel. The gels prepared in presence of non- electrolytes found to be homogeneous immediately after its preparation but in case of gels prepared in presence of electrolytes were not homogeneous and needed 24h after preparation to become homogeneous.

From viscosity studies, it was observed that viscosity is mainly affected in the order of ionic strength> pH> non-electrolytes.

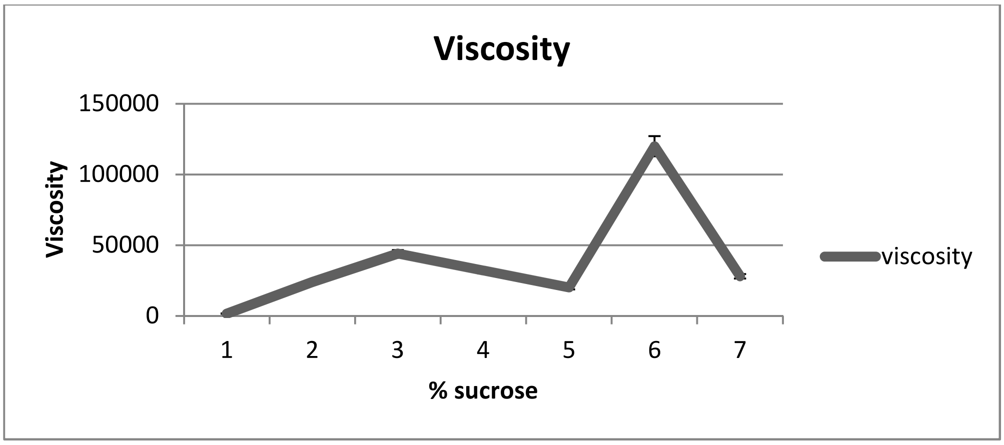

Figure 5: Viscosity (cps) in presence of different concentrations of sucrose

GEL MELTING TEMPERATURE
Gel melting temperature is the temperature at which gel liquefies. This is one of the important physicochemical parameter to determine the possibility to be available in the semisolid form at the site of application. This is useful to determine the drug release from gel matrices. If the gel melts at body temperature, it may drain out which may result in less contact time with mucosal membranes and probably sustained delivery of the drugs will not be possible resulting in less bioavailability.

Effect of electrolytes on gel melting temperature

As the concentration of sodium chloride increases, the gel melting temperature also increases. The observations have shown that the gel formed in the presence of sodium chloride does not melt at body temperatures.

Calcium chloride shows the steady increase pattern. As the concentration of calcium chloride increases, the gel melting temperature also increases. Observations have shown that the gel formed in presence of calcium chloride does not melt at body temperatures. As the concentration of magnesium chloride increases, the gel melting temperature also increases. The observations have shown that the gel formed in presence of magnesium chloride does not melt at body temperatures.

As the concentration of aluminium trichloride increases, the gel melting temperature also increases. The observations have shown that the gel formed in presence of aluminium trichloride does not melt at body temperature. Strong gels are formed due to trivalent cations so the melting point is much higher.

Figure 6 shows that gel melting temperature of gel prepared in presence of trivalent cation is higher than gels prepared in presence of monovalent and divalent cations. The gels prepared in presence of magnesium and calcium ions were having almost same gel melting temperature, but gel melting temperature of gel prepared in presence of monovalent sodium was less in comparison to divalent and trivalent cations. At concentration of 3%, gel prepared in presence of aluminium has much higher gel melting temperature (73^0C) than gels prepared in presence of rest of cations.

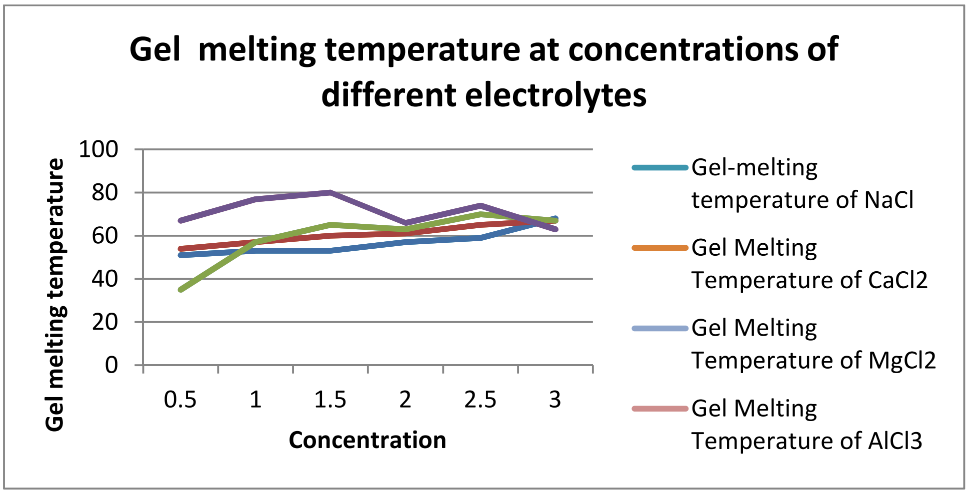

Figure 6: Gel melting temperature in presence of monovalent, divalent and trivalent ions

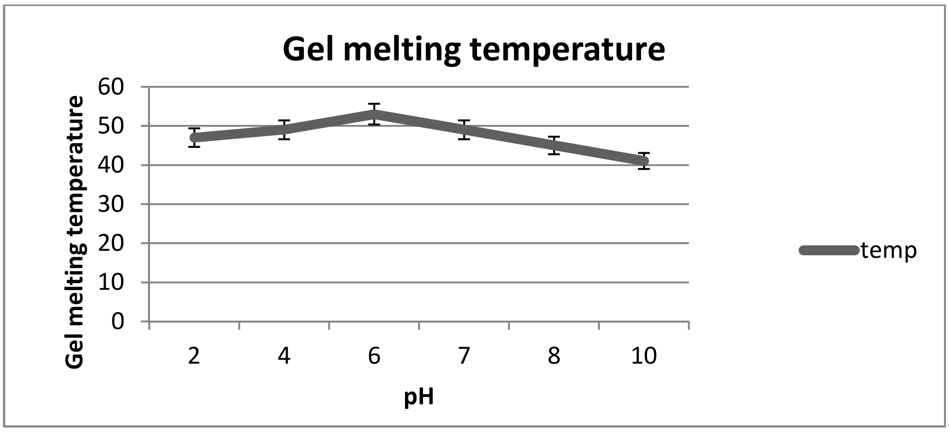

Figure 7: Gel melting temperature in presence of different pH

Effect of pH on gel melting temperature of gellan gum

As the pH increases, the gel melting temperature also increased upto pH 6 but in basic pH it was not depend on increase in pH. From the observations, it is shown that the gel formed in presence of pH will not melt at body temperatures. In acidic pH, from pH 2 to 6, gradual increase in gel melting temperature was observed (Figure 7) however; such a correlation was not observed in presence of basic pH [16].

Effect of sucrose on gel melting temperature

The gel melting temperature at different concentrations of sucrose was obtained. As the concentrations of sucrose increased, the gel melting temperature did not reflect in sharp rise. But all the values of gel melting temperature lies within 45^0-55^0C (Figure 8). The observations have shown that the gel formed in presence of sucrose does not melt at body temperature. As, sucrose has no any free ion participating in gel formation process, so, there was no any significant relation between concentration of sucrose with gel melting temperature.

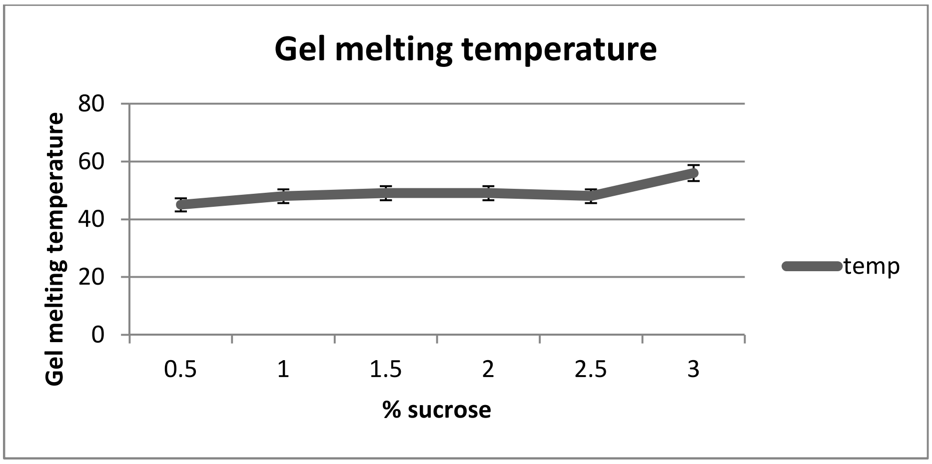

Figure 8: Gel melting temperature in presence of different concentration of sucrose

From the above mentioned results of the gel melting temperature, it has been shown that gel melting temperature of gel prepared in presence of trivalent cation is higher than gels prepared in presence of monovalent and divalent cations. The variables affect gel melting temperature in the order as, ionic strength> pH > non- electrolytes.

SWELLING FACTOR

The swelling index or swelling factor is the volume in ml taken up by the swelling of 1 g of polymer under specified conditions. The porosity of polymeric specimens is a dominant factor that controls their swelling behavior. Increased porosity leads to fast initial rates of weight uptake and high extent of equilibrium swelling. On the other hand, dissolution may result in some variations from the above-mentioned behavior. With respect to the application, the overall delivery rate from a polymeric specimen is expected to be a function of swelling. It is necessary to study the effect of increasing concentration of different physiological variables on swelling properties of gellan gum [17].

Effect of electrolytes on swelling factor

Swelling factor obtained from different concentrations of sodium chloride was not found directly proportional to the increasing concentrations of sodium chloride. It may be due to electrostatic repulsion between carboxyl groups present in gellan gum is shielded by increasing concentration of cations and as a result, helix formation and aggregation of molecules get promoted. So swelling factor is increased. However in coil formation of polymers, the electrostatic shield leads to reduction of coil dimensions, so there is decrease in swelling factor [15].

Calcium chloride shows the steady increase pattern in swelling factor. It may be due to gellan gum forms strong gel in presence of divalent cations, so it forms complex structural bonds with the water of hydration, so the swelling is increased as the concentration of divalent cations is increased. Swelling factor obtained from different concentrations of magnesium chloride was not directly proportional to the increasing concentrations of magnesium chloride. Swelling factor obtained from different concentrations of aluminium trichloride was not directly proportional to the increasing concentrations of aluminium trichloride. It has shown that gel formed in presence of small amount (0.5%) of aluminium trichloride had more swelling ability than rest of the solutions which tend to ooze water quickly.

Figure 9 shows that swelling factor of gel prepared in presence of trivalent cations was less than swelling factor of gels prepared in presence of monovalent and divalent cations. The gels prepared in presence of magnesium and calcium ions had not similar swelling factors, probably due to formation of brittle gel by magnesium ions in comparison with gel prepared in presence of calcium ions. The former may have less swelling ability than latter. Moreover, swelling factor of gel prepared in presence of monovalent sodium ions was less in comparison to divalent cations. At concentration of 3%, gel prepared in presence of calcium has shown much higher swelling factor than gel prepared in presence of rest of the cations.

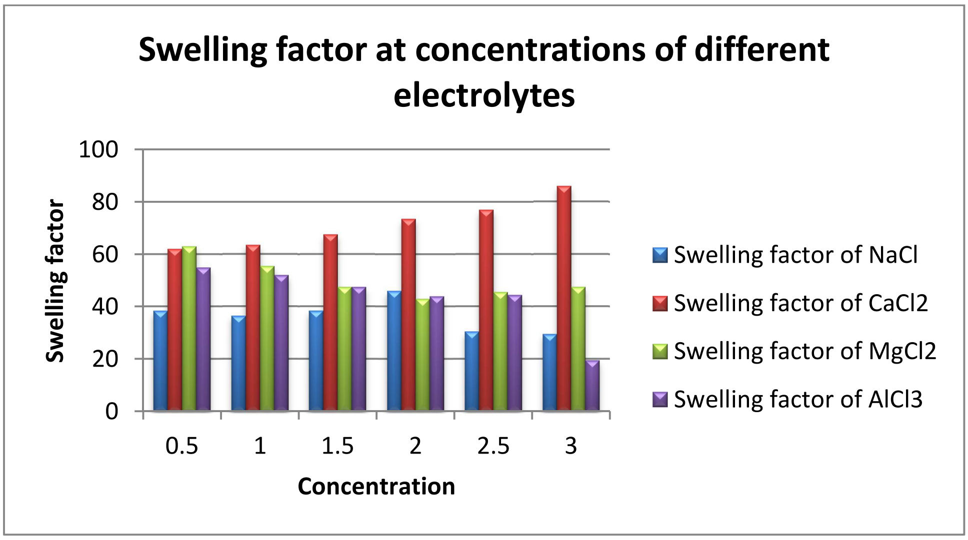

Figure 9: Swelling factor in presence of monovalent, divalent and trivalent cations

Effect of pH on swelling factor of gellan gum

Swelling factor obtained from different pH has not shown proportionality with the increasing pH. It shows that pH has not significant effect on swelling factor. Swelling factor shows the steady increase in the acidic environment but as it enters into the basic pH the graph didn't show any significant relation with increasing pH (Figure 10).

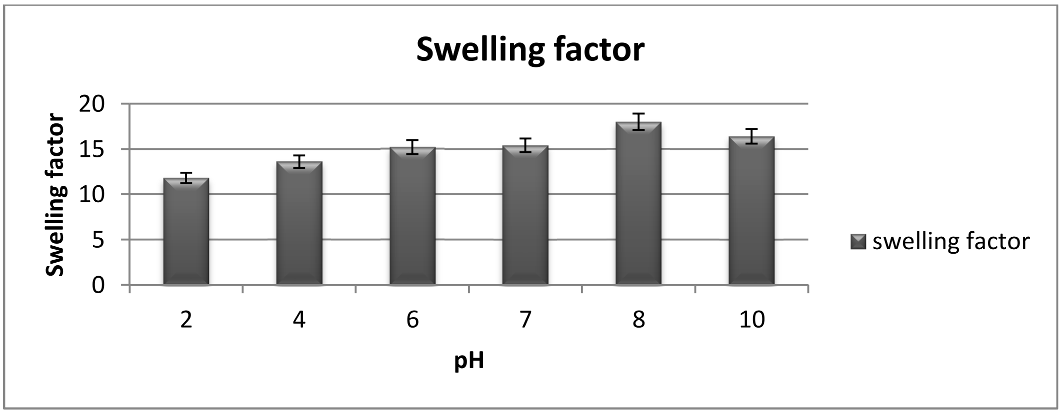

Figure 10: Swelling factor in presence of different pH

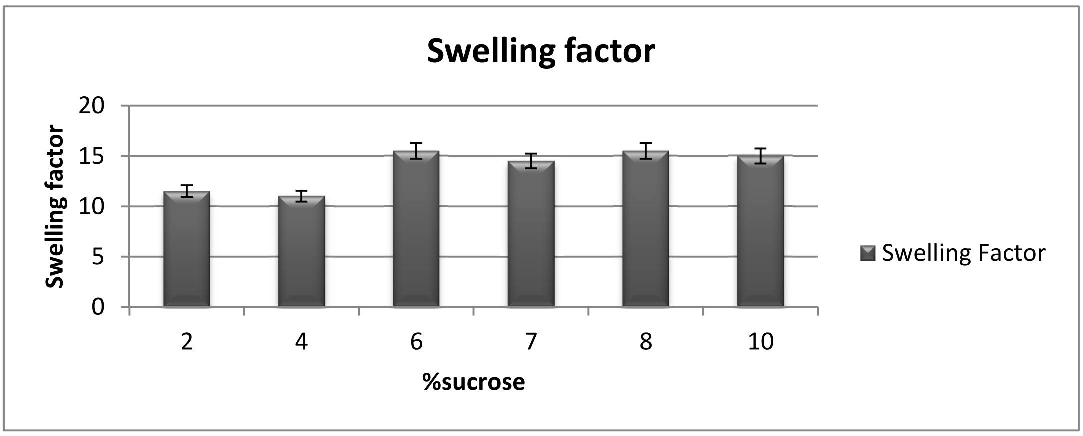

Figure 11: Swelling factor in presence of different concentration of sucrose

Effect of sucrose on swelling factor of gellan gum

Swelling factor obtained from different concentrations of sucrose does not found directly proportional to the increasing concentrations of sucrose as shown in Figure 11 and Table 1. It shows that increasing concentrations of sucrose have not any significant effect on the swelling factor.

Results obtained from the swelling factors showed that swelling factors were in the order of ionic strength> pH> non- electrolytes.

Table 1: Swelling factors in presence of different concentrations of electrolytes

Sr. No.	Conc. of electrolytes % w/v	Ionic strength (mg/ml)	Swelling Factor ±S.D. of NaCl	Swelling Factor ±S.D. of $CaCl_2$	Swelling Factor ±S.D. of $MgCl_2$	Swelling Factor ±S.D. of $AlCl_3$
1.	0.5	5	38.5±0.20	62±0.11	63±0.13	55±0.1
2.	1	10	36.5±0.22	63.5±0.16	55.5±0.19	52±0.21
3.	1.5	15	38.5±0.20	67.5±0.14	47.5±0.04	47.5±0.24
4.	2	20	46±0.15	73.5±0.15	43±0.24	44±0.22
5.	2.5	25	30.5±0.13	77±0.21	45.5±0.18	44.5±0.13
6.	3	30	29.5±0.19	86±0.24	47.5±0.16	19.5±0.18

Mucoadhesive strength

Adhesion of bioadhesive drug delivery devices to the mucosal tissue offers the possibility of creating an intimate and prolonged contact at the site of administration. This prolonged residence time can result in enhanced absorption and in combination with a controlled release of drug also improved patient compliance by reducing the frequency of administration [18].

Effect of electrolytes on mucoadhesive strength

The mucoadhesive force is important physicochemical parameter for prolonging retention time, assessment of mucoadhesive strength in terms of detachment stress showed that gel formed with increasing concentrations of sodium chloride possess good adhesion properties shown in Table 2. However, no relation between ionic strength and mucoadhesive strength was found. As the ionic strength contributed by calcium chloride increases; there is gradual increment in the mucoadhesive strength. Gel formed with increasing concentrations of calcium chloride.

Table 2: Mucoadhesive strength in presence different concentrations of electrolytes

Sr. no.	Conc. of electrolytes % w/v	Ionic Strength (mg/ml)	Mucoadhesive strength (dyne/cm^2) ±S.D. of NaCl	Mucoadhesive strength (dyne/cm^2) ±S.D. of $CaCl_2$	Mucoadhesive strength (dyne/cm^2) ±S.D. of $MgCl_2$	Mucoadhesive strength (dyne/cm^2) ±S.D. of $AlCl_3$
1	0.5	5	1295±0.24	2487±0.31	2260±0.24	1487±0.26
2	1	10	1335±0.36	2651±0.29	2351±0.26	1651±0.22
3	1.5	15	3901±0.28	2980±0.18	2420±0.18	1280±0.29
4	2	20	1989±0.17	3035±0.16	1780±0.14	1327±0.18
5	2.5	25	1335±0.25	3100±0.14	2100±0.16	1490±0.17
6	3	30	1785±0.05	3478±0.02	2478±0.06	1652±0.21

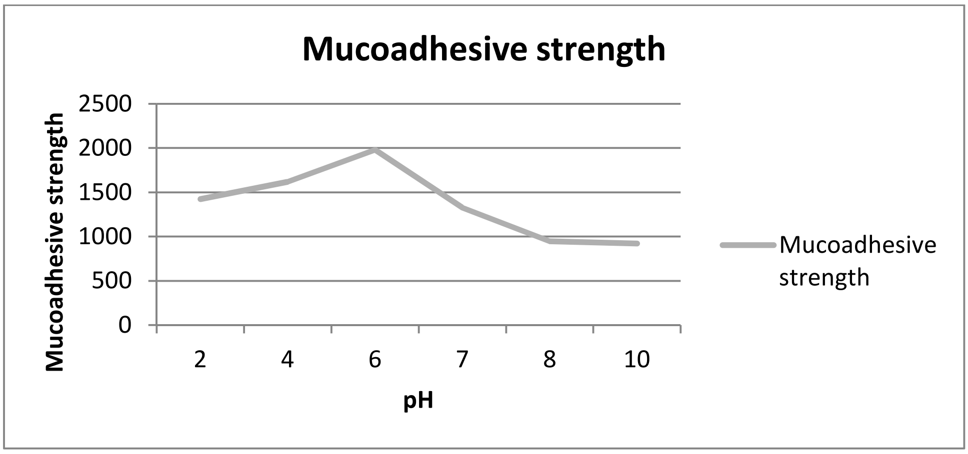

Figure 12: Mucoadhesive strength in presence of different pH

Effect of pH on mucoadhesive strength
It has been observed that gel formed with increasing pH possess adhesion properties. As the pH increases in the acidic medium; the mucoadhesive strength increases, but in the basic pH, the mucoadhesive strength decreases (Figure 12).

Effect of non-electrolyte on mucoadhesive strength
Although the concentration of sucrose increases; there is no relation of it with the mucoadhesive strength The mucoadhesive force is important physicochemical parameter for prolonging retention time, assessment of mucoadhesive strength of gel formed with increasing concentrations of sucrose possess good adhesion properties (Figure 13).

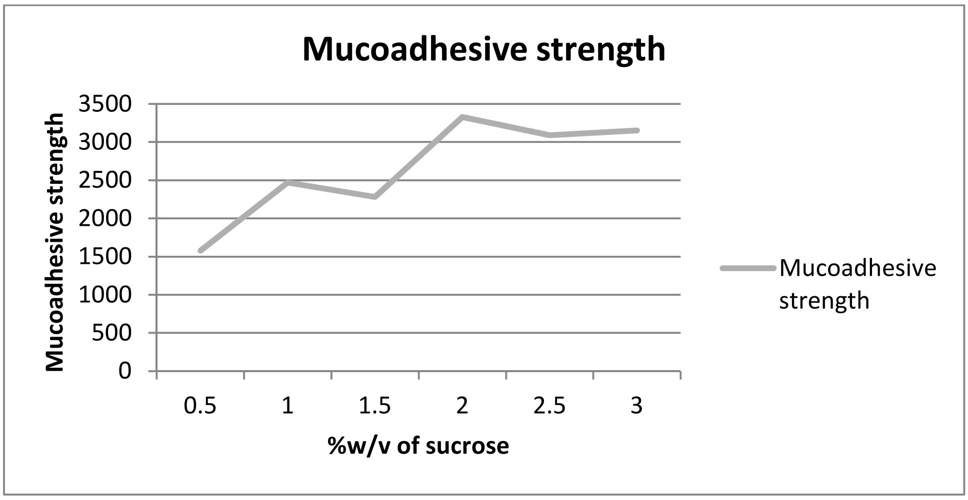

Figure 13: Mucoadhesive strength in presence of different concentrations of sucrose

Mucoadhesive strength is neither dependent on concentration of electrolytes nor on concentration of non-electrolytes. But, the order of variables affecting mucoadhesive strength was pH> ionic strength≈ non- electrolytes.

CONCLUSION

In present work characteristics of deacetylated gellan gum are measured in terms of viscosity, gel melting temperature, swelling factor and mucoadhesive strength (dependent variables). The effect of ionic strength, pH and non- electrolyte (independent variables) is correlated with dependent variables. The independent variables affect dependent variables in the order i) viscosity: ionic strength> pH> non- electrolytes, ii) gel melting temperature: ionic strength>pH> non- electrolytes iii) swelling factor: ionic strength>pH> non- electrolytes and iv) mucoadhesive strength: pH> ionic strength= non-electrolytes. Thus, ionic strength is the variable which dominantly affects viscosity, gel melting, swelling and mucoadhesive strength. Therefore, it is advisable to consider effect of independent variables and concentrating more on ionic strength while developing formulation of in-situ gel of gellan gum and while predicting in-vivo performance and in-vitro in-vivo correlation. As gellan gum has wide applications in the pharmaceutical industry, this study is helpful in understanding the relation of physiological variables with in situ gelling properties of gellan gum.

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