Polymer Bulletin 13, 83–88 (1985)

Polymer Bulletin

Static Measurements of Refractive Index Increments at 633 nm with a Modified Refractive Index Detector

V. Soria, A. Llopis, B. Celda, A. Campos, and J. E. Figueruelo*

Departamento de Química Física, Facultad de C. Químicas, Universidad de Valencia, Burjasot, Valencia, Spain

* To whom correspondence should be addressed

SUMMARY

A R 401 Waters Assoc. differential refractometer has been modified to obtain the variation of refractive index increments, dn/dc, of polymer solutions at 633 nm. The introduced modifications were in the sample injection system and in the light source. The performance of the modified refractometer both with aqueous and organic solutions is analyzed. In order to localize a conformational transition in lysozyme, the dependence of dn/dc on temperature for lysozyme solutions in phosphate buffer is studied. Similarly, $(dn/dc)_k$ values for the ternary n-alkane/butanone/poly(dimethyl siloxane) systems over the whole composition range of the binary solvent mixtures are evaluated.

INTRODUCTION

Differential refractometry (DR) is a widely used technique for the determination of specific refractive index increments, ν or dn/dc, of macromolecular solutions. The experimentally determined observable is the difference between refractive indices of a dilute macromolecular solution and the pure solvent, Δn, at a given wavelength and temperature (HUGLIN, 1972). DR is an auxiliar technique for light scattering measurements and it is also widely used as a detector in size exclusion chromatography (SEC). Besides, DR has also been used as an independent technique for the quantitative determination of binary mixture composition (SINOVEC and YEUNG, 1983), the localization of conformational transitions (KATIME and ROIG, 1974), and the evaluation of preferential solvation coefficients in polymer/mixed solvents ternary systems (ABAD et al., 1979), (SORIA et al., 1978).

Classically available differential refractometers allow the determination of dn/dc values at the wavelengths 366, 436 and 546 nm, as can be evidenced by reported data (HUGLIN, 1975). However, a light scattering photogoniometer with a He-Ne laser as the light source has been recently commercialized, which demands the evaluation of dn/dc values at its wavelength, 633 nm.

The refractive index detector (DRI) simultaneously arises with the development of high performance liquid chromatography, its monitorized signal being proportional to the solute refractive index and to its concentration.

The aim of this paper is the application of a modified DRI as DR at 633 nm. The necessary instrumental modifications are concerned with the injection of samples in the DRI microcell and the replacement of its standard light source with a monochromatic one at

633 nm. The modified equipment has been a R 401 detector from Waters and its versatility has been tested in this work. Several calibrations in organic and aqueous media have been repeatedly carried out through a year, using the equipment as a SEC detector and DR alternatively. Besides that, the validity of this technique for the localization of conformational transitions has been tested by dn/dc measurements at different temperatures of lysozyme in a buffer phosphate solution (pH=8), the transition of which has been evidenced by other techniques (COZZONE et al., 1975), (SAINT BLANCHARD et al., 1977), (JOLLES, 1975). Lastly, the specific refractive index increments at constant chemical composition, ν_k or $(dn/dc)_k$, for the ternary systems n-alkane/butanone(MEK)/poly (dimehtyl siloxane)(PDMS) over the whole range of compositions have been determined (CELDA, 1984).

EXPERIMENTAL

Static measurements of dn/dc of several aqueous and organic macromolecular solutions have been made using a R 401 differential refractometer from Waters Assoc. (IMS, 1976) modified in our laboratory. Solutions were injected using a Hamilton CR-700-200 syringe adapted to the inlet metal tubing of the microcell. The original light source was replaced by a Sargent-Welch, ChemAnal System (Illinois, USA) monochromatic source, fixed in a optical bench provided with levelling screws, that allowed the appropriate optical beam alignment. The so aligned beam enters into the optical module through a hole in which is inserted the holder for phenolic rod (IMS, 1976). The photoelectric cell of the R 401 optical module is not affected by the wavelength of the incident light but by its intensity.

Solvents used, benzene, carbon tetrachloride, tetrahydrofuran (THF), and methyl ethyl ketone (MEK) were distilled and purified by standard procedures (RIDDICK and BUNGGER, 1970). Alkanes used, n-hexane(HEX), n-heptane(HEP), n-octane(OCT), n-nonane(NON), n-decane (DEC), n-undecane(UND), n-dodecane(DOD) and n-hexadecane(HXD) were p.a. Merck grade. Water used for lysozyme solutions was previously purified by means of a MilliQ, Millipore Corp. (Bedford, Mass.,USA). Polystyrene(PS) and poly(methyl methacrylate) (PMMA) samples were narrow fractions prepared in our laboratory. PDMS has been provided by Siliconas Hispania S.A. (Barcelona, Spain) and lysozyme, crystallized three times, by Sigma Chem. Corp.(S. Louis, Mo. USA).

Two different calibrations in organic and aqueous solvents have been made. Calibrations in organic phases were made with Cl_4C solutions in benzene at 25.0°C in the concentration range 4×10^{-3} up to 2×10^{-2} g/ml. Values of Δn for each composition have been evaluated using the following expression

$$\Delta n = (n_{Cl_4C} - n_{C_6H_6})(m_{Cl_4C}/\rho_{Cl_4C})\ V_t$$

where n is the refractive index, m the mass in g, ρ the density in g/ml and V_t the total volume. n values at 633 nm were determined by extrapolation of reported data at 436, 546 nm (KRUIS, 1936) and the value obtained at 589 nm using a Pulfrich refractometer, through the Cauchy expression. Calibrations in aqueous medium were carried out with KCl solutions at 25.0°C covering the concentration range from

1.0×10^{-2} up to 3.5×10^{-2} g/ml.

RESULTS AND DISCUSSION

The R 401 signal was registered by means of a graphic recorder and it was linear in the whole range of sensitivities, its standard deviation being less than 2%, except for the 128× sensitivity, in which deviations from linearity amounted up to 10%. Four calibrations were performed with organic solutions and six with aqueous-salt ones. The respective mean calibration equations were:

$\Delta n\times10^{6} = 5.03\ L - 39$, for organic and

$\Delta n\times10^{6} = 12.0\ L + 5$, for aqueous solutions

where L stands for signal height.

Several polymer-solvent binary systems of known dn/dc have been measured in order to check the obtained data with the reported ones. The obtained results for PS and PMMA are listed in table 1. Column A values have been obtained using the calibration equation for organic solutions, whereas column B values by application of the calibration equation for aqueous. Values of correlation coefficient, τ, are referred to the linear least squares fit of Δn vs. c plots. Column C shows the extrapolated values through the Cauchy relation. Finally, the experimental values given by (MILLAUD and STRAZIELLE, 1979) and the results obtained using the Gladstone-Dale (GD) equation are shown in columns D and E, respectively.

TABLE 1

Comparison of dn/dc values (ml/g) at 633 nm to reported data for several polymer/solvent systems.

System	A	B	τ	C	D	E
Benzene/PS (M_w=265000)	0.110	0.104	0.997	0.107[a] 0.104[b]	0.104[f]	0.0995(GD)
Cl_4C/PS (M_w=265000)	0.149	0.141	0.998	0.143[a] 0.141[b]	——	——
THF/PS (M_w=470000)	0.191	0.180	0.999	0.189[c]	0.186[f]	0.184(GD)
THF/PMMA (M_w=125000)	0.093	0.088	0.995	0.086[d] 0.086[e]	0.087[f]	0.087(GD)

[a](WIGAND and WEITH, 1969); [b](ESKIN et al., 1965); [c](ELIAS and ETTER, 1963); [d](BODMAN, 1969); [e](CANTOW and BODMAN, 1955); [f](MILLAUD and STRAZIELLE, 1979).

A comparative study of our results (columns A and B) to literature ones (columns C, D and E) shows that dn/dc values obtained using the aqueous medium calibration (column B) are in accordance with those reported by other authors, while higher deviations are observed for values in column A. Therefore we can conclude that a greater accuracy is reached when using salt solutions as calibration standards than the Cl_4C/benzene ones.

The variation of dn/dc with temperature can be positive, negative or rarely zero, as for example polyacrylic acid, poly(methacrylic acid) and poly(methacrylamide) in water (SILBERBERG et al., 1957). More precisely, the value of d(dn/dc)/dT generally lies in the region 1×10^{-4} to 5×10^{-4} ml·g^{-1}/°C (HUGLIN, 1972). In some cases, however, this value changes considerably due to the possible existence of a conformational transition for the solute. This possibility has been confirmed by measuring the dn/dc variation with T for lysozyme in a phosphate buffer solution (pH=8), in which a conformational transition has been previously detected by other techniques (JOLLES, 1975). In figure 1, dn/dc variation for lysozyme in the temperature range 16 to 30 °C is shown. As it can be seen, dn/dc remains constant except in the 21-23°C range, where an approximate decrease of 8% with respect to the mean value of the dn/dc at the remaining temperatures is observed, in accordance with the temperature at which lysozyme suffers a structural transition in the solid phase (COZZONE et al., 1975). Nevertheless, for the quantitative evaluation of conformational transitions using the modified DRI R 401, it is necessary to take into account that the magnitude of error inferred from calibration is about 5%.

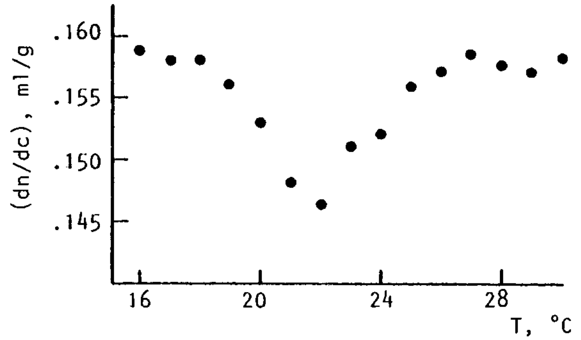

Fig.1. (dn/dc) dependence on temperature for lysozyme solutions in phosphate buffer.

On the other hand, specific volume and dn/dc value are experimental parameters of great interest when studyng multicomponent macromolecular solution systems. These magnitudes are important not only for the determination of molecular weights, but also for the evaluation of preferential solvation coefficients. These parameters can be obtained from refractive index increments at constant chemical potential, $(dn/dc)_\mu$, and constant chemical composition, $(dn/dc)_k$. The modified DRI R 401 has also been used to evaluate the $(dn/dc)_k$ of n-alkane/MEK/PDMS(M_w=125000) ternary systems covering a range of compositions (expressed as volume fraction, ϕ_{10}) from 0 to 1. The obtained values are shown in table 2, wherefrom the evaluation of preferential solvation coefficients is possible(CAMPOS et al., 1984 and in press a). Moreover, from these results it is possible to evaluate the partial specific volume of PDMS, $\bar{v}_3$, through GD, Lorenz-Lorentz(LL) and Eykman(EY) correspondingly linearized equations. Good linear correlations are obtained for all the systems, except for the HEP/MEK/PDMS one. The anomalous behaviour in that system is probably due to the similarity of polymer and solvent refractive indices, leading to large errors in the dn/dc evaluation and in its derived magnitudes, In this case it is not advisable to use RD. n_3 and $\overline{v}_3$ obtained values from GD, LL and EY equations are gathered in table 3.

TABLE 2

Variation of $(dn/dc)_k$ at 633 nm with solvent mixture composition for n-alkane/MEK/PDMS(M_w=125000) systems.

ϕ_{10}	n-alkane							
	HEX	HEP	OCT	NON*	DEC	UND*	DOD	HXD
0	0.0295	0.0295	0.0295	0.0295	0.0295	0.0295	0.0295	0.0295
0.10		0.0295		0.0275	0.0260	0.0260	0.0250	0.0240
0.20	0.0305	0.0295		0.0250	0.0236	0.0226	0.0206	0.0170
0.30		0.0293		0.0230	0.0209	0.0200	0.0178	0.0130
0.40		0.0287		0.0215	0.0184	0.0160	0.0140	0.008
0.50	0.0315	0.0280	0.0225	0.0205	0.0160	0.0115	0.0100	
0.60		0.0227	0.0220	0.0165	0.0124	0.0086	0.0030	-0.002
0.70		0.0265	0.019	0.0115	0.010	0.0017	0.0015	-0.010
0.80	0.0310	0.0250	0.017	0.0090		-0.0003	-0.0065	-0.0145
0.90		0.0240	0.016	0.0060	-0.007	-0.0098	-0.0100	
1	0.030	0.0190	0.008	-0.0100	-0.010	-0.0161	-0.0150	

*Partially reported by (CAMPOS et al., in press a and b)

TABLE 3

Refractive index, n_3, and partial specific volume at infinite dilution, $\overline{v}_3$, for PDMS(M_w=125000) at 25°C and 633 nm.

n-alkane	n_3^{GD}	$\overline{v}_3^{GD}$	n_3^{LL}	$\overline{v}_3^{LL}$	n_3^{EY}	$\overline{v}_3^{EY}$
Heptane	1.4056	1.085	1.4066	1.0466	1.4064	1.0519
Nonane	1.3994	1.3135	1.3994	1.322	1.3996	1.3204
Decane	1.4047	1.141	1.4036	1.2025	1.4036	1.190
Undecane	1.4035	1.1840	1.4035	1.189	1.4049	1.1683
Dodecane	1.4055	1.0473	1.4054	1.0723	1.4051	1.0679
Hexadecane	1.4023	1.0034	1.4020	1.0455	1.4023	1.0427

It is worthwhile to remark that, at polymer infinite dilution, $\overline{v}_3$, depends on the solvent nature. The deviations between $\overline{v}_3$ and v_3 (reciprocal of density) in a solvent indicate the swelling degree of the polymer in that solvent. $\overline{v}_3$ values in table 3 decrease with the increasing number of carbon atoms in the n-alkane, with a minimum value $\overline{v}_3^{EY}$= 1.0427 ml/g for n-hexadecane very close to v_3 = 1.0282 ml/g for the pure solute. Moreover, $\overline{v}_3$ variation is in agreement with the solvatant power of the solvent as expressed through the a exponent of the Mark-Howink-Sakurada equation. So, a values decrease with the number of carbon atoms of the n-alkane from 0.69 for n-heptane, down to a<0.50 for n-hexadecane; θ conditions (a = 0.50) being reached for n-pentadecane (CAMPOS et al., in press b).

From the comparison of the results obtained for $\overline{v}_3$ in all the systems, using different equations, it can be observed that GD results usually are lower than LL and EY ones, these last ones being similar to each other. With respect to n_3 values, they are very close and in accordance with the value 1.4056 at 589nm(CELDA, 1984).

The above results show the adequate performance of the modified DRI R 401 and its use, as conclusion, offers additional advantages such as: low cost, the simplicity of the assemblage and alignment of the beam and the small amount of sample required for the measurements.

REFERENCES

ABAD,C.,TRUEBA,M.ZATON,A. and CAMPOS,A.: An. Quim. 75, 993(1979)
BODMAN, O.: Makromol. Chem. 122, 196(1969)
CAMPOS, A., CELDA, B. TEJERO, R. and FIGUERUELO, J.E.: Europ. Polym. J. 20, 447(1984)
CAMPOS, A., CELDA, B., MORA, J. and FIGUERUELO, J.E.: Polymer (in press a)
CAMPOS, A., CELDA, B., MORA, J. and FIGUERUELO, J.E.: Europ. Polym. J. (in press b)
CANTOW, H.-J. and BODMAN, O.: Z. Phys. Chem. 3, 65(1955)
CELDA, B.: PhD These, Valencia University, Valencia(Spain)
COZZONE, P., OFELLA, J.J., JARDIZKY, O., BERTHOV, J. and JOLLES, P.: Proc. Natl. Acad. Sci. US 72, 2095(1975)
ELIAS, H.G. and ETTER, O.: Makromol. Chem. 66, 56(1963)
ESKIN, V.E., IZYUMNIKOV, A.L., ROGOZHKINA, E.D. and VYRSKII, YU.P.: Polym. Sci. USSR 7, 1310(1965)
HUGLIN, M.B.: Light Scattering from Polymer Solutions, London: Academic Press 1972
HUGLIN, M.B.: Polymer Handbook, 2nd Ed., New York: Wiley-Interscience 1975
IMS: Instruction Manual Series R 400, Waters Assoc. Inc. Mass. USA 1976
JOLLES, P.: Febbs Lett. 55, 165(1975)
KATIME, I. and ROIG, A.: An. Quim. 70, 853(1974)
KRUIS, A.Z.: Physik. Chem.B 34, 13(1936)
MILLAUD, B. and STRAZIELLE, C.: Makromol. Chem. 180, 441(1979)
RIDDIK, J.A. and BUNGGER, W.B.: Organic Solvents, Vol. 2, 3th Ed., New York: Interscience Publis. Inc. 1970
SAIN BLANCHARD, J., CLOCHARD, A., COZZONE, P.J. and JOLLES, P.: Biochim. Biophys. Acta 491, 354(1977)
SILBERBERG, A., ELIASSAF, J. and KATCHALSKY, A.: J. Polym. Sci. 23, 259(1957)
SORIA, V., CAMPOS, A. and FIGUERUELO, J.E.: An. Quim. 74, 1026(1978)
SYNOVEC, R.E. and YEUNG, E.S.: Anal. Chem. 55, 1599(1983)
WIGAND, G. and WEITH, J.J.: Past. und Kaut. 16, 671(1969)

Accepted December 7, 1984