

Polymer - collagen Biocomposites

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Natural polymers, or polymers derived from living creatures, are of great interest in the biomaterials field. Collagen is the most widely found protein in mammals (25% of our total protein mass!) and is the major provider of strength to tissue. The paper presents a study regarding obtaining and characterization of biocomposite based on vinyl-acrylic copolymers as latexes and collagen polypeptides, in the following variants: blends, intermolecular complexes and interpenetrated polymeric structures. The present work discusses the results obtained on the rheological behaviour of the fluid mixtures. Regression analysis of the experimental rheological data illustrates that the latex of vinyl-acrylic copolymer is characterized by a second-order polynomial model, while the composites including collagen hydrolysates are preferentially fitted with third-order polynomial model. It has been proved the influence of the molecular weight and the concentration of collagen hydrolysate aqueous solutions on the viscosity and flowing index of polymer mixture in fluid state.

Keywords: biocomposite, collagen, blends, acrylic biopolymer

Due to environment and sustainability issues, this century has witnessed remarkable achievements in green technology in the field of materials science through the development of biocomposites. The development of high-performance materials made from natural resources is increasing worldwide.

The field of biomaterials is multidisciplinary and the design of biomaterials requires the synergistic interaction of material science, biological science, chemical science, medical science and mechanical science. It is necessary to develop cross-disciplinary approach in designing new biomaterials.

Collagen is the most widely found protein in mammals (25% of our total protein mass!) and is the major provider of strength to tissue. A typical collagen molecule consists of three intertwined protein chains that form a helical structure (similar to a spiral staircase). These molecules polymerize together to form collagen fibers of varying length, thickness, and interweaving pattern (some collagen molecules will form ropelike structures, while others will form meshes or networks). There are actually at least 15 different types of collagen, differing in their structure, function, location, and other characteristics.

Composite materials from latexes (CML) define a special type of polymeric composite, characterized by the fact that its realization involves the participation of at least a compound in the form of latex [1, 2].

The quality of CML may be attributed to several polymeric systems, assuming a close correlation with the structure and processing methods of the latexes constituted of a single macromolecular compound:

- latexes with composite particles (LCP), frequently called "composite latexes", similar with the traditional latexes, from the point of view of the structure of the disperse system, i.e., with a single type of solid phase. Their particular character is due to the solid morphology, in which several polymeric phases (possessing different structural characteristics) with either adjacent or interpenetrated dispersion may be identified [3-6]. The composite latexes

result from chemical processes involving polyreactions between monomers or, respectively, polymers and monomers [7, 8];

- the solid-liquid disperse media based on latexes (DML) result when mixing a latex (with monophasic -LMP- or composite - LCP- particles) with another polymer. The second polymeric partner may occur in quite various forms, such as: powder, fibers, textile materials, leather, another latex or an aqueous solution of polymer. The specific character of the DML is given by the presence of the three major compounds of the resulted composite material: polymer 1, polymer 2 and water [9-11];

- the polymeric solid from latexes (LS) represents the composite material resulted by removing water from either LP or DML, via specific processing operations (drying or coagulation-drying). The intercoupled or IPN-type configurations result from reactions between the polymeric partners, either directly or by means of certain auxiliaries introduced in the system during the DML preparation [12].

The DML-composite materials and from that ones the LS-type, may be theoretically obtained by three different procedures [13]:

·the dilution method, which involves mixing the polymeric latex with polymer solutions less concentrated than the latex;

·the mixing method at constant concentration, when the polymer solution added to the latex has the same concentration as the liquid-solid disperse system;

·the concentration method, when mixing the polymer latex with a polymeric solid which will be dissolved by the water representing the latex dispersion medium.

The first of the above-mentioned procedures is applied in most of the applications, as the interaction effect between the two polymers in solid state is neglected and only the modification of the mixture viscosity is followed, in order to permit a certain profiling technology of it. In the case of the dilution method, the function of "thickener" is assured by polymers with high molecular weights (over 100,000 Da) and very good water solubility (cellulosic

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derivatives, gelatin, polyvinyl alcohol, etc.). The mass fraction of the polymer soluble in the ternary system being usually around 0.005-0.01, the final solid composite will contain less than 4% of thickener polymer.

The method of mixing at constant concentration is used to obtain disperse mixtures with a content of dry matter not exceeding 10%. The system viscosity (below 5,000 cP) recommends it exclusively for stratifications via pulverization, as well as for some methods involving application with cylinders.

The obtaining of mixtures via the concentration method may be applied only for macromolecular compounds having a high water affinity and molecular weights below 100,000 Da.

The present paper discusses the results of a complex study concerning the obtaining and characterization of some DML and LS-type composite materials, based on vinyl-acrylic copolymer latexes and collagen polypeptides, in the following variants: latex blends (LB), intermolecular complexes (ICL) and interpenetrated polymeric structures from latexes (IPN-L). This first note of our study presents the rheological behaviour of the fluid mixtures.

Experimental part

Materials and methods

The vinyl-acrylic copolymer latex (VACL1), used to form the composite materials, has been synthesized and characterized according to the methodology described elsewhere [14, 15]. The latex characteristics are listed in table 1.

The two collagen polypeptides (collagen hydrolysates), CH1 and CH2, used in the study, have been obtained by neutral hydrolysis of untanned leather wastes [16-19] and atomization of the solution obtained. Their characteristics are reported in table 2.

The rheological characterization of the fluid mixtures has been performed on a rheo-viscosimeter with coaxial cylinders (Rheotest 2, VEB MLM, Germany). The pair of cylinders has been selected taking into account that the device recorder should cover the range between 10 and 90, over the whole available shearing domain (0.17...1312 s⁻¹). Experimental determinations have been carried out at 20°C, in both increasing and decreasing direction of the shear rate (i.e., step-up and step-down measurements),

with a 5 min. pause among the 18 shear rate steps of the rheometer.

The rheological characteristics have been calculated with the following relations:

$$\tau = z \cdot \alpha \cdot [\text{dyn} / \text{cm}^2] \quad (1)$$

$$\eta = \frac{\tau}{\gamma} \cdot 100, [\text{dyn} \cdot \text{s} / \text{cm}^2] \quad (2)$$

where: τ -shear stress, α -indication of device, [Sk]; z -cylinders' constants [$\frac{\text{dyn}}{\text{cm}^2 \cdot \text{Sk}}$]; η -dynamic viscosity, [cP]; γ - shear rate, [s⁻¹].

The fluid disperse mixtures are generally considered as non-Newtonian fluids, presenting a shear rate dependence which may be approximated by means of several models. In order to explain their complex behaviour (similar to those observed for solutions with a viscosity higher than 1,000 cP), a polynomial model expressed by the relation:

$$\ln \eta = f_0(x_1, \dots, x_n) + \sum_1^k f_i(x_1, \dots, x_n) \ln(\gamma)^i \quad (3)$$

has been selected. Here, x_1, \dots, x_n represent the main factors (combination ratio, concentration, pH, temperature etc.) able to influence the fluid rheological constants, while their functions f_0, \dots, f_n appear as material coefficients. By substituting in relation (3) the functions corresponding to the material coefficients, one may obtain the following relation:

$$\ln \eta = c_0 + c_1 \ln \gamma + c_2 \ln^2 \gamma + \dots \quad (4)$$

Estimation of the c_0, c_1, c_2, \dots values involved polynomial regression. The degree of the rheological polynomial model controls the efficiency of the polynomial fitting, consequently, for each system the situation which generates a correlation coefficient higher than 0.85 has been selected. However, the necessity of reducing the correlation coefficient became obvious when we observed that, in most cases, there are great differences between the rheological behaviour of the pure copolymer latex and the latex/soluble polymer mixture. Thus, all fittings have been performed with polynomial models with a same degree, for all the combination ratios of a given type of mixture. The physical meaning of c_0 from equation (3) is

Characteristic	Unit	Value
1. Copolymer chemical composition		
- styrene	%	41.41
- butyl acrylate	%	49.49
- acrylic acid	%	5.05
- N- methylol acrylamide	%	5.05
2. Copolymer physico-chemical characteristics		
- intrinsic viscosity	dL/g	0.4886
- vitrification temperature	°C	14.5
3. Latex physico-chemical characteristics		
- content of solid substance	%	38.6
- superficial stress	dyn/cm	35.59
- pH	g NaCl	5.61
- stability	100g latex	0.427

Table 1
VACL1 CHARACTERISTICS

Type of CH	Content of mineral substances	N ₂	NH ₂ ^α	[η]	Humidity content	I.E.P.
	%	%	m.moles/g		%	
CH1	1.236	17.49	0.623	0.0688**	4.52	5.03
CH2	1.236	17.44	0.412	0.0989	3.87	4.74

Table 2
CH^{*} CHARACTERISTICS

*Characterization has been made according to data given in [16].

** in 1M KCl solution at 25°C.

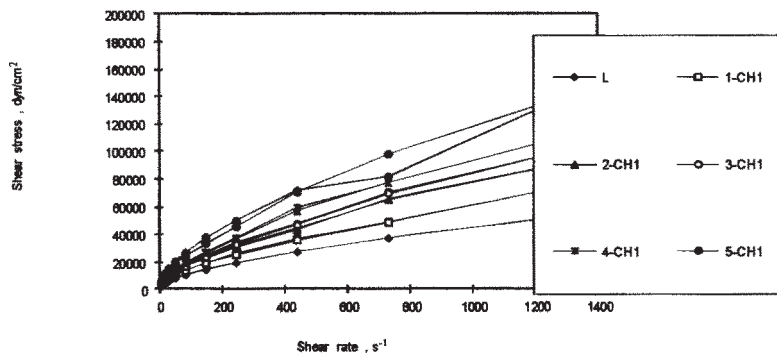


Fig. 1. Shear stress as a function of shear rate, in the case of the disperse mixtures L-CH₁

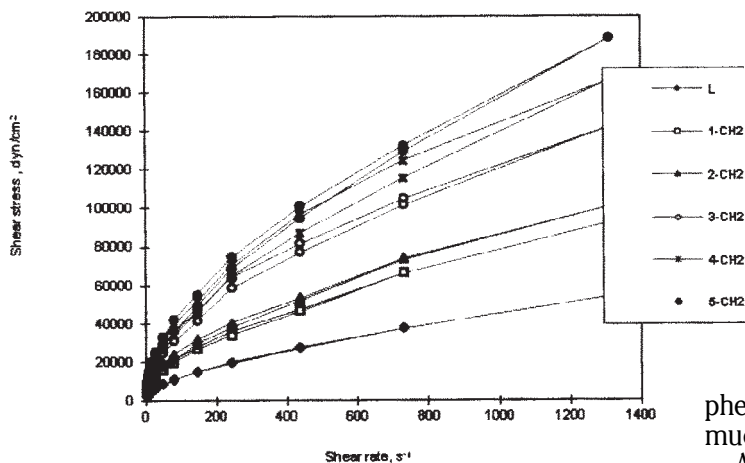


Fig. 2. Shear stress as a function of shear rate, in the case of the disperse mixtures L-CH₂

the logarithm of standard viscosity corresponding to a shear rate of 1 s⁻¹. In consideration of interpretation necessities, the rheological coefficient c_1 was converted into a flowing index (n), by employing the following relation:

$$n = c_1 + 1 \quad (5)$$

The DML-type composite material has been obtained by adding collagen hydrolysate powder, either CH₁ or CH₂, into the vinyl-acrylic copolymer latex and by mixing the entire system (with a stirrer with pallets - 60rpm), for 4 h, at a temperature of 30°C. The mixture obtained was left to rest for 12 h., then mixed again for 30 min. and again left to rest for 24 h.

Following the mentioned method, 10 different mixtures (called 1-CH₁...5-CH₁ and 1-CH₂...5-CH₂) with hydrolysate content varying between 1...5g at 100g latex (gravimetric fraction 0.0099...0.04761) have been obtained.

Results and discussions

The influence of the soluble polymer content on the shape of the rheological diagrams, describing the shear stress variation with the shear rate, is presented in figure 1 and 2.

Analysis of these diagrams indicates that all compositions taken into study behave as pseudo plastic, non-Newtonian fluids. The increase of the soluble polymer content generates an increase of the shear stress, for the same shear rate. The rheological diagrams also reveal that there is an influence of the molecular weight of the collagen hydrolysate, both from the point of view of the final shear stress and dependence shape, for a same shear rate and CH concentration.

By applying step-up and step-down measurements (with a 5 min. period between two different shear rates), it may be observed that the mixtures containing collagen hydrolysate on higher dose than 3g/100g latex become time-dependent, both tixotropic and rheopexic

phenomena being manifested. The time-dependence is much more significant in the case of CH₂, than for CH₁.

A very interesting observation is that, when using soluble polymer with higher molecular weight, the character of time-dependence is only tixotropic while, in the case of the lower molecular weight CH₁, a dilatation effect also appears. A possible explanation for such behaviour might be the cluster effect, when the soluble phase itself forms associates which include the polymeric solid from the latex. Shifting from one type of time-dependence to another is caused by the modification of the size distribution of the particles constituting the solid phase, fact evidenced by several studies on disperse media [19]. Mixtures containing large particles, with an average diameter larger than 5μm, present a dilated behaviour due to the penetration of the dispersion medium in the space between particles, as a consequence of increasing the shear rate. When the average size of the particles forming the solid phase is about 1μm or even lower, the fluids present a tixotropic behaviour.

In our case, the collagen hydrolysate with lower molecular weight, CH₁, is more easily adsorbed on the surface of the polymer particles forming the solid phase, fact that induces an increase of particle sizes and, implicitly, generation of the dilatation effect.

The CH₂ collagen hydrolysate presents a more reduced adsorption capacity, which means that it tends to remain in the dispersion medium and thus it does not modify the apparent particle size of the solid phase from the latex.

It has been demonstrated beyond any doubt [20] that the polymer absorbability on the surface of the solid phase from a latex is strongly affected by the molecular weight and superficial electrical potential of the solid. From this point of view, as a result of a higher content in carboxylic groups on the polypeptide chain, CH₂ will generate much stronger repulsion effects than CH₁, situation providing additional arguments in favour of the experimentally demonstrated phenomenology.

The regression analysis of the experimental data was performed by using a polynomial rheological model (table 3 and 4), considered the most suitable approximation of the viscosity dependence on the shear rate. This analysis

Mixture	ln k	a ₁	a ₂	a ₃	COR	SDA
	11.809	-0.502			0.9947	0.0757
Latex (L)	12.093	-0.676	0.0209		0.9997	0.0182
	12.044	-0.626	0.00723	0.0011	0.9998	0.0184
	12.044	-0.494			0.9925	0.0888
L - 1 CH ₁	12.362	-0.688	0.0234		0.9989	0.0348
	12.257	-0.581	-0.00603	0.00237	0.9991	0.0356
	12.208	-0.484			0.9932	0.0828
L - 2 CH ₁	12.515	-0.672	0.0227		0.9995	0.0234
	12.409	-0.564	-0.00727	0.00241	0.9997	0.0193
	12.242	-0.482			0.9922	0.0863
L - 3 CH ₁	12.548	-0.671	0.0233		0.9995	0.0212
	12.431	-0.553	-0.00938	0.00263	0.9998	0.0138
	12.204	-0.452			0.9929	0.0793
L - 4 CH ₁	12.487	-0.626	0.0209		0.9989	0.0318
	12.466	-0.604	0.0149	0.00048	0.9989	0.0335
	12.634	-0.489			0.993	0.0852
L - 5 CH ₁	12.898	-0.65	0.0195		0.9975	0.0533
	12.968	-0.721	0.039	-0.00157	0.9976	0.0557

COR= correlation coefficient, SDA = standard deviation of assessment.

Mixture	ln k	a ₁	a ₂	a ₃	COR	SDA
	11.809	-0.502			0.9947	0.0757
Latex (L)	12.093	-0.676	0.0209		0.9997	0.0182
	12.044	-0.626	0.00723	0.0011	0.9998	0.0184
	12.640	-0.543			0.9959	0.0718
L - 1 CH ₂	12.884	-0.693	0.01808		0.9991	0.0355
	12.713	-0.519	-0.0298	0.00386	0.9995	0.0283
	12.796	-0.551			0.9963	0.0698
L - 2 CH ₂	13.055	-0.7103	0.0191		0.9997	0.0197
	13.000	-0.657	0.00442	0.001186	0.9997	0.0195
	13.278	-0.569			0.9969	0.0651
L - 3 CH ₂	13.509	-0.711	0.017		0.9995	0.0272
	13.393	-0.593	-0.0153	0.0026	0.9996	0.0235
	13.189	-0.528			0.9978	0.0509
L - 4 CH ₂	13.360	-0.633	0.0126		0.9994	0.026
	13.323	-0.596	0.00224	0.00084	0.9995	0.027
	13.177	-0.514			0.9978	0.05
L - 5 CH ₂	13.362	-0.627	0.136		0.9998	0.0151
	13.284	-0.548	-0.0081	0.0017	0.9999	0.0114

COR= correlation coefficient, SDA = standard deviation of assessment.

Table 3
REGRESSION ANALYSIS OF THE
RHEOLOGICAL CHARACTERISTICS FOR THE
LATEX/CH₁ MIXTURES

Table 4
REGRESSION ANALYSIS OF THE
RHEOLOGICAL CHARACTERISTICS FOR THE
LATEX/CH₂ MIXTURES

indicates that the vinyl-acrylic copolymer latex is characterized by a second-order polynomial model, while the composites including collagen hydrolysates are preferentially fitted with third-order polynomial models. The accuracy of our rheological model, which assumes the intervention of the 2nd and 3rd order terms, may be discussed in the case of stratifications with thickness below 0.1 mm, performed by means of devices with cylinders. The rougher stratification systems, i.e. the devices with knives, remain practically insensible to the higher-order coefficients, as the shear stress generated in the material mass is reduced.

The rheological models we have chosen permitted to fit the experimental data quite well [21] (correlation coefficient higher than 0.999), which means that the linear models are sufficient for the objectives of the present study.

The accuracy of the linear rheological models for the mixtures under study is exemplified in figure 3. The rheological diagrams plotted in figure 3 show that the addition of soluble polymer in the latex of vinyl-acrylic copolymer does not affect significantly the rheological behaviour of the initial aqueous dispersion. This affirmation is based on the observation that the linearity is maintained, some modifications appearing only with respect to the slope of the line and to the interception value. These two characteristics are affected by the differences of molecular weight and polarity between the two collagen hydrolysates selected for the exemplification of the rheological behaviour.

Considering the quantification concept, one may state that CH₂ is much more pseudoplastic than CH₁,

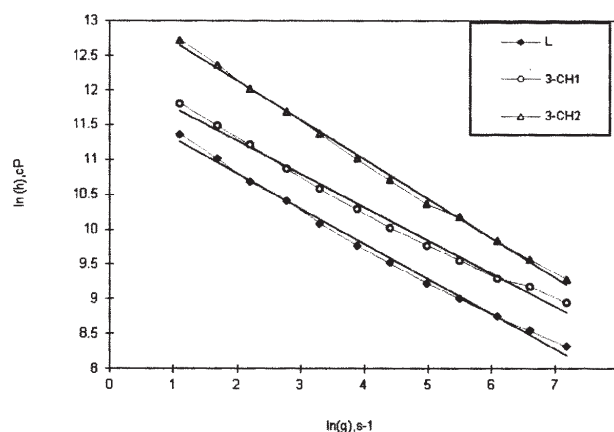


Fig. 3. Viscosity evolution as a function of shear rate, for fluids based on vinyl-acrylic copolymer latex

comparatively with the vinyl-acrylic copolymer latex. The influence of the soluble polymer content on the standard viscosity of the composites containing vinyl-acrylic latex is shown in figure 4.

The general effect of the increasing of the collagen hydrolysate content into the latex/CH mixture is an increase of the mixture viscosity, according to the specific dependence of each type of soluble polymer used. For instance, the CH₁ assures an approximately linear increase of viscosity, easy to control and adjust for profiling necessities. The CH₂ presents a more complicated effect: up to 3 % concentration, it assures a much more pronounced increase of viscosity of the mixture; above

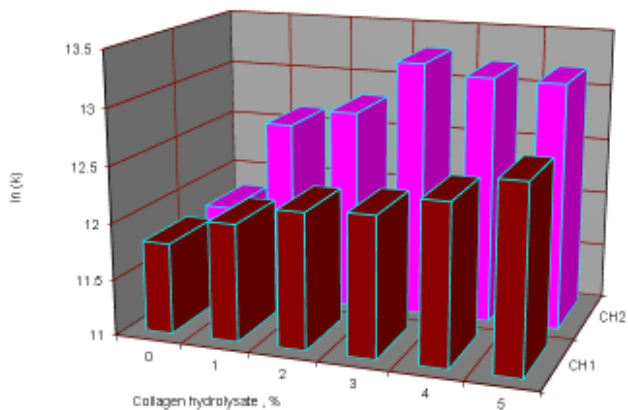


Fig. 4. Influence of the concentration and the HC molecular weight on the consistency index (or the coefficient of standard viscosity) of the L - CH mixtures

this concentration, the CH₂ tends to decrease the system viscosity. According to the theories [22] regarding the flow of disperse systems, the increase of the solid phase content (when the characteristic particle size remains constant) should generate a monotonous increase of viscosity. The fact that, in the case of CH₂ mixtures, the content of dry matter increase, while viscosity decreases, may have two causes, as follows: some modifications of the granulometric characteristics of the solid or the occurrence of a phase separation phenomenon. The phase separation, via flocculation, is accompanied by formation of some large associates, which tend to sedimentate, thus practically reducing the content of solid phase of the latex as such.

In order to elucidate which of these two phenomena causes the decrease of the viscosity of the mixtures with more than 3% CH₂, the composites have been subject to centrifugation (5,000 rpm, for 60 min., at 20°C), in order to detect the existence of any sediment induced by flocculation. In the applied experimental conditions, flock formation could not be evidenced.

Consequently, the mixtures containing more than 3% CH₂ are characterized to a compression effect, due to the electrostatic repulsion induced by the excess of polypeptides dissolved in water. By compacting the external layer, the particle size decreases and that generates the decreasing of viscosity. The compacting effect is felt through the reduction of the water content in the double diffuse layer and also through the occurrence of a false dilution phenomenon.

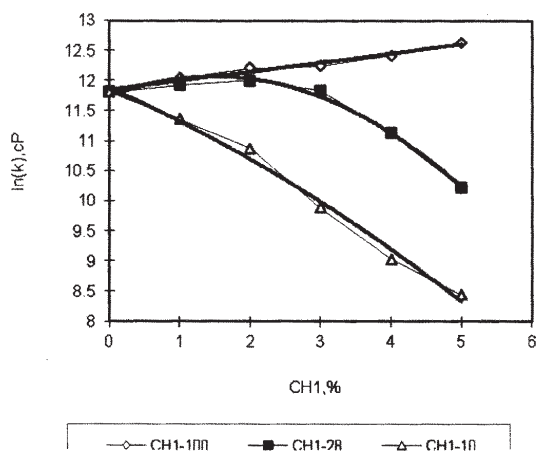


Fig. 6. Influence of the concentration of the CH1 solution on the variation of the consistency index, as a function of soluble polymer content

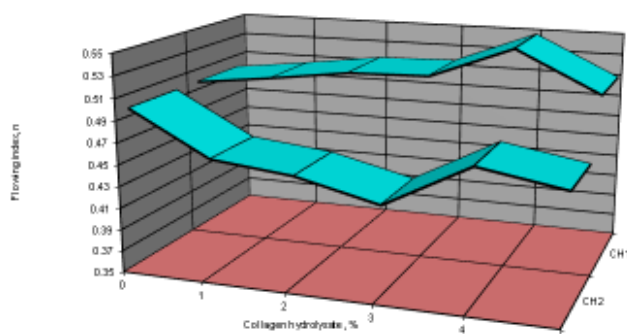


Fig. 5 Influence of the concentration and the CH molecular weight on the flowing index of the L - CH mixtures

This hypothesis is supported by the results presenting the influence of the concentration and the molecular weight of the soluble polymer added in the latex, on the flowing index of the mixture (fig. 5).

The evolution of the flowing index, as a function of the soluble polymer content, shows that the CH₁ leads to mixtures which tend to diminish their non-Newtonian character (increase of the *n* value), while CH₂ has an opposite behaviour, i.e., intensification of the pseudo-plasticity. The deviation from the ideal state is due to the fluid morphology, especially to the granulometric characteristics of the disperse phase. As already mentioned, passing from a pseudo plastic fluid towards a dilatation one occurs due to a gradual increase of the particles size and to a simultaneous modification of granulometric distribution (unimodal @ bimodal @ unimodal). In our case, the non-uniform increase of the particle size may appear only if clusters are formed. This phenomenon is easy to detect in the case of low concentrated latex/CH₁ (1..4%), and, respectively, in the case of latex/CH₂ mixtures with more than 3% soluble polymer.

The complex phenomenology that may be evidenced when the latex/CH mixtures are obtained via the concentration method becomes irrelevant when applying the dilution method, as shown in figure 6.

When using CH₁ solutions with concentrations higher than 25%, the effect of viscosity increase may be attained only for low doses (up to 3%). That is in reason of the pure effect of polypeptide adsorption on the surface of the synthetic polymer particles and, implicitly, of the influence induced by size modifications of the disperse phase. When the quantity of the CH₁ solution 28% added to the latex is increasing, the additional contribution of water introduced in the system exercises a much more significant effect than the one corresponding to the increase of the particle size and, consequently, the mixture viscosity decrease.

The viscosity of mixtures prepared with a 10% solution of CH₁, is decreasing continuously, when increasing the ratio of soluble polymer solution versus the latex. Obviously, the result is due to the additional contribution of water, which cannot be compensated by the reduced amount of polypeptide absorbed by the synthetic polymer.

However, the effect of increasing viscosity may be significant in the case of the dilution method, as well, by a careful upgrading of the effect of the molecular weight and of the polymer polarity. This observation is supported by the experimental data plotted in figure 7.

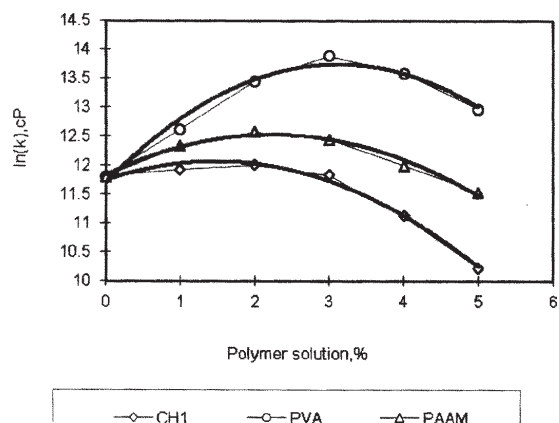


Fig. 7. Influence of the soluble polymer nature and the proportion of solution of 28% concentration added into acrylic latex, on the consistency index of the composite (PVA=polyvinyl alcohol; PAAM=polyacrylamide)

Conclusions

The synthesized latexes of vinyl-acrylic copolymers may be used for mixing with aqueous solutions of water-soluble macromolecular solids, such as collagen hydrolysates, without any phase separation over the whole domain of mixing ratio under study.

The procedure of "thickening" of the latex by means of collagen hydrolysate powders leads to pseudo plastic, non-Newtonian fluids. By increasing the content and average molecular weight of the soluble polymer, the system viscosity increases, concomitantly with the occurrence of a time-dependence (either tyxotropic or rheopexic) behaviour.

Over the whole concentration domain of the collagen hydrolyzates taken into study (1..5g CH/ 100g latex), the rheological behavior of the mixtures is well approximated by polynomial models of the second and third order.

The research results can be used in the field of obtaining biomaterials with application in industry [24], medicine [25, 27], cosmetics, agriculture [26], etc.

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