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Enantiomeric separations of benzodiazepines by electrochemically modulated liquid chromatography

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Abstract

Electrochemically modulated liquid chromatography (EMLC) has been applied to the enantiomeric separation of a group of benzodiazepines (i.e., oxazepam, lorazepam and temazepam) using a porous graphitic carbon (PGC) stationary phase and β-cyclodextrin (β-CD) as a chiral mobile phase additive. The basis of the separation derives from the reversible electrosorption of the additive onto PGC, resulting in a new approach for manipulating the chirality and selectivity of the stationary phase. Changes in the voltage applied (E_{appl}) to the stationary phase were found to alter the retention and the enantioselectivity of the separation, both of which reflect the dependence of the electrosorption of the additive on E_{appl}. By exploiting these dependencies, mixtures of each of the three racemates could be resolved to differing extents while using the same isocratic elution condition. A general description of the retention process and the role of the apparent enantiomerization of oxazepam and lorazepam are briefly discussed.

Keywords: Enantiomer separation; Electrochemically modulated liquid chromatography; Porous graphite carbon; Liquid chromatography, electrochemically modulated; Stationary phases, LC; Benzodiazepines; Oxazepam; Lorazepam; Temazepam

1. Introduction

The separation and identification of enantiomeric compounds represents one of the most challenging tasks in separation science [1–9]. The most common approaches using high-performance liquid chromatography (HPLC) include: derivatization of enantiomers to form diastereomers which are subsequently separated at an achiral column; separations at an achiral column of the diastereomers which are formed by complexation with a chiral mobile phase additive; and separations of enantiomers which are carried out on chiral stationary phases (CSPs) [10–12].

In this paper, we demonstrate the ability to manipulate the retention and efficiency of chiral separations by a coupling of electrochemical and liquid chromatographic techniques [13–31]. This novel separation strategy derives from the ability to alter the retention characteristics of conductive stationary phases [e.g., porous graphitic carbon (PGC)] through changes in the potential applied (E_{appl}) to a LC column that is configured as an electrochemical cell. We have termed this approach electrochemically modulated liquid chromatography (EMLC). As reported by several groups [13–22], including our own [23–31], alterations in E_{appl} have a marked influence on the retention of a wide range of analytes. More recently [31], we have shown that EMLC can be applied to separations of the enantiomeric pharmaceutical compounds hexobarbital and

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mephenytoin by using PGC as a stationary phase and β-cyclodextrin (β-CD) as an electrosorbed chiral additive.

This paper continues this new facet of our investigations on the range and scope of EMLC by exploring the enantiomeric separation of the three benzodiazepines shown in Fig. 1. Since their discovery in 1957 [32], benzodiazepines have become among the most widely prescribed drugs for the treatment of anxiety, insomnia, convulsion and related disorders. The therapeutic efficacy of these compounds has, however, been shown to be enantiomeric specific [33,34]. The enantiomeric separation of these compounds, generally carried out by using synthetically-created CSPs [35–39] or by adding a chiral selector in mobile phase and an achiral stationary phase [40], is therefore an important part of the analytical methodologies in pharmaceutical laboratories. We demonstrate herein that an EMLC-based separation can be applied to the enantiomeric separations of the three benzodiazepines by manipulating the reversible electrosorption of β-CD from the mobile phase onto PGC. Indeed, by exploiting the ability to alter the extent of the electrosorption of β-CD through changes in E_{appl}, we show that the enantiomers for all three compounds can be at least partially resolved under the same isocratic elution conditions. A general description of the retention process and the role of the apparent enantiomerization of oxazepam and lorazepam are also briefly discussed.

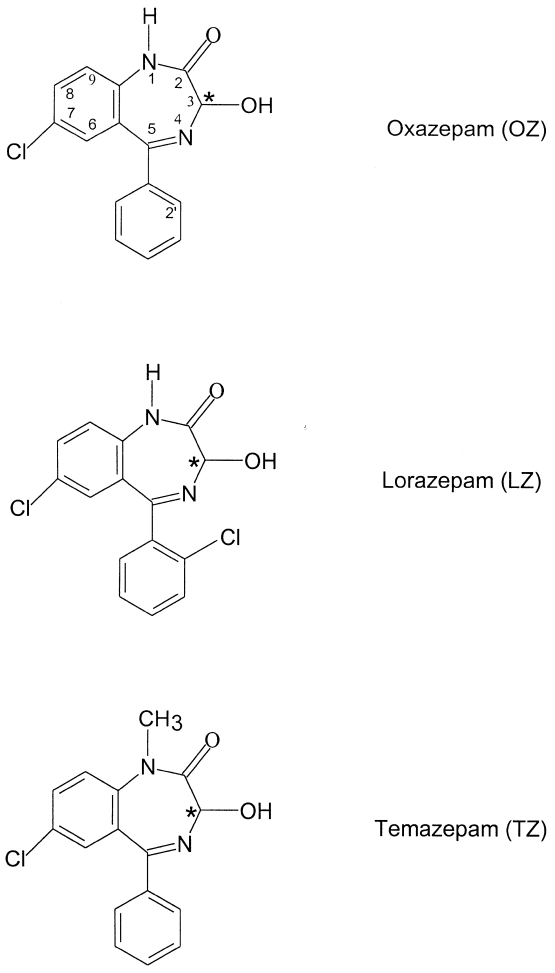

Fig. 1. Chemical structures of the benzodiazepines.

2. Experimental

2.1. Reagents and chemicals

The chemical structures for each of the benzodiazepines are shown in Fig. 1. Oxazepam (OX), lorazepam (LZ), and temazepam (TZ) were purchased from Sigma (St. Louis, MO, USA); lithium perchlorate from Aldrich (Milwaukee, WI, USA); acetonitrile (HPLC grade), sodium phosphate monobasic (NaH_2PO_4), orthophosphoric acid (H_3PO_4), and sodium hydroxide from Fisher Scientific (Fair Lawn, NJ, USA); dibromomethane from Eastman Kodak (Rochester, NY, USA); and ethanol from Quantum Chemical (Newark, NJ, USA). The β-CD was kindly supplied by Cerestar USA, (Hammond, IN, USA). All chemicals were used as received, with aqueous solutions prepared using water obtained from a Millipore Milli-Q purification system (Bedford, MA, USA). Before use, all solutions were filtered through a 0.20-μm filter (Alltech, Deerfield, IL, USA) and thoroughly degassed by sparging with helium.

2.2. Instrumentation

Chromatographic experiments were performed using an HP 1050 series module equipped with a

solvent cabinet, pumping system and diode array detector (Hewlett-Packard, Santa Clara, CA, USA). Sample solutions were injected via a Rheodyne Model 7125 injector with a 5-μl loop (Cotati, CA, USA). A HP 1047A refractive index detector was used for assessing the retention characteristics of β-CD. The pH of the buffer was determined before mixing with the organic component of the mobile phase by using an Orin Model 520 A pH meter (Boston, MA, USA); all pH values are reported as the pH of the unmixed aqueous component of the mobile phase. The voltage applied to the stationary phase was controlled by a Princeton Applied Research Model 173 potentiostat–galvanostat (Princeton, NJ, USA).

2.3. Chromatographic operation

The general construction of the EMLC column, which has a length of 9.2 cm and an internal diameter of 0.3 cm and was packed with ~7 μm PGC spheres (Hypersil, Runcon, UK), has been described elsewhere [28]. Between changes in E_{appl}, the column was equilibrated with mobile phase at a flow-rate of 0.90 ml/min until a stable detector baseline at 230 nm was obtained (~30 min). The mobile phase was composed of two components: 75% water [0.1 *M* $LiClO_4$, 6 m*M* β-CD, 20 m*M* phosphate buffer (pH~1.8)] and 25% acetonitrile (0.1 *M* $LiClO_4$). This mobile phase was selected to provide an inert solution that was effective in solubilizing both β-CD and the supporting electrolyte as well as in providing a sufficient acidity to protonate the analytes. The operational back pressure was ~2800 p.s.i. (1 p.s.i.=6894.76 Pa) The dead volume of the column (0.63 μl) was determined by the injection of water. The open circuit potential of the column was +0.18 V with respect to Ag/AgCl/saturated NaCl electrode; all values of E_{appl} are given herein with respect to this reference. The voltage window for E_{appl} was held between +0.50 and −1.00 V; these limits reflect concerns about the possible oxidation of PGC above +0.50 V and the reduction of the solvent below −1.00 V [28,41,42]. The racemates were dissolved in methanol at total concentrations of ~100 ppm. Injection volumes were 5 μl. All experiments were conducted at 25°C.

2.4. Data analysis

The effectiveness of the enantiomeric separations was assessed by estimations of the separation factor (α), where $\alpha = k_2'/k_1'$ and k_1' represents the capacity factor for the more weakly retained enantiomer and k_2' the capacity factor for the more strongly retained enantiomer. The values for k_1' were calculated from the expression: $k_1' = (t_1 - t_0)/t_0$ where t_1 is the retention time for enantiomer 1 and t_0 is the column dead time. The same approach was used in the determination of k_2' for enantiomer 2.

3. Results and discussion

3.1. Cyclodextrin-based chiral separations

β-CD is a neutral, chiral compound composed of seven D-(+)-glucopyranose units that are interconnected via α-1,4 linkages. β-CD is shaped like a hollow truncated cone in which the rim of the torus with the larger circumference has 14 secondary hydroxyl groups and the rim on the smaller side of the torus has seven primary hydroxyl groups. The secondary hydroxyl groups are directly connected to chiral carbon centers. The interior of the cavity is relatively hydrophobic when compared to the strongly hydrophilic rims [43]. Because of favorable hydrophobic and/or hydrogen-bonding interactions, many organic molecules form inclusion complexes with β-CD. As a consequence of the high spatial conformity that is central to inclusion complexation, there can be appreciable differences in the binding strengths for each of the complexes formed from an enantiomeric pair of guests [44,45] which serves as the mechanism for an enantioseparation. Thus, by controlling the amount of electrosorbed β-CD through E_{appl}, EMLC can be used to manipulate reversibly the chirality of the PGC surface [31], which affects the efficiency of an enantiomeric separation. To this end, the next section examines the electrosorption of β-CD on PGC.

3.2. Retention dependence of β-CD as a function of E_{appl}

In a preliminary study [31], we attributed the

dependence of the electrosorption of β-CD on PGC to the effect of E_{appl} on the donor interactions of the primary alcohols on the small side of the cavity. This assertion is similar to that proposed for the electrosorption of β-CD on mercury [46]. Fig. 2 shows a structural representation of β-CD and outlines the key aspects of the electrosorption of β-CD onto PGC. As E_{appl} moves positively, PGC becomes a stronger acceptor, and the amount of electrosorbed β-CD increases [27]. On the other hand, the amount of electrosorbed β-CD decreases as E_{appl} becomes more negative because the acceptor strength of PGC decreases.

Fig. 3 shows the results of chromatographic experiments that test the above assertion. These experiments were conducted by injecting 5-μl solutions of a 6 m*M* aqueous solution of β-CD in mobile phase devoid of β-CD and by using refractive index detection. The injection peak is observed at ~0.8 min in all four cases. As proposed, the more positive the value of E_{appl}, the greater the retention of β-CD. Indeed, β-CD is very weakly retained by PGC at

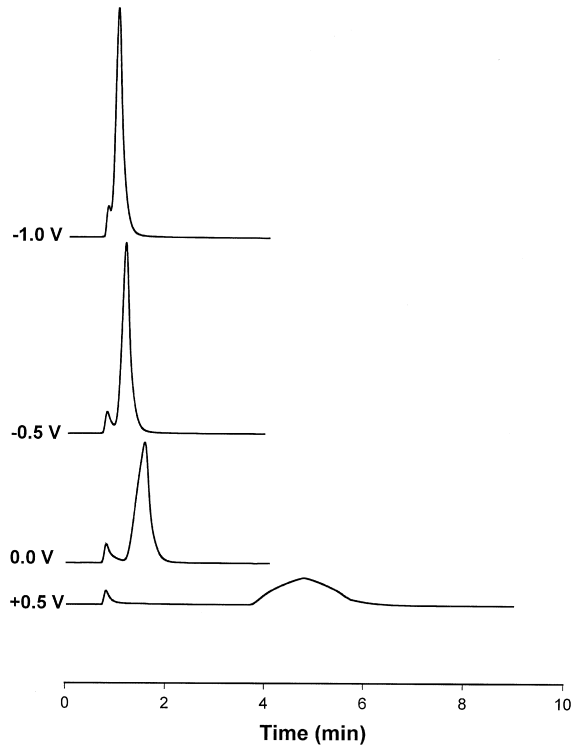

Fig. 3. Retention behavior of β-CD on a PGC stationary phase as a function of E_{appl}: +0.5 V, 0.0 V, −0.5 V and −1.0 V. All the values of E_{appl} are given with respect to a Ag/AgCl/saturated NaCl electrode. The mobile phase was composed of two components: 75% water [0.1 *M* $LiClO_4$, 20 m*M* phosphate buffer (pH~1.8)] and 25% acetonitrile (0.1 *M* $LiClO_4$). The flow-rate was 0.9 ml/min. The *y*-axis is the change in refractive index in arbitrary units. The peak at ~0.8 min is the solvent injection peak.

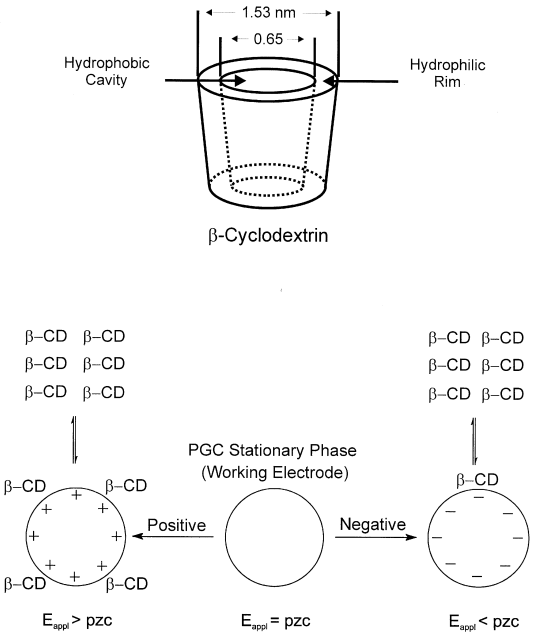

Fig. 2. Schematic diagram of β-CD structure and an idealized depiction of the electrosorption process of β-CD onto PGC (pzc-potential of zero charge).

−1.0 V, but is more strongly retained at +0.5 V. It follows that a chiral separation at the more negative values of E_{appl} will likely be dominated by interactions with β-CD dissolved in the mobile phase. In contrast, the interactions of analytes with β-CD electrosorbed on the stationary phase will become increasingly important as E_{appl} becomes more positive, with the enantiomeric elution order reflecting the competition between the solubilized and electrosorbed forms of β-CD and the enantiomeric pair. This dependence, while insufficient for developing more fundamental insights into the retention process (e.g., assessment of the mode of interactions, spatial orientation and fraction of electrosorbed β-CD operative in chiral selectivity), offers a new parame-

ter to manipulate when developing chiral separations [31].

3.3. Effect of E_{appl} on the retention of benzodiazepines

As shown in Fig. 1, OZ, LZ and TZ have a common chiral center, the carbon at the 3-hydroxy-substituted position of the benzodiazepine ring. The distinguishing feature among these compounds is the 2-chloro substituent that is present at the 5-phenyl group in LZ but is absent in OZ and TZ, and the methyl substituent that is present at the C-1 ring position in TZ but is absent in OZ and LZ. Interestingly, the subtle structural differences for the three compounds suggest that the conditions required for the HPLC-based separation of their enantiomers should be similar. Recent reports, however, show that this expectation is not realized. In a study on enantiomeric separations of benzodiazepines using a chiral column of α_1-acid glycoprotein [37], the enantiomers of OZ and LZ were effectively resolved, but not those of TZ. Use of a chiral cellulose triacetate column marginally resolved the enantiomers of TZ, but failed to resolve those of OZ and LZ [40]. On the other hand, only the enantiomers of OZ were partially resolved using a β-CD derivatized column [40]. In addition, attempts to exploit β-CD as mobile phase additive with a PGC column were successful only in resolving the enantiomers of OZ and LZ [40]. These complications motivated us to investigate the enantiomeric separations of these compounds as another benchmark for assessing the range and scope of EMLC.

As a starting point, chromatograms of racemic mixtures of OZ (Fig. 4), LZ (Fig. 5) and TZ (Fig. 6) are presented at three different values of E_{appl} using mobile phase devoid of β-CD. Fig. 7 summarizes these results through plots of log k' vs. E_{appl}; the error bars for log k' are roughly represented by the physical size of the symbols used to denote the data points, and represent the range of three or more replicate separations. There are four observations that can be drawn from these results. First, the retention of all three compounds increases monotonically as E_{appl} becomes more negative. These trends are consistent with the strong acceptor character of

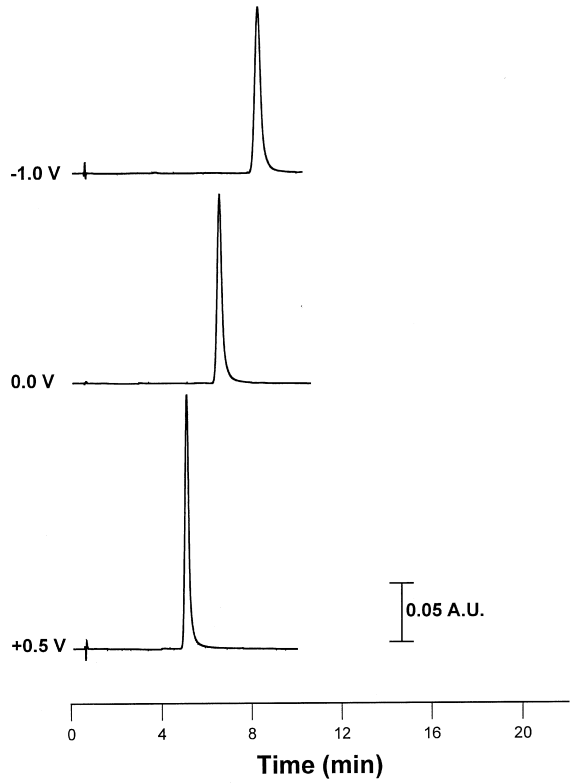

Fig. 4. Separation of OZ on a PGC stationary phase as a function of E_{appl}: +0.5 V, 0.0 V and −1.0 V. All the values of E_{appl} are given with respect to a Ag/AgCl/saturated NaCl electrode. The mobile phase was composed of two components: 75% water [0.1 *M* $LiClO_4$, 20 m*M* phosphate buffer (pH~1.8)], and 25% acetonitrile (0.1 *M* $LiClO_4$). The flow-rate was 0.9 ml/min. The detection wavelength was 230 nm.

the analytes, which results from the protonation of their nitrogen atoms at the 4-position of the diazepine ring under our elution conditions [47]. Second, the elution order (i.e., OZ<LZ<TZ) is the same at all values of E_{appl}. The elution order, which may partially reflect differences in hydrophobicity [48], is generally the same as that reported using PGC and a slightly different mobile phase [29]. Third, the enhancements in retention as E_{appl} becomes more negative differ somewhat for the three compounds. While presently uncertain as to the origin of these differences, the enhancement as E_{appl} moves negatively is TZ>LZ>OZ. Forth, and most importantly, there is no detectable enantiomeric resolution in any of the separations. This result is

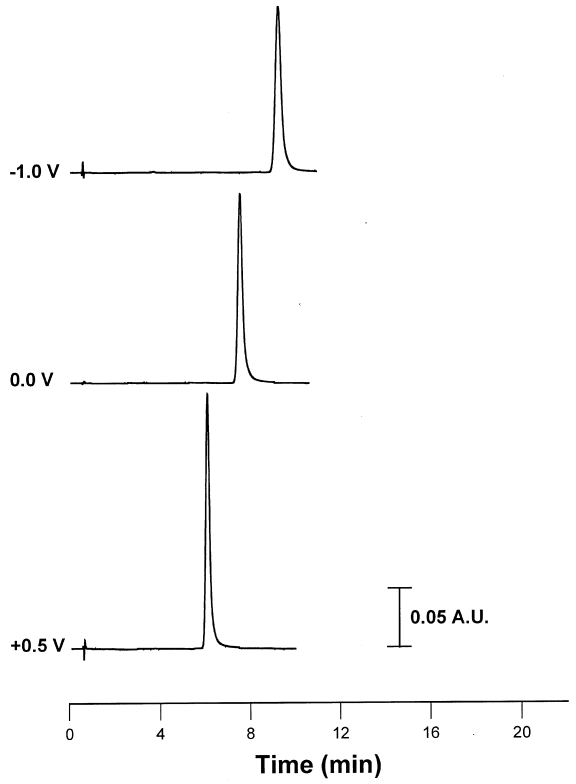

Fig. 5. Separation of LZ on a PGC stationary phase as a function of E_{appl}. Conditions as in Fig. 4.

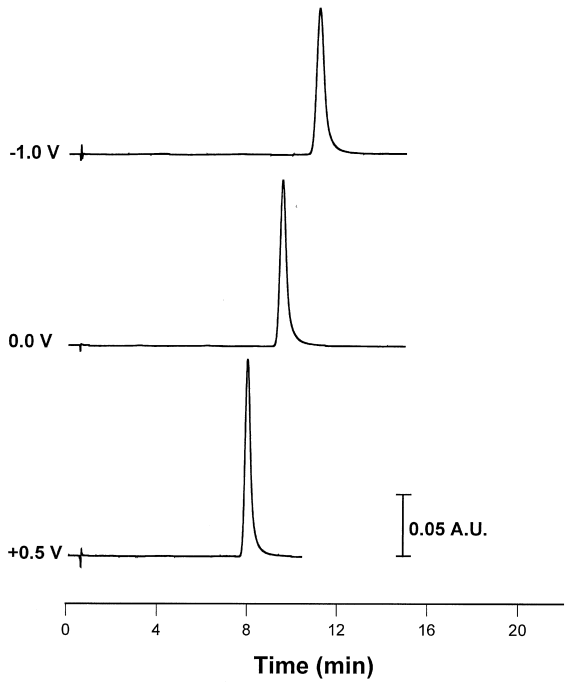

Fig. 6. Separation of TZ on a PGC stationary phase as a function of E_{appl}. Conditions as in Fig. 4.

expected for a separation system devoid of a chiral selector.

3.4. Effect of β-CD as an additive to the mobile phase

Marked differences in the above separations are found when β-CD is added to the mobile phase. Fig. 8 shows the chromatograms of a racemic mixture of OZ with the chiral selector β-CD present in the mobile phase at five different values of E_{appl}: +0.5 V, +0.3 V, 0.0 V, −0.5 V and −1.0 V. Importantly, an enantiomeric separation is observed at several, but not all values of E_{appl}. Enantiomeric separations are clearly evident at +0.5 V, and +0.3 V, with corresponding α values of 1.20, and 1.16. There are only hints of enantioseparations at −0.5 V and −1.0 V, and no observable enantioseparation at 0.0 V. We also note that the separations at +0.3 V and +0.5 V exhibit characteristics diagnostic of the partial (10–15%) enantiomerization of OZ. This on-column conversion has been observed for OZ as well as LZ (see below), requiring, for example, the use of lower separation temperatures to minimize the intraconversion and enhancing sample quantitation [49–51].

The same experiments were performed for separating the racemic mixtures of LZ and TZ, as shown in Figs. 9 and 10, respectively. As with OZ, enantioseparations of both LZ and TZ are realized at several values of E_{appl}. The α values for LZ are 1.24 at +0.5 V and 1.20 at +0.3 V; the α values for TZ are 1.10 at −1.0 V and 1.08 at −0.5 V. We suspect that the distortion of the first elution band at +0.5 V arises from the use of methanol for solubilizing LZ; methanol is a stronger eluent than the mobile phase which can result in a momentary, partial distortion of the injection plug [52]. This issue, along with the apparent enantiomerization of LZ, will require further examination before developing a more exacting analysis of the results.

It is interesting to compare the results in Figs.

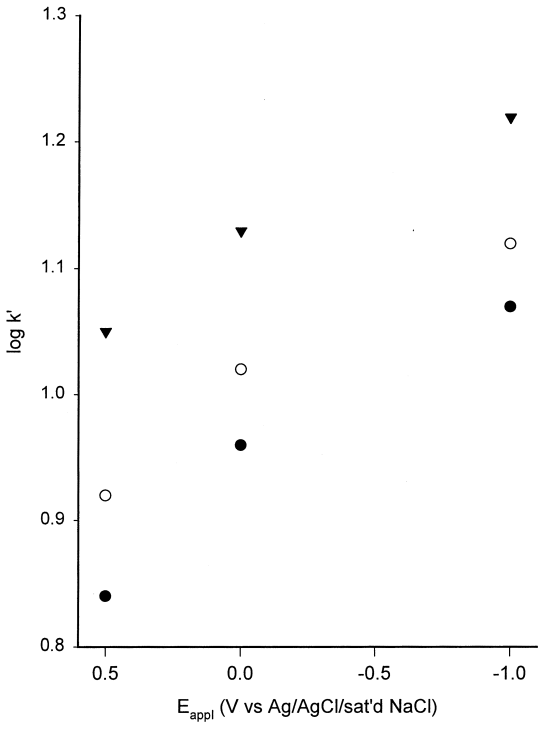

Fig. 7. Plots of log k' vs. E_{appl} for the separation of racemates of OZ (▼), LZ (○) and TZ (●) from Figs. 4–6.

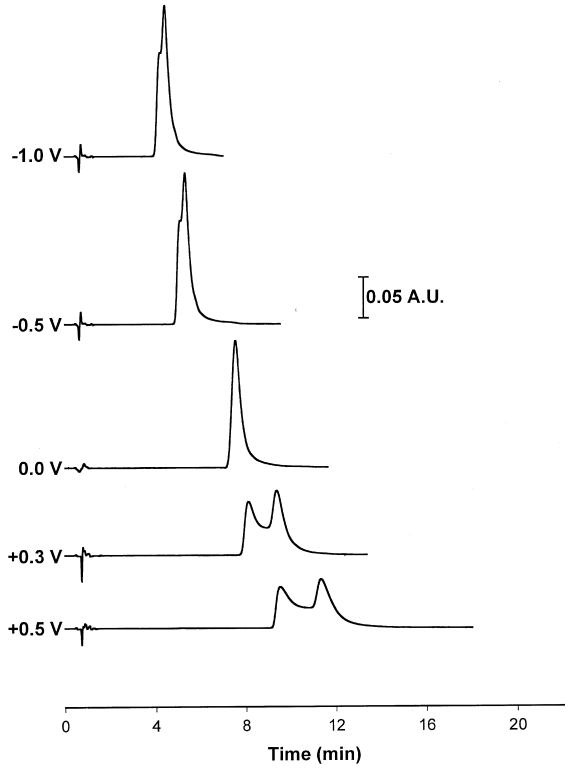

Fig. 8. Separation of OZ on a PGC stationary phase as a function of E_{appl}: +0.5 V, +0.3 V, 0.0 V, −0.5 V and −1.0 V. All the values of E_{appl} are given with respect to a Ag/AgCl/saturated NaCl electrode. The mobile phase was composed of two components: 75% water [0.1 *M* $LiClO_4$, 6 m*M* β-CD, 20 m*M* phosphate buffer (pH~1.8)], and 25% acetonitrile (0.1 *M* $LiClO_4$). The flow-rate was 0.9 ml/min. The detection wavelength was 230 nm.

8–10 with those reported for these compounds using β-CD as a mobile phase additive [40]. The latter separation proved only partially effective in resolving the enantiomers of OZ and LZ, but totally ineffective in resolving those of TZ. Figs. 8–10 not only demonstrate an improved effectiveness in resolving the enantiomers of all three compounds, but a decrease in the overall elution time by more than 50%. These results testify to the potential value of EMLC as a new tool in the arsenal of chromatographic techniques.

Figs. 8–10 also show that changes in E_{appl} affect the retention of OZ and LZ differently than that of TZ. The retention for TZ with and without β-CD increases as E_{appl} becomes more negative. The situation for OZ and LZ is somewhat different. Like TZ, the retention of OZ and LZ increases as E_{appl} moves negatively when using mobile phase without of β-CD. With β-CD in the mobile phase, however, the retention of OZ and LZ decreases as E_{appl} becomes more negative, whereas that of TZ increases as E_{appl} increases negatively. The next section examines these observations by developing a preliminary description of the retention process.

3.5. Insights into the EMLC-based chiral separation mechanism

This section briefly examines the possible factors that affect the enantiomeric separation of the three racemic mixtures via EMLC. The intent is to assess qualitatively how changes in E_{appl} affect these separations in order to build a foundation for advancing applications as well as for designing more in-depth mechanistic investigations. To this end, Fig. 11 presents the possible equilibria involved in the

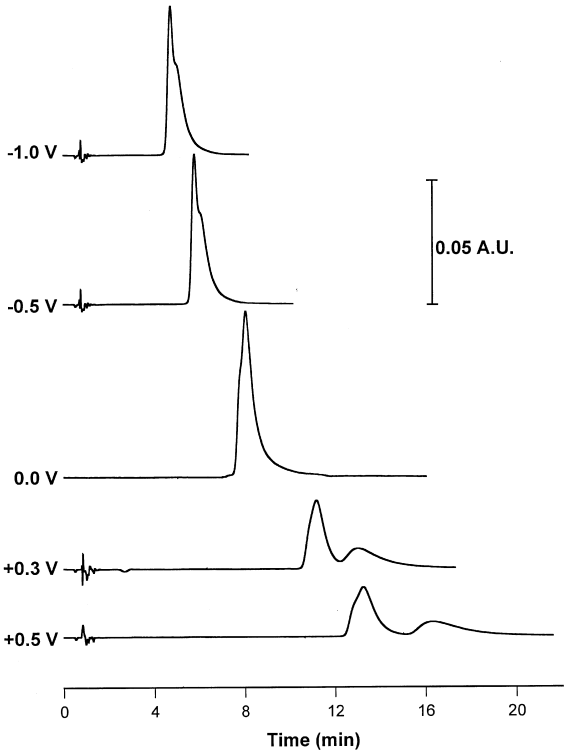

Fig. 9. Separation of LZ on a PGC stationary phase as a function of E_{appl}. Conditions as in Fig. 8.

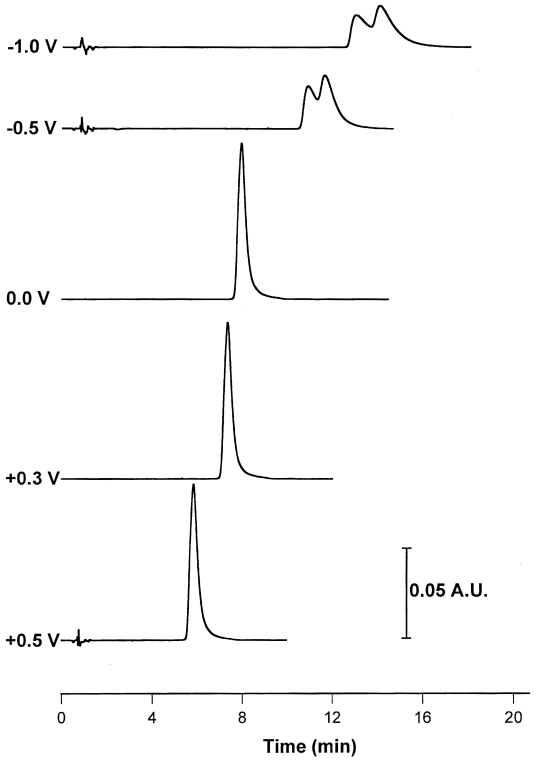

Fig. 10. Separation of TZ on a PGC stationary phase as a function of E_{appl}. Conditions as in Fig. 8.

separation of an enantiomerically pure analyte A on PGC using β-CD as a mobile phase additive, with (m) and (s) denoting whether the species is present in the mobile phase or electrosorbed onto PGC. Drawing from our previous treatment [31], we will assume that: (1) the electrosorption of the inclusion complex A:β-CD formed solely in the mobile phase plays a minor role in the overall retention process, an assertion that reflects the pre-equilibration of the column with β-CD; and (2) the binding of β-CD from the mobile phase to electrosorbed A is negligible, an argument based on steric considerations. As such, the equilibria viewed initially as contributing to the overall retention process are: (1) electrosorption of the chiral selector β-CD; (2) inclusion complexation of A(m) with β-CD(m) or with β-CD(s); and (3) electrosorption of A(m) onto PGC. In all cases, it is the chiral selectivity of β-CD that controls the effectiveness of the enantiomeric separation, with the elution order for a pair of enantiomers determined primarily by the competition between the inclusion of A(m) with either β-CD(m) or β-CD(s).

As demonstrated, the presence of β-CD has dramatic but differing effects on the retention of OZ and LZ with respect to TZ. The dependence of the retention for TZ on E_{appl} when β-CD is present follows that as found using mobile phase devoid of β-CD. In contrast, the retention dependencies of OZ and LZ on E_{appl} when using mobile phase containing β-CD are opposite of those found when β-CD is absent from the mobile phase. We attribute these observations to the differences in the binding strength of the inclusion complexes formed by the

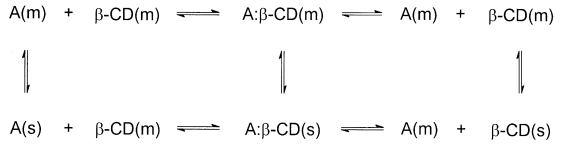

Fig. 11. Possible equilibria (see Section 3.5).

analytes with β-CD and how these differences compete with the interactions of the analytes directly with PGC. Although the stability constants (K) for the binding of β-CD with the enantiomers of OZ, LZ and TZ were not found, an examination of the structural differences and similarities of the three compounds that draws on literature precedents [49–51,53,54] provides a rough assessment of relative magnitudes. This examination suggests that K for TZ is smaller than those for OZ and LZ because of the steric hindrance to complexation posed by the methyl substituent at the C-1 position of the benzodiazepine ring of TZ.

As a consequence of the differences in K, the concepts that we used previously to describe the enantiomeric separations of hexobarbital (HE) and mephenytoin (ME) on PGC coated with β-CD(s) [31] can be applied as a starting point for unraveling the basis of the retention trends for OZ, LZ and TZ. That is, the overall retention is determined by the competition between analyte interacting directly with PGC and analyte interacting with either β-CD(m) or β-CD(s). For OZ and LZ, the larger values of K argue that β-CD plays a more significant role in the retention process than in the case for TZ. This importance is reflected by the decrease in the retention of OZ and LZ at the more negative values of E_{appl} when β-CD is in the mobile phase. Similarly, the stronger retention of OZ and LZ at the more positive values of E_{appl} arises from their apparently larger affinity for β-CD(s) than for unmodified PGC. This analysis is consistent with that discussed for the enantioseparation of HE using electrosorbed β-CD and PGC [31]. In the latter case, we also found that the elution order of the D- and L-forms of HE reversed depending upon whether β-CD(s) or β-CD(m) controlled the enantiomeric separation.

The reverse arguments can be applied to describe the trends in the separations of TZ which parallel those observed in our separation of ME [31]. With TZ, we believe that the weaker retention found at positive values of E_{appl} when β-CD is present in the mobile phase originates from a lower affinity of TZ for electrosorbed β-CD than for unmodified PGC. We also view that the stronger retention of TZ at negative values of E_{appl} when β-CD is present is from a contribution of the sorption of the TZ:β-CD complex onto PGC. Though only speculative at present, the increased retention suggests that this equilibrium may play a more significant role in the overall process than was originally believed. We are presently devising experiments to evaluate this possibility.

4. Conclusions

This paper has demonstrated the application of EMLC to the chiral separation of a group of closely related enantiomeric benzodiazepines (i.e., oxazepam, lorazepam, and temazepam) on a PGC stationary phase. This capability is realized through the manipulation of the selectivity of PGC by the electrosorption of a chiral additive from the mobile phase. Though not discussed, we also found that the extent of electrosorption of β-CD was reversible. That is, after flowing mobile phase devoid of β-CD through the column used for the separation in Fig. 8 for ~30 min while holding E_{appl} at −1.0 V, the retention times for OZ at uncoated PGC were effectively the same as in Fig. 4. This observation suggests that electrosorption may provide as a means to alter the surface composition of PGC where, for example, an achiral column could be readily converted to a column with reversed-phase character by electrosorption of long chain alcohols and amines. Efforts to extend the capability of EMLC to other chiral compounds as well as to explore the use of electrosorption to create other types of chromatographic phases are underway. Studies to further our insights into the EMLC-based chiral separation mechanism, as well as solutions needed for overcoming the observed enantiomerization of OZ and LZ, are also being pursued.

Acknowledgements

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References

[1] V.A. Davankov, Adv. Chromatogr. 18 (1980) 139.
[2] D.W. Armstrong, T.J. Ward, R.D. Armstrong, T.E. Beesley, Science 232 (1986) 1132.
[3] W.L. Hinze, T.E. Riehl, D.W. Armstrong, W. Demond, A. Alak, T. Ward, Anal. Chem. 57 (1985) 237.
[4] A.M. Stalcup, A.-C. Chang, D.W. Armstrong, J. Chromatogr. 540 (1991) 113.
[5] D. Sybilska, J. Zukowski, J. Bojarski, J. Liq. Chromatogr. 9 (1986) 591.
[6] M. Gazda, G. Szepesi, L. Huszar, J. Chromatogr. 436 (1988) 31.
[7] D.W. Armstrong, J. Liq. Chromatogr. 7 (1984) 591.
[8] T.J. Ward, Anal. Chem. 66 (1994) 633A.
[9] M. Novotny, H. Soini, M. Stefansson, Anal. Chem. 66 (1994) 646A.
[10] J.G. Dorsey, W.T. Cooper, B.A. Siles, J.P. Foley, H.G. Barth, Anal. Chem. 68 (1996) 515R.
[11] R.K. Gilpin, L.A. Pachla, Anal. Chem. 69 (1997) 145R.
[12] R.E. Boehm, D.E. Martire, D.W. Armstrong, Anal. Chem. 60 (1988) 522.
[13] R.F. Antrim, R.A. Scherrer, A.M. Yacynch, Anal. Chim. Acta 164 (1984) 283.
[14] R.F. Antrim, A.M. Yacynch, Anal. Lett. 21 (1988) 1085.
[15] A.R. Ghatak-Roy, C.R. Martin, Anal. Chem. 58 (1986) 1574.
[16] H. Ge, G.G. Wallace, Anal. Chem. 61 (1989) 2391.
[17] T. Nagaoka, M. Fujimoto, Y. Uchida, K. Ogura, J. Electroanal. Chem. 336 (1992) 45.
[18] T. Nagaoka, M. Fujimoto, H. Nakao, K. Kakuno, J. Yano, K. Ogura, J. Electroanal. Chem. 350 (1993) 337.
[19] T. Nagaoka, M. Fujimoto, H. Nakao, K. Kakuno, J. Yano, K. Ogura, J. Electroanal. Chem. 364 (1994) 179.
[20] P. Lam, P.R. Elliker, G.E. Wnek, T.M. Przybycien, J. Chromatogr. A 707 (1995) 29.
[21] T. Suzuki, R.D. Noble, C.A. Koval, Inorg. Chem. 36 (1997) 136.
[22] C.K. Chambliss, M.A. Oldham, C.M. Morales, C.R. Martin, S.H. Strauss, Anal. Chem. 70 (1988) 757.
[23] R.S. Deinhammer, K. Shimazu, M.D. Porter, Anal. Chem. 63 (1991) 1889.
[24] R.S. Deinhammer, E.Y. Ting, M.D. Porter, J. Electroanal. Chem. 362 (1993) 295.
[25] R.S. Deinhammer, K. Shimazu, M.D. Porter, J. Electroanal. Chem. 387 (1995) 35.
[26] R.S. Deinhammer, E.Y. Ting, M.D. Porter, Anal. Chem. 67 (1995) 237.
[27] E.Y. Ting, M.D. Porter, Anal. Chem. 69 (1997) 675.
[28] E.Y. Ting, M.D. Porter, Anal. Chem. 70 (1998) 94.
[29] E.Y. Ting, M.D. Porter, J. Chromatogr. A 793 (1998) 204.
[30] E.Y. Ting, M.D. Porter, J. Electroanal. Chem. 44 (1998) 180.
[31] M.K. Ho, S. Wang, M.D. Porter, Anal. Chem., in press.
[32] S. Garattini, E. Mussini, L.O. Randall (Eds.), The Benzodiazepines, Raven Press, New York, 1973.
[33] H. Möhler, T. Okada, Science 198 (1977) 849.
[34] W.E. Müller, U. Wollert, Mol. Pharmacol. 11 (1975) 52.
[35] P. Salvadori, C. Bertucci, E. Domenici, G. Giannaccini, J. Pharm. Biomed. Anal. 7 (1989) 1735.
[36] X.-L. Lu, S.K. Yang, J. Chromatogr. 535 (1990) 229.
[37] I. Fitos, J. Visy, M. Simonyi, J. Hermansson, J. Chromatogr. A 709 (1995) 265.
[38] J. Liu, J.T. Stewart, Anal. Lett. 30 (1997) 1555.
[39] A.M. Stalcup, W. Wu, K.L. Williams, Biomed. Chromatogr. 11 (1997) 325.
[40] A.F. Fell, T.A.G. Noctor, J.E. Mama, B.J. Clark, J. Chromatogr. 434 (1988) 377.
[41] R.C. Engstrom, Anal. Chem. 54 (1982) 2310.
[42] R.L. McCreery, in: A.J. Bard (Ed.), Electroanalytical Chemistry, Vol. 17, Marcel Dekker, New York, 1991, p. 221.
[43] J. Szejtli, T. Osa, Cyclodextrins, in: J.L. Atwood et al. (Eds.), Comprehensive Supramolecular Chemistry, Vol. 3, Pergamon, New York, 1996.
[44] D.W. Armstrong, W. Demond, J. Chromatogr. Sci. 22 (1984) 411.
[45] C.E. Dalgliesh, J. Chem. Soc. 137 (1952) 3940.
[46] R.K. Jaworski, M. Goledzinowski, Z. Galus, J. Electroanal. Chem. 252 (1988) 425.
[47] J. Barrett, W. Franklin Smyth, I.E. Davidson, J. Pharm. Pharmacol. 25 (1973) 387.
[48] C. Hansch, A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1979.
[49] N. Takashi, Y. Hayashi, S. Suzuki, H. Kubo, H. Ohtani, J. Chromatogr. A 767 (1997) 93.
[50] K. Cabrera, M. Jung, M. Fluck, V. Schurig, J. Chromatogr. A 731 (1996) 315.
[51] K. Cabrera, D. Lubda, J. Chromatogr. A 666 (1994) 433.
[52] J.W. Dolan, LC·GC 15 (1997) 612.
[53] A. Berthod, S.-C. Chun, D.W. Armstrong, Anal. Chem. 64 (1992) 395.
[54] Y. Inoue, T. Hakushi, Y. Liu, L.-H. Tong, B.-J. Shen, D.-S. Jin, J. Am. Chem. Soc. 115 (1993) 475.