Polymer Bulletin 48, 361-366 (2002)

Polymer Bulletin

Interpolymeric complexes of poly(itaconic acid) and chitosan

Cristóbal Lárez V (✉), Freddy Canelón, Enrique Millán and Issa Katime[1)]

Universidad de Los Andes, Facultad de Ciencias, Departamento de Química,Grupo de Polímeros, Mérida 5101, Venezuela
[1)]Universidad del País Vasco (UPV/EHU), Facultad de Ciencias (Campus de Leioa), Departamento de Química Física, Grupo de Nuevos Materiales, Bilbao, España.

email: clarez@ciens.ula.ve, Fax: +58-274-2401286

Received: 1 April 2002 / Accepted: 3 May 2002

Summary

Polycomplexes of poly(itaconic acid) have been synthesized in the presence of small amounts of chitosan (eq. NH_2/eq. COOH between 0 and 1.31%). We have studied the effect on the yield and polydispersity of the final materials when increasing quantities of chitosan are employed. Under the reported experimental conditions, we have found that chitosan is quantitatively incorporated into the new material to produce dramatic effects on the system behavior. It has been established a critical R-value beyond which a hidrogel is obtained. The electrostatic interactions between polyelectrolytes turn from irreversible to reversible when pH is made strongly basic.

Introduction

In recent years considerable attention has been paid to the study of interactions among polyelectrolytes of opposite charges. These materials can exhibit a range of interesting properties and applications such as: controlled drug release, including anticancer compounds [1] and proteins [2], cell and islet immunoisolation [3], natural or industrial waters purification systems [4], modification of the final properties of existing materials [5], carriers for genetic modifiers [6]. Since this variety of application, an important effort has been dedicated to develop simple models, which may lead to a complete understanding of the fundamental processes occurring during polycomplexation [7]. These models may be used to explain complex systems like autoduplication of the DNA, selective reactivity of enzymes, antigens/antibody reactions, etc.

Experimental verification of proposed models has been difficult. Nevertheless, it is important to emphasize that these difficulties can be partly overcome by using noncomponent of the polyelectrolyte system may facilitate a possible route to understand the characteristics of these materials.

In this work we present the results obtained during *in situ* non stoichiometric polycomplexes formation between the cationic polyelectrolyte, named chitosan, and the polyanion poly(itaconic acid).

Experimental

Reagents: Itaconic acid (IA, Sigma) use without further purification and characterized by FTIR and ^{1}H-RMN. Chitosan (FLUKA) was characterized by ^{13}C-RMN, FTIR and by conductimetric titrations to determine its deacetylation degree (da), which resulted to be around 84%. Solutions were prepared by using water from Millipore Milli-QTM. Oxygen was removed by warming solutions to boiling and then cooling under flow of nitrogen, just before beginning the reaction. Potassium persulfate (Baker 99.4 %), acetone (ACS 99.5%), sodium nitrate (Merck 99%), acetic acid (Riedel de Haen 99.8%), potassium biphthalate (Riedel de Haen 99.5%) and other common reagents were used as they come from the supplier.
Equipments: Thermogravimetric studies were carried out by using a Perkin Elmer thermobalance, model TGA-7; ^{1}H-RMN and ^{13}C-RMN spectra were obtained from spectrophotometer Bruker, model DRX of 300 MHz; a Perkin Elmer System 2000 spectrophotometer was used to infrared studies. pH measurements were realized with Metrohm model 691; conductimetric measurements were obtained by a conductimeter Hanna Instruments, model 8033. SEC measurements were realized with in a Perkin Elmer HPLC chromatograph, which posses a Chrom Hitachi L-7110 pump and a Rheodyne 7125 injector; the detection system is a differential refractometer Knauer. Separation columns Shodex OH Pak SB 806, arranged in series, were used for analysis of ionic polymers. Samples of polyacrylamide of known molar masses, in aqueous $NaNO_3$, were used to obtaining the calibration curve.
Synthesis: Polycomplexes were synthesized by using a previous described procedure to synthesize poly(itaconic acid) [9]. 10 mL of deionised water was warmed up to boiling and then cooled up to environment temperature under constant stirring and bubbling nitrogen. Once achieved the environment temperature, 200 μL of acetic acid and the corresponding amount of chitosan are added, the system is stirred until the dissolution of the chitosan is observed; once the chitosan is dissolved the itaconic acid and the potassium persulfate are added, the pH of the solution measured and the system is thermostated at 60°C, under continuous stirring for 48 hours. The experimental conditions used in this stage are summarized in table 1. Initially the itaconic acid is not dissolved completely but after around 5 minutes, the mixture of reaction become transparent. Most of the reactions where chitosan was used produced cloudy solutions after 2 hour of polymerization, which is maintained until the end of the process. Synthesis of PIACH-H was difficult due to an increasing viscosity of the medium. After 2 hour of reaction, the viscosity of the medium becomes to increase producing a gel material that absorbs most of the solvent. Due to this, polymerization was stop after 15 hours.

Table 1. Experimental conditions for the synthesis of the PIA in presence of chitosan. T = 50 °C.

Sample	IA initial		Chitosan initial		pH		Time	PIA yield	
	(g)	(meq CO_2H)	(g)	(meq NH_3^+)	initial	final	(h)	(g)	(%)
PIA-D	3.0	45.8	---	---	2.1	1.7	48	0.3883	13
PIACH-E	3.0	45.8	0.025	0.15	2.4	2.1	48	0.3600	12
PIACH-F	3.0	45.8	0.050	0.30	2.4	2.1	48	0.7676	25
PIACH-G	3.0	45.8	0.075	0.45	2.5	2.0	48	1.0078	32
PIACH-H	3.0	45.8	0.100	0.60	2.5	---	~15	1.2280	39

Soluble samples were purified in water as previous work [9]. The insoluble samples were placed in acetone and then dialyzed in deionised water. These materials were placed in a dessicator for 15 hours at room temperature and then under reduced pressure at 50 °C for 90 hours, a yellow solid was finally obtained. All the materials synthesized showed a fragile glassy aspect. The yellow color is intensified when the contents of chitosan is increased.

Attempts to homopolymerise itaconic acid in the presence of chitosan (without other acids to solubilise chitosan) failed due to the low solubility of chitosan in water. Polymerization of itaconic acid in the presence of chitosan and several dicarboxylic acids, i.e. malonic, maleic and adipic acid was also useless. This behavior has been attributed in the literature to the formation of intermolecular bonding between chitosan molecules and dicarboxylic acids by means of electrostatic interactions [10,11].

Discussion

A relevant experimental fact in the synthesis of polycomplexes of itaconic acid is the appearance of a slightly turbidity after around 2 hour reaction. This may be attributed to complexation of the preexisting polycation with the growing polyanion. After this reaction time, the polyanion reach a critical size and sufficient concentration to produce a turbidity. When the chitosan concentration is increased over a critical concentration, i.e. PIACH-H, complexation may occurs all over the system, producing a highly viscous solution which seen to appears as a gel. Under these experimental conditions, it is important to recognize that the amount of functional groups in the polycation always remains minor than the amount of carboxylate groups (table 2.)

Despite of the lack of IR spectroscopy evidence of the NH_3^+/COO^- interaction, due to the low chitosan concentration present in these polycomplexes, we have obtained several experimental evidence that the synthesized materials contain proportional amounts of chitosan. For example, figure 1 shows the relationship between final carboxylic acids and initial chitosan equivalents. A linear relationship is obtained from these data, which agrees with the assumption that chitosan is quantitatively incorporated into the polycomplex.

Moreover, table 2 shows the size exclusion chromatography (SEC) studies for the PIA-D and the soluble polycomplexes with different amount of chitosan.

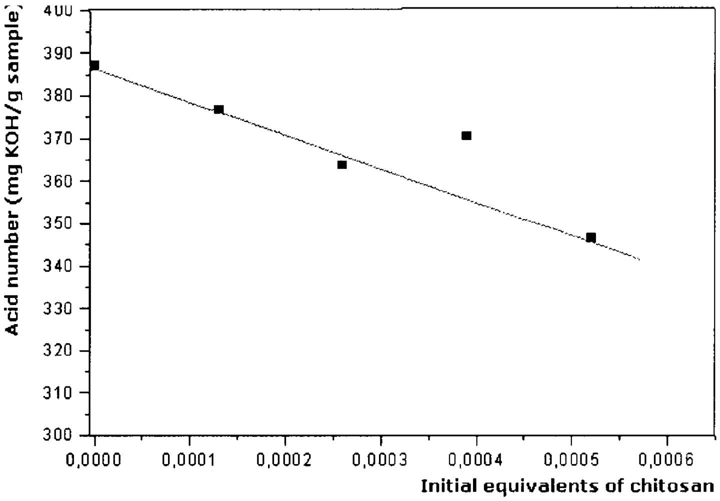

Figure 1. Correlation among carboxylic acid equivalents and initial chitosan equivalents in samples of polycomplexes.

Table 2. Average molar mass and polydispersity of the synthesized materials.

Sample	$M_v x10^5$	M_w x10^5	M_n x10^5	Polydispersity	$R_{initial}$	R_{final}
PIA-D	5.4	5.6	5.0	1.12	---	---
PIACH-E	5.3	5.4	4.7	1.14	305.4	38.3
PIACH-F	6.1	6.7	5.0	1.30	152.6	15.4
PIACH-G	8.6	10.0	5.7	1.74	119.8	19.3
PIACH-H	---	---	---	---	76.4	8.6

R = (meq. COOH/meq. NH_2)

As can be seen the average molar mass and polydispersity of samples increases with the amount of chitosan added. This behavior may be attributed to the formation of polycomplexes with the growing chains of poly(itaconic acid). Incorporation of the polycation in the soluble samples is achieved by a simple electrostatic interaction between opposite polyelectrolytes, which generates a fraction of reversible crosslinked molecules. This is reflected as bigger average molar mass and polydispersity. This is also observed as an increasing yields (table 1). Figure 2 (a) shows a possible scheme for this type of reversible interaction, based on a model previously reported by Tsuchida [8].

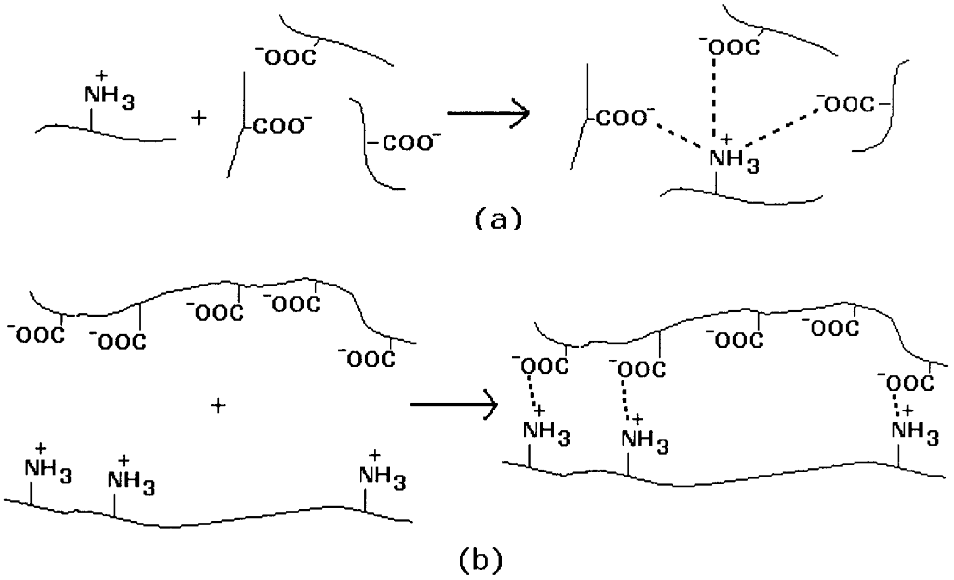

Figure 2. Scheme of reaction to complex (a) soluble and (b) insoluble polyelectrolytes.

On the other hand, a gel like system (insoluble samples) was observed at R_i = 76.4. This may implies the existences of a critical ratio over which gelification occurs. For this particular system, this ratio will be between 119.8 – 76.4.

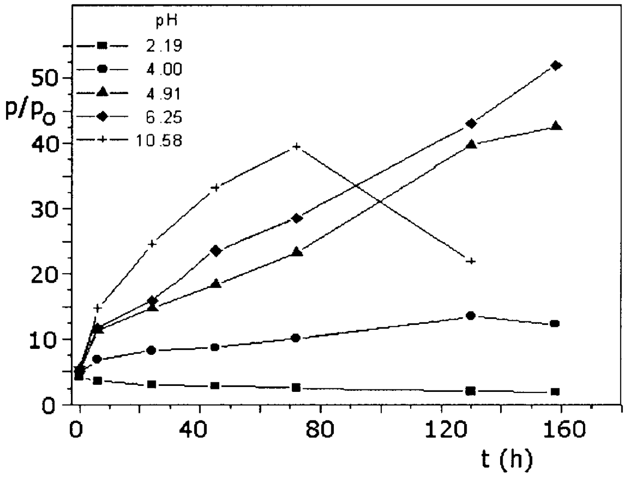

Figure 3. Behavior of PIACH-H on swelling at several pHs.

The driving force to generate samples with high molar mass is the electrostatic cooperative interactions (Figure 2(b)). Tsuchida et al [8] proposed that an irreversible union is obtained on light acid or basic conditions when the hydroxyl groups of the chitosan are in contact with the carboxylic groups of PIA. However, we have found that this interaction may become reversible under strong alkaline medium as it is estimated from the swelling studies of the sample PIACH-H (Figure 3). The drop down observed at pH 10.58 has been assigned to the disrupt the electrostatic bonds in the hydrogel and desegregate it in fragments of small sizes, specially those at the surface, with the consequent lost of molar mass that could be mistaken with a deswelling process. This fragmentation was observed after around 80 hours with the probably dissolution of the small disrupted fragments.

Conclusions

It has been established that itaconic acid is capable to polymerize in presence of small quantities of chitosan, when amino groups have been protonated with acetic acid. Macromolecular soluble species have been obtained employing a critical concentration of chitosan (approximately 1 % equivalent of groups NH_2 in relation to initials equivalent of COOH groups). We have found that molar mass, yield and polydispersity decrease when the initial concentration of chitosan is diminished. SEC and acid number studies have proved that chitosan has been quantitatively incorporated to the polymer. On the other hand, when the chitosan concentration is increased up to 1.31 % of NH_3^+/ initial COOH equivalent ratio, the system becomes a gel after approximately 15 hours. The material obtained hereby shows the typical hydrogel properties when it is studied in lightly acid or alkaline conditions; nevertheless, when the studies are carried out in more drastic alkaline conditions the hydrogel seems to suffer descomplexation reactions, generating products according to the following balance:

$$\text{—}NH_3 + {}^-OOC\text{—} + NaOH \Leftrightarrow {}^-NH_2 + Na^+ \, {}^-OOC\text{—} + H_2O$$

Acknowledgements. The authors would like to thank Consejo de Desarrollo Científico, Humanístico y Tecnológico de la Universidad de los Andes (CDCHT-ULA) project C-933-99-08. We are also grateful to Grupo de Nuevos Materiales del Departamento de Química Física de la Universidad del País Vasco for FC support and assistance.

References

1. Mi, F., Shyu S., Kuan C., Lee S., Lu K., Jang S. **J. Appl. Polym. Sci., 74** (7), 1868-1879 (1999).
2. Dumitriu, S., Chornet, E. **Adv. Drug Delivery Rev., 31 (3),** 223-246 (1998).
3. Prokop, A., Hunkeler, D., DiMari, S., Haralson, M., Wang, T. **Adv. Polym. Sci., 136**, 1-51 1998
4. Pinotti, A., Bevilacqua, A., Zaritzky, N. **J. Food Engineering, 32** (1), 69-81 1997
5. Amiji, M. **Carbohydrate Polymers, 32** (3-4), 193-199 (1997).
6. Murata, J., Ohya, Y., Ouchi, T. **Carbohydrate Polymers, 32** (2), 105-109 (1997).
7. Haronska, P. and Vilgis, T. **Physical Review E, 50**, 1, 325-334 (1994).
8. Tsuchida, E. **JMS-Pure Appl. Chem., A31** (1), 1-15 (1994).
9. Lárez, C., Canelón, F., Millán, E., Perdomo G., Katime, I. Send to Polymer Bulletin.
10. Demarger-Andre, S. and Domard, A. **Carbohydrate Polymers, 23**, 211-219 (1994).
11. Rogovina, S., Vikhoreva, G., Apokova, T., Gorbacheva, I. **J. Appl. Polym. Sci., 67**, 616-622 (2000).