Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1034 (2004) 213–220
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

# Separation of quaternary ammonium diastereomeric oligomers by capillary electrophoresis

Bin Zhang [a,1], Ira S. Krull [a,*], Aharon Cohen [b,*], David L. Smisek [b,2], Alla Kloss [b], Bing Wang [b], Andre J. Bourque [b]

[a] *Department of Chemistry, 102 Hurtig Hall, Northeastern University, 360 Huntington Avenue, Boston, MA 02115 USA*
[b] *Analytical R&D Department, GelTex Pharmaceuticals, Inc., Genzyme Corporation, 153 Second Avenue, Waltham, MA 02451 USA*

Received 17 July 2003; received in revised form 18 November 2003; accepted 4 February 2004

**Abstract**

The separation of novel diastereomeric trimers (3M) and pentamers (5M), derived from quaternary ammonium salts, was studied in conventional, uncoated and coated capillaries using capillary zone electrophoresis (CZE) with a variety of buffers and additives. Resolution of 5M diastereomers was best achieved using gamma-cyclodextrin (gamma-CD) as a chiral selector, while no diastereomeric resolution was realized for the 3M material.


*Keywords:* Diastereomeric resolution; Quaternary ammonium oligomers; Cyclodextrins

## 1. Introduction

Quaternary ammonium compounds represent an important class of pharmaceuticals. The analysis of these compounds and strongly cationic, low molecular weight (MW) species, in general, has usually been done using ion-exchange chromatography or ion-paired, reversed phase liquid chromatography (RPLC). Fig. 1 illustrates the two particular quaternary ammonium oligomers of interest, here termed trimer (3M) and pentamer (5M), these being formed by coupling the dipyridine precursor, A, with a diepoxide comonomer, B. These two compounds (3M and 5M) differ in their degree of copolymerization and MW. These permanent cationic materials exhibit strong positive charges at any pH. Both compounds are mixtures of diastereomers due to the presence of two or more chiral centers. For the 3M, two chiral centers lead to four possible diastereomers (three unique diastereomers accounting for molecular symmetry), while for the 5M, there are six chiral centers leading to sixty-four possible diastereomers (38 unique). Depending on how these compounds are synthesized and the enantiomeric excess (chiral purity) of the precursors, not all of these diastereomers might be formed. Attempts to resolve these species by conventional RPLC have almost always led to a single peak for the main component, for

*Abbreviations:* A6-28, HPLC purified 5M sample; CIA, capillary ion analysis; CD, cyclodextrin; cIEF, capillary isoelectric focusing; CE, capillary electrophoresis; CGE, capillary gel electrophoresis; CMC, critical micellar concentration; CTAB, cetyltrimethylammonium bromide; CZE, capillary zone electrophoresis; Da, Dalton; DTAB, dodecyltrimethylammonium bromide; EIC, extracted ion chromatogram; EOF, electroosmotic flow; EPF, electrophoretic force; ESI, electrospray ionization; gamma-CD, gamma-cyclodextrin; HP, Hewlett Packard; HPCE, high-performance capillary electrophoresis; HPLC, high-performance liquid chromatography; *I*, current; IEC, ion exchange chromatography; IPLC, ion-paired liquid chromatography; LC, high-performance liquid chromatography (HPLC); *m/z*, mass-to-charge ratios; MS, mass spectrometry; MSD, mass selective detector; MW, molecular weight; 3M, trimeric, cationic, quaternary ammonium analyte; 5M, pentameric, cationic, quaternary ammonium analyte; MEKC, micellar electrokinetic chromatography; MECC, micellar electrokinetic capillary chromatography; PVA, polyvinyl alcohol; RPLC, reversed phase (liquid chromatography); R.S.D., relative standard deviation; %R.S.D., percent relative standard deviation; SDS, sodium dodecyl sulfate; SIM, selected ion monitoring; TIC, total ion current; TMDP, trimethylene dipyridine

* Corresponding authors. Tel.: +1-617-373-2862; fax: +1-617-373-8795.
*E-mail address:* irask@aol.com (I.S. Krull).
[1] Department of Chemical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA.
[2] Analytical Support, Genta Incorporated, Two Connell Drive, Berkeley Heights, NJ 07922, USA.

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.02.039

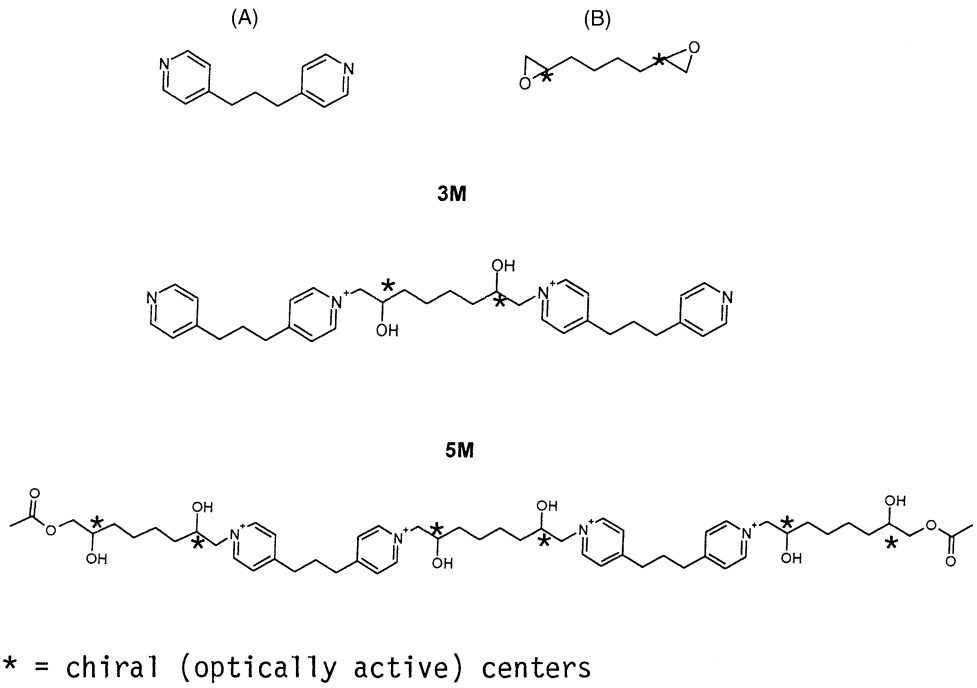


Fig. 1. Diagram of the two oligomers derived from a dipyridine monomer (A) and a diepoxide monomer (B), leading to a trimer, 3M and a pentamer, 5M.

both the 3M and 5M materials. Because chirality may play an important role in the pharmaceutical properties of such compounds, it is important to analyze, when possible, these diastereomers.

In general, capillary electrophoresis (CE) is a complementary separation technique to augment or replace high-performance liquid chromatography (HPLC) approaches, especially for small molecules [1–3]. The approach we have taken is to utilize CE, an analytical separation technique for both small and large molecules [4–14]. There are numerous literature reports on the application of CE for chiral (enantiomeric) resolutions [4–11,15–23]. Because CE operates on the basis of differences in mass-to-charge (*m*/*z*) ratios resulting in different electrophoretic forces or mobilities along with a constant (though variable) electroosmotic flow (EOF), capillary zone electrophoresis (CZE) is perhaps ideally suited to resolve species showing differences in *m*/*z* similar to mass spectrometry (MS) [12]. For higher molecular weight (MW) analytes, it has become quite common to utilize either fixed gels or replaceable polymer solutions in the capillary, thereby leading to capillary gel electrophoresis [14].

Here we describe a series of CE-based approaches for both the 3M and the 5M, including the use of conventional CZE buffers at various pHs, MS-compatible CZE buffers, and a variety of cyclodextrin (alpha-, beta-, and gamma, underivatized) additives [15–23]. Although we have not exhausted all possible buffer conditions for these cationic species, we have been able to achieve resolution of 5M diastereomers. We have also determined the nature of individual peaks in CZE using both on-line and off-line MS approaches. All of these separation studies are described for the 5M, and to a lesser degree for the 3M. The 5M is clearly the more complex analyte with the potential for up to 33 unique diastereomers present in a single sample.

## 2. Experimental

### 2.1. Chemicals and reagents

Phosphoric acid, sodium dihydrogen phosphate, sodium hydroxide 1N solution, sodium dodecyl sulfate, acetonitrile and methanol were obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA). Dodecyltrimethylammonium bromide (DTAB) and polyoxyethylene 23 lauryl ether (Brij 35), ammonium hydroxide, cholic acid sodium salt, deoxycholic acid sodium salt, ammonium formate, and gamma-cyclodextrin (gamma-CD) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Octanesulfonic acid sodium salt, formic acid and sodium tetraborate decahydrate were from Aldrich Chemical Co. (Sigma–Aldrich, Milwaukee, WI, USA). The alpha and beta-cyclodextrins were from Fluka Chemical Co. (Sigma–Aldrich, Milwaukee, WI, USA). Sodium acetate was from J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). Water used for all experiments was de-ionized and distilled from a Corning Glass Works (Corning, NY, USA) Megapure Mgamma-1 water purification system. For HPLC experiments HPLC grade water and acetonitrile from JT Baker were used.

Formic acid (99% pure) from EM Science was used for pH adjustment.

### 2.2. HPLC instrumentation and conditions

HPLC was performed with a Synergi Polar-RP column (80 Å, 4 μm, 150 mm × 2 mm; Phenomenex Corporation, Torrance, CA, USA). The mobile phase was chosen to be suitable for on-line MS analysis: 50 mM ammonium formate buffer, pH 4.8 with acetonitrile. Separations were carried out in a linear gradient mode: 10–20% acetonitrile in 40 min at a flow rate of 0.3 ml/min. UV (254 nm) and MS detection were carried out simultaneously.

### 2.3. LC-MS instrumentation and conditions

LC-MS (on-line and off-line) was carried out using a Waters/Micromass Model ZMD single quadrupole system (Waters Corporation, Milford, MA, USA) supported with Masslynx 3.4 software (Micromass Corporation, Danvers, MA, USA). The system included a Waters 2690 separations module and a Waters 2487 dual wavelength absorbance UV detector. MS detection was achieved by electrospray ionization (ESI) in the positive ion mode. The capillary voltage was set to 4 kV, the cone to 20 V, and extractor to 5 V. The source block temperature was set at 100 °C with desolvation at 300 °C. For the off-line MS measurements the parameters controlling the instrument resolving power were adjusted to achieve the isotope resolution sufficient for the analytes' charge determination. When performing on-line LC-MS, after passing through the UV detector the flow was split, so that the HPLC eluent was introduced into the MS ion source at 35 μl/min. When performing off-line analysis, the solution was typically infused into the ion source at 20 μl/min. In direct infusion experiments, the 0.02 mg/ml analyte solutions were prepared using a 9:1 acetonitrile and water mixture as solvent. For LC-MS experiments, approximately 1 mg/ml analyte solutions in water were used. The *m*/*z* was typically scanned between 100 and 500. For on-line LC-MS analysis, the acquisition time was 1–2 s. For the direct infusion MS experiments, typically 30 scans were averaged.

### 2.4. Capillary electrophoresis, instrumentation and conditions

CE was carried out on a Waters Model Quanta 4000E Capillary Electrophoresis System (Waters Corporation, Milford, MA, USA) and a Beckman Model P/ACE 5500 System (Beckman Coulter Instruments, Inc., Fullerton, CA, USA). Bare, fused-silica capillaries (Polymicro Technologies, Phoenix AZ, USA) of dimensions 50 μm × 36.5 cm (29 cm effective length), 50 μm × 27 cm (20 cm effective length) and a 50 μm × 32 cm (24.5 cm effective length) polyvinyl alcohol (PVA) coated capillary (Agilent Technologies, Andover, MA, USA) were used. Direct UV detection was performed at either 254 or 214 nm. The voltage applied across the capillary was from positive to negative (anode to cathode, normal polarity). A pressure injection mode was chosen for the Beckman CE instrument, and a hydrostatic injection mode was chosen for the Waters instrument. Buffer of pH 2.5, 50 mM phosphate was prepared from phosphoric acid and sodium dihydrogen phosphate. Buffers of 50 mM ammonium formate at pH 3.0, 4.8, and 8.0 were prepared by the addition of 50 mM ammonium hydroxide to 50 mM formic acid. Run buffers with different additives were filtered through 0.45 μm PTFE filters (Scientific Resources Inc., Eatontown, NJ, USA).

Before first use, a new, bare capillary was preconditioned by rinsing for 15 min (each) with 1N NaOH, water, and 50 mM $H_3PO_4$, in that order, followed by a 10 min rinse with water and 15 min with 50 mM phosphate (pH 2.5). At the start of each day, the capillary was conditioned with 50 mM $H_3PO_4$ for 15 min, water for 10 min and 50 mM phosphate (pH 2.5) for 15 min. Between the introduction of samples, the capillary was subjected to a 2-min rinse (each) with 50 mM $H_3PO_4$, water and run buffer, in that order. The relative standard deviation (R.S.D.) of migration times for three injections was within 1.5%. The PVA-coated capillary was flushed with water for 10 min before each use. Between runs, the PVA-coated capillary was subjected to a two-minute rinse with run buffer. The R.S.D. of migration time was within 1.4% for the PVA-coated capillary.

### 2.5. CE-MS, instrumentation and conditions

All samples for CE-MS were run in duplicate. The conditions were as follows: fused silica capillary, 59 cm (total length) × 50 μm (i.d.) PVA coated; electrolyte, 50 mM ammonium formate (pH 3.0) with and without gamma-CD; +20 kV (current ~12 μA). The CE-MS experiments were performed using a homemade CE-MS system built at Southeastern Oklahoma State University (Laboratory of Dr. T. Smith, Department of Chemistry, Southeastern Oklahoma State University, Durant, OK, USA), using a Spellman (Plainview, NY, USA) 30 kV power supply and a custom-built autoinjector with controlled pressure and time for injections. The MS instrument was a Hewlett Packard (Agilent) Model 1946A Mass Selective Detector (MSD). This single quadrupole instrument was equipped with electrospray ionization (ESI) and the HP CE-MS spray adapter. The sheath fluid was 50/50 isopropanol-$H_2O$ with 5 mM formic acid pumped at 5 μl/min. The nebulizer was off for the injection step and was turned on at 0.5 min into the CE separations and maintained at 8 psi. The MS parameters were as follows: dry gas temperature, 90 °C; drying gas flow rate 8 l/min; capillary voltage 3500 V; fragmentor voltage 70 V. The analyses were performed in two modes: first the selected ion monitoring (SIM) mode using a selected *m*/*z*. The experiments were done with and without the gamma-CD, and they were also repeated in the SIM mode scanning from 170 to 300 *m*/*z*.

### 2.6. Samples

The 5M and the 3M were dissolved in water at concentrations of 1.0–1.5 and 0.2–0.5 g/l, respectively, for all CE analyses.

## 3. Results and discussion

### 3.1. HPLC and LC-MS analysis of 3M and 5M

Analysis of oligo-ionene samples is complicated due to the presence of various sizes of oligomers, fragments and acetylation products. These species have a wide range of molecular weights and varying hydrophobicity, polarity, charge, and reactivity. Consequently, development of a suitable HPLC method brings about certain challenges. Fig. 1 illustrates the structures of compounds 3M and 5M, for which these HPLC and CZE methods have been developed and optimized. The masses of 3M and 5M are 540.8 and 947.2 Da, respectively.

We have developed an HPLC method utilizing a Synergi Polar-RP column for the separation of our highly polar analytes. In this column, an ether-linked phenyl phase with hydrophilic end capping was designed specifically for optimal retention and selectivity of polar aromatic compounds. On-line LC-MS analyses are possible using an MS-compatible mobile phase. Two chromatographic profiles (LC-UV at 254 nm and LC-MS-TIC (total ion current)) were simultaneously recorded. This allowed us to distinguish between sample components with and without pyridine groups. Sample components generating peaks in the TIC chromatographic trace were identified using the associated MS-spectrum. Identity of the oligomers was confirmed by their mass-to-charge ratios (*m*/*z*) and by the isotope peak separation. For this purpose, an off-line, direct infusion MS analysis was carried out in a continuous mode to achieve the best MS resolution. The results are shown in Figs. 2 and 3. For simplicity we choose to show only the UV chromatographic traces and the mass spectrum of the main sample component (peak) in Figs. 2 and 3. Fig. 2a illustrates a typical LC-UV chromatogram for the 3M using previously described (Experimental) conditions. Fig. 2b is the MS spectrum of the main peak (24.9 min) in Fig. 2a. Fig. 3a is the LC-UV chromatogram of crude 5M, while Fig. 3b is a similar LC-UV chromatogram for a purified sample of the main peak (ca. 7 min) in Fig. 3a. Fig. 3c is the MS spectrum of the purified 5M of Fig. 3b.

As shown in Figs. 2b and 3c, the measured *m*/*z* values of 270.27 and 236.75 matched closely those expected for 3M and 5M at 270.37 and 236.82 *m*/*z*, respectively. Notice that the number of charges, *z*, is 2 for 3M and 4 for 5M. At pH 4.8, the majority of oligomeric molecules are not protonated on their terminal pyridine rings. Apparently, the 3M derives its charges mostly from the two quaternary ammonium groups and appears predominantly as doubly charged ions in the gas

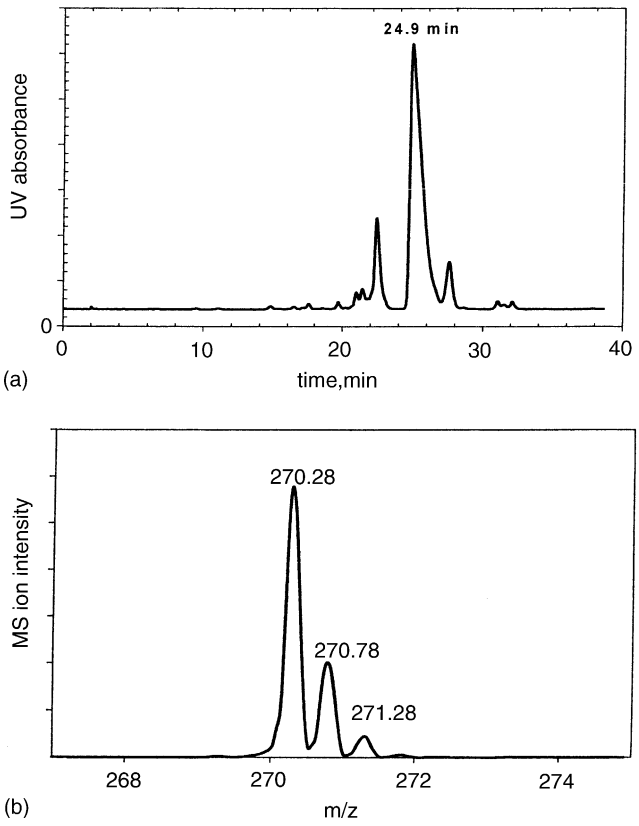


Fig. 2. (a) LC-UV chromatographic trace (254 nm) obtained for the 3M sample; (b) MS spectrum of the main sample component (peak eluted at 24.9 min). HPLC conditions: Synergi Polar-RP column (80 Å, 4 μm, 150 mm × 2 mm), mobile phase 50 mM ammonium formate buffer, pH 4.8 with acetonitrile; linear gradient mode: 10–20% acetonitrile in 40 min, flow rate 0.3 ml/min.

phase. That the 3M appears as doubly charged ions can be directly confirmed by the *m*/*z* spacing between its monoisotopic peak of the quasi-molecular ion and that for 13 °C. Because the mass difference is a constant 1 Da, the spacing is charge number dependent. For a doubly charged ion, $z = 2$ and $m/z = 0.5$. As shown in Fig. 2b, the monoisotopic and 13 C peaks of 3M are indeed separated by 0.5 Da. The protonation state of the 5M is not pH dependent, and these species are, therefore, always quadruply charged. This is clearly seen in Fig. 3c where the separation between the monoisotopic peak and that of the 13 °C isotope for the 5M is 0.25 Da, which is consistent with the expectation of $z = 4$.

The excellent separations achieved using the above-described HPLC conditions allowed us to separate pure, major compounds from the impurities by fraction collection. Thus, using HPLC we can isolate pure sample components different in their structures and nature. However, using this method we were not able to resolve the diastereomers of either the 3M or the 5M material.

### 3.2. CE analysis of 3M and 5M using CZE conditions

The analysis of basic compounds using a phosphate buffer combined with low UV wavelength detection is well

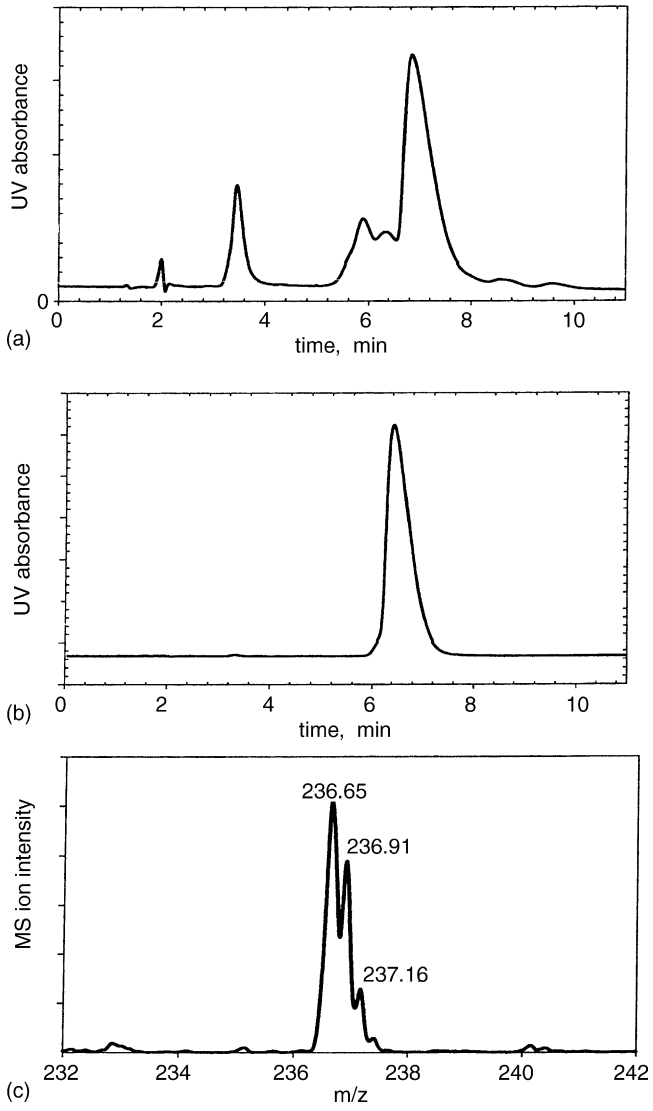


Fig. 3. (a) LC-UV chromatographic trace (254 nm) of the crude 5M sample; (b) LC-UV chromatographic trace (254 nm) of the RP-HPLC purified 5M sample; (c) MS spectrum of the main component of the purified 5M sample. HPLC conditions: Synergi Polar-RP column (80 Å, 4 μm, 150 mm × 2 mm), mobile phase 50 mM ammonium formate buffer, pH 4.8 with acetonitrile; linear gradient mode: 10–20% acetonitrile in 40 min, flow rate 0.3 ml/min.

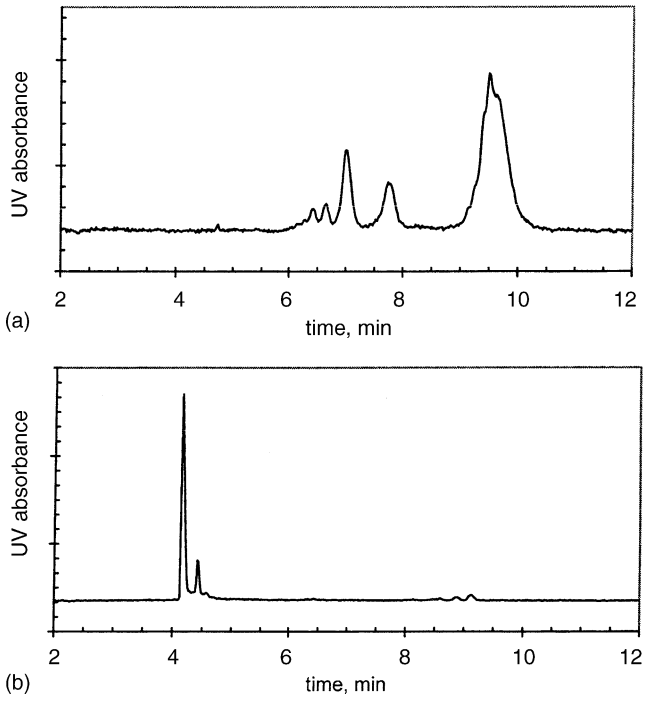


Fig. 4. CZE separation of (a) 5M and (b) 3M. Conditions: pH 2.5, 50 mM phosphate, 36.5 cm × 50 μm bare fused capillary, +14 kV, 15 s hydrostatic injection; 214 nm; 25 °C.

established. Thus, 50 mM phosphate buffer (pH 2.5) and a bare, fused-silica capillary were first chosen to separate 3M and 5M. As shown in Fig. 4a, the main component of the 5M appeared as a split peak, while the main component of the 3M was a sharp and homogeneous peak (Fig. 4b). The HPLC-UV and HPLC-MS analyses of these same samples indicated that the largest peaks in those chromatograms gave the largest UV and MS responses. It is possible that the largest peaks in both these samples by HPLC and HPCE are identical, suggesting that the largest CE peaks are indeed the main components. However, such extrapolations are suggestive and not demanding at all times. At least four impurity peaks migrating prior to the main component of the 5M and only one minor impurity peak after the main component of the 3M were observed. The peak shapes and resolutions in Fig. 4a may have been partly due to the specific buffer and/or capillary wall conditions. Both peak shapes and resolutions improved, Fig. 6a, when buffer and capillary wall conditions were changed (vide infra).The 5M and the 3M can be diastereomeric mixtures according to their structure, depending on the number of chiral centers present. As such, they might be able to be resolved into different diastereomers by CE-MS, all having the same molecular weight and MS patterns (vide supra). Common buffer additives, such as methanol, acetonitrile, 1-octanesulfonic acid, dodecyltrimethylammonium bromide (DTAB), sodium dodecyl sulfate (SDS) and polyoxyethylene 23 lauryl ether (Brij 35), were tried in an effort to enhance the separation. These attempts yielded the following results: the addition of 2 mM DTAB in the pH 2.5 phosphate buffer improved the resolution of the 5M (although it did not change the resolution of the 3M as shown in Fig. 5). The critical micellar concentration (CMC) values for DTAB, as determined in pure water and in a phosphate buffer (70 mM) at pH 6.0, are 15.6 mM [24] and 11.0 mM [25], respectively. Therefore, DTAB is in a monomeric state (below the CMC) at 2 mM concentration.

The improvement of resolution with the addition of a low amount of DTAB resulted from the adsorption of cationic surfactants onto the charged silica surface of the capillary. Consequently, a slow anodic EOF was achieved, which increased the differences in effective electrophoretic mobility of the diastereomers. However, it is difficult to maintain

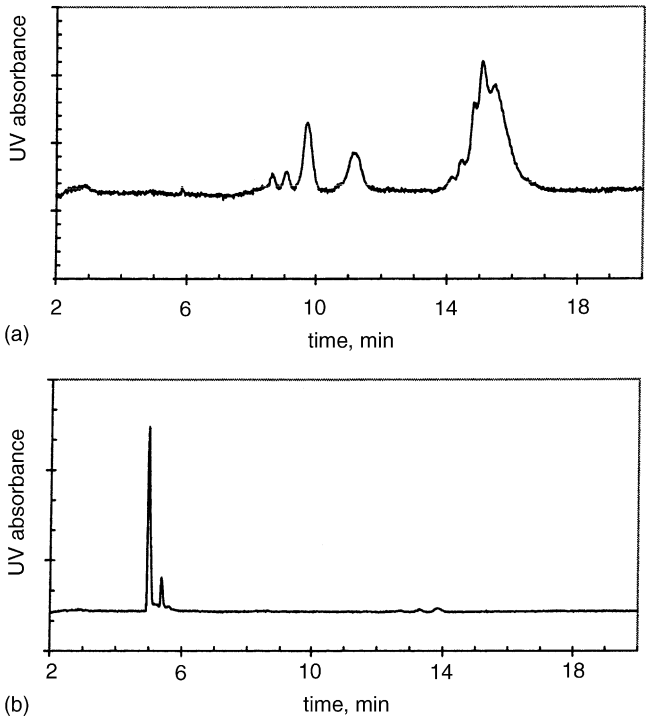


Fig. 5. CZE separation of 5M and 3M with 2 mM DTAB added. Conditions: 50 mM phosphate (pH 2.5) with 2 mM DTAB; 36.5 cm × 50 μm bare fused capillary; +14 kV, 15 s hydrostatic injection; 214 nm; 25 °C.

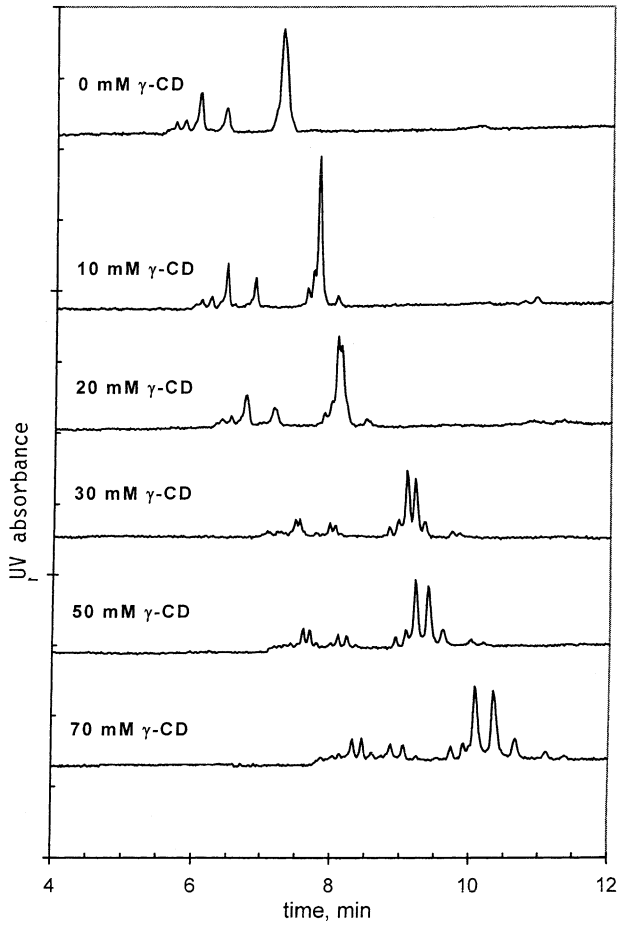


Fig. 6. CZE separation of 5M using different gamma-CD concentrations in 50 mM ammonium formate (pH 3.0) buffer; 32 cm × 50 μm PVA coated capillary, +5 kV, 5 s hydrostatic injection; 214 nm; 25 °C.

the reproducibility of migration times at 2 mM DTAB in the presence of other additives in the run buffer or after washing the capillary with 1N NaOH because the dynamic coating created by DTAB was not stable. The migration times of the 5M increased with increasing DTAB concentration in the run buffer. With 9 mM DTAB in the pH 2.5 phosphate buffer, the 5M became a very broad peak while the main 3M component remained a sharp, homogeneous peak. No peak was detected within 20 min at 20 mM DTAB, 220 V/cm, likely due to a large anodic EOF (reversed direction of EOF because of larger dynamic coating on capillary surface). The 5M was also separated using 30 mM bile salts (sodium cholate and sodium deoxycholate), pH 7.0, 25 mM phosphate-borate buffer, but no diastereomeric separation was observed (data not shown).

To develop MS-compatible conditions, we changed the buffer composition to more volatile components. Ammonium formate was used instead of phosphate. The influence of run buffer pH on the separation of 5M was investigated using a PVA-coated capillary. The best results in terms of resolution and peak symmetry were obtained with ammonium formate at pH 3.0. Three different cyclodextrins were evaluated (alpha, beta, and gamma) with gamma-CD giving the best resolution for the 5M diastereomers. Fig. 6 shows the effect of different gamma-CD concentrations on the chiral separation of the 5M. The 5M could not be separated into several peaks until the gamma-CD concentration was increased to 30 mM. As a function of capillary temperature, the 5M chiral resolution varied as follows (temperature/resolution (average ± S.D.), $n = 3$): 12 °C/1.66 ± 0.04; 20 °C/1.62 ± 0.07; 25 °C/1.64 ± 0.06; 37 °C/1.43 ± 0.06. The CE conditions for these results are given in Section 2 and Fig. 6. The influence of the gamma-CD concentration on the 5M chiral resolutions was also determined as follows (gamma-CD concentration/resolution (*average* ± *S.D.*), $n = 3$): 30 mM/1.01 ± 0.08; 50 mM/1.43 ± 0.00; 70 mM/1.89 ± 0.10. Thus, diastereomeric resolution increased with increasing gamma-CD concentration in the run buffer from 30 to 70 mM. This resolution remained relatively constant between 12 and 25 °C. Fig. 7 compares crude and RPLC-purified 5M under the conditions indicated.

On-line CE-MS was performed to provide additional evidence that gamma-CD caused the diastereomeric separation of the 5M. Fig. 8a shows a selected ion chromatogram of 5M prior to the addition of gamma-CD. Upon the addition of gamma-CD, the single peak derived from *m/z* 236.8 ion was resolved into four distinct peaks (Fig. 8b), mirroring the electropherogram by CE-UV (Figs. 6 and 7). Although, in theory, these four peaks could also be due

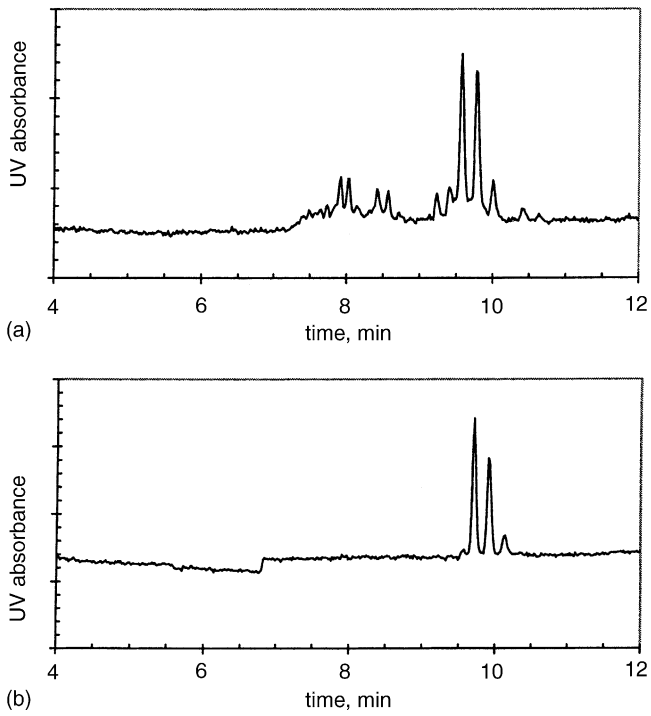


Fig. 7. CZE separation of (a) crude 5M and (b) RPLC-purified material with conditions of 50 mM ammonium formate (pH 3.0) buffer with 50 mM gamma-CD; 32 cm × 50 μm PVA-coated capillary; +5 kV, 5 s hydrostatic injection; 214 nm; 25 °C.

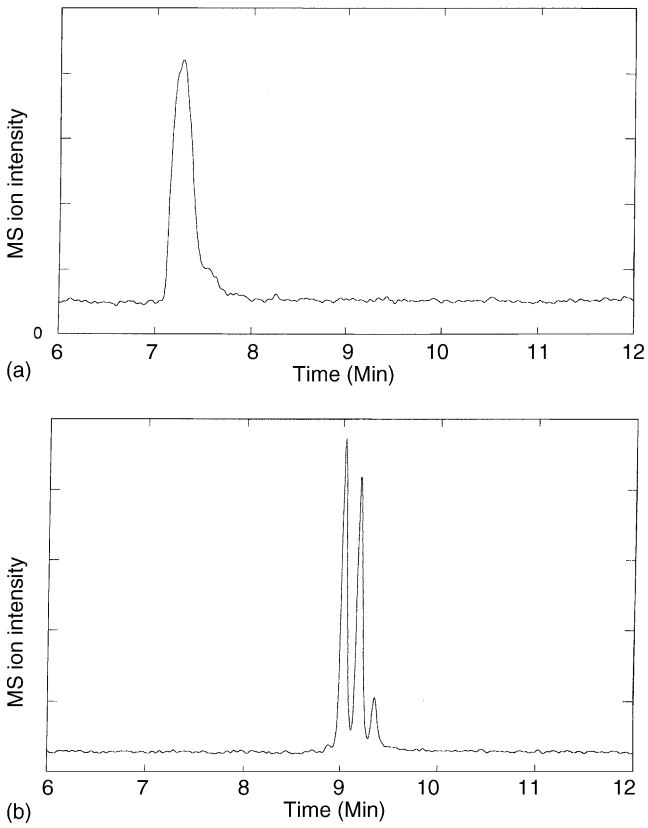


Fig. 8. CZE-MS of the purified 5M sample. Conditions: MS SIM mode (236.8 *m/z* EIC); CE capillary, 50 μm × 60 cm PVA coated; +20 kV; buffer; injection, 0.5 psi for 3 s; sample diluted 1:50 in run buffer. (a) 50 mM ammonium formate (pH 3.0) buffer; (b) 50 mM ammonium formate (pH 3.0) buffer with 50 mM gamma-CD.

to structural isomers of 5M, off-line MS-MS analysis has shown that the predominant structure of the 5M was consistent with the expected product (data not shown). The four mass spectra obtained from each peak in Fig. 8b were identical.

The use of micelles, bile salts, or CDs has not been fully explored for strongly cationic analytes, such as the 3M or the 5M (15–23). It would appear that for the 5M, these species can fit into the gamma-CD cavity quite well, and therefore, be resolved, but not into 33 distinct peaks. The formation of two to four peaks (two major peaks) suggests that each of these peaks consist of numerous diastereomers that are not yet resolved. It is possible that only one part of the 5M molecule can fit into the gamma-CD cavity, to different extents or with different distribution constants (KD), thus leading to a separation of only one pair of diastereomers. That is, perhaps only one chiral center enters the cavity and resolution occurs at that single terminal center. Resolution of the other diastereomers in each of the two major peaks would require another mechanism of recognition or differentiation, which gamma-CD cannot seem to provide. This might be accomplished by using mixed-CDs or gamma-CD together with other chiral additives such as chiral micelles. These might then interact with chiral centers other than those embedded in the gamma-CD cavity, perhaps leading to further resolution of the diastereomers believed present. Such work is under consideration for future studies.

## 4. Conclusions

We have demonstrated the ability of CZE with gamma-CD to resolve some of the diastereomers of the 5M and to resolve impurities from both the 3M and the 5M. These resolutions appear to be superior to those yet possible by RPLC. In addition, for the first time, we have been able to resolve some of the diastereomers of the 5M under optimized CZE conditions that are compatible with MS studies.

## Acknowledgements

Northeastern University acknowledges with deep appreciation and gratitude the financial support provided by Gel-Tex Pharmaceuticals (Genzyme Corporation) for the work described here. Dr. Bin Zhang was a Postdoctoral Fellow at Northeastern University (2001–2002).

## References

[1] V.R. Meyer, Practical High-Performance Liquid Chromatography, third ed., Wiley, New York, 1998 (Chapter 10).

[2] A.M. Krstulovic and P.R. Brown, Reversed-Phase High-Performance Liquid Chromatography, Theory, Practice, and Biomedical Applications, Wiley-Interscience, New York, 1982 (Chapters 6 and 7).
[3] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, Wiley-Interscience, New York, 1997 (Chapters 6 and 7).
[4] C.E. Evans, A.M. Stalcup, Chirality 15 (2003) 709.
[5] I.S. Lurie, R.F.X. Klein, T.A.D. Cason, M.J. LeBelle, R. Brenneisen, R.E. Weinberger, Anal. Chem. 66 (1994) 4019.
[6] C. Dette, S. Ebel, S. Terabe, Electrophoresis 15 (1994) 799.
[7] M. Blanco, I. Valverde, Trends Anal. Chem. 22 (7–8) (2003) 428.
[8] G.K. Scriba, Electrophoresis 24 (15) (2003) 2409.
[9] B. Chankvetdaze, Capillary Electrophoresis in Chiral Analysis, Wiley, New York, 1997.
[10] P. Camilleri (Ed.), Capillary Electrophoresis, Theory and Practice, CRC Press, Boca Raton, 1993 (Chapter 5).
[11] R. Weinberger, Practical Capillary Electrophoresis, second ed., Academic Press, San Diego, 2000 (Chapter 4).
[12] R. Weinberger, Practical Capillary Electrophoresis, second ed., Academic Press, San Diego, 2000 (Chapters 3 and 9).
[13] R. Weinberger, Practical Capillary Electrophoresis, second ed., Academic Press, San Diego, 2000 (Chapter 7).
[14] R. Weinberger, Practical Capillary Electrophoresis, second ed., Academic Press, San Diego, 2000 (Chapter 6).
[15] J.P. Quirino, S. Terabe, Electrophoresis 21 (2000) 355.
[16] J.G. Clothier Jr., L.M. Daley, S.A. Tomellini, J. Chromatogr. B 683 (1996) 37.
[17] W.L. Ding, J.S. Fritz, Anal. Chem. 70 (1998) 1859.
[18] C.Y. Quang, M.G. Khaledi, Anal. Chem. 65 (1993) 3354.
[19] X.Q. Ren, Y.Y. Dong, A.J. Huang, Y.L. Sun, Z.P. Sun, Chromatographia 49 (1999) 411.
[20] F. Bressolle, M. Audran, T.-N. Pham, J.-J. Vallon, J. Chromatogr. B 687 (1996) 303.
[21] H. Matsunaga, J. Haginaka, Electrophoresis 22 (2001) 3382.
[22] H. Wang, J.L. Gu, H.F. Hu, R.J. Dai, T.H. Ding, R.N. Fu, Anal. Chim. Acta 359 (1998) 39.
[23] C.L. Ng, C.P. Ong, H.K. Lee, S.F.Y. Li, J. Chromatogr. A 680 (1994) 579.
[24] M. Chorro, C. Chorro, O. Dolladille, S. Partyks, R. Zana, J. Colloid Interf. Sci. 210 (1999) 134.
[25] C.E. Lin, T.Z. Wang, T.C. Chiu, C.C. Hsueh, J. High Resolut. Chromatogr. 22 (1999) 256.