Physico-Chemical Properties of Two Poly(carboxybetaines) Based on Poly(4-vinylpyridine)

ANCA GIORGIANA GRIGORAS[1]*, STEFANIA RACOVITA[1], SILVIA VASILIU[1], ALINA DIACONU[1], VASILE CRISTIAN GRIGORAS[1], LACRAMIOARA OCHIUZ[2]

[1] Petru Poni Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Allet, 700487, Iasi, Romania

[2] Gr. T. Popa University of Medicine and Pharmacy, Faculty of Pharmacy, 16 Universitatii Str., 700115, Iasi, Romania

The poly(betaines) have been still intensively studied due to them biomedical potential. In the present work, two poly(carboxybetaines) derived from poly(4-vinylpyridine) P4VP that have methylene (P4VPB-1) or ethylene (P4VPB-2) spacer between N^+ and COO^- groups were investigated in order to evaluate its physico-chemical properties in aqueous salted solutions with NaCl or $CaCl_2$. The values of refractive index increment dn/dc, weight-average molecular weight $\overline{M_w}$, hydrodynamic radius R_h, and particle size distribution depend on the solvent type and balance between molecular forces as refractometric and laser light scattering measurements showed.

Keywords: differential refractometry, laser light scattering, poly(carboxybetaine)

P4VP is an interesting polymer with remarkable properties, making it available for many applications. Chemical modification of this polymer with suitable low molecular compounds lead to macromolecular chains which contain both positive and negative charges in the same monomer unit [1]. Generally, the poly(betaines) were synthesized by (1) free radical polymerization of betaine monomers and (2) polymer analogous reactions of polymers containing tertiary amino groups [2]. Nowadays, poly(betaines) are synthesized by using new polymerization techniques: controlled radical polymerization [5], group chain-transfer polymerization [6], reversible addition-fragmentation chain transfer polymerization [7, 8] and ring opening metathesis polymerization [9].

In commercial terms, the poly(betaines) are described by their ability to provide a macromolecular support for the preparation of various materials, such as: sorbents, oil recovery agents, fungicides, flame-retardant polymers, wetting agents, dyeing agents in textile industry and cryoprotectors [10, 11], as well as drug delivery systems or cosmetic formulations [12-16]. Other research groups were focused on antimicrobial properties of derivatives of 4-vinylpyridine. Park et al. [17] studied copolymers of styrene with 4-vinyl pyridine quaternized with *n*-octyl iodide. Also Zhang et al. [18] grafted P4VP onto halloysite nanotubes, and than immobilized silver ions on P4VP.

Looking ahead to biomedical potential of poly(betaines), this paper reveals that the physico-chemical properties of P4VPB-1 and P4VPB-2 are influenced by the environment, so that finding a *good* solvent will be a desiderate for maximum interaction between poly(betaines) and charged structural components of microorganisms.

Experimental part

Material, solution preparations and methods

P4VP ($\overline{M}_v$ = 60000 g mol^{-1}), acrylic acid, sodium chloroacetate, sodium chloride and calcium chloride were purchased from Aldrich Chemical Co. The acrylic acid was distilled in vacuum prior to use. P4VPB-1 was obtained by a nucleophilic substitution reaction of P4VP with sodium chloroacetate, and P4VPB-2 from a nucleophilic addition reaction of acrylic acid to P4VP. The polymerization of P4VP and synthesis of poly(carboxybetaines) derived from P4VP and having the maximum degree of quaternization of 95 %, were presented in other works [19, 20]. In addition, by dialysis and lyophilization, these poly(carboxybetaines) were highly purified. The chemical structures of precursor and the two derivatives were presented in figure 1.

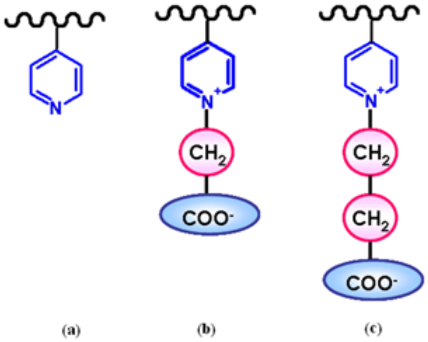

Fig. 1. Structural units of poly(4-vinylpyridine) (a), P4VPB-1(b) and P4VPB-2(c)

The salted aqueous solutions of poly(carboxybetaines) were prepared with purified water from a Millipore (Billerica, MA, USA) Simplicity UV apparatus, at room temperature, and then stirred about 24 h for homogenization. In all cases, the solvent and the stock solutions were filtered by 0.02 μm, and 0.2 μm Whatman filters, respectively. The dilute solutions were gravimetrically prepared for refractometric and laser light scattering measurements in range of 2 x 10^{-3} – 2.5x10^{-4} g mL^{-1}. The quartz scintillation vials (Quartz SUPRASIL) used for static light scattering measurements were provided by Hellma GmbH & Co. KG, Germany.

The refractometric and laser light scattering measurements were made on unfractionated samples, holded 2 days for degassing in scintillation vials, following the already established protocol [21-23]. *dn/dc* values were recorded at 25 °C and 633 nm using an Optilab-rEX differential refractometer (Wyatt Technology, Santa Barbara CA, USA) in off-line mode. The refractive index increment influences the precision and final results of laser light scattering measurements. The multiangle laser light scattering (MALLS) measurements in batch mode were

---

* email: angrig@icmpp.ro; Phone: (+40) 742943519

Dedicated to the 150th anniversary of the Romanian Academy realized at 25 ^{o}C with a DAWN DSP laser photometer (Wyatt Technology). The scattered light intensities were measured at 633 nm, between 14^{o} and 152^{o} to the incident beam. The photometer was calibrated with HPLC-grade toluene (Sigma Aldrich) and detectors were normalized with poly(ethylene glycol), (M_n = 3930 g mol^{-1}, Polymer Laboratories, Stretton, UK; standard for GPC). The laser light scattering data processing and calculations of M_w and second virial coefficient A_2 were realized by Astra 4.90.07 software based on Zimm equation (eq.1), where K is optical constant for vertical polarized incident light (eq. 2), $R_θ$ Rayleigh ratio, θ scattered intensity angle, λ_0 wavelength of the incident light, c concentration (g mL^{-1}) of the scattering species, R_g radius of gyration, n refractive index of macromolecular solution, and N Avogadro number. Than the Zimm plots were computed using Debye formalism (eq. 3).

$$\frac{Kc}{R_\theta} = \frac{1}{\overline{M}_w} + 2A_2c + \frac{16\pi^2}{3\lambda_0}\frac{R_g{}^2}{\overline{M}_w}\sin^2\frac{\theta}{2} + \ldots \tag{1}$$

$$K = \frac{4\pi^2 (dn/dc)^2 n^2}{N\lambda_0{}^4} \tag{2}$$

$$\frac{R_\theta}{Kc} \quad versus \quad \sin^2\left(\frac{\theta}{2}\right) \tag{3}$$

The second virial coefficient A_2 quantifies the thermodynamic interaction between solute molecules at a specific temperature. If $A_2 > 0$, the polymer-solvent interactions dominate the polymer-polymer interactions, and solvent is *good*. In case of $A_2 = 0$, the both interactions are energetically equivalent (specific case for a *theta* solvent). For $A_2 < 0$, polymer-polymer is preferred to polymer-solvent contact, and the solvent is a *poor* for the given polymer [24, 25].

The particle size distribution was determined by dynamic light scattering (DLS) technique, using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Malvern, UK) working with a He-Ne red laser (633 nm) and clear disposable zeta cells. Based on measurements realized at 173^{0} scattering angle, the Nano software provided R_h values according to Stokes-Einstein relation:

$$R_h = \frac{k_BT}{6\pi\eta D} \tag{4}$$

where k_B is Boltzman constant, T absolute temperature, h solvent viscosity and D traslational diffusion coefficient of molecules.

Results and discussions

P4VPB-1 was insoluble in water, but soluble in salted water. In contrast, P4VPB-2 was soluble in water with and without salts. Therefore all experiments were realized with salted solutions (0.1M and 0.5 M).

Refractometric and light scattering data

Laser light scattering results for poly(carboxybetaines) (table 1) completes the older viscosity and solubility studies [26] in order to evaluate the solution properties. For either of two poly(carboxybetaines) dissolved in salted solutions, R_h increased with increasing of salt concentration. This behavior is due to salting-in phenomenon (known as anti-polyelectrolyte effect) specific to zwitterionic polymers like poly(betaines).

According to DLS measurements, R_h recorded lower values in solutions based on $CaCl_2$, than in NaCl solutions, both for P4VPB-1 and P4VPB-2. Also, a smaller R_h of P4VPB-1 than of P4VPB-2 is related with shorter methylene spacer between $–R_4N^+$ and $–COO^-$ groups, compared with $-CH_2-CH_2-$ spacer.

Additionally, $\overline{M}_w$ values of P4VPB-1 were higher than those of P4VPB-2. This result was related to the higher number of aggregates developed in solution by P4VPB-1, compared with P4VPB-2.

Generally, it was observed that for the same concentration of salt in solution, M_w values were lower in solutions containing $CaCl_2$ than in those containing NaCl. The exception was record for P4VPB-2 in 0.1 M solution because the particles size distribution was bimodal in 0.1 M $CaCl_2$ solution, but momomodal in 0.1 M NaCl solution (fig. 2).

For same polymer and same salt, generally $\overline{M}_w$ values decreased with increasing of salt concentration in solution due to dissociation of inter-chain agglomerated structures occurred with increasing of environmental ionic strength. The dissociation of inter-chain associations allows the weakening of electrostatic attraction between $-R_4N^+$ and $-COO^-$ groups, too. Such results were not recorded for P4VPB-2 in NaCl solutions, where the inter-chain aggregations still not damaged due to increasing of R_h values with NaCl concentration increasing. In this case, the presence of inter-chain aggregations was confirmed by bimodal distribution of particle sizes in solution 0.5 M NaCl.

Generally, an increase of A_2 values with salt concentration increasing was observed, meaning that dissolution of polymeric chains become easier in a solution with a larger amount of salt, due to fulfillment of the thermodynamically conditions for solvent. Surprisingly, for P4VPB-2 in solutions with $CaCl_2$, the polymer-solvent

Sample	Salt	type	dn/dc [mL g^{-1}]	$M_w x10^{-5}$ [g mol^{-1}]	$A_2 x10^3$ [mol mL g^{-2}]	R_h [nm]
P4VPB-1						
	NaCl	0.1 M	0.208	1.5860	1.3450	12.2
		0.5 M	0.202	1.2260	1.6040	18.8
	$CaCl_2$	0.1 M	0.202	0.9205	0.9945	9.5
		0.5 M	0.197	0.8772	1.0250	10.5
P4VPB-2						
	NaCl	0.1 M	0.191	0.8001	1.5310	16.0
		0.5 M	0.186	1.1050	2.2920	20.0
	$CaCl_2$	0.1 M	0.187	0.8333	1.1500	10.4
		0.5 M	0.181	0.6205	0.4382	12.9

Table 1
REFRACTOMETRIC AND LASER LIGHT SCATTERING DATA

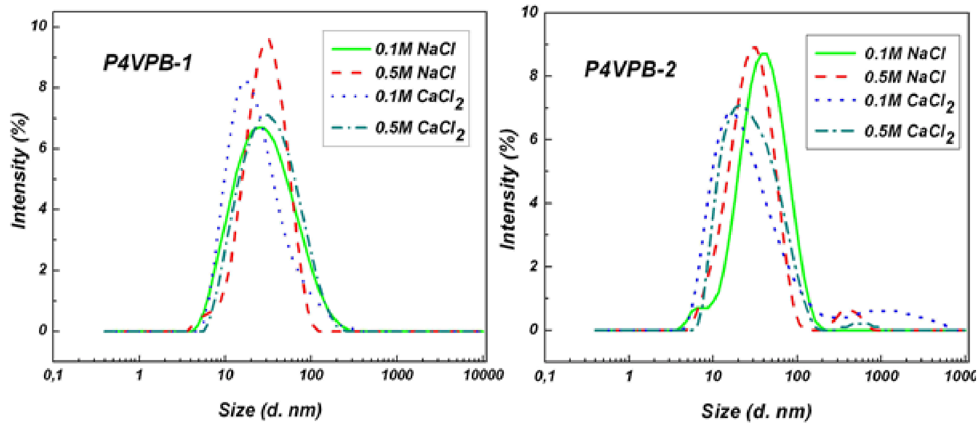

Fig. 2. Particle size distribution for poly(carboxybetaines) in different salted aqueous solutions

interactions were not favored in solutions containing higher concentration of $CaCl_2$. In this case, electrostatic attraction tends to be favorable, more than intra-chains associations (based on interactions of a single Ca_2^+ ion with two COO^- groups) that numerically dominated the inter-chains associations.

Conclusions

The poly(carboxybetaines) P4VPB-1 and P4VPB-2 were soluble in aqueous solutions with low molecular salt addition (NaCl or $CaCl_2$) and exhibited an *antipolyelectrolyte effect*. The salted aqueous solutions were *good* solvents for these poly(betaines) as the positive A_2 values showed.

The particle size distribution of macromolecular chains in solution was monomodal or bimodal depending on the solvent type and balance between intramolecular and intermolecular forces. Poly(carboxybetaines) have dominant globular conformation of derived from aggregates with dimensions of 10-20 nm.

Generally, the molecular masses and refractive index increments of studied poly(carboxybetaines) decreased with increasing of low molecular salt concentration in solutions due to a better screening of polymeric charged parts.

References

1. KUDAIBERGENOV, S., JAEGER, W., LASCHEWSKYL, A., Adv. Polym. Sci., vol. 201, 2006, p. 157

2. DRAGAN, E.S., Focus on Ionic Polymers, Research Singpost, Kerala, 2005, p.117–152

3. LANDENHEIM, H., HORAWETZ, H., J. Polym. Sci. Part A: Polym. Chem., 26, nr.113, 1957, p. 251

4. HART, R., TIMMERMAN, D., J. Polym. Sci., 28, nr. 118, 1958, p.638

5. LOWE, A.B., McCORMICK, C.L., Chem. Rev., 102, nr. 11, 2002, p.4177

6. LOWE, A.B., BILLINGHAM, N.C., ARMES, S.P., Chem. Commun., 13, 1996, p.1555

7. AROTCARENA, M., HEISE, B., ISHAYA, S., LASCHEWSKY, A., J. Am. Chem. Soc., 124, nr. 14, 2002, p. 3787

8. VIRTANEN, J., AROTCARENA, M., HEISE, B., ISHAYA, S., LASCHEWSKY, A., TENHU, H., Langmuir, 18, nr. 14, 2002, p. 5360

9. RANKIN, D.A., LOWE, A.B., Macromolecules, 41, nr. 3, 2008, p. 614

10. COLAK, S., TEW, G.N., Macromolecules, 41, nr. 22, 2008, p. 8436

11. LOYD, A.W., BAKER, J.A., SMITH, G., OLLIFF, C.J., RUTT, K.J., J. Pharm. Pharmacol., 44, nr. 6, 1992, p. 507

12. PEREZ, J.H., CARDOSO, J., MANERO, O., Polymer, 39, nr. 26, 1998, p. 6969

13. GAL, Y.S., JIN, S.H., LIM, K.T., KOH. K., Current Appl. Phys., 5, nr. 1, 2005, p. 38

14. KUDAIBERGENOV, S.E., DIDUTH, A.G., ZHUMADILOVA, G.T., KOIZHAIGANOVA, R.B., BIMENDINA, L.A., JUNG-GYUN, N., GECKELER, K.E., Macromol. Symp., 207, nr. 1, 2004, p. 153

15. JOHNSON, K.M., FEVOLA, M.J., McCORMICK, C.L., J. Appl. Polym. Sci., 92, nr. 1, 2004, p. 647

16. NAKAYA, T., LI, Y.L., Prog. Polym. Sci., 24, nr. 1, 1999, p. 143

17. PARK, E.S., KIM, H.S., KIM, M.N., YOON, J.S., Eur. Polym. J., 40, nr. 12, 2004, p. 2819

18. ZHANG, J., ZHANG, Y., CHEN, Y., DU, L., ZHANG, B., ZHANG, H., LIU, J., WANG, K., Ind. Eng. Chem. Res., 51, nr. 7, 2012, p. 3081

19. BARBOIU, V., STREBA, E., LUCA, C., SIMIONESCU, C.I., J. Polym. Sci. Part A: Polym. Chem., 33, nr. 3, 1995, p. 389

20. BARBOIU, V., HOLERCA, M.N., STREBA, E., LUCA, C., J. Polym. Sci. Part A: Polym. Chem., 34, nr. 2, 1996, p. 261

21. GRIGORAS, A.G., RACOVITA, S., VASILIU, S., NISTOR, M.T., DUNCA, S., BARBOIU, V., GRIGORAS, V.C., J. Polym. Res., DOI: 10.1007/s10965-012-0008-1, 2012

22. GRIGORAS, A.G., CONSTANTIN, M., GRIGORAS, V.C., DUNCA, S.I., OCHIUZ, L., React. Funct. Polym., 73, nr. 9, 2013, p. 1249

23. PODZIMEK, S., Light scattering, size exclusion chromatography and asymmetric flow field flow fractionation. Powerful tools for characterization of polymers, proteins and nanoparticles, John Wiley & Sons, Inc., Hoboken, New Jersey, 2011, p.37–98

24. CZECHWSLKA-BISKUP, R., WOJTASZ-PAJAK, A., SIKORSKI, J., HENKE, A., ULANSKI, P., ROSIAK, J.M., Polish Chitin Society Monograph XII, P-1, 2007, p.187

25. JENKINNS, A.D., Polymer Science Library, vol. 5, Elsevier, Amsterdam, 1987

26. RACOVITA S., VASILIU S., RADU C.D., PROFIRE L., CHECHERITA L., FOIA L., Rev. Chim. (Bucharest), **64**, no. 12, 2013, p.1422

Manuscript received: 24.06.2015