Journal of Chromatography A, 1218 (2011) 237–241

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Separation of statistical poly[(N-vinyl pyrrolidone)-co-(vinyl acetate)]s by reversed-phase gradient liquid chromatography

Guanglou Cheng*, Jim Cullen, Chi-san Wu

International Specialty Products, 1361 Alps Road, Wayne, NJ 07470, USA

ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form 30 October 2010
Accepted 10 November 2010
Available online 21 November 2010

Keywords:
Reversed-phase liquid chromatography
N-vinylpyrrolidone-vinylacetate copolymers
Gradient elution

ABSTRACT

Although size exclusion chromatography (SEC) has been used successfully to determine the molecular weight distribution (MWD) of statistical poly[(N-vinyl pyrrolidone)-co-(vinyl acetate)]s [PVPVAs], SEC cannot separate the copolymers according to their chemical composition. In this article, the separation of commercial PVPVAs with varying chemical compositions is reported, by aqueous reversed-phase gradient liquid chromatography (RPLC) using polystyrene-divinylbenzene-based wide pore columns. RPLC–SEC cross-fractionation indicates the presence of molar mass dependant effects during RPLC separation due to broad MWD for the copolymer studied; therefore the width of the RPLC peak could not be associated entirely with chemical composition distribution of the copolymer. Coupling of RPLC with online FTIR spectroscopy reveals the increase of VA content with increasing THF gradient, an indication of interaction mechanism between VA repeating units and the stationary phase for water soluble PVPVAs. Separation of water insoluble PVPVAs and PVAs by the RPLC are possibly based on both interaction and precipitation/redissolution mechanisms.

1. Introduction

Copolymers of N-vinyl pyrrolidone-vinyl acetate or PVPVAs are commercially important products. Depending on their chemical composition and molecular weight (MW), these products can find wide applications in various fields such as cosmetic and pharmaceutical industries [1,2]. Size exclusion chromatography has been used successfully to characterize the molecular weight distribution of these products, but revealed no information on their chemical composition [3].

Chemical composition characterization of synthetic polymers by column HPLC was first reported in 1979 by Teramachi et al. for p(S-co-MA) [4]. Since then, a great variety of polymers, including polymer blends, statistical copolymers, and graft copolymers, have been characterized by HPLC techniques [5–16]. HPLC methods could provide not only average chemical composition like other conventional methods (e.g. NMR, FTIR, UV–vis, Titration, etc) do, but also chemical composition distribution (CCD), which is directly related to manufacturing process and product performance. To the best of our knowledge, separation of PVPVAs by eluent gradient reversed-phase HPLC has never been reported.

In the present work, separation of commercial PVPs, PVPVAs, and PVAs is studied by using aqueous gradient reversed-phase HPLC with wide pore polystyrene supports. The usefulness of the HPLC method is exemplified in quantifying (<1 wt%) homo-polymer contamination such as PVP in the PVPVAs.

2. Experimental part

2.1. Materials

Samples of commercial PVPVA copolymers were obtained from *International Specialty Products* (Wayne, NJ), and their molecular weight by SEC method [3] and composition data are summarized in Table 1. Fig. 1 also illustrates an overlay of SEC traces for PVPVAs and PVP homopolymers. The PVP K-30, K-60, and K-90 PVP homopolymers were provided by *International Specialty Products* (Wayne, NJ). The PVA homopolymers were obtained from *Acros Organics* (Fair Lawn, NJ) and *Polysciences Inc* (Warrington, MA). Molecular weights of PVP by SEC method [3] and PVA by providers are listed in Table 2. All HPLC solvents were used as received from *Thermo-Fisher Scientific* (Waltham, MA).

2.2. Measurements

HPLC separations were performed on Waters Alliance 2695 Module with a column heater at 35 °C. Photodiode array detector PDA 996 (Waters) and Waters 2424 evaporative light-scattering detector (ELSD) were coupled sequentially to the HPLC instrument. The ELSD nebulizer was heated at 75% power level, with drift tube at

* Corresponding author. Tel.: +1 973 6283625; fax: +1 973 6283633.
E-mail address: gcheng@ispcorp.com (G. Cheng).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.11.017

Table 1
Weight-average molecular weight, polydispersity (PD), and chemical composition of commercial VP–VAc copolymers.

PVP/VA	Lot #	% Solid (in ethanol)	Composition (VP/VA, w/w)	Mw (relative to PEO)	PD
E-335	05700182778	48.9	30/70	23,800	3.9
E-535	05700183001	48.6	50/50	36,200	4.3
E-635	05700193585	48.9	60/40	45,100	4.9
E-735	05800204923	51.4	70/30	39,800	4.1

SEC Conditions: 1.0 mg/ml, 50 μl injection, Shodex OHPak SB806MHQ (4.6 × 250 mm, 10 μm, 5 μm), 0.5 ml/min, run time 30 min, H_2O/MeOH 50/50 with 0.1 M LiNO3, RI detector (see Ref. [3] for other details).

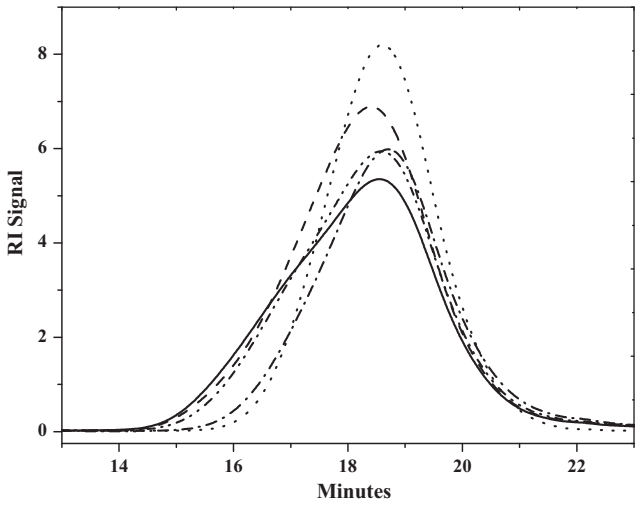

Fig. 1. Overlaid SEC chromatograms for PVP K30 (. . .), E-335 (– – –), E-535 (–··), E-635 (–), E-735 (–·–). SEC Conditions: Shodex OHPak SB806MHQ (8.0 × 300 mm, 13 μm), H_2O/MeOH 50/50 with 0.1 M $LiNO_3$, 0.5 ml/min, run time 30 min, 1.0 mg/ml, 50 μl injection, RI detector (see Ref. [3] for more details).

75 °C, and nitrogen pressure at 50 psi. Data acquisition and process were performed with *Empower* software. The HPLC column used was polystyrene-divinylbenzene-based PLRP-S columns (Polymer Laboratories, now a part of Agilent Technologies). Linear mobile phase gradient (5–75% THF in water over 5 min, 75% THF isocratic for 3 min, for a 50 × 4.6 mm column) with a flow rate of 1.0 ml/min was used for all gradient separations. The HPLC samples were prepared as 0.1 ∼ 0.2 wt% solution in HPLC grade water, except THF for PVA and 20 wt% THF aqueous solution for E-335. Full sample recovery was confirmed by absence of any ELSD peak from a blank injection of pure THF after each sample injection. HPLC with online FTIR detector was performed on a *DiscovIR-LC* system by *Spectra Analysis Inc* (Marlborough, MA). The nebulizer of *DiscovIR* was set at 15–18 W with carrier gas at 400 cc, disk speed at 3 mm/min, disk temperature at −5 °C, pressure chamber/cyclone at 3.2/160 torr, condenser (single) temperature at 0 °C, and cyclone temperature at 170 °C. The VA mol% of the samples was based on peak heights at 1740 cm^{-1} (for VA) and 1680 cm^{-1} (for VP), as well the VP/VA molar IR absorptivity ratio (1.22) determined from PVPVA standards with known compositions.

Table 2
Weight-average molecular weight and polydispersity (PD) of PVP and PVAc.

Sample	Provider	Lot #	Mw (relative to PEO)	PD
PVP K30	ISP	#05900219368	24,930	2.7
PVP K60		#03700177552	127,710	4.6
PVP K90		#03700186201	703,770	5.1
PVAc1	Acros Organics	#A0251821	170,000[a]	N.A.
PVAc2	Polysciences	#519165	90,000[a]	N.A.

[a] Relative to PS standards.

3. Results and discussion

PVPVA copolymers are amphiphilic due to hydrophilic VP repeating units and hydrophobic VA repeating units. The copolymers are water soluble except very hydrophobic copolymers with high VA content (e.g. E-335 with 70 wt% VA). These properties make PVPVAs excellent candidates for traditional aqueous gradient RPLC which utilizes mainly enthalpic interactions between small molecular solutes and stationary phases. Initial RPLC trials with C8/C18-based wide-pore silica columns were unsuccessful, possibly due to H-bond interactions between VP units in the copolymers and residual silanol groups in the stationary phase. Therefore we switched to silanol-free polystyrene (PS) based RPLC columns. Wide pore (1000 Å or 4000 Å) packing was chosen to minimize the SEC effects and maximize the contact between the VP–VA copolymers (hydrodynamic radius ∼10 nm) and packing surface. A water/THF gradient was used, since water is a weak RPLC solvent, while THF is a strong and displacing RPLC solvent, in addition to its good solubility for PVPs, VP–VA copolymers, and PVAs.

Fig. 2 presents an overlay of RPLC traces for PVP K30, E-735, E-635, E-535, E-335, and PVA homopolymers, using a polystyrene-based RPLC column (PLRP-S from Polymer Labs) and H_2O/THF gradient. Unlike their SEC traces with no separation (Fig. 1), the current RPLC method demonstrates separation between homopolymers and copolymers with varying chemical compositions (Fig. 2). PVP homopolymer without any VA content elutes at 2.2 min, indicating that there are weak interactions between VP repeating units of the polymer and PS stationary phase (V_0 = 0.8 min for the 50 × 4.6 mm column used). The retention increases to 3.2 min for 30 wt% VA (E-735), and to 3.8 min for 50 wt% VA (E-535). The increase of VA content in the copolymers increases interac-

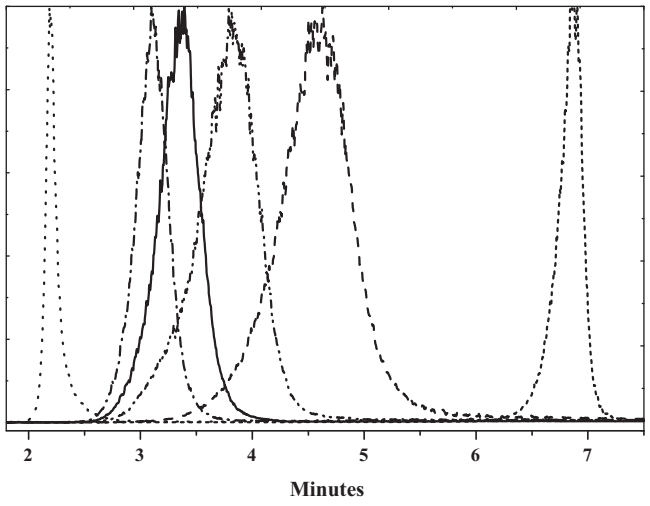

Fig. 2. Normalized overlaid RPLC chromatograms of PVP K30 (. . .), E-335 (– – –), E-535 (–··), E-635 (–), E-735 (–·–) and PVA (—, short dash). Column: PLRP-S; 1000 Å; 5 μm; 50 × 4.6 mm. Eluent: THF/H_2O, 1.0 mg/ml (sample concentration), 20 μl (injection volume), 1.0 ml/min, THF/H_2O gradient from 5/95 to 75/25 linearly in 5 min followed by 75/25 isocratic for 3 min.

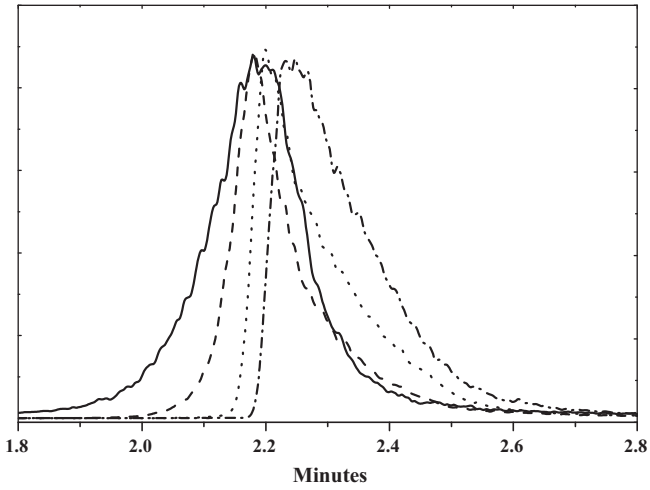

Fig. 3. Normalized overlaid RPLC traces of PVP K15 (–), K30 (– – –), PVP K60 (. . .), PVP K90 (–.–), conditions as in Fig. 2.

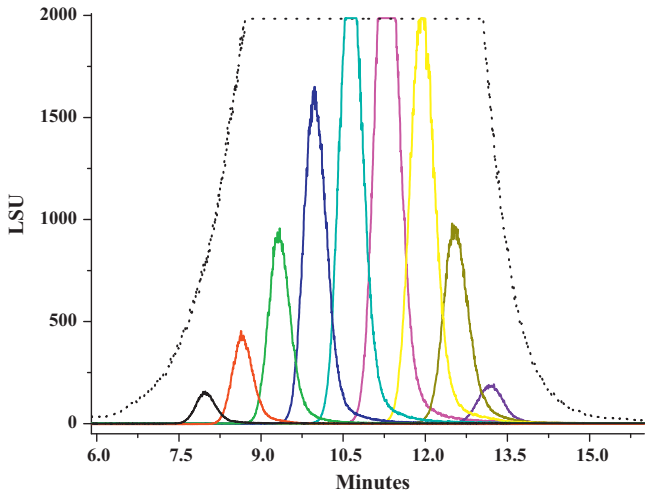

Fig. 4. RPLC Fractions collected for E-635 copolymers. Colors for fractions: black for 7.96 min, red for 8.65 min, green for 9.31 min, blue for 9.98, cyan for 10.63 min, magenta for 11.30 min, yellow for 11.97 min, dark yellow for 12.52 min, violet for 13.2 min, and black dots for E-635. RPLC conditions: PLRP-S 30 μm 4000 Å, 250 × 4.6 mm, 1.0 ml/min, 5–75%/15 min THF in water followed by 3 min 75%THF isocratic, 11.8 mg/ml (sample concentration), 100 μl (injection volume), fractions collection interval 20 s.

tions between hydrophobic VA units and PS stationary phase, and in-turn increases the RPLC retention. Therefore, for water soluble PVPVA copolymers, separation based on chemical compositions of the copolymers is achieved by utilizing mainly the strong interactions between hydrophobic VA units and hydrophobic PS stationary phases. For water insoluble copolymers like E-335 copolymers and PVAs, the separation is most likely due to a combination of enthalpic interactions and precipitation/redissolution controlled by solubility limit [17].

It is also observed in Fig. 2 that each later eluting chromatographic peak of the copolymer is broader than the one eluting earlier. This should not be interpreted as increasing CCD with increasing elution time, since the peak width could be affected by many factors besides CCD, such as MW/MWD, band broadening, polymer solubility, etc. For example, MW and MWD have been found to affect peak width of even PVP homopolymers, as in Fig. 3. Although the peak elution times of K15/K30/K60/K90 are very close (2.18–2.25 min), their width/distribution profiles are different (Fig. 3). Significant fronting at earlier elution time for low MW PVP (e.g. K15) and considerable tailing at later elution time for high MW PVP (e.g. K90) are observed. In this case, the peak width varies due to the differences in MW/MWD of various homo-PVP grades. Besides, as in RPLC of small molecules, basic peak dispersion processes (e.g. Eddy diffusion and mobile phase mass transfer) are present in RPLC of polymers, and will contribute to band broadening, and as a result, peak width will increase with retention time [18,19]. Interplay between band broadening, interactions, and MW effects during RPLC of PVPVAs needs to be studied further.

To further investigate the MW dependent separation during RPLC of PVPVA copolymers, RPLC–SEC cross-fractionation was performed. Fractions were collected during RPLC of E-635 (Fig. 4) and were then injected into SEC using ELS detector (Fig. 5). MW was not calculated relative to PEO because unlike an RI detector an ELSD is not a linear detector. Based on retention times at peak maxima, early RPLC fractions always contain mostly low MW copolymers which would be subject to Martin rules under isocratic conditions [20,21]. The MW of the RPLC fraction increases with elution time until ~11.6 min, after which the MW of RPLC fractions plateaus with elution time. MW independent separation of PVPVA copolymers on wide-pore packing happens for significantly large macromolecules, in line with theories of gradient separation at critical point of absorption [11,22].

The effect of column pore size on retention was also investigated as in Fig. 6. RPLC Peak of the E-635 sample on 300 Å column elutes 0.4 min later than on the 4000 Å, possibly due to higher surface area of the 300 Å packing. The overall peak widths for all three columns are similar, indicating similar mass transfer for these large pore packing materials.

To further confirm the presence of chemical separation of the PVPVAs according to the VA content, an online FTIR detector was coupled to the RPLC to characterize chemical compositions of individual peak fractions. Fig. 7 shows the FTIR spectra for the peak fractions eluted at 2.2 min for PVP K30, 3.2 min for E-735, 4.6 min for E-335, and 6.8 min for PVA, respectively. The FTIR spectra at 2.2 min and 6.8 min are typical of PVP (e.g. 1680 cm^{-1} for carbonyl group in PVP) and PVA homo-polymers (e.g. 1740 cm^{-1} for carbonyl groups in PVA), while FTIR spectra at 3.2 and 4.6 min are characteristic of VP–VA copolymers (e.g. both 1740 cm^{-1} and 1680 cm^{-1}). Fig. 8 illustrates an increase of VA content with the increase of retention during RPLC, indicating increasing interactions between hydrophobic VA units and PS stationary phase with increasing time,

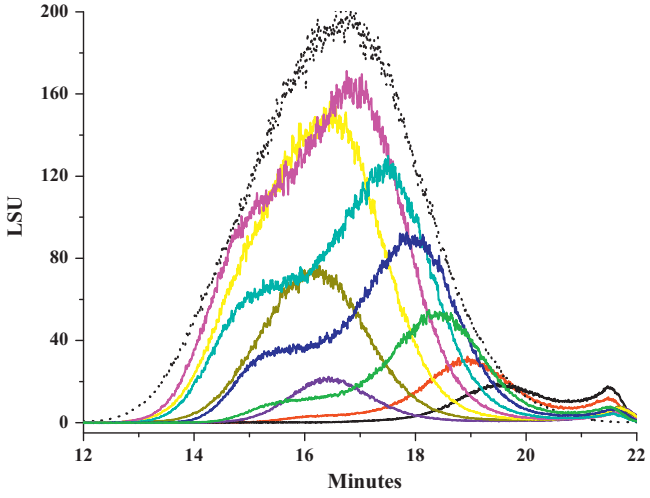

Fig. 5. SEC chromatographs of RPLC fractions (as is) in Fig. 4. Color code same as Fig. 4. SEC conditions: H_2O/MeOH 50/50 with 0.5% TFA, 0.5 ml/min, Shodex OHPak SB806 MHQ, injection volume 100 μl.

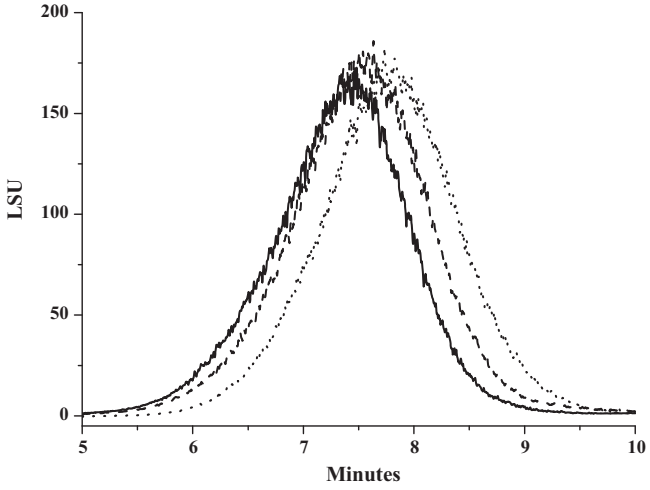

Fig. 6. Overlaid RPLC chromatographs of E-635 using columns with different pore sizes. 300 Å (. . .), 1000 Å (–·–), and 4000 Å (–). Conditions: 10 μm; 150 × 4.6 mm. Eluent: THF/H_2O, 1.0 mg/ml (sample concentration), 40 μl (injection volume), 1.0 ml/min, THF/H_2O gradient from 5/95 to 75/25 linearly in 15 min followed by 75/25 isocratic for 5 min.

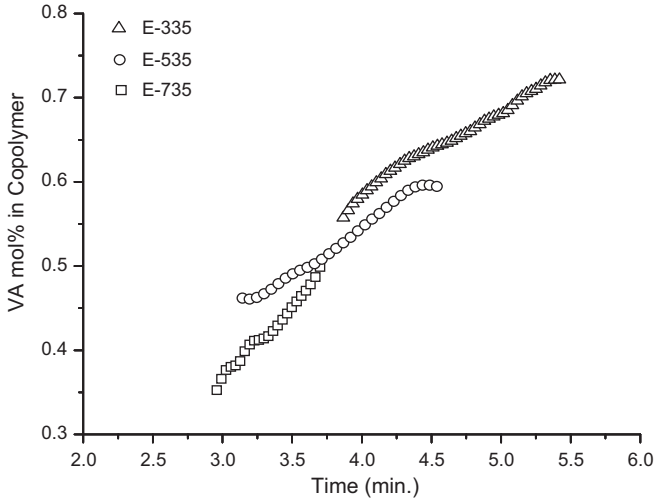

Fig. 8. VA mol% vs. elution time for E-335, E-535, and E-735, RPLC conditions the same as in Fig. 2.

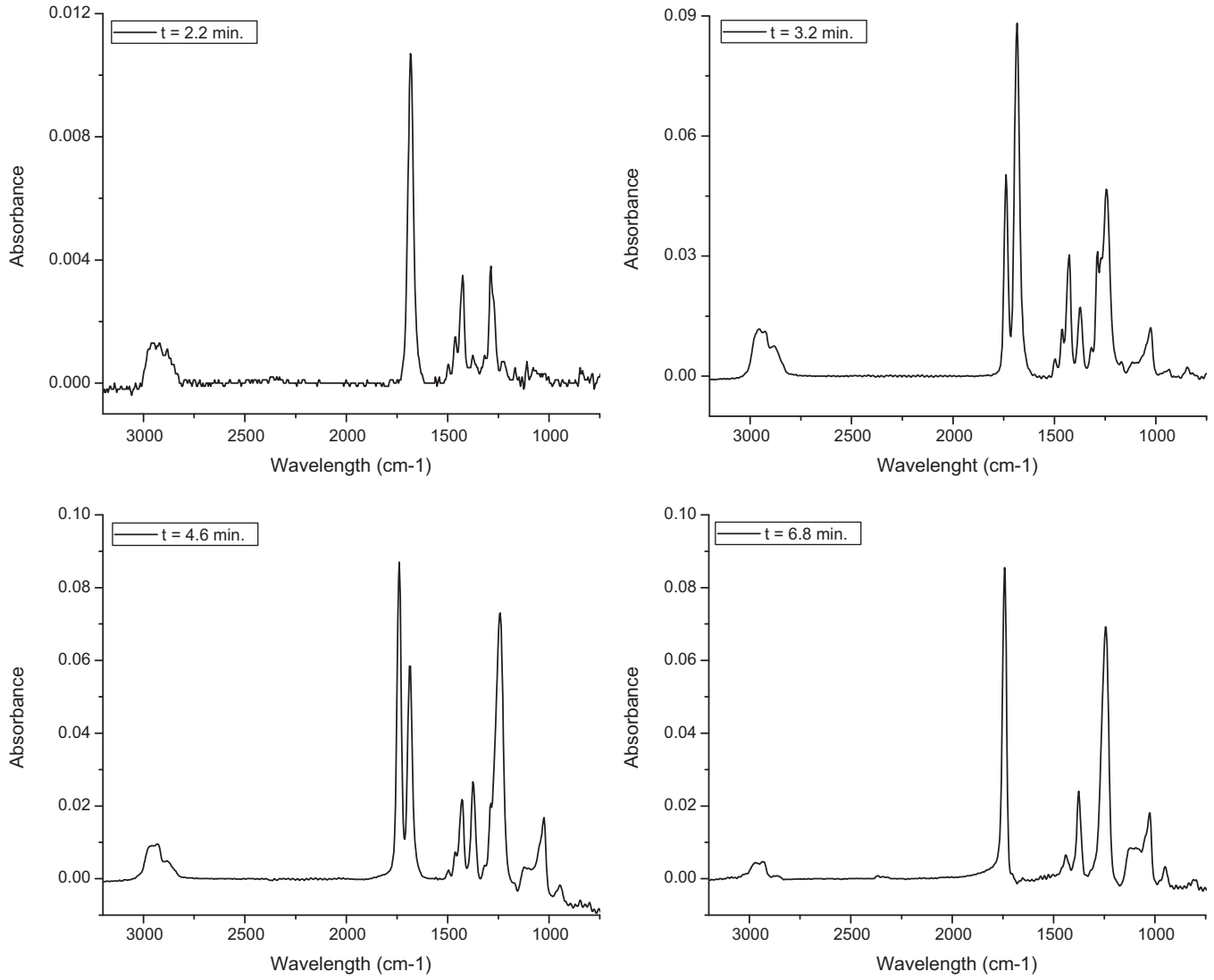

Fig. 7. FTIR spectrum for elutions at 2.2 min for PVP K30, 3.2 min for E-735, 4.6 min for E-535, and 6.8 min for PVA, RPLC conditions the same as in Fig. 2.

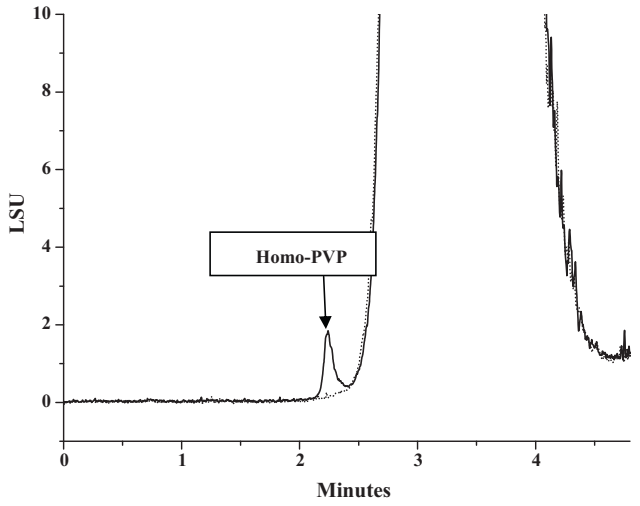

Fig. 9. RPLC traces of E-635 and E-635 spiked with 1% homo-PVPs, RPLC conditions the same as in Fig. 2.

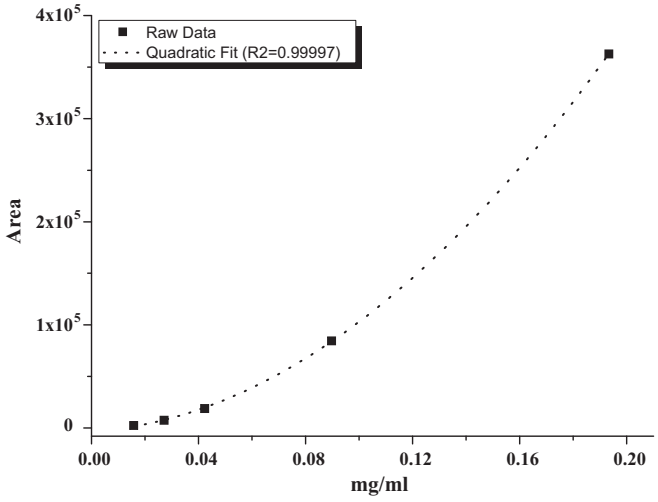

Fig. 10. ELSD calibration by PVP K30 standards in water, RPLC conditions the same as in Fig. 2.

therefore confirms the presence of chemical separation. Furthermore, observation of different VA content at the same elution time for different samples reflects the differences in MW distribution or even blockiness for these samples. This observation was also reported previously in HPLC of ethylene-vinyl acetate copolymers [23]. Therefore, for CCD evaluation, a calibration curve by copolymer standards with known chemical compositions in this study would not be as practical as in GPEC, which based primarily on precipitation/redissolution mechanism [12,24,25].

An immediate application of current method is to quantify minor (~1.0%) homo-PVP contamination in PVPVAs. Such a task has been thought impossible traditionally by conventional techniques such as SEC, NMR, FTIR, etc. Fig. 9 illustrates HPLC traces for the E-635 sample spiked with 1.0 wt% PVP homopolymer. After calibration the ELS detector by external PVP K30 standards (Fig. 10), the PVP K30 level in the E-635 sample is calculated at 0.97 wt%.

4. Conclusion

The separation of commercial PVPVAs with varying chemical compositions is reported, by reversed-phase gradient liquid chromatography (RPLC) using polystyrene-divinylbezene-based wide pore columns and water/THF gradient. The RPLC peak width could not be associated entirely with CCD of the copolymer, due to MW dependent interactions. The HPLC method demonstrates efficacy in separating minor PVP contaminants (< 1% wt %) from PVPVA copolymers.

Acknowledgements

We thank Dr. Ming Zhou and Mr. David Dunn at *Spectra Analysis Inc* for the LC-FTIR analysis.

References

[1] E.W. Flick, Cosmetics additives: An Industrial Guide, William Andrew, 1991.
[2] V. Buehler (Ed.), Polyvinylpyrrolidone Excipients for Pharmaceuticals, Springer, Berlin, Heidelberg, 2005, p. 179.
[3] C.S. Wu, J. Curry, L. Senak, J. Liq. Chromatogr. 14 (1991) 3331.
[4] S. Teramachi, A. Hasegawa, Y. Shima, M. Akatsuka, M. Nakajima, Macromolecules 12 (1979) 992.
[5] D. Berek, Prog. Polym. Sci. 25 (2000) 873.
[6] G. Gloeckner, Adv. Polym. Sci. 79 (1986) 159.
[7] H.J.A. Philipsen, J. Chromatogr. A 1037 (2004) 329.
[8] A. Albrecht, R. Brull, T. Macko, P. Sinha, H. Pasch, Macromol. Chem. Phys. 209 (2008) 1909.
[9] S. Teramachi, A. Hasegawa, T. Matsumoto, K. Kitahara, Y. Tsukahara, Y. Yamashita, Macromolecules 25 (1992) 4025.
[10] A.M. Striegel, J. Chromatogr. A 971 (2002) 151.
[11] Y. Brun, P. Alden, J. Chromatogr. A 966 (2002) 25.
[12] P. Cools, F. Maesen, B. Klumperman, A.M. vanHerk, A.L. German, J. Chromatogr. A 736 (1996) 125.
[13] S. Mori, Y. Uno, M. Suzuki, Anal. Chem. 58 (1986) 308.
[14] H. Sato, K. Mitsutani, I. Shimizu, Y. Tanaka, J. Chromatogr. 447 (1988) 387.
[15] T.H. Mourey, J. Chromatogr. 357 (1986) 101.
[16] T.C. Shunk, J. Chromatogr. A 656 (1993) 591.
[17] R.A. Shalliker, P.E. Kavanagh, I.M. Russell, J. Chromatogr. 543 (1991) 157.
[18] P. Schoenmakers, F. Fitzpatrick, R. Grothey, J. Chromatogr. A 965 (2002) 93.
[19] L.R. Snyder, J.J. Kirkland, J.W. Dolan, Introduction to Modern Liquid Chromatography, John Wiley & Sons., Publication, Hoboken, NJ, 2009.
[20] Y. Kim, S. Ahn, T. Chang, Anal. Chem. 81 (2009) 5902.
[21] H.J.A. Philipsen, H.A. Claessens, H. Lind, B. Klumperman, A.L. German, J. Chromatogr. A 790 (1997) 101.
[22] H. Pasch, B. Trathnigg, HPLC of Polymers, Springer, Berlin, 1997.
[23] A. Albrecht, R. Brull, T. Macko, H. Pasch, Macromolecules 40 (2007) 5545.
[24] A.M. Striegel, J. Chromatogr. A 996 (2003) 45.
[25] I. Kramer, W. Hiller, H. Pasch, Macromol. Chem. Phys. 201 (2000) 1662.