

RHEOLOGICAL RESEARCH OF SOME POLYSACCHARIDE GELS LOADED WITH *NIGELLA SATIVA* EXTRACTS

ANDREI ROȘU,* SIMONA BISTRICEANU,* CONSTANTA IBĂNESCU,**
OANA MARIA DARABA *** and MARIA LUNGU*,***

*“Gheorghe Asachi” Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, 71 A, Prof.dr.doc. Dimitrie Mangeron Blvd., Iasi, Romania

**“Petru Poni” Institute of Macromolecular Chemistry, 41A, Grigore Ghica Voda Alley, Iasi, Romania

***“Apollonia” University of Iasi, 2, Muzicii Str., Iasi, Romania

Received July 19, 2012

Rheological properties of xanthan and carrageenan polysaccharides were investigated by means of dynamic shear oscillation measurements. These hydrogels were tested both with and without *Nigella sativa* extracts of different pH values. Although no major difference was noticed between the control samples and those loaded with the active ingredients, some of the new gels showed better rheological behavior.

Keywords: *Nigella sativa* extracts, xanthan and carrageenan, viscoelastic domain, cosmetic vehicles, polysaccharide gels

INTRODUCTION

Recent development of environmental legislation as well as the consumer demands resulted in a renewed interest in natural materials and biodegradable polymers.

The carrageenans belong to the group of linear sulfated polysaccharides, obtained from seaweeds. There are several types of carrageenans, of different chemical structure and properties, the most common ones being iota, kappa and lambda carrageenan. Both kappa and iota carrageenan are able to form strong firm gels, at low polymer concentrations, in the presence of potassium and calcium salts.¹

The chemical structure of each type of carrageenan greatly influences the physical properties of the resulting gel. Different kinds of these polysaccharides differ only by the position and numbers of ester sulfate groups.

The applications of this product range from food industry, where it is used as a fat substitute or as a stabilizer, to personal care and pharmaceutical industry, as a thickener, stabilizer or as a binder.² The recent researches over these

polysaccharides placed their application in the biomedical domain, for drug delivery, wound dressing or as medium for cell encapsulation and enzyme immobilization.^{3,4}

Xanthan is a natural polysaccharide produced by the bacterium *Xanthomonas campestris*. Its structure is based on repeated pentasaccharide units formed by two glucose units, two mannose units and one glucuronic acid unit.⁵ The main uses of xanthan gums include domains like food and cosmetics industry. Its applications range from stabilizer for emulsions to thickener for a wide variety of vehicles.^{6,7}

Hydrogels are a particular class of materials based on three-dimensional networks made out of polymers, able to absorb large quantities of water.⁸ Their capacity to respond to external stimuli like pH changes, chemical changes, thermal and magnetic stimuli has drawn the attention of the researchers.⁹ In the biomaterials domain, the hydrogels are used as carriers for active ingredients,¹⁰ as wound dressing and skin grafts¹¹ or as rheological modifiers.¹²

Cellulose Chem. Technol., **47** (5-6), 359-367 (2013)

The rheological characterization of carrier systems with and without active ingredient helps choose the best solution, due to the qualitative and quantitative indicators of deformation and flow behavior, stability over time and influence of different factors on the efficacy of the products. The correlation of rheological properties with the attributes of efficiency, perception and use of formulated products is particularly important for their choice for characterization. As an example, the absorption of many personal care products, which are to be applied by spraying, depends on how well they would remain on the affected area of skin. Studies¹³ demonstrated that the addition of thickeners can increase dramatically the efficacy of the spray, as they impart a higher low shear rate viscosity.

Another noticeable example is that of the hand creams tailored in such a way as to have high viscosity at low shear rates and low viscosity at high shear rates. The high viscosity at low shear rates gives the cream a good “standup” appearance in the pot, resulting in a rich creamy product. When a lotion allowed to rest has a low viscosity it might be unstable by storage till the phase separation. A low viscosity at high shear rates allows the product to be absorbed into the skin when rubbed. Viscosity profiles of the lotions can influence the product stability and its end use. The viscosity profile will also dictate how the material can be pumped and its appearance at rest.

Rheological characterization under shear oscillation best simulates the conditions a product is subjected to during manufacture, transport, storage and use, and thus, it can be an effective tool to control the product properties during all these operations.

EXPERIMENTAL

Materials

Nigella sativa (*Ns*) oilseeds were supplied by Ulrich Walter GmbH, sodium hydroxide and hydrochloric acid were supplied by Sigma-Aldrich Chemie GmbH, carrageenan and xanthan gums were

supplied by Kelco Co. All reagents were used as received without further purification.

Methods

Nigella sativa extracts (*Ns*) were prepared in three stages, as described below.

In the first stage, three extract types were obtained with *Ns* seeds in different pH media:

- an acid extract prepared by mixing 5 grams of *Ns* seeds with 20 mL of 50% HCl solution, followed by storage for 24 hours at 50 °C under shaking,

- an alkaline extract prepared by mixing 5 grams of *Ns* seeds with 20 mL of 50% NaOH solution, followed by storage for 24 hours at 50 °C under shaking,

- an ethanolic extract prepared by mixing 5 grams of *Ns* seeds with 50 mL of 50% ethanol solution, followed by storage for 24 hours at 50 °C under shaking.

The second stage consisted in mixing the alkaline and the acid extracts with 30 mL of 50% ethanol solution. The mixtures were filtered after four hours.

The hydrogel mixtures were prepared as follows: for the control samples, 1 gram of carrageenan or xanthan gum powder was mixed mechanically, at room temperature, with 99 grams of distilled water, until clear and stable gels were obtained. For the gels with the active ingredient (*Ns* extract), the following recipe was applied: 2 grams polysaccharide gum, 3 grams of extract and 1 gram of poly(oxyethylene) (20) sorbitan monolaurate were mechanically mixed with 94 grams of distilled water until obtaining clear and stable gels. The mass of all the samples was kept constant. For the sake of simplicity, the samples were noted according to Table 1.

Measurements

The rheometric investigations were performed in controlled-stress and controlled-strain modes on an Anton Paar, Physica MCR 501 modular rheometer provided with a Peltier system for temperature control. A solvent trap was used to avoid solvent evaporation. All measurements were carried out in parallel-plate geometry with a diameter of 50 mm, and a gap of 1 mm. The temperature of the tested samples was maintained at 25 °C for the amplitude (AS) and frequency (FS) sweep tests. For the temperature tests, a constant strain was chosen, in accordance with the linear viscoelastic domain, a constant frequency of 1 Hz, and the temperature was swept with 1 °C per minute between 20 and 50 °C.

Table 1
Abbreviations used for the studied samples

Xanthan gel	Xanthan gel with ethanolic extract	Xanthan gel with acid extract	Xanthan gel with alkaline extract	Carrageenan gel	Carrageenan gel with ethanolic extract	Carrageenan gel with acid extract	Carrageenan gel with alkaline extract
Xanth	XanthE	XanthA	XanthB	Carag	CaragE	CaragA	CaragB

RESULTS AND DISCUSSION

For all the studied samples, amplitude sweep (AS) tests were performed, in order to determine the linear viscoelastic (LVE) domain. Within the limits of this domain, the structure of the sample remains stable, not being affected by the increased effort or strain applied. Under these determined conditions, frequency sweeps can be applied at constant values of stress or strain within the LVE limits. Also, the values of the limits of the linear viscoelastic domain give an insight into system stability, against syneresis, for instance¹⁴. A relatively quick amplitude sweep experiment can indicate the strength of a gel by means of storage modulus (G') characteristic values. This can therefore be used to optimize dosing of gelation agents and other components.

The graphical representation of G' , G'' curves against strain, for all the studied samples, allowed a comparison of their characteristics. Furthermore, the inclusion of curve families of the systems without active ingredients helped highlight the influence of active ingredient addition on the rheology of the overall system, since the same concentration of polysaccharide was used for all the samples.

Xanthan and carrageenan gels present G' and G'' curves characteristic of well-structured systems with large LVE domains, extended up to 10% strain and decreasing trends over this threshold. For all the studied cases, G' values higher than G'' values could be observed, which showed a stable gel, even after adding Ns extract in the carrier. Even so, an obvious contrast appears between the maximum storage modulus values corresponding to carrageenan gels (between 2103 and 104 Pa) and the values corresponding to xanthan gels (between 130 and 5 Pa). This is a first indication that carrageenan gels have a more compact structure and are more stable at rest (Figure 1). It is well-known that the viscoelastic materials with high values of the G' modulus show a higher stability over time¹⁵.

On the other hand, at strains above 10%, carrageenan gels show a faster dissolution, so that at a deformation exceeding this value, the $G' = G''$ condition is reached, so the transition from a predominantly solid viscoelastic material to a predominantly viscous one is made. Xanthan gels present the tendency of approaching the dynamic moduli, but the cross-over point appears to be at a higher deformation than the one applied.

Table 2 presents the results of measurements obtained by sweeping the amplitude for all

studied samples, with the exact values for the linear viscoelastic range limit and cross-over points for $G'(\gamma)$ and $G''(\gamma)$ curves. It can be observed that adding the active ingredient (Ns extract) causes a slight decrease in the gel stability. The limiting values of the linear viscoelastic domain (Table 2) support this finding. The lowest value determined for the LVE domain was determined for the carrageenan gel that included acid extract, but this gel had the highest limiting values for cross-over point. This can be translated as a shift from a stiff gel to a more flexible one. Adding Ns extracts to the xanthan gels had an effect only on the limits of the LVE domain, the lowest registered value being for the alkaline extract and the highest one being for the acid one. It must be clearly specified that for both xanthan and carrageenan gels, the dominant behavior that could be observed is similar for a viscoelastic solid with G' values over G'' (gels, emulsions, asphalt).¹⁶

Similar considerations have been made on gel strength and stability based on the analysis of the graphs obtained for frequency sweeps for both groups of gels. A frequency sweep (FS) in the sample LVE range can be used to characterize the nature of a gel or solution. When a material has strong intermolecular associations it will give a gel-like structure and the elastic modulus is dominant over the viscous modulus. A gelled system is characterized by little change in the viscoelastic properties with frequency.

Figure 2 presents the variation of the viscoelastic features of the carrageenan samples as a function of frequency of oscillations and Figure 3 presents the same variation for the xanthan gels. Both the variation of dynamic moduli, G' , G'' (Figures 2a, 3a) and complex viscosity, η^* (Figures 2b, 3b) have been monitored. Frequency sweep measurements were performed at constant amplitude of oscillations, chosen from the appropriate range of LVE domain (Table 2).

As a first observation, it could be noticed that for all the samples, slightly increasing $G'(\omega)$ curves are all over $G''(\omega)$ curves, which is an indicative of the gel character for all the studied systems over the entire frequency domain applied ($0.01\div 1000\text{ s}^{-1}$). So, it could be assumed that the frequency of oscillations has a low influence on the viscoelastic properties of the studied samples. It can be observed that for carrageenan gels, there is only an approaching tendency between $G'(\omega)$ and $G''(\omega)$, which can be noticed at levels

exceeding the maximum frequency applied. For xanthan gels, this approximation takes place at lower frequencies, of hundreds s^{-1} , see Table 2. This means¹⁷ that xanthan-containing systems

behave as reversible networks and are able to pass from a gel state at low frequencies to a very viscous liquid state at high frequencies.

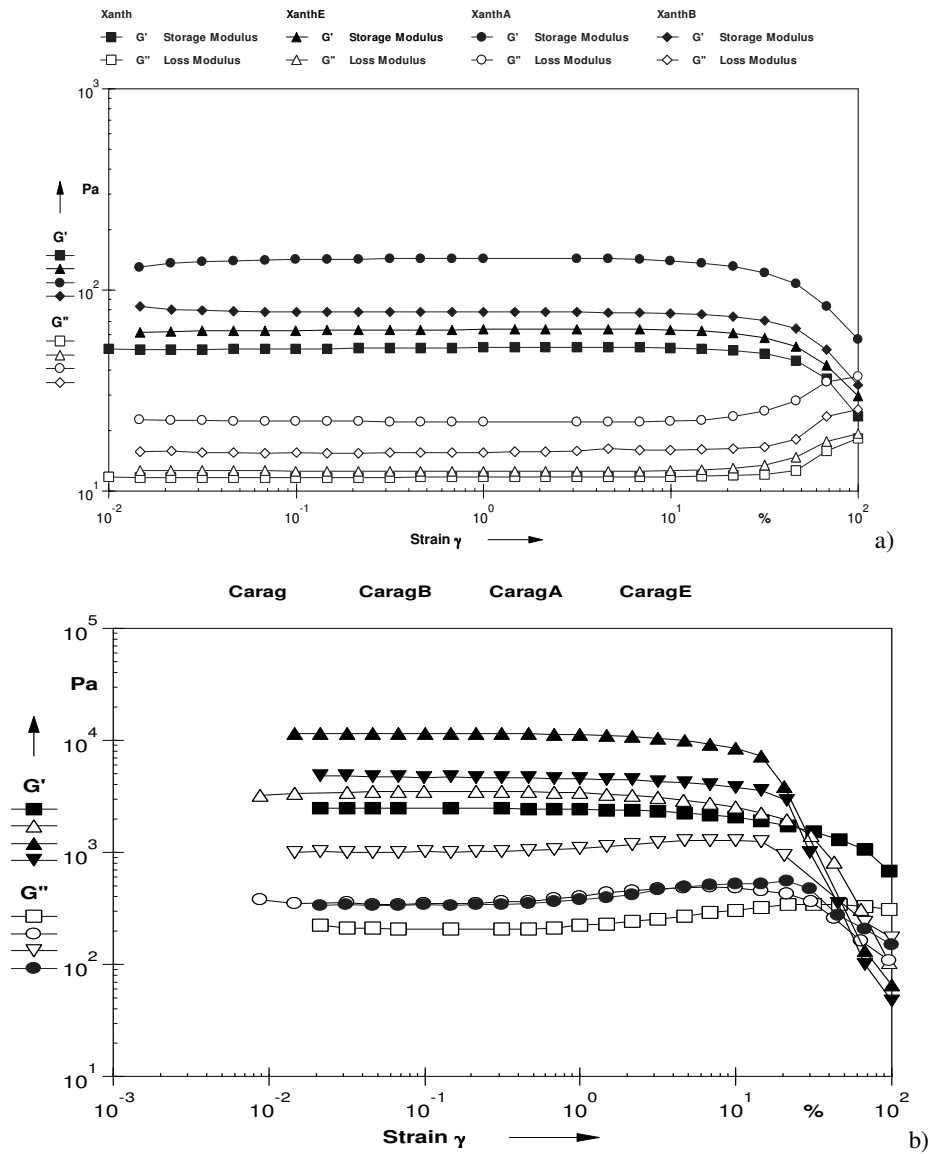


Figure 1: Amplitude sweep for: a) xanthan gel (Xanth), xanthan with ethanolic extract (XanthE), xanthan with alkaline extract (XanthB) and xanthan with acid extract (XanthA); b) carrageenan gel (Carag), carrageenan gel with ethanolic extract (CaragE), carrageenan gel with acid extract (CaragA), carrageenan gel with alkaline extract (CaragB)

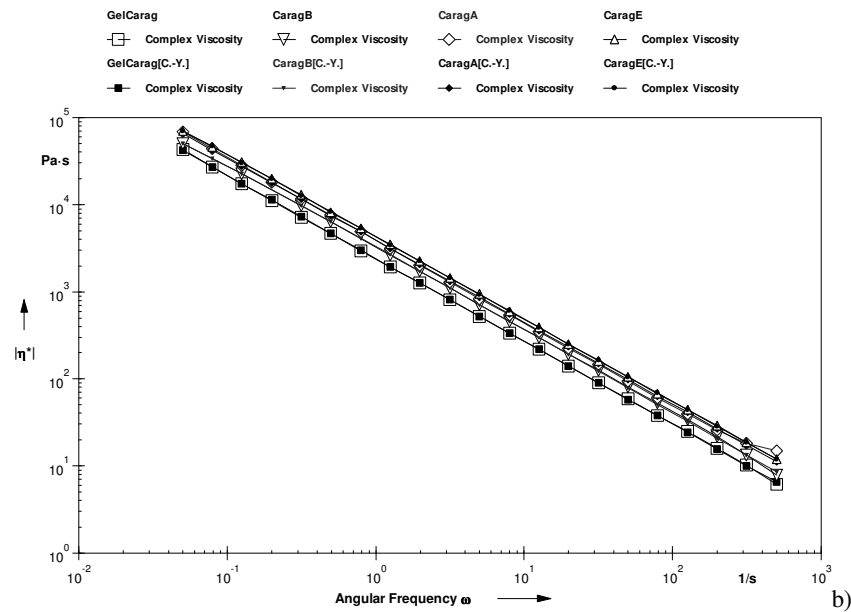
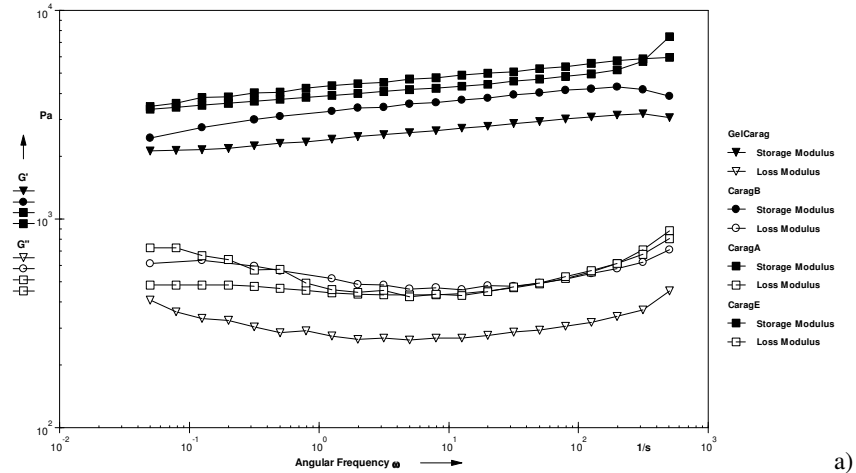
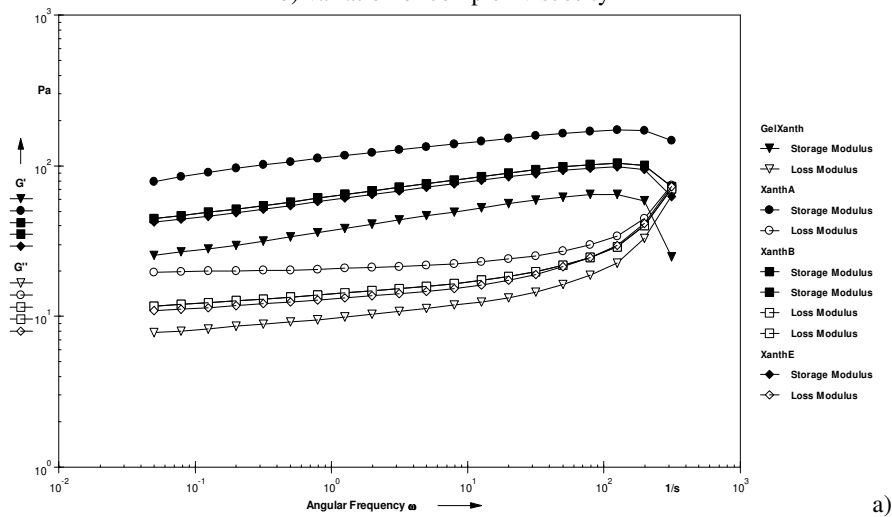


Figure 2: Frequency sweep for carrageenan gel (Carag), carrageenan gel with ethanolic extract (CaragE), carrageenan gel with acid extract (CaragA), carrageenan gel with alkaline extract (CaragB) with a) variation of dynamic moduli, and b) variation of complex viscosity



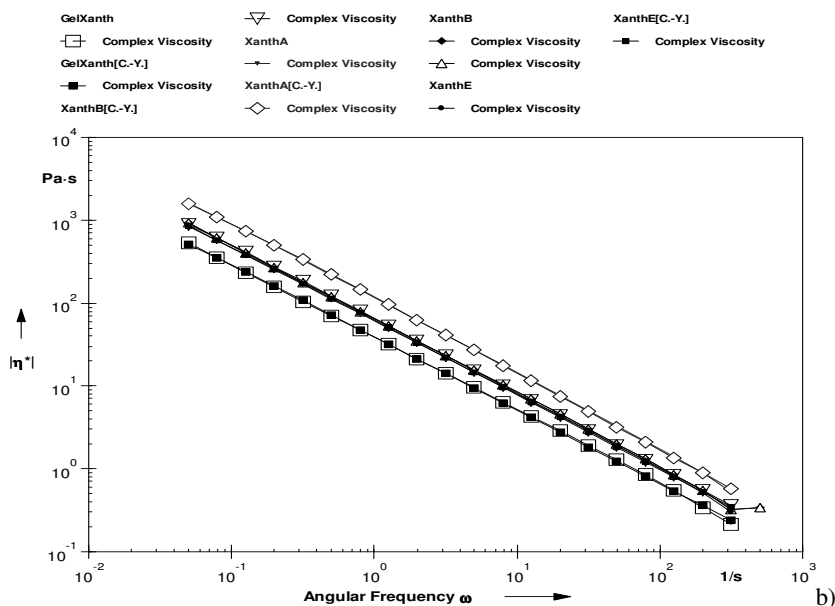


Figure 3: Frequency sweep for xanthan gel (Xanth), xanthan with ethanolic extract (XanthE), xanthan with alkaline extract (XanthB) and xanthan with acid extract (XanthB) with a) variation of dynamic moduli; and b) variation of complex viscosity

The advantage of this behavior is that such a formulation has good long-term stability ($G' > G''$ at low values of frequencies). If the formulation is meant to be applied by spraying, it is recommended that at very high frequencies occurring in a spray system the condition $G'' > G'$ be fulfilled. These systems can be described by the Maxwell and Kelvin models, or their more

complex derivatives. If a material is required to have good storage stability, it will generally need to be predominantly elastic at low frequencies. Similarly, if a material is to be sprayed, it should not be too elastic at high frequencies, otherwise it might exhibit undesirable properties, such as 'cobwebbing'.

Table 2

Viscoelastic parameters calculated based on rheological measurements under oscillatory shear regime for carrageenan gel (Carag), carrageenan gel with ethanolic extract (CaragE), carrageenan gel with acid extract (CaragA), carrageenan gel with alkaline extract (CaragB), xanthan gel (Xanth), xanthan with ethanolic extract (XanthE), xanthan with alkaline extract (XanthB) and xanthan with acid extract (XanthB)

Parameters		Sample	Reference sample		Gel with NaOH extract of N_s		Gel with HCl extract of N_s		Gel with ethanolic extract of N_s	
			Carag. gel	Xanth. gel	Carag. gel	Xanth. gel	Carag. gel	Xanth. gel	Carag. gel	Xanth. gel
Amplitude sweep data	LVE data	γ (%)	2.89	9.59	1.14	6.32	1.58	7.66	2.06	7.05
		γ_{rec} (%)	1	5	0.5	5	1	5	1	5
		τ (Pa)	24.3	2.66	17.5	3.95	114	7.21	45.5	3.24
	Cross-over data	$G'=G''$ (Pa)	-	-	197.1	-	285.4	-	187.4	-
		γ (%)	-	-	61.87	-	47.78	-	49.6	-
		τ (Pa)	-	-	178.4	-	285.4	-	187.4	-
Frequency sweep data	Storage stability	η_0 (Pas)	184570	3344.4	262420	6018.9	284130	12792	202650	5697.5
	Cross-over data	$G'=G''$ (Pa)	-	42.33	-	70.82	-	-	-	66.73
	ω (s^{-1})	-	237.1	-	319	-	-	-	-	293.8

The decrease of complex viscosity frequency, for all samples studied, is an important feature in the use of products based on these gels. Thereby, the products having a very high viscosity at low frequencies might show a firm, stable, well-bodied products, with good standup properties, while at higher frequencies the viscosity falls dramatically and the samples would therefore be applied and absorbed into the skin easily.

It must be mentioned that one of the most important rheological parameters in assessing the

capacity of storage and use of the studied materials is the zero shear viscosity, η_0 . This parameter shows the material behavior at rest or at a minimum stress. Zero shear viscosity can be determined by applying the Carreau-Yasuda model.¹⁸ As shown in Table 2, η_0 values for carrageenan gels are very high, of the 10^5 Pa·s order, which confirms the strength and stability of carrageenan-based gels, given also by other rheological parameters.

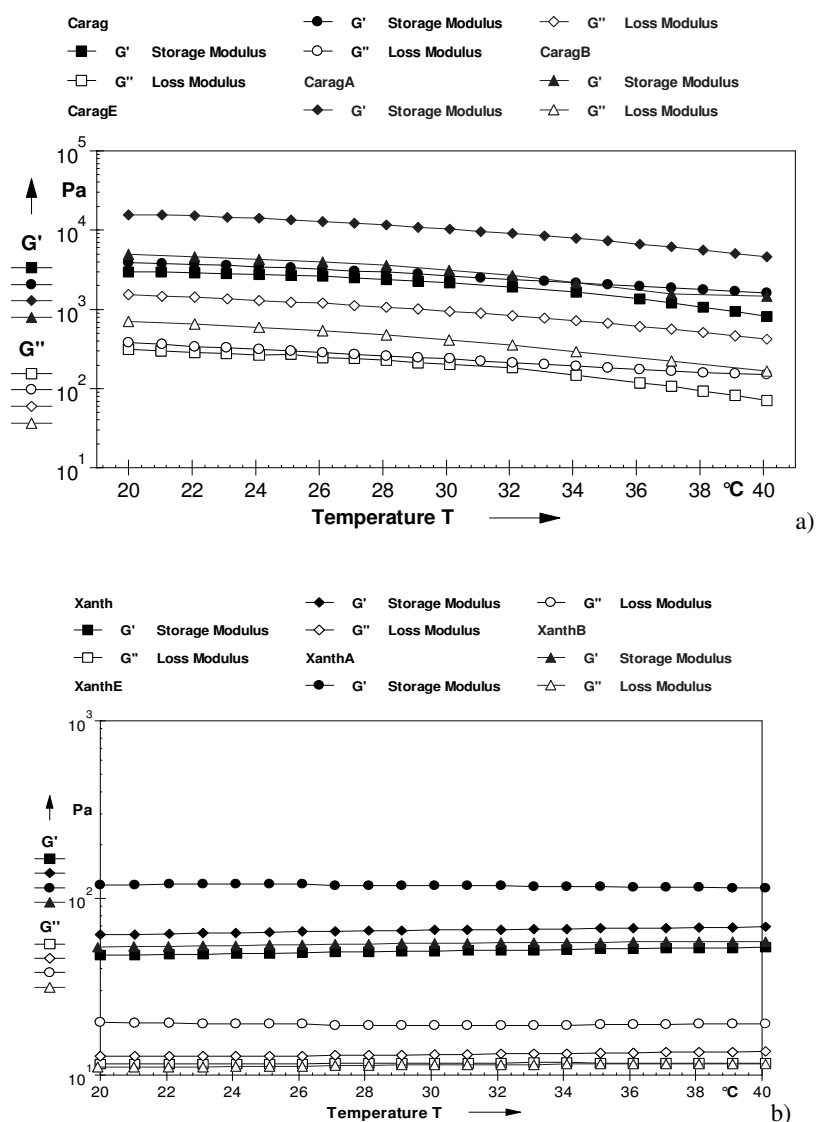


Figure 4: Variation of dynamic moduli with temperature for a) carrageenan gel (Carag), carrageenan gel with ethanolic extract (CaragE), carrageenan gel with acid extract (CaragA), carrageenan gel with alkaline extract (CaragB); b) xanthan gel (Xanth), xanthan with ethanolic extract (XanthE), xanthan with alkaline extract (XanthB) and xanthan with acid extract (XanthB)

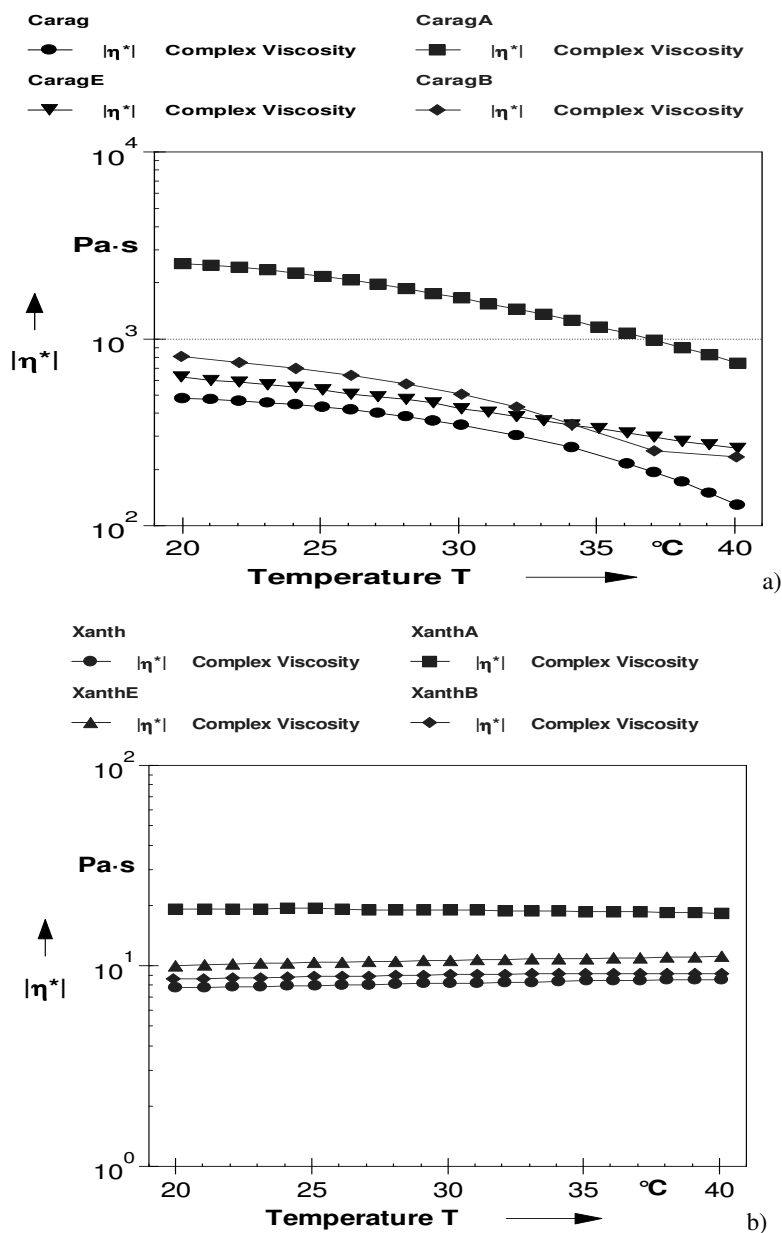


Figure 5: Variation of complex viscosity for a) carrageenan gel (Carag), carrageenan gel with ethanolic extract (CaragE), carrageenan gel with acid extract (CaragA), carrageenan gel with alkaline extract (CaragB); b) xanthan gel (Xanth), xanthan with ethanolic extract (XanthE), xanthan with alkaline extract (XanthB) and xanthan with acid extract (XanthB)

Although the zero shear viscosity values for xanthan gels are low, of 10^3 Pa·s order, these systems can be considered strong gels, structured well enough to have good stability at rest and during their storage.

One can appreciate that the addition of the active ingredient (extract of *Ns*) induces an increase of the zero shear viscosity, thus it can be considered a thickening element for the tested samples under low shear and so it cannot be

considered a negative influence from the rheological point of view. The most significant increase of η_0 can be noticed when HCl extract of *Ns* is added, in both groups of samples, which might be explained by the formation of new physical links between polymer chains.

The influence of temperature on defining viscoelastic characteristics was made evident by plotting the dynamic moduli and complex viscosity against temperature at constant values of

strain, according to Table 2, and frequency ($f = 1$ Hz). Figures 4 and 5 present the results for the temperature sweep tests with storage and loss moduli variation for carrageenan (a) and xanthan (b) samples. Temperature sweep tests were performed in the interval between 20 and 40 °C, at 1 °C/min, considered suitable for simulating the use of skin care products.¹⁹

One can appreciate that if for carrageenan gels a very slight decrease in elastic and viscous moduli occurs over the applied temperature range, xanthan gum gels seem to be unaffected by the temperature. These facts allowed the conclusion that temperature changes within the temperature range considered have no influence on the characteristics of the studied systems.

CONCLUSION

Carrageenan and xanthan gels were successfully tested with and without active ingredient. Amplitude sweeps showed large limiting values for the viscoelastic domain in the xanthan gels and a gel character over the entire domain of deformation. Even if carrageenan gels presented lower limiting values of the linear viscoelastic domain and cross-over points could be found for all the tested samples, it seems that adding acid extract of *Ns* would improve the rheological properties of the gels.

On the other hand, it turned out that carrageenan gels possess a greater stability on storage, with or without active ingredient. Furthermore, it could be observed that adding an extract of *Ns* to carrageenan gels improves their stability, as proven by the higher values of G' modulus. For xanthan gels, only acid and alkaline extract of *Ns* improves their stability, but even so, they still do not match the carrageenan ones. Temperature variation does not affect any of the studied gels, within the applied domain.

Finally, the assumption could be advanced that adding an active ingredient, such as an acid, alkaline or alcoholic extract of *Ns*, to xanthan or carrageenan gels generally results in improving their rheological properties.

ACKNOWLEDGMENTS: This paper was realized with the support of EURODOC “Doctoral Scholarships for Research Performance at European Level” (ID-59410) project, financed by the European Social Fund and Romanian Government. The rheological tests were performed in the Rheology Laboratory on the Interdisciplinary Training and Research Platform

“High performance multifunctional polymeric materials for medicine, pharmacy, microelectronics, energy/information storage, environmental protection” in the Natural and Synthetic Polymers Department of “Gheorghe Asachi” Technical University of Iasi.

REFERENCES

- ¹ W. Helbert, in “Gums and Stabilizers for the Food Industry”, edited by P. A. Williams and Glyn O. Philips, RSC Publishing, 2011, pp. 19-27.
- ² A. Imeson, in “Handbook of Hydrocolloids”, edited by G. O. Philips and P. A. Williams, CRC Press, 2000, pp. 106-121.
- ³ L. Kong and G. R. Ziegler, *Food Hydrocolloid.*, **46**, 25 (2011).
- ⁴ J. A. Fernández-Romero, C. J. Abraham, A. Rodriguez, L. Kizima, N. Jean-Pierre *et al.*, *Antimicrob. Agents Ch.*, **56**, 1 (2012).
- ⁵ F. G. Ochoa, V. E. Santos, J. A. Casas and E. Gomez, *Biotechnol. Adv.*, **18**, 7 (2000).
- ⁶ S. M. T. Gharibzahedi, S. M. Mousavi, M. Hamed, F. Khodaiyan and S. H. Razavi, *Carbohydr. Polym.*, **87**, 2 (2012).
- ⁷ I. E. Raschip, E. G. Hitruc, A. M. Oprea, M. C. Popescu and C. Vasile, *J. Mol. Struct.*, **887**, 1-3, (2011).
- ⁸ M. V. Dinu, S. Schwartz, I. A. Dinu and E. S. Dragan, *Colloid. Polym. Sci.*, **396**, 1 (2012).
- ⁹ J. Liu, H. Liu, H. Kang, M. Donovan, Z. Zhu and W. Tan, *Anal. Bioanal. Chem.*, **402**, 1 (2012).
- ¹⁰ H. Hosseinzadeh, *J. Chem. Sci.*, **122**, 4 (2010).
- ¹¹ R. Yoshida and T. Okano, in “Biomedical Applications of Hydrogels Handbook” edited by R. M. Ottenbrite, K. Park and T. Okano, Springer, 2010, pp. 19-43.
- ¹² M. Dogan, O. S. Toker, T. Aktar and M. Goksel, *Food Biophys.*, **6**, 4 (2011).
- ¹³ A. S. Harris, E. Svensson, Z. G. Wagner, S. Lethagen and I. M. Nilsson, *J. Pharm. Sci.*, **77**, 5 (1988).
- ¹⁴ I. G. Mandalaa and E. D. Palogou, *Int. J. Food Prop.*, **6**, 2 (2003).
- ¹⁵ T. G. Mezger (ed.), “The Rheology Handbook for Users of Rotational and Oscillatory Rheometers”, Coatings Compendia, 2006, pp. 80-88.
- ¹⁶ V. Sahni, T. A. Blackledge and A. Dhinojwala, *Nat. Commun.*, **1**, 1 (2010).
- ¹⁷ M. Lungu, L. G. Halițchi, O. Darabă, A. Roșu, “Elemente de formulare și caracterizare a unor preparate cosmetice și de îngrijire” (in Romanian), edited by M. Lungu, Editura Apollonia, 2012, pp. 65-75.
- ¹⁸ L. Shena, X. J. Lia, Jia-Cai Lib and K. M. Yub, *J. Macromol. Sci. Phys.*, **51**, 2 (2012).
- ¹⁹ M. Nikoo, X. Xu, S. Benjakul, G. Xu, J. C. Ramirez-Suarez *et al.*, *Int. Aquat. Res.*, **3**, 2 (2011).