STRUCTURAL PROPERTIES OF XANTHAN GUM HYDROGELS: PRELIMINARY STUDY FOR FUTURE INCORPORATION OF B12 VITAMIN

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Growing social concern about health has increased interest in new functional foods. To meet this demand, hydrogels represent a promising alternative: semisolid structures with a high water-retention capacity that can act as drug-encapsulating systems. Hydrogels are composed of structuring agents, generally biopolymers, capable of promoting gelation. Xanthan gum (XG) can increase the medium's viscosity through molecular interactions with other components. Thus, evaluating its gelling behavior in the incorporation of B12 vitamin into hydrogels becomes essential. Hydrogels were prepared using four concentrations of xanthan gum: XG1 (0.5% w/v), XG2 (0.75% w/v), XG3 (1.0% w/v), and XG4 (1.5% w/v). Samples were analyzed for moisture content, gel fraction, swelling index, and Raman spectroscopy, and statistical significance was assessed. The moisture content of all samples remained between 98% and 99%. The highest gel fraction values were observed for XG2 and XG3—85.33% and 82.4%, respectively, indicating more efficient network formation. The swelling index ranged from 25.64 to 28.66 g/g across samples. Raman spectroscopy revealed characteristic peaks for all formulations, including C–H vibrations, ester groups, and asymmetric and symmetric CH_2 and CH_3 stretching modes. Overall, intermediate concentrations of xanthan gum proved more efficient for producing hydrogels suitable for future vitamin incorporation.

KEYWORDS: BIOPOLYMER; RAMAN SPECTROSCOPY; GEL PROPERTIES; RHEOLOGY.

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1. INTRODUCTION

There is a growing demand for new functional foods, driven by concerns related to health issues and emerging dietary habits (HAM et al., 2025; ISMAIL; HWANG; JOO, 2020). As a result, the food industry has developed new alternatives, including hydrogels, semisolid structures capable of retaining large amounts of water (HAM et al., 2025; FAN et al., 2026).

Hydrogels consist of hydrophilic polymer chains with high water-retention capacity (BONINO et al., 2026). These chains may be formed through different routes; one approach involves using a biopolymer compatible with the intended application (HOSSEINI; ZAHABI, 2023). Commonly used hydrogel-forming biopolymers include starches and cellulose derivatives, although careful selection is required to ensure adequate mechanical properties. A recurring challenge in hydrogel development is their relatively low mechanical strength (HAM et al., 2025; HOSSEINI; ZAHABI, 2023).

Xanthan gum (XG) is a polysaccharide produced by *Xanthomonas campestris*. It is water-soluble and interacts with other polysaccharides, increasing viscosity and making it a potential gelling agent (NSENGIYUMVA; ALEXANDRIDIS, 2022). XG is supplied as a white powder that must be dissolved to exert its gelling behavior, forming double-helix structures through strong molecular interactions (AKHTAR et al., 2025; NSENGIYUMVA; ALEXANDRIDIS, 2022).

Gelation occurs predominantly via hydrogen bonding between hydroxyl groups in XG and water molecules. Additional hydrogen bonds may form through oxygen atoms in glycosidic linkages in aqueous solutions (NSENGIYUMVA; ALEXANDRIDIS, 2022). Hydrogels can act as drug-delivery systems, owing to their encapsulation capacity and the controlled release triggered by water absorption (ZHANG et al., 2022; ZHENG et al., 2025). A key advantage of using XG as a gelling agent is that its β-1,4 glycosidic bonds are not hydrolyzed in the stomach, enabling drug delivery to the small intestine with enhanced absorption (MEHRAB POURMADADI; MALEKI; MAJID ABDOUSS, 2025).

Cobalamin, or simply vitamin B12, is an essential macronutrient for humans that is predominantly found in animal foods. Vegans and people who have a diet rich in plant-based foods may be deficient in this vitamin, often requiring supplementation (SANTOS et al., 2023). Vitamin B12 deficiency can cause symptoms such as tiredness, weakness, memory problems, and tingling in the extremities, which highlights its importance for quality of life and overall body balance (DAWCZYNSKI, 2021). Given the above and in order to contribute to the development of nutritious foods, this study aims to evaluate the potential of xanthan gum as a gelling agent in hydrogels intended for the incorporation of vitamin B12.

2. MATERIALS AND METHODS

All procedures were performed at the Laboratory of Thermodynamics and Separation Processes (LATOS) of the Department of Chemical Engineering at the Federal University of Paraná (UFPR), Curitiba.

2.1 Hydrogel preparation

Hydrogel preparation followed Cortez-Trejo (2021), with modifications. Hydrogels were formulated with 50 mL of distilled water (pH ≈ 7.4) and xanthan gum (XG) concentrations of 0.5, 0.75, 1.0, and 1.5%. Table 1 presents the experimental design.

TABLE 1 – EXPERIMENTAL DESIGN OF HYDROGELS

Amostra	Water (mL)	XG (%)
XG1	50	0.5
XG2	50	0.75
XG3	50	1.0
XG4	50	1.5

Figure 1 illustrates the methodological flowchart. First, 50 mL of distilled water was heated to 60 °C while stirring continuously. Once the temperature reached, xanthan gum gradually added while maintaining vigorous agitation for 30 minutes. The dispersion was then allowed to cool to room temperature (≈ 22 °C) to complete dissolution. Hydrogels were stored at 4 °C until analysis.

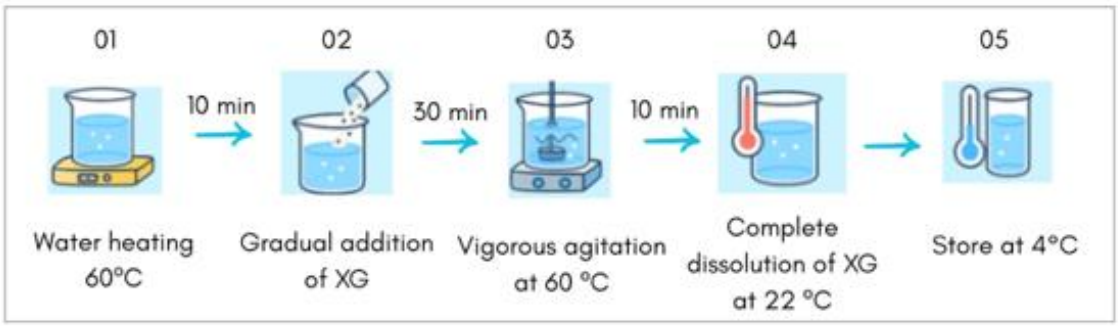

FIGURE 1 - FLOWCHART OF THE METHODOLOGY FOR HYDROGEL PREPARATION

2.2 Moisture content

The moisture analysis was performed using the oven-drying (thermogravimetric) method. Approximately 5 g of the wet sample was weighed. The sample was then placed in an oven at 105 °C until all water had evaporated and the dry mass reached a constant weight (IAL, 2008). Moisture content was calculated according to Equation 1.

$$U(\%) = \frac{m_i - m_f}{m_i} \quad (1)$$

Where m_f is the weight of the dried sample, and m_i is the weight of the initial sample.

2.3 Gel fraction

Gel fraction (GF) was determined according to Alshehri et al. (2016) with adaptations. Samples were dried at 60 °C for 24 h and weighed. They were then placed in a mesh filter, immersed in deionized water at 80 °C for 4 h, dried again at 60 °C for 72 h, and weighed. Gel fraction was calculated using Equation 2.

$$GF\ \%\ (m/m) = \frac{WE}{WO} * 100 \quad (2)$$

Where WO is the initial mass of dried hydrogel, and WE is the final mass after immersion.

2.4 Swelling index

Dried hydrogels were cut into 1 × 1 cm² pieces (≈1 g), immersed in 40 mL of a buffer solution (NaCl, KCl, Na_2HPO_4, KH_2PO_4; pH 7.4) for 48 h at 20 °C, then filtered and weighed. The swelling index was calculated using Equation 3 (ALSHEHRI et al., 2016).

$$GI\ (\%) = \frac{(mf - mi)}{mi} * 100 \quad (3)$$

Where m_i is the initial dry mass, and m_f is the final filtered mass.

2.5 Raman spectroscopy

Raman spectroscopy was performed using a confocal Raman microscope, Alpha300 R (WITec, Germany), equipped with a 785-nm laser and a CCD detector. Spectra were collected at multiple points using a 50 × objective (NA 0.50) over the 100–3100 cm^{-1} range.

2.6 Statistical analysis

All analyses were performed in triplicate. Results were expressed as mean ± standard deviation. Data were analyzed using ANOVA and Tukey's test ($p < 0.05$) using Statistica v.10 and SASM-Agri v.8.2.

3. RESULTS AND DISCUSSION

3.1 Macroscopic appearance

Figure 2 illustrates the hydrogels after complete solubilization of xanthan gum. It can be observed that as concentrations increase, the gel expands. Sample XG1 (0.5% w/v) presented a translucent gel with low turbidity and no lumps due to the low concentration. Sample XG2 (0.75% w/v) exhibited a dense appearance, suggesting good polymer hydration and chain expansion in water. Samples XG3 (1.0% m/v) and XG4 (1.5% m/v) showed a denser network than XG2, indicating that as the concentration increases, the amount of free water decreases, homogenization occurs, and possible lumps may form during gelation. However, in XG4, visual uniformity is greater than in XG3, while opacity indicates greater retention of structured water or a greater content of the polymeric phase diffused in the matrix.

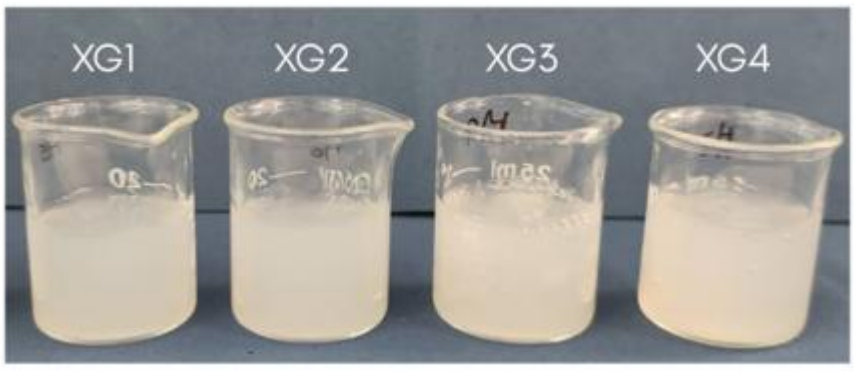

FIGURE 2 – MACROSCOPIC VIEW OF XANTHAN GUM HYDROGELS: XG1, XG2, XG3, AND XG4

Lopes (2017) developed a hydrogel with modified xanthan gum for the controlled release of vitamin B12, in which he used 50 mg to 5 mL (1.0% (m/v) of distilled water in the formation of the hydrogel, indicating that, analyzing only the macroscopic aspect, it is noticeable that medium concentrations, such as 0.75 and 1.0% of xanthan gum, would be more suitable for

stabilizing vitamins. Knowing that low concentrations can lead to component dissolution and high concentrations can result in excessively rigid gels, making it challenging to release vitamins.

In the work of Huang and collaborators (2025), for the development of biodegradable antibacterial hydrogels with XG for the controlled release of ciprofloxacin, polymer concentrations of 0.4 to 0.76% were used. These concentrations provided good interaction between the hydrogel components, efficient drug encapsulation, and greater thermal stability of the formed networks, which justifies the use of intermediate concentrations.

3.2 Hydration properties and gel structure

Table 2 presents the gel fraction, swelling index, and moisture values of the hydrogel formulations. In general, the different compositions significantly influenced only the gel fraction, while swelling and moisture remained statistically similar across treatments. The moisture analysis (U) did not indicate significant differences among the samples, ranging from 98.33 to 99.72%, suggesting that, regardless of xanthan gum concentration, all formulations contained a high amount of water, typical of hydrated hydrogels (NEVES et al., 2021).

TABLE 2 – SWELLING INDEX, GEL FRACTION, AND MOISTURE CONTENT OF HYDROGELS

Sample	Gel fraction (%)	Swelling (g/g)	Moisture (%)
XG1	69.5±0.71^{c}	28.66±1.65^{a}	99.68±0.41^{a}
XG2	85.33±0.47^{a}	28.48±0.74^{a}	99.46±0.34^{a}
XG3	82.4±0.14^{a}	25.69±4.19^{a}	99.72±0.21^{a}
XG4	76.0±0.94^{b}	25.64±2.19^{a}	98.33±0.18^{a}

NOTE: Values presented as mean ± standard deviation (n = 3). Values followed by different letters differ significantly from each other using the Tukey test ($p < 0.05$). The data met the assumptions of normality and homoscedasticity.

Gel fraction is the amount of effectively cross-linked polymeric material, that is, the part that forms the insoluble network in the structuring of the gel (PACHECO; PALACIO, 2016). When evaluating the samples, it was noted that in XG2 (85.33%) and XG3 (82.4%), the three-dimensional network formed most efficiently, whereas in XG1 (69.5%), GF was lowest, suggesting less cross-linking between the polymeric components. Therefore, higher gel fractions indicate better incorporation into the network, resulting in less soluble hydrogels. Samples 2 and 3 have better organization between XG and water.

The swelling index ranged from 25.64 to 28.66 g/g; however, there were no significant differences between the samples. The fact that XG1 has the lowest GF, but similar swelling, indicates that its lower crosslinking was not enough to increase its swelling index, in the same way that XG2, being the most crosslinked sample, there was no significant reduction in water absorption, therefore the network density did not reach a threshold capable of restricting swelling (LEE; HAN; CHANG, 2025). It is possible to correlate the decrease in swelling at higher concentrations with the lower mobility of the aqueous phase, as denser networks tend to swell less, thereby trapping water (SILVA, 2015).

Mirmasoudi and collaborators (2025) developed a hydrogel based on polysaccharides, including xanthan gum, in which intermolecular interactions increased the network density and reduced the swelling capacity. However, the increase in the gel fraction is associated with the

structural reinforcement promoted by xanthan gum, interfering with the efficiency of chemical crosslinking. Therefore, both studies showed lower swelling at higher XG concentrations.

3.3 Raman spectroscopy

Figure 3 illustrates the Raman spectra of the hydrogels, divided into two shifts. The first displacement varies from 0 - 1750 cm-¹ (Figure 3a), where it is possible to analyze a peak at 1250 - 1750 cm-¹ in all samples, indicating C-H vibrations and ester groups, in addition to bands associated with the structure of biopolymers (SAMPAIO, 2021). Furthermore, the common C-O-C stretching of polysaccharides and saccharide ring bands and the second shift of 1800 - 3100 cm-¹ (Figure 3b) can be seen in a slightly more spaced band, indicating the presence of asymmetric and symmetric stretching of CH_2 and CH_3 (SOUZA, 2022).

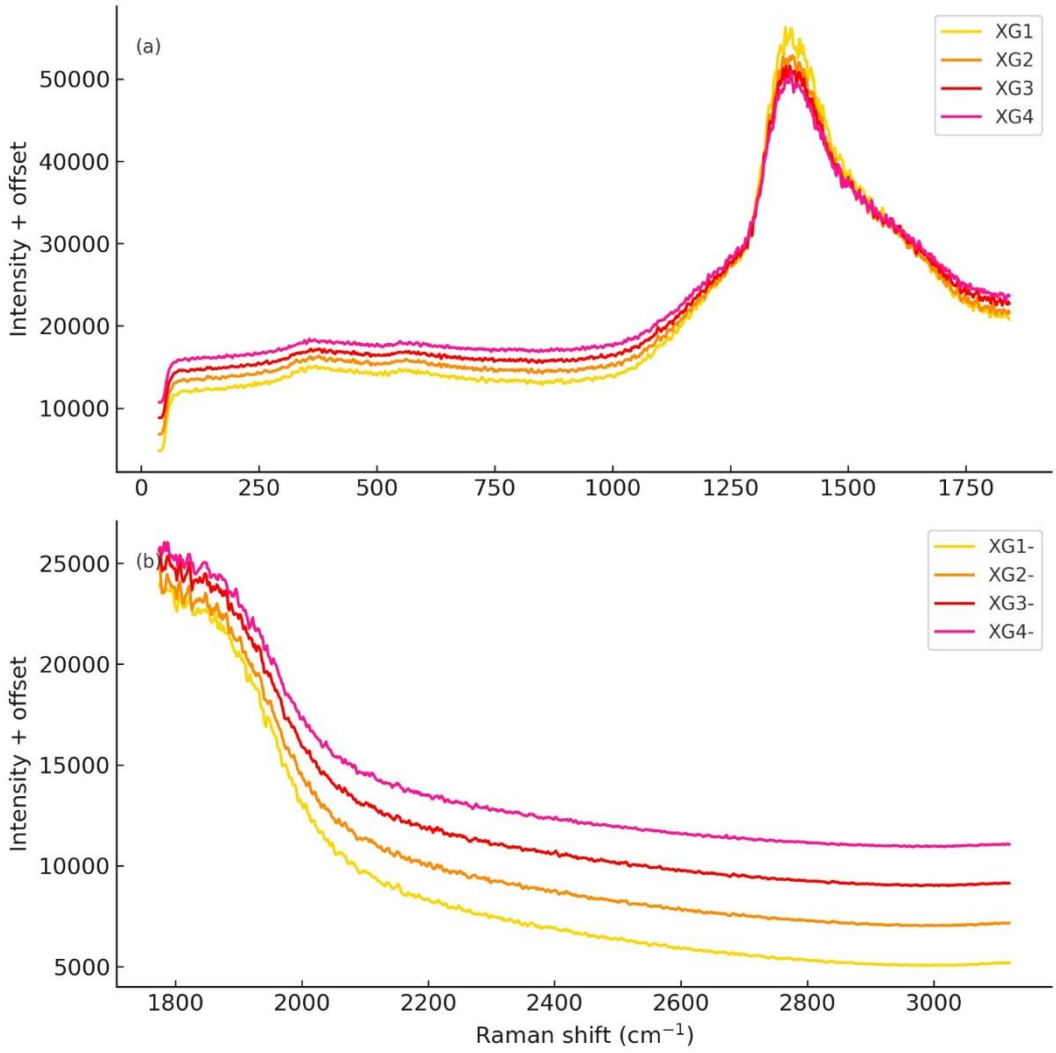

FIGURE 3 – RAMAN SPECTRA OF HYDROGELS: (A) 0–1750 CM⁻¹; (B) 1800–3100 CM⁻¹

As the concentration of xanthan gum increases, the polymer network compacts and the molecular density increases. It reduces free space and intensifies interactions, mainly hydrogen bonds, causing structural changes, modifying the vibrational environment of the functional groups present in XG, directly reflecting the intensity of the bands (PRUDENTE, 2025).

The Raman analysis obtained in the present work illustrates a behavior like that described for xanthan gum and modified cyclodextrin hydrogels. In the study mentioned above, shifts and broadening of the O-H and C=O bands were interpreted as evidence of increased hydrogen-

bonding interactions and structural reorganization of the network (SUN et al., 2025). Furthermore, the structural analysis was limited to Raman spectroscopy and did not include other techniques, such as Fourier transform infrared spectroscopy (FTIR) and confocal microscopy.

4. CONCLUSION

The evaluation of xanthan gum as a gelling agent for the possible incorporation of vitamins into hydrogels demonstrated that the concentration of the biopolymer directly influences the structure, appearance and three-dimensional organization of the formulations. Macroscopic analysis showed that intermediate concentrations (0.75–1.0% w/v) yielded more uniform hydrogels, with lower turbidity and improved polymer chain hydration, whereas very low concentrations yielded poorly structured gels, and high concentrations led to increased opacity and possible lump formation. These results corroborate the literature, which indicates that moderate concentrations of XG are efficient at stabilizing and incorporating bioactive compounds.

The gel fraction, a parameter directly associated with the degree of crosslinking, presented the highest values in XG2 and XG3, indicating greater network organization and more efficient polymer incorporation. Although the swelling index and moisture did not vary significantly between treatments, all hydrogels exhibited high water content, characteristic of highly hydrated systems.

Raman spectroscopy was the only structural analysis carried out and reinforced the conclusion that all formulations have a similar chemical composition, with intensity differences reflecting the concentration of xanthan gum, highlighting characteristic bands of C–H vibrations, ester groups, C-O-C of polysaccharides, and modes associated with biopolymers. In general, the results demonstrate that intermediate concentrations of xanthan gum are most efficient for forming hydrogels suitable for vitamin incorporation like B12, as they promote good structural organization, adequate cross-linking, and maintenance of high hydration capacity, without compromising the system's visual uniformity or stability.

Therefore, a more in-depth characterization of the hydrogel is necessary, including texture analysis, differential scanning calorimetry (DSC) and thermogravimetry (TGA), techniques that will allow us to understand better the organization of the polymeric network and the thermal stability of the system, fundamental parameters to evaluate the viability and efficiency of xanthan gum as a gelling agent for the future incorporation of vitamins.

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