

Optimizing Rheological Behavior of Pectin Gels: A Response Surface Methodology Approach

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Abstract

Pectin, a natural polysaccharide widely used in the food and pharmaceutical industries, forms gels in the presence of sugar and divalent ions such as calcium. Its rheological behavior is strongly influenced by extrinsic factors including pH, calcium chloride, and sugar concentration. This study aimed to optimize the viscosity of pectin gels prepared in aqueous medium, targeting applications in edible films and biodegradable packaging. Gel formulations were prepared with varying calcium chloride concentrations (5-15% w/v), pH levels (4.5-8.0), and sugar concentrations (5-20% w/v). Viscosity was measured using a Brookfield viscometer and analyzed by response surface methodology (RSM). Results showed that pH had the strongest effect, with viscosity increasing from 1734.2 cP at pH 4.5 to a maximum of 2681.2 cP at pH 8.0, reflecting enhanced ionization and cross-linking. Calcium chloride exhibited an optimum at 10% w/v (1097.6 cP), while sugar enhanced viscosity up to 15 g (2747.2 cP) but declined at higher levels due to excessive dehydration. ANOVA confirmed the model's significance ($p < 0.001$), with strong quadratic effects but negligible interactions among factors. Optimal viscosity was obtained under near-neutral pH, 10% w/v calcium chloride, and 15% w/v sugar, highlighting the potential of pectin gels as sustainable biomaterials for food and pharmaceutical applications.

Keywords: Pectin, Viscosity, Calcium Chloride, Sugar, Brookfield Viscometer, Rheocalc software.

Introduction

Pectin, widely used in the food industry, is valued for its ability to form gels in the presence of sugar and acid, a property essential for producing jams, jellies and marmalades (1). Its rheological behavior is influenced by intrinsic factors such as botanical origin, degree of methylation, distribution of non-methylated galacturonic acid (GalA) residues and degree of acetylation, as well as extrinsic factors including temperature, pH, concentration and the presence of divalent ions (2).

Citrus peels are among the richest sources of pectin, containing approximately 20-30% more than apple peels. In addition to their higher yield, citrus-derived pectin typically exhibits a lighter cream color, whereas pectin obtained from apple peels tends to be darker (3). Other widely utilized commercial sources include sugar beet pulp, mango processing waste and sunflower heads, owing to their high pectin content and availability as agro-industrial byproducts (4). Literature reports indicate that pectin content varies significantly across fruits and vegetables. On a dry matter basis, carrots (6.9-18.6%), orange pulp (12.4-28.0 %) and sugar beet pulp (10.0-30.0%) are particularly rich in pectin, while fresh fruits such as apples (0.5-1.6%), bananas (0.7-1.2%) and peas (0.9-1.4%) contain comparatively lower amounts (5).

In the food sector, one of the major challenges lies in the packaging of food products. To meet the increasing demand for packaged foods, numerous synthetic polymers have been developed and employed due to their cost-effectiveness, flexibility and versatility.

These materials fulfill diverse industrial requirements, offering protection against biotic and abiotic factors while maintaining product freshness (6). However, the use of synthetic polymers poses significant environmental risks, as their disposal contributes to persistent waste management problems. Consequently, research has increasingly focused on the development of biodegradable materials for food packaging. Among natural polymers, polysaccharides have garnered particular interest due to their versatility, biodegradability and broad availability at relatively low cost (7).

Edible films and coatings are commonly produced from various natural polysaccharides, including starch, chitosan derivatives, alginate, cellulose and seaweed extracts (8). Among these, pectin is one of the most important natural polymers, occurring widely in foods and frequently employed as a packaging material. In addition, pectin serves functional roles in the food industry as an emulsifier and stabilizing agent (9).

In the pharmaceutical sector, pectin has demonstrated significant therapeutic potential. It has been shown to reduce blood cholesterol levels in both *in vivo* and *in vitro* studies (10). Mechanistically, pectin promotes bile acid production, facilitates the removal of cholesterol from the bloodstream, and lowers low-density lipoprotein (LDL) levels, which are associated with cardiovascular diseases (11). Furthermore, pectin contributes to blood glucose regulation by modulating glucose absorption, thereby decreasing pancreatic insulin secretion.

The primary aim of this study was to investigate the rheological characteristics of aqueous pectin gels, with a focus on evaluating the influence of calcium chloride concentration, pH and sugar content on gel viscosity and flow behavior. By systematically varying the calcium chloride concentration, adjusting pH, and incorporating different sugar concentrations, the work sought to identify optimal formulation conditions for achieving desirable gel properties. These findings are intended to support the development of pectin-based edible films,

coatings and packaging materials as sustainable, biodegradable alternatives to synthetic polymers, with potential applications in both the food and pharmaceutical sectors.

Materials and Methods

Viscometer (Model: DV2TLVTJ0) was manufactured by Ametek Brookfield. pH Analyser (Model: LP139SA) was manufactured by Polmon, India. Sodium Hydroxide, Calcium Chloride and Hydrochloric Acid were procured from Fisher Scientific, Mumbai. Pectin (Extra pure) was purchased from Loba Chemie, Mumbai. Sugar was obtained from local market.

Preparation of gels with different volumes of calcium chloride solutions (10% w/v)

Gels were prepared by dispersing 2 g of pectin in 90 mL of water. The dispersion was heated until the pectin was completely dissolved, then allowed to cool. Subsequently, 10 mL of 10% w/v calcium chloride solution was added, maintaining pectin dispersion in calcium chloride solution (12). In a similar manner, gels were prepared with calcium chloride solution of 5 and 15% w/v.

Preparation of gels with varied pH

The effect of pH on pectin gel rheology was evaluated by adjusting samples to pH 4.5, 6.8, 7.2 and 8.0 using dilute hydrochloric acid or sodium hydroxide. These values were selected to represent acidic, near-neutral, and slightly alkaline conditions. Rheological measurements were conducted to assess changes in gel strength and viscosity across the pH range (13).

Preparation of gels with different concentrations of sugar

The effect of sugar concentration on pectin gel rheology was evaluated by incorporating sucrose at 5-20% w/v. This range was chosen to reflect typical levels in jam and jelly formulations. Rheological measurements were conducted to assess changes in gel strength and viscosity with increasing sugar content (14).

Table 1: Rheological data observed from the pectin gel formulated with different concentrations of calcium chloride

Speed (RPM)	Calcium Chloride (%w/v)					
	5		10		15	
	Viscosity (cP)	Torque (%)	Viscosity (cP)	Torque (%)	Viscosity (cP)	Torque (%)
25	1875.0	25.0	2205.0	29.4	1313.0	17.5
50	956.3	25.9	1234.0	32.9	1140.0	30.4
75	725.0	29.0	837.5	33.5	785.0	31.4
100	646.9	34.5	663.8	35.4	618.8	33.0
125	591.0	39.4	547.5	36.5	555.0	37.0

Rheological study

The viscosities of pectin gel fractions prepared with varying calcium chloride concentrations (5-15% w/v), sugar concentrations (5-20% w/v), and adjusted pH values (4.5 to 8.0) were measured using a Brookfield viscometer. Measurements were performed with spindle T-D and spindle SC4-18, operating at a torque range of 10-90%, employing both a small sample adapter (8 mL capacity) and a large sample adapter (T spindle). Viscosity values were analyzed using Rheocalc software, and the data were fitted to Bingham's model (15). For comparison, viscosities were evaluated at the same shear rate and maintained at room temperature.

Optimization of rheological properties using response surface methodology

Optimization of the rheological properties of the gel was carried out using response surface methodology (RSM) with a face-centered composite design. The design space was selected for pH 4.5-8.0, calcium chloride concentration 5-15% w/v, and sugar concentration 5-20% w/v (16). Experimental conditions were generated using Minitab software, and a total of 40 gel formulations were prepared.

Results and Discussion

The influence of various factors on rheological properties of pectin gel was studied and the results are discussed here.

Table 2: Average viscosities of pectin gel formulated with various concentrations of calcium chloride solution

Calcium Chloride (%w/v)	Average Viscosity(cP)
5	958.84
10	1097.56
15	882.36

Influence of calcium chloride on viscosity of pectin gel

The viscosity of pectin gels was significantly affected by calcium chloride concentration and detailed rheological data was mentioned in (Table 1). Maximum viscosity was observed at 10% w/v (1097.56 cP), compared with lower (5% w/v, 958.84 cP) and higher (15% w/v, 882.36 cP) concentrations and it is shown in (Table 2). This indicates that moderate calcium levels promote optimal cross-linking of pectin chains, while excessive calcium leads to over-aggregation and structural weakening of the gel network.

Influence of pH on viscosity of pectin gel

The results shown that pH strongly affected pectin gel viscosity. At acidic pH 4.5, viscosity was lowest (1734.2 cP) due to limited ionization of carboxyl groups. With increasing pH, viscosities rise significantly, reaching 2452.6 cP at pH 6.8 and 2560.3 cP at pH 7.2, indicating enhanced calcium cross-linking. At pH 8.0, viscosity peaked at 2681.2 cP and it was shown in (Fig. 1). Overall,

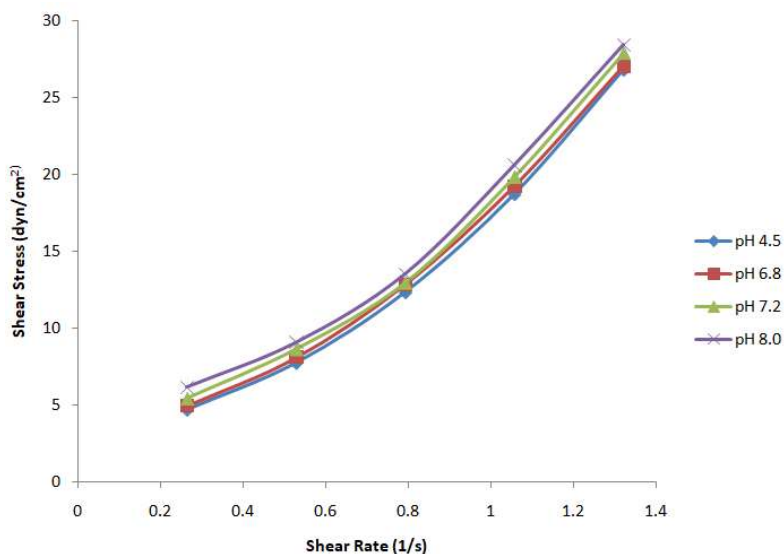


Fig 1: Rheogram of pectin gel formulated with different pH's

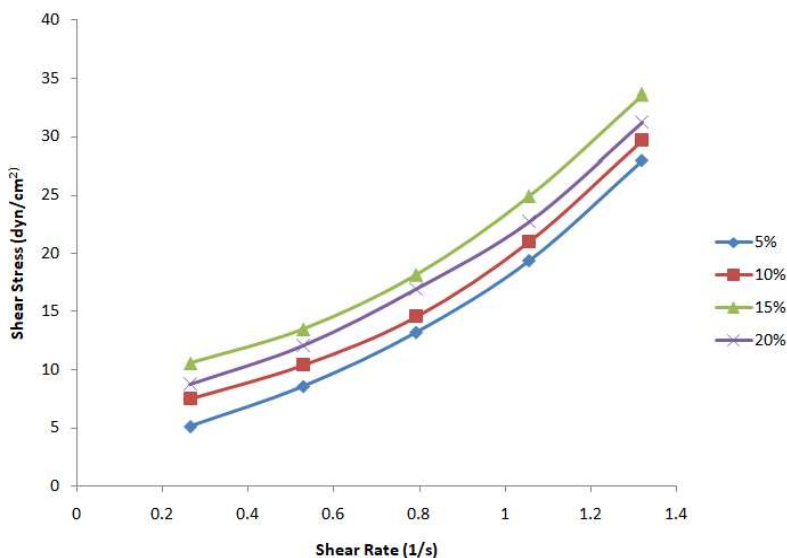


Fig 2: Rheogram of pectin gel formulated with different concentrations of sugar

higher pH favored stronger gel network formation and viscosity development.

Influence of Sugar on viscosity of pectin gel

In the Figure 2 observed that the viscosity of pectin gels increased with sugar concentration, reaching a maximum at

15%w/v (2747.2 cP). Sugar promotes dehydration of pectin chains, enhancing molecular interactions and cross-linking. At 20%w/v, viscosity declined (2452.0 cP) due to excessive dehydration and reduced water availability, weakening the gel. Thus, 15%w/v sugar was optimal for maximum viscosity.

Optimization of pectin gel viscosity revealed that all three factors i.e pH, calcium chloride, and sugar had clear optimum levels. Viscosity increased steadily with pH, peaking at 2681.2 cP under near-neutral to slightly alkaline conditions (pH 7.2-8.0). Calcium chloride showed maximum gel strength at 10% w/v (1097.56 cP), as both lower and higher concentrations weakened the network. Sugar enhanced viscosity up to 15%w/v (2747.2 cP), beyond which excess reduced gel strength. Thus, optimal gel formation was achieved at near-neutral pH, 10%w/v calcium chloride, and 15%w/v sugar.

Optimization of rheological properties using response surface methodology

The viscosities of 40 gels prepared as per the DOE and the observed results are tabulated

in (Table 3). The resulting data were analyzed using analysis of variance (ANOVA).

The ANOVA results demonstrated that the quadratic regression model was highly significant ($p < 0.001$), confirming its appropriateness for describing and predicting the system under study. Among the linear effects, pH was the dominant factor, contributing the most to the variation in the response, followed by sugar concentration, whereas calcium chloride exhibited only a minor yet significant effect. The quadratic terms were also highly significant ($p < 0.001$), with pH^2 and $sugar^2$ accounting for the largest contributions, thereby highlighting the nonlinear influence of these variables on the response. Conversely, the interaction terms ($pH \times$ calcium chloride, $pH \times$ sugar, and calcium chloride \times sugar) were weak and statistically

Table 3: Analysis of variance for viscosity(cps)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	1562838	1562838	173649	890.96	0.000
Linear	3	696498	696498	232166	1191.21	0.000
pH	1	467587	467587	467587	2399.11	0.000
Calcium Chloride(%w/v)	1	1222	1222	1222	6.27	0.018
Sugar(%w/v)	1	227689	227689	227689	1168.24	0.000
Square	3	864532	864532	288177	1478.59	0.000
pH*pH	1	539247	34675	34675	177.91	0.000
Calcium Chloride(%w/v)*Calcium Chloride(%w/v)	1	109801	17717	17717	90.90	0.000
Sugar(%w/v)*Sugar(%w/v)	1	215483	215483	215483	1105.61	0.000
Interaction	3	1808	1808	603	3.09	0.042
pH*Calcium Chloride(%w/v)	1	603	603	603	3.09	0.089
pH*Sugar(%w/v)	1	603	603	603	3.09	0.089
Calcium Chloride(%w/v)*Sugar(%w/v)	1	603	603	603	3.09	0.089
Residual Error	30	5847	5847	195		
Lack-of-Fit	5	1006	1006	201	1.04	0.417
Pure Error	25	4841	4841	194		
Total	39	1568685				

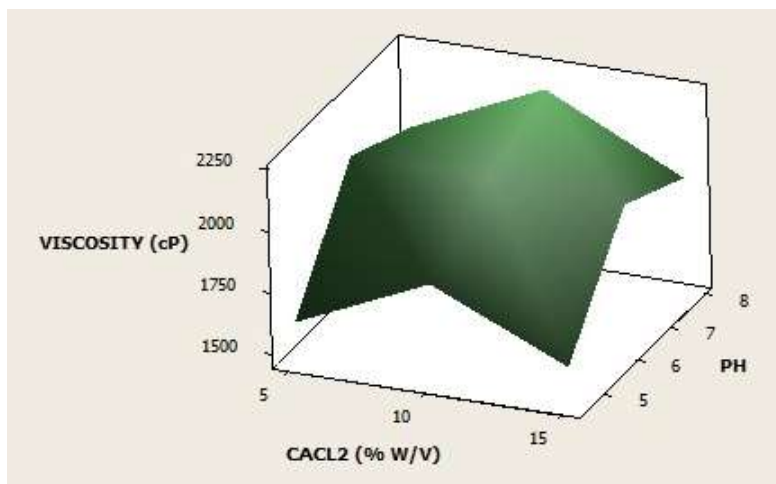


Fig 3: Surface plot graph for viscosity, pH and calcium chloride

non-significant ($p > 0.05$), indicating that the variables largely influenced the response independently within the studied range. The lack-of-fit test was non-significant ($p = 0.417$), while the residual error remained low, further confirming that the quadratic model provided an excellent fit to the experimental data. Collectively, these results underscore the robustness and predictive capability of the response surface methodology, with pH and sugar concentration emerging as the most critical factors governing the response behavior.

The relationship between viscosity and the independent variables pH (x_1), calcium chloride concentration (x_2), and sugar concentration (x_3) was described using a second-order polynomial regression model as typically applied in response surface methodology. The general form of the model is expressed as:

$$\hat{Y} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

The above equation was rewrite by insert values and the resulting equation is representing below

$$\hat{Y} = 2098.23 + 152.90x_1 - 7.82x_2 + 106.70x_3 - 79.40x_1^2 - 56.76x_2^2 - 197.94x_3^2 - 6.14x_1x_2 - 6.14x_1x_3 + 6.14x_2x_3$$

where \hat{Y} represents the predicted viscosity, β_0 is the intercept, $\beta_1, \beta_2, \beta_3$ are the linear coefficients, $\beta_{11}, \beta_{22}, \beta_{33}$ are the quadratic coefficients, and $\beta_{12}, \beta_{13}, \beta_{23}$ are the interaction coefficients. This model form captures both the linear and nonlinear effects of each factor, as well as their pair wise interactions, allowing for an accurate prediction and optimization of viscosity in relation to pH, calcium chloride, and sugar levels.

The 3D surface plot Figure 3, highlights the combined effect of pH and calcium chloride concentration on the viscosity of pectin gels. Viscosity increased markedly as pH values approached neutrality, reaching maximum levels around pH 7, which can be attributed to optimal ionization of carboxyl groups that enhances calcium-mediated cross-linking within the pectin matrix. At more acidic or alkaline conditions, insufficient or excessive ionization disrupted network formation, resulting in lower viscosity. In comparison, calcium chloride concentration showed only a modest influence, with viscosity changes remaining relatively small across the examined range. This pattern suggests that while CaCl_2 contributes to cross-link stabilization, pH is the predominant determinant of gel rheology, consistent with the statistical findings of the regression and ANOVA analyses.

Temperature reduces the viscosity and hence the studies are conducted at room temperature. However the viscosity of pectin gel was altered by incorporating the calcium chloride and sugar. The palatability of gels is effected with calcium chloride and hence sugar is also incorporated to achieve both rheological properties and palatability. The influence of aging on rheological properties and microbial stability should be studied for further exploitation of the observed research findings.

Conclusion

The study demonstrated that the rheological properties of pectin gels are significantly influenced by pH, calcium chloride concentration, and sugar content. Optimal gel viscosity was achieved at near-neutral to slightly alkaline pH (7.2–8.0), 10% w/v calcium chloride, and 15% w/v sugar, highlighting the importance of these parameters in promoting effective cross-linking and network formation. pH emerged as the most critical factor, followed by sugar concentration, while calcium chloride had a moderate yet significant effect. Excessive calcium or sugar reduced viscosity due to over-aggregation or dehydration, respectively. The response surface methodology and quadratic regression model accurately described the system and confirmed the independent, nonlinear effects of each variable. These findings provide a scientific basis for formulating pectin-based edible films and coatings, supporting the development of sustainable, biodegradable alternatives to synthetic polymers in both food and pharmaceutical applications.

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