DOI: 10.1002/masy.200650710

Interaction of Maleic Acid Copolymers with Collagen

1. Homogeneous and Phase Separating Systems

Adina Graziella Anghelescu-Dogaru,[1,2] *Gabrielle Charlotte Chitanu,*[*1]
Heikki Tenhu[2]

Summary: One of the present trends to increase the efficiency of the chrome tanning process is the use of chemicals which change the affinity and induce a better binding of the chromium ions in the hides. Recently, some anionic polyelectrolytes based on poly(acrylic acid) or maleic acid copolymers were developed as additives in the chrome tanning. The effect of polyelectrolytes could be explained by their interaction with the collagen from hide. In our work four maleic acid copolymer/collagen systems were investigated by turbidimetric, conductometric and potentiometric titrations. The interaction between the two macromolecular compounds was clearly evidenced, and its dependence on the chemical structure of the maleic acid copolymer and on the amount of added chromium ions was studied. Homogeneous systems or turbid dispersions were observed depending on the hydrophilic or hydrophobic character of the maleic copolymer, on the [CH]/[MP] ratio, and on the concentration of the chromium ions.

Keywords: chrome tanning; collagen; maleic acid copolymers; polyelectrolytes; proteins; supramolecular structures

Introduction

One of the most important challenges of the contemporary world is the environment protection in order to preserve the balance of the ecosystem. The leather industry is one of domains encountering difficulties related to the requirements concerning the discharge of wastewater and the treatment of sludge. Chromium salts are among the most popular tanning agents. Chrome tannage is distinguished by its effectiveness conferring high thermal stability due to the coordination of the carboxyl groups of protein to chromium complexes.[1] In the chrome tanning the chromium salt is used together with masking agents. Such masked chrome solutions are characterized by their mild and slow tanning action and further, their tanning ability at high pH.[2,3] There are also some studies regarding a new technology of an ultra-rapid sulpho-syntan tannage consisting of the successive action of a basic and then acid liquor without pre-tanning. This technology leads to a significant reduction in the total tanning time. The tanning liquors are recycled after simple adjustment of parameters.[4] Previously the interaction in heterogeneous systems between the synthetic additives of sulpho-syntan type and the collagen was studied as a specific case of interaction between two oppositely charged polyelectrolytes, producing a polyelectrolyte complex formation.[5]

Recently, some anionic polyelectrolytes based on poly(acrylic acid) or maleic acid copolymers were developed as additives in the chrome tanning. It was shown that the quality and the properties of the tanned hides are dependent on the chemical struc-

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[1] ''Petru Poni'' Institute of Macromolecular Chemistry, Bioactive and Biocompatible Polymers Department, Al. Gr. Ghica Voda 41A, 700487 Iasi, Roumania
Tel.: +40 232 217454, Fax +40 232 211299.
E-mail: chita@icmpp.ro

[2] Laboratory of Polymer Chemistry, University of Helsinki, PO BOX 55, FIN 00014, Helsinki, Finland

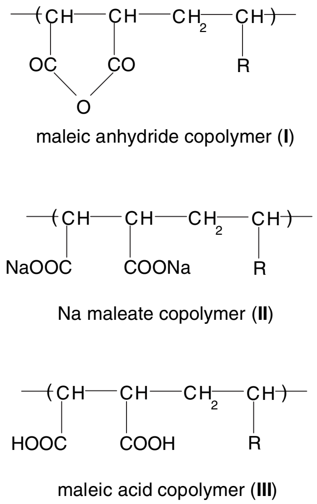
ture of the polymeric additive. [6,7] The formation of polyelectrolyte complexes (PEC) of collagen hydrolysates with carboxymethyl cellulose has also been investigated[8] as well as the properties of the insoluble complexes.[9,10] An amphoteric copolymer of acrylic acid and methacrylethyltrimethylammonium chloride has been proposed as a performant retanning agent.[11] The system tanning float/hide/polyelectrolyte is a complex one and the data concerning the mechanism of action of the polymeric additives are rather scarce. In this paper new results concerning the interaction between collagen hydrolysate (CH) and several maleic acid copolymers (maleic polyelectrolytes – MP) with various hydrophilicity/hydrophobicity are presented. We have also investigated the effect of the chromium ions on the interaction between MP and CH. The results are rationalised in terms of the formation of PEC between CH and MP.

Experimental

Polymer Synthesis

The copolymers of maleic acid (maleic polyelectrolytes – MP) were obtained from maleic anhydride copolymers according to the reactions summarized in the Scheme 1. Maleic anhydride (MAn) copolymers (**I**) with vinyl acetate (VA), N-vinylpyrrolidone (NVP), styrene (St), or methyl methacrylate (MMA), were synthesized by radical polymerization as described elsewhere.[12–15] The monomers, the solvents, and the initiators (benzoyl peroxide, azobisisobutironitrile) were carefully purified according to the standard methods. The copolymerization was carried out at 70–80 °C, which results in an alternating distribution of the comonomers along the chain.[16] The copolymers were purified by repeated precipitation or by extraction, washed several times with diethyl ether and dried at 40 °C under reduced pressure for 48 hours. They were characterized by conductometric titration with aqueous 0.1N NaOH in 1:1 (vol) acetone-water mixture[17] and viscometric measurements in acetone at 30 °C[18–20] or in aqueous HCl solution having pH = 2.1 at 25 °C.[21] The characteristics of maleic anhydride copolymers are summarized in Table 1.

The copolymers (**II**) of sodium maleate (NaM) were obtained from **I** by hydrolysis with diluted aqueous NaOH at 20–60 °C for 12–24 hours. The solutions were desalted and the oligomers were removed by diafiltration, and the polymeric salts **II** were recovered by freeze-drying. From **II**



where R: $-N(CO)(CH_2)_3$, $-OCOCH_3$, $-(CH_3)COOCH_3$, or $-C_6H_5$

Scheme 1.
The obtention of maleic acid copolymers and their salts.

the copolymers (**III**) of maleic acid (MAc) were obtained by passing dilute solutions of **II** through an ion exchange column (for details see [22]). The aqueous solution of **III** was used as such.

Collagen hydrolysate (CH), produced by SC ICPI SA Bucharest, was obtained by alkaline hydrolysis of calf skin and used without additional purification. Its molecular weight estimated by viscometric measurements[23] was 40.000. Water was twice distilled and chromium acetate monohydrate was analytical grade reagent from Chimopar SA Bucharest, Roumania.

Methods

The conductometric titrations were carried out using a Radiometer Copenhagen conductivity meter type CD210 and a CDC865 cell. pH measurements were performed with a Metrohm automatic instrument type Titrino 716 DMS equipped with a combined glass electrode 6.0218.010. The titrating agent was added in portions of the same size (Monotonic End-Point Titration - MET).[24] Turbidimetric titrations were performed using a Brinkmann PC 900 apparatus equipped with a 20-23-634-5 probe tip.

Results and Discussion

Previously we have tested the sodium salt of maleic acid-vinyl acetate copolymers with various molecular weights as additives in the tanning of cattle hides with basic chromium salts. Our results, covered by patent applications, showed that using this polyelectrolyte the used concentration of basic chromium salts is lowered.[25,26] The Cr_2O_3 content in the waste liquor has also drastically decreased. The physico-chemical properties of the tanned leather fitted the quality standard. The results regarding the favorable effect of the maleic acid copolymers in the chrome tanning raised the question on the mechanism of action of the polymeric additive. In our previous papers[27,28] we studied the interaction in solution between maleic acid-vinyl acetate copolymer and collagen hydrolysate solutions in the presence or absence of chromium ions, varying the concentration of collagen solutions and reversing the order of the compounds addition. The results suggested that the interaction between collagen and maleic copolymer took place by both electrostatic and weak hydrophobic forces.

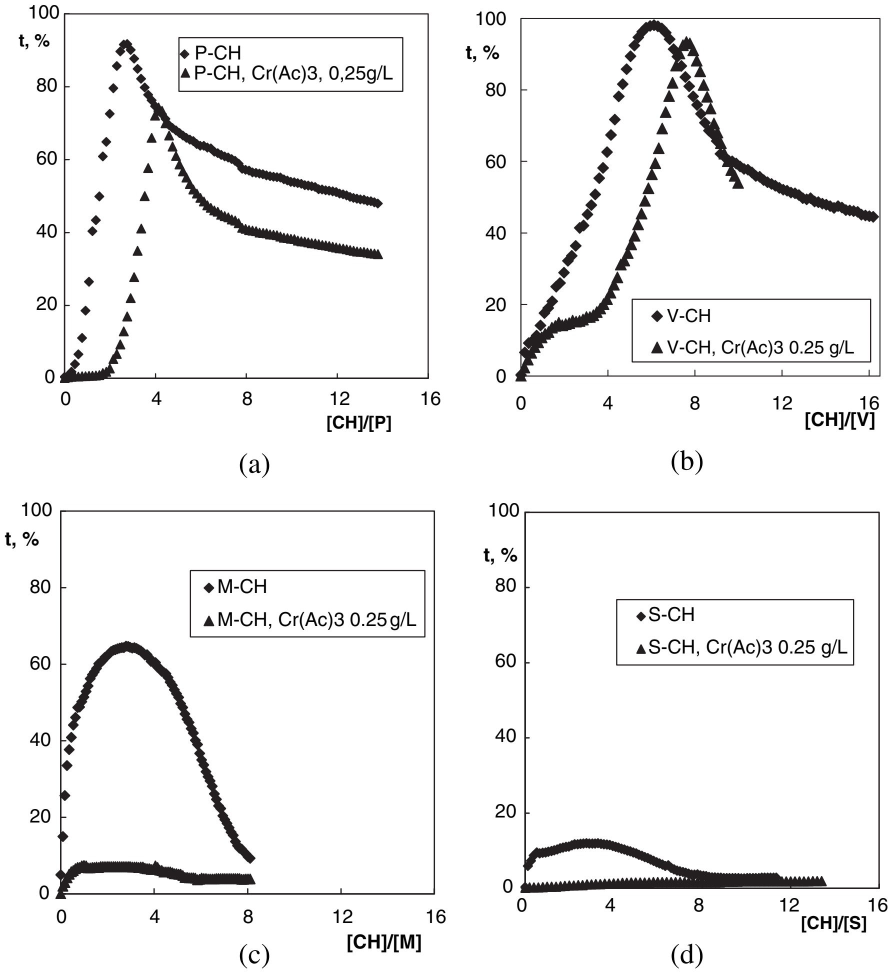
In order to verify this supposition maleic acid copolymers with hydrophilic or hydrophobic comonomers were synthesized whose characteristics were presented in Table 1. Aqueous CH solution with concentration of 10 g/L was added into maleic acid copolymer solutions with concentrations between 0.505 and 0.74 g/L. The changes of the systems were followed by turbidimetric, conductometric and potentiometric measurements, taking into account that this set of methods is recommended in the study of PEC systems in which a phase separation can be expected.[29] Low values of turbidity mean an interaction with keeping a homogeneous system, while the increase of turbidity indicate a phase separation following the interaction.

The Influence of the Chemical Structure of Maleic Polyelectrolyte

The turbidimetric titrations of maleic acid copolymers with aqueous CH solution are presented in Figure 1.

Table 1.
Maleic anhydride (MAn) copolymers

Sample code	Chemical structure	Chemical composition MA: comonomer (moles)	Molecular weight, M_v
P	MAn-NVP	1:1	28.000
V	MAn-VA	1:1	102.000
M	MAn-MMA	1:2	49.460
S	MAn-St	1:1.57	141.000



(a) (b) (c) (d)

Figure 1.
Turbidimetric titration of maleic acid copolymers P (a), V (b), M (c) and S (d) with aqueous CH solution with/without added chromium ions.

A very different behaviour can be noticed depeding on the nature of the comonomer in the maleic acid copolymers and on the presence or absence of chromium ions. Without added chromium ions, in all cases a sharp increase of turbidity is observed, followed by a decrease when further collagen solution was added. It should be mentioned that by titration of hydrophobic copolymers M and S the decrease of turbidity is due at least partially to a macroscopic phase separation, which is not observable with the other two copolymers. By titration of P or V copolymers in the presence of $Cr(Ac)_3$ we notice a slight increase of the turbidity, then a flat part, followed by an noticeable increase and a marked decrease. In the case of M or S copolymers only a slight increase of the turbidity may be observed then a flat part, the values of the turbidity being much smaller than those obtained with P or V copolymers. That suggests that the presence of chromium ions results in more homogeneous systems due to the interaction of CH with hydrophobic maleic acid copolymers.

The interaction of CH with MP was investigated also by conductometric and potentiometric titrations. In Figure 2 are

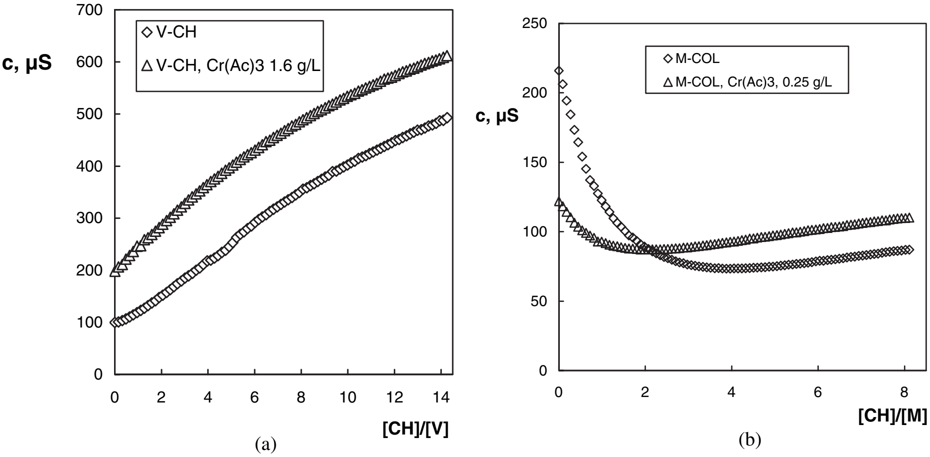


Figure 2.
Conductometric titration of V (a) and M (b) copolymers with aqueous CH solutions with/without added chromium ions.

presented two typical examples of conductometric titration: of hydrophilic copolymer V and of hydrophobic copolymer M.

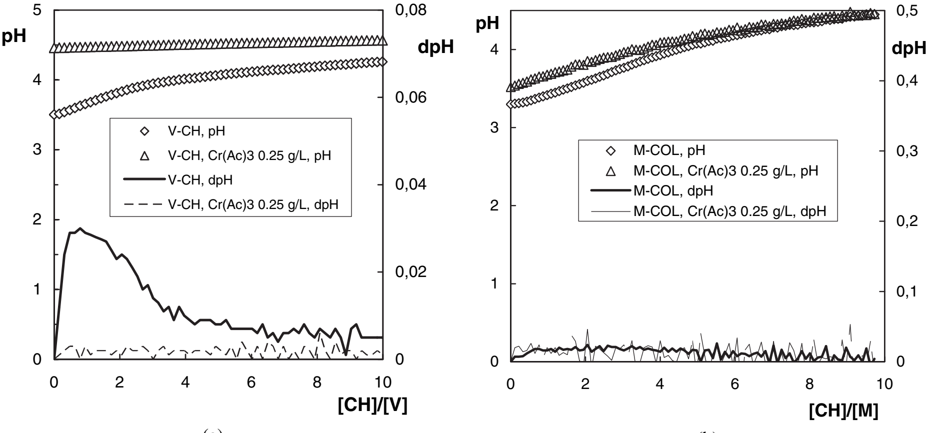
In the case of the hydrophilic copolymer V an end-point is not observed, neither in the presence or absence of the chromium ions. In this case the increase of the conductivity may be attributed to the higher concentration of CH solution which hides the effect of the dilution. The other hydrophilic copolymer P behaves similarly. The result is not unexpected while in literature are mentioned systems for which significant differences were observed between conductometric and turbidimetric end-points, for example with branched polyelectrolytes.[29] In the case of hydrophobic copolymer M (Fig. 2b) the conductometric curves show an end-point which is sharper in the presence of chromium ions. In this case we can assume that a neutralisation of the acid groups of M copolymer with amino groups from CH takes place. The titration curves of S copolymer have the same shape.

In Figure 3a and 3b are exemplified the potentiometric curves of V and M copolymers.

It can be observed that the interaction of MP with CH is influenced by both the nature of comonomer and the presence of chromium ions. So, in the absence of chromium ions an end-point is not observed on the potentiometric curves of M copolymer, while an inflection point can be found on the titration curve of V copolymer. In the presence of chromium ions the potentiometric curves of both V and M copolymers do not show inflection points. Table 2 summarizes the values of maleic copolymer/CH ratio corresponding to the turbidity, conductivity and pH end-points, if observable, on the titration curves.

The Influence of Added Chromium Ions on the Interaction of MP with CH

As we mentioned, the motivation of our work is to obtain new results which can help to explain the favorable effect of MP in tanning of hides with chromium salts. In this view a series of experiments were performed in which MP solutions were titrated with a CH solution, with various added amounts of chromium ions (as chromium acetate) from 0.05 up to 2.00 g/L. The changes of the systems were followed by measuring their turbidity. The results are presented in Figures 4a, 4b, 5a and 5b in which the copolymers are grouped together according to their hydrophilic/hydrophobic character.



(a) (b)

Figure 3.
Potentiometric titration of V (a) and M (b) copolymers with aqueous CH solution with/without added chromium ions.

It can be clearly seen that the concentration of the chromium ions strongly influences the shape of the turbidimetric curves depending on the chemical structure of the copolymer. In the case of the hydrophilic copolymers, the presence of increasing amounts of chromium ions produces a shift to higher values of the ratio [CH]/[MP] at which the turbidity is developed and/or reaches its maximum. This behavior is more marked in the case of P copolymer having in the chain the very hydrophilic N-vinylpyrrolidone unit. During these titrations no macroscopic phase separation was observed. At a certain amount of chromium ions (more than 1 g/L) no turbidity is produced at any ratio [CH]/[MP].

A different behaviour can be observed in the case of hydrophobic copolymers M and S. Here the chromium ions have the same effect to make the systems CH/MP more homogeneous, but the increase of chromium concentration results in lowering of ratio [CH]/[MP] at which the turbidity starts to develop. At lower chromium concentrations the phase separation occurred. It has been observed that the homogeneous systems are formed at chromium acetate amounts of 1–2 g/L which was the range of typical used concentration in our experiments of chromium hide tanning with MP as additives.

The results above are somewhat qualitative but they show clearly an interaction

Table 2.
Maleic copolymer titration with collagen hydrolysate

Anionic component	PE-CH ratio at the end-point, g/g		
	Conductometric titration	Potentiometric titration	Turbidimetric titration
V	*	0.79	6.11
V (Cr 0.25 g/L)	*	*	7.6
P	*	*	2.75
P (Cr 0.25 g/L)	4.40	*	4.13
S	0.93	*	2.61–3.24
S (Cr 0.25 g/L)	0.72	1.20	4.6–10.6
M	1.40	*	2.79
M (Cr 0.25 g/L)	1.20	*	0.8–4.14

*end point not measurable.

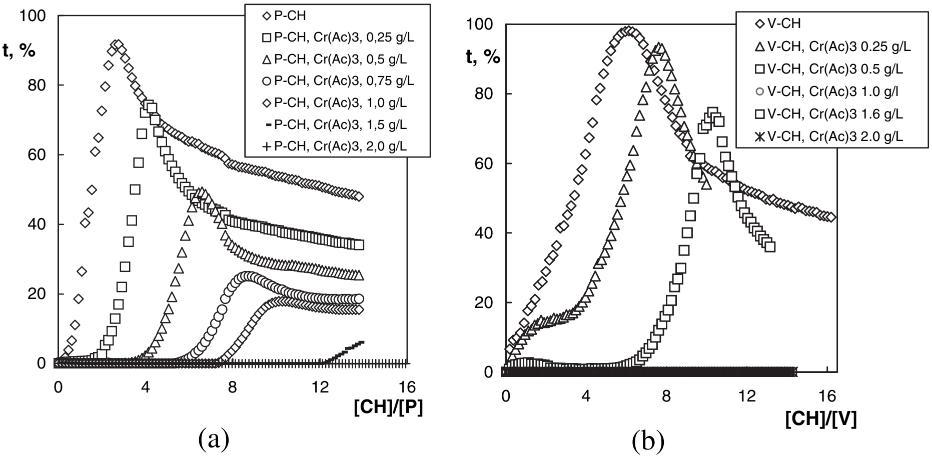


Figure 4.
Turbidimetric titration of P (a) and V (b) copolymers with aqueous CH solution with various amounts of added chromium ions.

in the binary systems CH/MP or in the ternary systems CH/MP/chromium ions. This interaction could be explained according to the theory of polyelectrolyte complexes (PEC) taking into account the interplay between the factors determining this interaction: the specific properties of the collagen hydrolysate, the forces acting in the complex formation (electrostatic, hydrophobic, hydrogen bonds[30]), the presence of metal ions which can result in metal-ligand interactions, pH, and the presence of low molecular salts. The increase of the turbidity could be connected to the formation of scarcely soluble complexes of the charged macromolecular partners with or without participation of chromium ions. The presence of metal ions increases the solubility of the complex, either by blocking the interacting sites or by promoting the formation of new particles rather than the growing of the PEC particles already formed.[31] By investigation of the interaction of poly(acrylic acid)

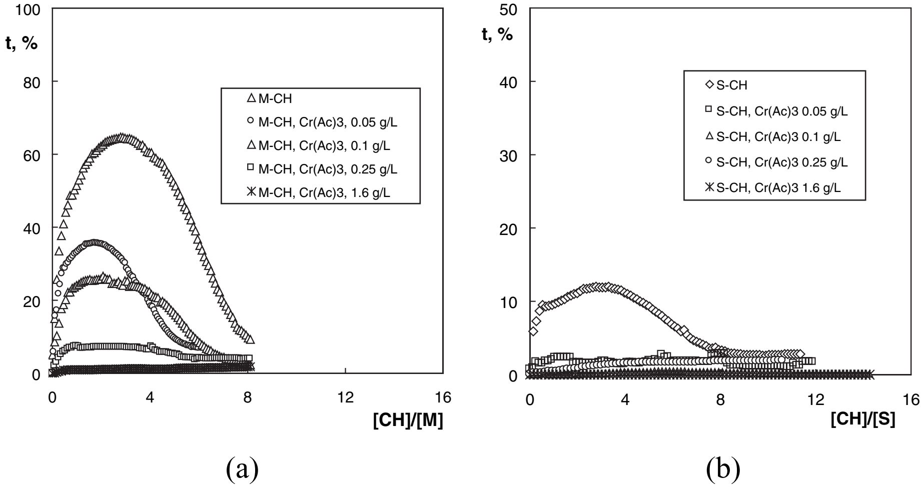


Figure 5.
Turbidimetric titration of M (a) and S (b) copolymers with aqueous CH solution with various amounts of added chromium ions.

(PAA) with trivalent metal cations such as iron (III) or aluminum in form of sulfates it was found that the cations are linked by COOH groups of PAA forming supramolecular structures in which more anionic and cationic groups are involved.[32]

In our experiments we found that the ratio between the macromolecular partners at the end-point was in the most cases different from the value 1:1, observed for many PECs formed of synthetic polyelectrolytes. We identified in the most cases a difference between the conductometric and turbidimetric end-point, the latter requiring a larger volume of titrant than the former. That suggests that the PEC particles consist of a neutral core surrounded by a stabilizing shell of the excess CH. The decrease of turbidity when the [CH]/[MP] ratio exceeds the value 1 in the case of hydrophilic copolymers seems to confirm this conclusion. Further studies are necessary in order to get a complete picture about the interaction of CH with MP. Experiments are now in progress about the pH and added salt influence and about the characterization of some CH/MP complexes in solid state.

Conclusion

Our aim was to offer a work tool for the investigation of the interactions occurring during the tanning process of hides with chromium salts using maleic polyelectrolytes as additives. Experiments have been conducted regarding the interaction between collagen hydrolysate as hide model and maleic polyelectrolytes, in the presence or absence of chromium ions. The chemical structure of the maleic copolymers and the amount of added Cr ions were varied.

The investigation of maleic polyelectrolytes-collagen hydrolysate systems by turbidimetric, conductometric and potentiometric titration evidenced clearly the interaction between the macromolecular partners. Homogeneous systems or turbid dispersions were observed depending on the hydrophilic or hydrophobic character of the maleic copolymer, on the [CH]/[MP] ratio, and on the concentration of the chromium ions. In all cases, the increase of the chromium concentration led to homogeneous systems. The influence of the [CH]/[MP] ratio on the turbidity was correlated with that of the chemical structure of the maleic copolymer. Differences between the conductometric and turbidimetric end-points were observed the latter requiring a larger volume of titrant. These first results could be qualitatively understood in the frame of polelectrolyte complexes theory.

Acknowledgements: The authors are grateful to the Academy of Finland and to the Romanian Academy for the financial suport in the frame of interacademic exchanges.

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