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Temperature effects on chiral microemulsion electrokinetic chromatography employing the chiral surfactant dodecoxycarbonylvaline

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Abstract

Dodecoxycarbonylvaline (DDCV) microemulsions (1% and 4%, w/v) were employed to evaluate the retention mechanism of a series of enantiomers over a temperature range of 15–35 °C. From the acquired retention data, van't Hoff plots were constructed and enthalpy and entropy of transfer were calculated from the slope and intercept, respectively. Resolution, enantioselectivity, distribution coefficients and Gibb's free energy were also calculated, as well as between enantiomer differences in enthalpy, entropy and Gibb's free energy. Finally, comparisons were made between the microemulsion thermodynamic data and a corresponding set of micellar data. While the 4% DDCV microemulsion did not provide a linear van't Hoff relationship, the 1% DDCV microemulsion was linear over a temperature range of 15–30 °C. For the 1% DDCV microemulsion, the enthalpic contribution to retention was consistently favorable ($\Delta H < 0$), whereas the entropic contribution varied from compound to compound. Finally, while the achiral attraction of the analytes was greater for the micellar phase, the microemulsion seemed to provide a suitable difference in entropy (and Gibb's free energy) between enantiomers to achieve chiral discrimination.

Keywords: Microemulsion electrokinetic chromatography; van't Hoff; Dodecoxycarbonylvaline; Chiral separation; Enantiomers; Capillary electrophoresis; Enthalpy/entropy compensation; Beta-blockers

1. Introduction

Capillary electrokinetic chromatography (EKC) has proven to be an invaluable tool for providing chiral separations. Its merits include fast, high efficiency, high resolution separations, which produce very little waste, consume minimal chemicals and are significantly more cost effective than its key competitors (namely SFC, GC, and HPLC). In general, enantiomeric separations are most often achieved in chromatography by eliciting temporary, diasteriomeric interactions between the enantiomers and some form of chiral selectand. This is referred to as the *direct method* of chiral separation [1]. In EKC, this is accomplished by supplementing the run buffer with a chiral additive or pseudostationary phase (PSP). For these purposes, various combinations of micelles, cyclodextrins, antibiotics, bile salts and crown ethers have all been explored in great detail [2–8]. In contrast, with only six published papers to-date [9–13b], chiral microemulsion electrokinetic chromatography (chiral MEEKC) has been investigated very little in this capacity.

An oil-in-water microemulsion is a spherical aggregate comprised of surfactant, co-surfactant and oil in a ratio such that a single, optically transparent, thermodynamically stable liquid is formed. The chemicals typically used for these purposes include short-chain linear alcohols as co-surfactants and hydrocarbons or moderately polar organic compounds as water immiscible oils. The result is a structure exhibiting a surfactant-enveloped oil core, with the co-surfactant acting to ease interfacial tension and electrostatic repulsion.

The use of a chiral microemulsion offers several distinct advantages with respect to other chiral PSPs. First, there are a greater number of parameters that can be manipulated when preparing a microemulsion. Variations in the concentration and identity of surfactant, co-surfactant and oil, as well as the

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pH and concentration of the background electrolyte have all proven to be important parameters in MEEKC [14–20]. In addition, it has been theorized that the microemulsion structure offers increased fluidity, aiding in analyte penetration and mass transfer [14,21]. Moreover, the presence of the oil core in microemulsions results in an aggregate that is better able to solubilize a wide array of analytes and additives. In effect, this extends CE towards more hydrophobic compounds for which it is not currently a preferred methodology. Hydrophobic compounds for which separations were either difficult or unachievable using conventional *micellar* EKC have been successfully separated via MEEKC [22,23] and we believe this will be the case with respect to chiral MEEKC as well. Last, and of great importance, is the ability to extend the elution range of the separation by changing the surfactant concentration and subsequently altering the charge density of the aggregate [24,25]. This is usually not an option with other PSPs, where elution ranges are largely fixed.

Four of the six previous chiral MEEKC publications dealt specifically with a chiral microemulsion based on a *chiral surfactant*, dodecoxycarbonylvaline (DDCV), a low interfacial tension oil (ethyl acetate) and 1-butanol as the co-surfactant [10–13a]. The DDCV-based chiral microemulsion provided many rapid, highly selective separations. Most notably, when compared to an analogous DDCV-based micellar system, the microemulsion separations exhibited equal or slightly larger enantioselectivities, a greater than two-fold increase in the elution range, and analysis times that were more than three-fold lower [10].

Several studies have been conducted to better understand the DDCV microemulsion and which variables are the most important with respect to chiral discrimination and chromatographic figures of merit. The effect of the identity and concentration of the oil, the identity and concentration of the background buffer, the incorporation of cyclodextrins as a secondary separation mechanism, separation voltage, and the effect of surfactant concentration have all been explored in great detail. These experiments have resulted in the knowledge that parameters such as oil identity play a somewhat minor role with respect to analytical performance, whereas background buffer, separation voltage and surfactant concentration all play a much more significant role in providing optimal separation conditions.

One variable that has yet to be explored with the DDCV microemulsion is temperature. In chiral separations, small differences in the enthalpy or entropy of solute transfer play an important role in chiral selectivity. The easiest way to elucidate these quantities is via van't Hoff analysis, where a linear plot of the natural logarithm of the distribution coefficient versus inverse temperature provides the enthalpy and entropy of transfer via the slope and intercept, respectively. While there have been a few publications [18,26–29] which have examined the effects of temperature on resolution, selectivity and efficiency in MEEKC, there have been relatively few which have performed a more rigorous van't Hoff analysis [30,31]. Due to the complex nature of microemulsion aggregates, it is important to gain an understanding of how the mechanism of solute–aggregate interaction changes with temperature and whether or not this relationship is linear. Further, this relationship was previously investigated for DDCV *micelles* [32] and it would be valuable to compare the micellar results to those obtained using an analogous DDCV microemulsion system. In this work, a 1% and 4% DDCV microemulsion are used to separate a variety of pharmaceutical compounds over a temperature range of 15–35 °C.

2. Materials and methods

2.1. Instrumentation

All separations were performed on an Agilent ^{3D}CE electrophoresis system (Agilent Technologies, Waldbronn, Germany) over a temperature range of 15–35 °C. The detection wavelength was 215 nm, although detection wavelengths of 236, 254 and 280 nm were also stored and monitored. Each microemulsion was evaluated on a fresh, fused silica capillary (L_d = 23.6 cm, L_t = 32 cm, i.d. = 50 μm) (Polymicro Technologies, Phoenix AZ, USA). The fresh capillaries were rinsed with 1 M NaOH for 10 min, 0.1 M NaOH for 5 min, HPLC grade water for 3 min and microemulsion for 15 min. In between analyses, capillaries were rinsed with HPLC grade water for 10 min, 0.1 M NaOH for 10 min, HPLC grade water for 3 min and microemulsion for 15 min. Sample injection was performed hydrodynamically by applying 25 mbar of pressure for 2 s. All sample injections were performed in triplicate. Voltages were applied such that a power of 0.3 W was observed, resulting in Joule heat values of 1.0 W/m. These voltages ranged from 7.5 to 8.0 kV. Analytical data from the Agilent ^{3D}CE were collected and processed on a Hewlett Packard Kayak XA system using ChemStation software (v. A.08.03).

2.2. Reagents

Dodecoxycarbonylvaline (DDCV), marketed under the name Enantioselect, was provided by Waters Corporation (Milford, MA, USA). Ethyl acetate, tetrapropylammonium hydroxide (TPAH), octanophenone, valerophenone, butyrophenone, acetophenone, pseudoephedrine, ephedrine, methylephedrine, norphenylephrine and atenolol were purchased from Aldrich (Milwaukee, WI, USA). 1-Butanol, propiophenone, metoprolol, indapamide, synephrine and epinephrine were purchased from Sigma (St. Louis, MO, USA).

2.3. Microemulsion preparation

Table 1 compares the electroosmotic flow (μ_{eo}), the electrophoretic mobility of the PSP ($\mu_{ep,PSP}$) and the elution range for each of the systems compared. The DDCV mi-

Table 1
Electrophoretic parameters and elution range for DDCV surfactant aggregates[a]

Microemulsion	$\mu_{ep,me}$(10^4 cm^2/V s)[d]	$\eta\mu_{ep,me}$ (10^4)	μ_{eo} (10^4 cm^2/V s)[e]	$\eta\mu_{eo}$ (10^4)	$t_{me}/t_{eo} = \mu_{eo}/\mu_{me}$
DDCV micelle [b]	−3.98 ± 0.02	−3.54	5.76 ± 0.03	5.13	3.2
1% (w/v) DDCV microemulsion [c]	−2.99 ± 0.01	−2.94	3.78 ± 0.17	3.72	4.0
4% (w/v) DDCV microemulsion [c]	−2.74 ± 0.03	−3.52	3.51 ± 0.04	4.50	6.5

[a] Based on results obtained at 25 °C, using normal (generating ≥ 1.5 W/m) operating voltages.
[b] 25 mM DDCV, 100 mM CHES buffer, pH 8.5 [32].
[c] Microemulsion components in addition to surfactant include 1.2% (v/v) 1-butanol and 0.5% (v/v) ethyl acetate in 50 mM phosphate buffer, pH 7.0.
[d] Octanophenone was used as a t_{me} marker. Values based on the average of four injections.
[e] Values based on the average of 24–50 injections.

celles were prepared using 25 mM DDCV in a 100 mM CHES buffer, pH 8.5 (as previously reported by Peterson and Foley [32]). In contrast, the microemulsions were prepared by weighing and combining the appropriate amount of surfactant (1% or 4% (w/v) DDCV) and buffer (50 mM sodium phosphate monohydrate) in a beaker and dissolving them in a volume of HPLC grade water equivalent to 75% of the final volume. The resulting solutions were then pH adjusted to 7.0 with 1.0 M tetrapropylammonium hydroxide (TPAH). Once the pH adjustment was complete, the ethyl acetate and 1-butanol were added and the contents were sonicated, while covered, for approximately 30 min. Once sonicated, the microemulsions were transferred to volumetric flasks, diluted to volume and allowed to rest for 1 h prior to use.

2.4. Sample preparations

Each pharmaceutical enantiomer was dissolved in the appropriate microemulsion at a concentration of 0.5 mg/mL, with the exception of indapamide (0.25 mg/mL). At the pH's employed in this study, all analytes are cationic with the exception of indapamide which was neutral. A negligible amount of methanol was added to the t_{me} marker, as well as to each sample to serve as a t_0 marker. The t_{me} marker (migration time of the microemulsion) was prepared by dissolving 1 μL of octanophenone in 3 mL of the appropriate microemulsion. A solution of homologous alkylphenones (acetophenone, propiophenone, butyrophenone and valerophenone) was similarly created by mixing 1 μL of each alkylphenone in 3 mL of the appropriate microemulsion.

2.5. Calculations

Each set of results was evaluated for resolution (R_s), retention factor (k), distribution coefficient (K_{eq}) and enantioselectivity (α_{enant}). Because the resolution was less than baseline in some cases, resolution was calculated using the respective half-height equation (Eq. (1)) via ChemStation software (v. A.08.03).

$$R_s = \frac{1.18(t_{r(b)} - t_{r(a)})}{W_{50(b)} + W_{50(a)}} \quad (1)$$

where W_{50} is the peak width at half-height (min), t_r is the peak retention time (min), and "a" and "b" denote peaks 1 and 2, respectively. Electroosmotic flow (μ_{eo}) was calculated using the equation:

$$\mu_{eo} = \frac{L_d L_t}{t_0 V} \quad (2)$$

where L_d and L_t are the length to the detector and total column length, respectively, t_0 signifies the retention time of methanol and V is the applied voltage. When calculating retention factors in EKC, both the electrophoretic mobility of the analytes and the retention characteristics must be taken into account. The electrophoretic mobility of the analytes (μ_{ep}) in the microemulsion was estimated by obtaining the electrophoretic mobilities under CZE conditions (phosphate buffer, pH 7.0) and applying a correction factor to adjust for viscosity differences between the CZE buffer and the microemulsion [33]. The viscosity correction factor was calculated for each of the microemulsions investigated. The retention factor of each enantiomer was then calculated by the equation:

$$k = \frac{t_r(1 - \mu_r) - t_0}{t_0 - ((\mu_{eo} - \mu_{ep,me})/\mu_{eo})t_r} \quad (3)$$

where μ_{me} is calculated using Eq. (2) with the substitution of t_{me} for t_0, $\mu_{ep,me} = \mu_{me} - \mu_{eo}$, and the relative electrophoretic mobility (μ_r) is defined as the ratio $\mu_{ep,analyte}/\mu_{eo}$. From the retention factors, the enantioselectivity can then be determined:

$$\alpha_{enant} = \frac{k_2}{k_1} \quad (4)$$

where k_2 and k_1 are the retention factors of the second and first eluting peaks, respectively. The retention factor can be related to the distribution coefficient (K_{eq}) by Eq. (5):

$$k = K_{eq}\left(\frac{V_{psp}}{V_{aq}}\right) \quad (5)$$

where (V_{psp}/V_{aq}) is the phase ratio (β) and can be determined as per Eq. (6):

$$\beta = \frac{\Sigma_i^n \bar{V}_i(c_i - c_{crit,i})}{1 - \Sigma_i^n \bar{V}(c_i - c_{crit,i})} \quad (6)$$

where $\bar{V}_i$ is the partial molar volume of the ith microemulsion component, $c_{crit,i}$ is the critical aggregate concentration of ith microemulsion component (assumed to be zero for 1-butanol and ethyl acetate), and c_i is the concentration of the ith microemulsion component.

The phase ratio of the DDCV microemulsion is somewhat difficult to ascertain, in part due to lack of information with respect to the partial molar volume of DDCV and the cac of the DDCV microemulsion aggregate. For these purposes, an estimate of the partial molar volume of DDCV was calculated based on the McGowan's characteristic volume (0.327 L/mol). This value, in conjunction with the volumes of 1-butanol and ethyl acetate in the microemulsion preparation, was then used to estimate the volume of microemulsion pseudostationary phase and the corresponding phase ratio. In addition, a cac value of 0.5 mM was utilized, corresponding to the cmc of DDCV micelles [32]. Ultimately, the phase ratio for the 1% DDCV microemulsion was calculated to be 0.028 and the phase ratio for the 4% DDCV microemulsion was calculated to be 0.060.

Once the distribution coefficient was calculated, a plot of $\ln K_{eq}$ versus inverse temperature ($1/T$) was used to acquire the enthalpy and entropy of transfer through the van't Hoff equation (7):

$$\ln K_{eq} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (7)$$

where the enthalpy is calculated from the slope ($-\Delta H^\circ/R$), and the entropy is calculated from the y-intercept ($\Delta S^\circ/R$).

3. Results and discussion

3.1. Changes in resolution, enantioselectivity and retention with temperature for analyses using 1% and 4% DDCV microemulsion

Fig. 1 displays comparative chromatograms of the separation of (±)-ephedrine employing 1% DDCV microemulsion over the range of temperatures studied (15–35 °C). As expected, resolution (Table 2) and retention (Figs. 2 and 3) decreased with increasing temperature. Using 1% DDCV, resolution ranged from 0.62 to 3.17 at 15 °C and 0 to 2.02 at 35 °C. In contrast, while a similar trend was noted with the 4% DDCV microemulsion, the compounds exhibited resolution values no lower than 0.84 over the range of temperatures studied. Using 4% DDCV, resolution ranged from 1.29 to 6.20 at 15 °C and 0.84 to 4.11 at 35 °C.

In terms of enantioselectivity (Table 3), the decrease in values with increasing temperature was similar. Moreover, using 1% DDCV the enantioselectivity (with increasing temperature) decreased to <1.02 for three out of nine compounds, resulting in a complete loss of resolution. Employing the 1% DDCV microemulsion, enantioselectivity ranged from 1.04 to 1.25 at 15 °C and 1.00 to 1.19 at 35 °C. In contrast, employing the 4% DDCV microemulsion always provided some

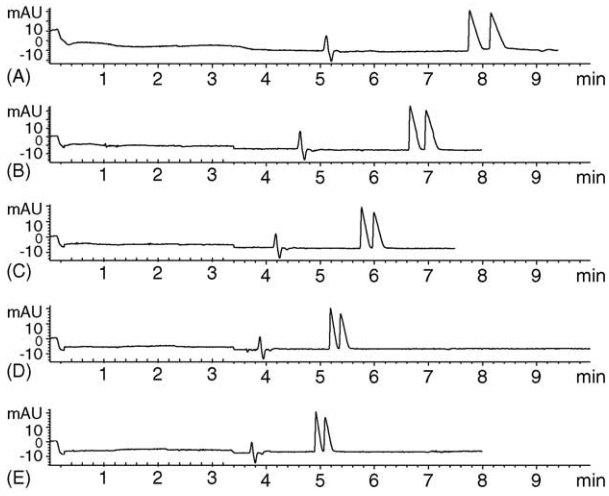

Fig. 1. Representative chromatograms displaying the separation of (±)-ephedrine employing 1% DDCV over a temperature range of 15–35 °C: (A) 15 °C, N_{ave} = 15,000, R_s = 1.70; (B) 20 °C, N_{ave} = 17,000, R_s = 1.52; (C) 25 °C, N_{ave} = 18,000; R_s = 1.38; (D) 30 °C, N_{ave} = 21,000, R_s = 1.34; and (E) 35 °C, N_{ave} = 23,000, R_s = 1.30.

degree of enantioselectivity for each of the compounds investigated over the entire temperature range. Using 4% DDCV, enantioselectivities ranged from 1.05 to 1.31 at 15 °C and 1.05 to 1.18 at 35 °C.

The bottom of Table 2 displays results for the elution range calculated via two different methods: the iterative homolog approach [34] and employing octanophenone as a t_{me} marker. Previous work with the DDCV microemulsion [11] found the iterative approach to be non-linear, resulting in the subsequent use of a t_{me} marker. It was later discovered [12,13a] that the background buffer used in those experiments (ACES) was the cause of the non-linearity, and the situation was subsequently corrected by replacing ACES with phosphate buffer. In this study, the iterative approach was re-examined to yield information on both the elution range and the methylene se-

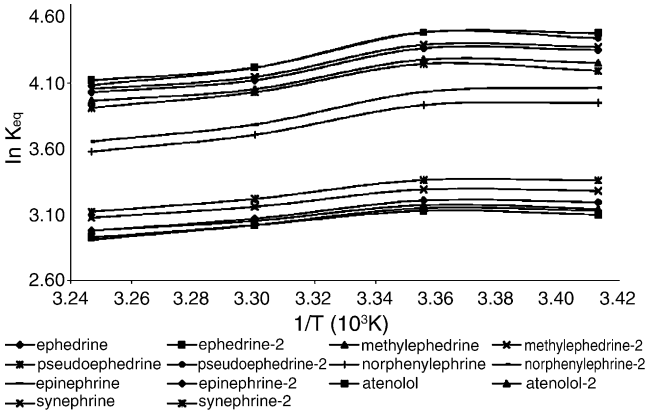

Fig. 2. van't Hoff plot for low-to-moderately retained solutes using 4% DDCV microemulsion over a temperature range of 20–35 °C. Microemulsion preparation noted in Table 1. Voltage ranged from 7.5 to 8.0 kV. Detection wavelength: 215 ± 5 nm, capillary dimensions: L_d = 23.6 cm, L_t = 32 cm, i.d. = 50 μm, injection: hydrodynamic (25 mbar × 2 s).

Table 2
Resolution, elution range and methylene selectivity vs. temperature using 1% and 4% DDCV microemulsion

Compound	1% DDCV[a]					4% DDCV[a]				
	15 °C	20 °C	25 °C	30 °C	35 °C	15 °C	20 °C	25 °C	30 °C	35 °C
Epinephrine	0.62	0.45	0.45	0.00	0.00	1.34	1.19	0.98	1.08	0.97
Ephedrine	1.65	1.51	1.39	1.37	1.29	3.22	2.91	2.46	2.86	2.21
Atenolol	0.63	1.04	0.30	0.40	0.00	1.65	1.37	1.07	1.19	0.96
Methylephedrine	1.56	1.46	1.32	1.35	1.07	3.03	2.67	2.38	2.73	2.14
Metoprolol	1.40	1.27	1.05	0.99	0.74	2.43	2.12	1.38	2.03	1.42
Synephrine	0.83	0.68	0.62	0.00	0.00	2.21	1.90	1.61	1.79	1.01
Norphenylephrine	1.38	1.22	1.02	0.76	0.74	2.60	2.32	1.96	2.05	1.68
Indapamide	1.07	0.95	0.93	0.82	0.79	1.29	1.29	0.62	1.25	0.84
Pseudoephedrine	3.17	2.84	2.52	2.14	2.02	6.20	5.61	4.77	5.02	4.11
Elution range[b]	36.5	14.0	10.6	8.5	7.5	55.7	10.5	8.9	7.9	6.9
Elution range[c]	5.1	5.3	4.7	4.3	3.9	6.1	5.5	6.6	5.5	5.1
α_{CH_2}	2.314	2.334	2.349	2.360	2.370	2.237	2.301	2.308	2.318	2.339

[a] Microemulsion preparations as noted in Table 1.
[b] Elution range obtained using the iterative homolog method (acetophenone through valerophenone).
[c] Elution range obtained using octanophenone as a t_{me} marker.

Table 3
Enantioselectivity vs. temperature using 1% and 4% DDCV microemulsion

Compound	1% DDCV[a]					4% DDCV[a]				
	15 °C	20 °C	25 °C	30 °C	35 °C	15 °C	20 °C	25 °C	30 °C	35 °C
Epinephrine	1.08	1.04	1.04	<1.02	<1.02	1.06	1.06	1.06	1.05	1.07
Ephedrine	1.12	1.11	1.11	1.10	1.10	1.13	1.14	1.13	1.10	1.10
Atenolol	1.04	1.08	1.02	1.03	<1.02	1.05	1.05	1.04	1.04	1.05
Methylephedrine	1.11	1.11	1.11	1.10	1.10	1.12	1.13	1.12	1.10	1.09
Metoprolol	1.09	1.09	1.08	1.08	1.07	1.11	1.13	1.12	1.07	1.07
Synephrine	1.09	1.10	1.07	<1.02	<1.02	1.09	1.08	1.07	1.06	1.05
Norphenylephrine	1.12	1.11	1.10	1.09	1.08	1.13	1.12	1.11	1.08	1.08
Indapamide	1.08	1.07	1.07	1.06	1.06	1.17	1.16	1.21	1.06	1.05
Pseudoephedrine	1.25	1.23	1.21	1.20	1.19	1.31	1.28	1.27	1.20	1.18

[a] Microemulsion preparations as noted in Table 1.

lectivity (α_{CH_2}) of the microemulsions. Interestingly, the elution range is significantly larger when calculated using the iterative approach. It should be further noted that the correlation for each of the iterative results was very good (r^2 no less than 0.9999 and F values > 18,000) and the precision was similarly high with respect to the uncertainty of the slope (relative uncertainty of 0.1%). Because α_{CH_2} is directly proportional to the slope, this precision is reflected in the methylene selectivity as well. To err on the side of caution, however, the migration time observed employing the t_{me} marker was used for all subsequent calculations. While this value may represent a worst-case scenario (by being smaller than the true value), it would be more prudent than employing a value that might be falsely inflated. A cautious stance of this nature is further supported by reports that the iterative approach can result in an overestimation of PSP migration times [35] when lower carbon homologs are used in the iteration, as was the case here.

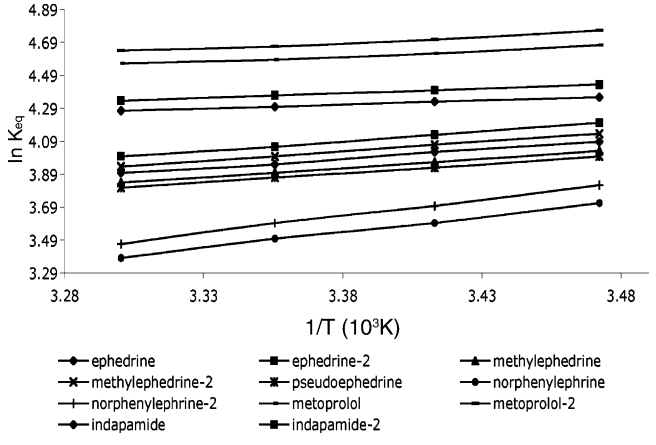

Fig. 3. van't Hoff plot for moderate-to-highly retained solutes using 1% DDCV microemulsion over a temperature range of 15–30 °C. Microemulsion preparation noted in Table 1. Voltage 8.0 kV. Detection wavelength: 215 ± 5 nm, capillary dimensions: L_d = 23.6 cm, L_t = 32 cm, i.d. = 50 μm, injection: hydrodynamic (25 mbar × 2 s.).

3.2. van't Hoff plots for 1% and 4% DDCV microemulsions

A plot of ln K_{eq} versus 1/T will be linear provided that the analyte–selectand interaction occurs via a single mechanism over the entire temperature range studied. Understandably then, this further requires that the heat capacity change upon transfer is zero and the phase ratio is independent of temperature [32]. Typical van't Hoff plots for the test analytes

Table 4
Enthalpies and entropies of transfer for chiral compounds using 1% DDCV microemulsion[a]

Solute	Enantiomer	r^2	Enthalpy		Entropy	
			ΔH° (kJ/mol)	Uncertainty	ΔS° (J/mol K)	Uncertainty
Epinephrine	1	0.94	−7.42	0.22	−1.64	0.76
	2	0.98	−10.66	0.19	−12.28	0.63
Ephedrine	1	1.00	−9.23	0.05	1.93	0.17
	2	1.00	−9.95	0.03	0.35	0.11
Atenolol	1	1.00	−7.87	0.04	−2.58	0.14
	2	0.95	−8.37	0.32	−3.91	1.07
Methylephedrine	1	1.00	−9.26	0.00	1.34	0.02
	2	1.00	−9.68	0.02	0.78	0.06
Metoprolol	1	0.99	−5.37	0.08	20.14	0.25
	2	0.99	−5.96	0.08	18.84	0.26
Synephrine	1	0.96	−6.64	0.17	2.30	0.58
	2	0.99	−11.29	0.15	−12.87	0.50
Norphenylephrine	1	1.00	−15.86	0.06	−24.21	0.21
	2	1.00	−17.14	0.06	−27.71	0.21
Indapamide	1	1.00	−4.01	0.01	22.31	0.03
	2	1.00	−4.80	0.01	20.20	0.02
Pseudoephedrine	1	1.00	−9.14	0.02	1.48	0.06
	2	1.00	−11.26	0.02	−3.99	0.06

[a] Microemulsion preparations as noted in Table 1. Data represents the linear range of 15–30 °C.

are displayed in Figs. 2 and 3 and the thermodynamic results for the 1% DDCV microemulsion are displayed in Table 4. Of particular interest is the lack of linearity when employing the 4% DDCV microemulsion (Fig. 2). The r^2 values ranged from 0.71 to 0.97 over the temperature range studied and the correlation improved only slightly (0.82–0.96) when the 15 °C data were excluded. Since the 4% DDCV contained a large concentration of surfactant compared to the 1% DDCV microemulsion (without a proportional increase in oil and/or co-surfactant), the temperature change most likely had a much more dramatic effect with respect to either the phase ratio or the respective microemulsion conformation. Due to the observed lack of fit, these data were not used to elucidate thermodynamic quantities.

In contrast, the 1% DDCV microemulsion exhibited linearity superior to the 4% DDCV microemulsion (Fig. 3), and the linearity was further improved when the temperature range was narrowed to 15–30 °C. The r^2 values exhibited an average of 0.79 when the entire temperature range was examined, and subsequently improved to an average of 0.99 when the highest temperature (35 °C) was excluded. For the latter set of data, the most linear relationships were observed with moderate-to-highly retained compounds (Table 4), whereas the correlation was somewhat less for compounds which were only slightly retained (epinephrine, atenolol and synephrine). The lack of fit in this case may have more to do with the assignment of migration times via Chemstation software than a change in the mechanism of interaction. This is because the lesser-retained enantiomers transitioned from being moderately resolved ($R_s \geq 0.62$) to being unresolved ($R_s < 0.4$) as the temperature increased. In chromatography, as peaks increasingly overlap their center of gravity will shift inward and the actual peak maximum will be shifted from its true value [36,37]. This discrepancy in integration would have understandably affected the retention factors and resulting van't Hoff data analysis.

As displayed in Table 4, the values for ΔH° are all negative, indicating an enthalpic preference of the enantiomers for the microemulsion pseudostationary phase. The values themselves ranged from −4.01 to −17.14 kJ/mol, with the less-negative values corresponding to solutes which were strongly retained (metoprolol and indapamide). In contrast, ΔS° displayed both positive and negative values, with the most *favorable* values (large, positive values) corresponding to metoprolol and indapamide, the compounds which had previously exhibited less favorable enthalpies.

The entropy of transfers ranged from −27.7 to 22.3 J/mol K, with all enantiomeric pairs displaying the same *relative* sign except synephrine and pseudoephedrine. Understandably, the larger the magnitude of the entropy value, the greater the difference in "order" that the solute has experienced in transferring from the aqueous environment to the microemulsion environment. The positive entropy values can be explained through the hydrophobic effect. When a comparatively hydrophobic solute is present in the aqueous phase, water molecules will orient themselves around the solute via a network of hydrogen bonds to compensate for the energetically unfavorable interaction. However, upon solute transfer into the microemulsion phase the converse is true because water molecules have no appreciable presence within

the microemulsion droplet, allowing for less rigid order and a subsequent increase in the entropy of the system.

In contrast, the solutes that displayed a negative ΔS° transitioned into an environment where they were forced into a more ordered state than the aqueous surroundings. If an analyte is significantly polar in nature, then the hydrophobic effect in the aqueous phase will not be as strong, resulting in the analyte actually assuming an increased order in the microemulsion phase than the aqueous phase. Further, more polar, cationic analytes will most likely be more attracted to the polar, anionic surfactant head groups than into the microemulsion core. This would lead to an interaction that was more *adsorbed* onto the microemulsion droplet than *absorbed* within the microemulsion droplet. Consequently, the increased rigidity caused by the more polar/electrostatic interactions would result in reduced and/or negative entropic values. Two of the compounds that displayed negative entropy values, epinephrine and norphenylephrine, are indeed polar analytes. In fact, similar behavior was noted for these two compounds during van't Hoff analysis using DDCV micelles [32]. With respect to the negative entropy values for synephrine, the aforementioned micellar study observed positive entropies for synephrine but negative entropies for octopamine. At this point in time, the reason behind the observed negative entropy value for synephrine is unknown. For this particular study, octopamine was not evaluated, however in previous work [11–13a] it had been found that both octopamine and synephrine behaved almost identically in all microemulsion experiments performed. So similar, in fact, that synephrine was chosen for evaluation over the octopamine in this work to narrow the scope of compounds. The two compounds differ only by a beta amino methyl group, so it is possible that this slight difference in structure had more of an impact when using the micellar phase than noted with the microemulsion phase. In terms of atenolol, the negative entropy value may have been a combination of the increased hydrogen bonding capability and steric bulkiness of the molecule preventing it from penetrating deeply into the microemulsion.

The previous two scenarios argue for the hydrophobic effect or lack thereof and serve only to elucidate the driving force behind *achiral* solute/PSP interactions. Importantly, there must be a difference in Gibb's free energy of transfer ($\Delta\Delta G^\circ$) between enantiomers for chiral differentiation to occur. Further, the change in Gibb's free energy between enantiomeric pairs must be brought about by differences in enthalpy and/or entropy. Table 5 displays a compilation of the selectivity (α_{enant}), resolution (R_s), distribution coefficients (K_{eq}), Gibb's free energy (ΔG°) and the change in Gibb's free energy ($\Delta\Delta G^\circ$) at 25 °C. Importantly, it also shows the differences in enthalpy ($\Delta\Delta H^\circ$) and entropy ($\Delta\Delta S^\circ$) of transfer between enantiomeric pairs. The entropy of transfer contribution is indeed significant, averaging approximately 72% of the enthalpic contribution. This makes sense if one considers the 3-point interaction rule of chiral discrimination [38]: both enantiomers will share two common *achiral* points of favorable interaction when interacting with a favorable chiral species. In contrast, only one of the two enantiomers will interact through a third preferential point which will result in the overall *chiral* discrimination. Essentially, this dictates that if chiral differentiation is observed, then one enantiomer must be interacting more strongly than the other, and thus

Table 5
Comparison of selectivities, resolution, distribution coefficients, and thermodynamic parameters using the 1% DDCV microemulsion[a] at 25 °C

Solute	Enantiomer	α_{enant}	R_s	K_{eq}	ΔG° (kJ/mol)	$-\Delta\Delta G^\circ$ (kJ/mol)	$-\Delta\Delta H^\circ$ (kJ/mol)	$-T(\Delta\Delta S^\circ)$ (kJ/mol)
Epinephrine	1	1.04	0.45	16.0	−6.93	0.07	3.24	3.17
	2			16.7	−7.00			
Ephedrine	1	1.11	1.39	51.9	−9.80	0.28	0.72	0.47
	2			57.7	−10.06			
Atenolol	1	1.02	0.30	17.4	−7.10	0.10	0.50	0.40
	2			17.8	−7.20			
Methylephedrine	1	1.11	1.32	49.4	−9.66	0.25	0.42	0.17
	2			54.5	−9.91			
Metoprolol	1	1.08	1.05	98.1	−11.38	0.19	0.59	0.17
	2			106	−11.57			
Synephrine	1	1.07	0.62	19.3	−7.32	0.13	4.65	4.52
	2			20.7	−7.45			
Norphenylephrine	1	1.10	1.02	33.1	−8.65	0.23	1.28	1.04
	2			36.3	−8.88			
Indapamide	1	1.07	0.93	73.7	−10.66	0.16	0.79	0.63
	2			78.8	−10.82			
Pseudoephedrine	1	1.21	2.52	47.9	−9.58	0.49	2.12	1.63
	2			58.1	−10.07			

[a] Microemulsion preparations as noted in Table 1.

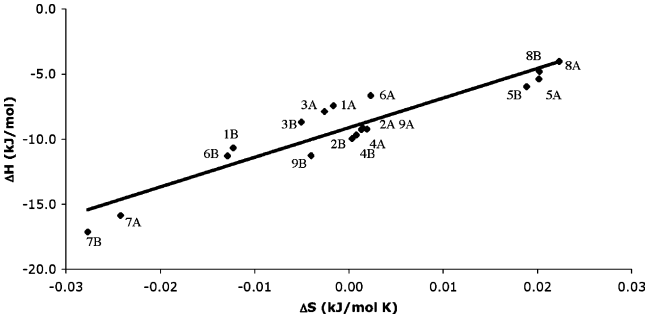

Fig. 4. Compensation plot for enantiomers analyzed using 1% DDCV microemulsion over a temperature range of 15–30 °C: (1A and B) epinephrine enantiomers; (2A and B) ephedrine enantiomers; (3A and B) atenolol enantiomers; (4A and B) methylephedrine enantiomers; (5A and B) metoprolol enantiomers; (6A and B) synephrine enantiomers; (7A and B) norphenylephrine enantiomers; (8A and B) indapamide enantiomers; and (9A and B) pseudoephedrine enantiomers. Voltage ranged from 7.5 to 8.0 kV. Detection wavelength: 215 ± 5 nm, capillary dimensions: L_d = 23.6 cm, L_t = 32 cm, i.d. = 50 μm, injection: hydrodynamic (25 mbar × 2 s).

must be held more rigidly, resulting in the larger observed differences in entropy over enthalpy.

3.3. Enthalpy/entropy compensation

Enthalpy/entropy compensation behavior is exhibited when the Gibb's free energy at a given compensation temperature (T_c) is equal for all solutes. Importantly, the existence of a relationship of this kind supports the idea of a similar retention mechanism for all of the solutes involved. Compensation behavior is signified by a linear correlation between ΔH° and ΔS°, where the slope of the line provides the compensation temperature (T_c), or the temperature around which the relationship holds true and ΔG° for all compounds is similar. Fig. 4 displays a graph of ΔH° versus ΔS° for all of the enantiomers studied using the 1% DDCV microemulsion. The correlation coefficient (r^2) was 0.87, somewhat lower than anticipated but still a good indication that enthalpy/entropy compensation occurred. Further, a plot of $\Delta\Delta H$ versus $\Delta\Delta S$ (not shown) yields an r^2 of 0.99. Ultimately, the ΔH° versus ΔS° graph yielded a slope, or compensation temperature, of 227 ± 22 K. A previous report of Peterson and Foley [32] about DDCV micelles noted slightly higher values for T_c (295 and 288 for the two groupings, respectively), however it should be pointed out that the compounds used in that set of experiments were somewhat different. Further, the compensation plots were segregated according to hydrophobic and hydrophilic analyte groupings, whereas in this case they were left as one singular group. Similar to the micellar results, however, our compensation temperature is somewhat lower than what would typically be observed in reversed-phase liquid chromatography (e.g. between 500 and 750 K) [39]. While the ΔG° values noted in Table 5 were calculated above the compensation temperature (298 K versus 227 K) a similarity in magnitude is still apparent, indicating with high probability that these compounds undergo a comparable retention mechanism.

3.4. Comparison of DDCV MEKC and MEEKC thermodynamic data for like compounds

Table 6 displays a comparison of thermodynamic data acquired for the compounds that were analyzed using both the DDCV micellar and microemulsion aggregates. In terms of ΔH°, a comparison of the results reveal that the data are evenly split, with half of the more favorable enthalpy values obtained with the DDCV microemulsion and half obtained with the DDCV micelle. In contrast, both ΔS° and ΔG° clearly favor the micellar phase. Again, these values show the overall *achiral* preference of the analyte for the aggregate. From that standpoint this particular set of analytes is more attracted (exhibits a greater increase in Gibb's free energy) to the DDCV micelles. This may have to do with the difference in background buffer employed with the two different phases. The micellar experiments were conducted using a zwitterionic background buffer (CHES), whereas the microemulsion experiments were conducted using an inorganic buffer (phosphate). Previous microemulsion experiments employing a zwitterionic buffer (ACES) exhibited greater analyte retention and migration times, as well as a larger elution range [11–13a]. This was largely attributed to (i) penetration of the ACES buffer species into the microemulsion aggregate and (ii) a lesser degree of microemulsion counterion association. The penetration of zwitterionic species can create a

Table 6
Comparison of thermodynamic data between DDCV micellar (MC) and microemulsion (ME) surfactant aggregates

Compound	Average ΔH°[a] (kJ/mol)		$\Delta\Delta H^\circ$ (kJ/mol)		Average ΔS°[a] (J/mol K)		$\Delta\Delta S^\circ$ (J/mol K)		Average ΔG°[a] (kJ/mol)		$\Delta\Delta G^\circ$ (kJ/mol)	
	MC[b]	ME[c]	MC[b]	ME[c]	MC[b]	ME[c]	MC[b]	ME[c]	MC[b]	ME[c]	MC[b]	ME[c]
Ephedrine	−7.33	−9.59	1.88	0.72	33.31	1.14	5.40	1.58	−17.4	−9.93	0.24	0.28
Atenolol	−6.52	−8.28	3.35	0.82	28.11	−3.82	12.00	2.48	−15.0	−7.14	0.10	0.08
Metoprolol	−7.07	−5.67	1.89	0.59	39.86	19.49	1.89	1.30	−19.1	−11.48	0.17	0.19
Synephrine	−10.21	−8.97	1.00	4.65	15.01	−5.29	0.17	15.17	−14.8	−7.39	0.17	0.13
Norphenylephrine	−18.25	−16.50	2.09	1.28	−9.34	−25.96	0.21	3.50	−15.6	−8.77	0.21	0.23
Pseudoephedrine	−9.98	−10.20	3.26	2.12	27.16	−1.26	0.51	5.47	−18.2	−9.83	0.51	0.49

[a] The average of enantiomer peaks 1 and 2.
[b] Micelle preparation as noted in Table 1.
[c] Microemulsion preparations as noted for 1% DDCV microemulsion in Table 1.

comparatively more hydrophobic aggregate overall. In fact, this was similarly recognized by Peterson and Foley [33] in a separate manuscript evaluating counterion effects. Had the microemulsion data been acquired using a zwitterionic buffer, the retention factors and subsequent magnitude of the thermodynamic data may have been more comparable. Unfortunately, the lack of reproducibility encountered when using the DDCV microemulsion in conjunction with zwitterionic buffers precluded a direct comparison of this nature.

With respect to chiral differentiation, one must compare $\Delta\Delta H^\circ$, $\Delta\Delta S^\circ$ and $\Delta\Delta G^\circ$ between the two systems. From this standpoint, it appears that entropy is the deciding factor between the two. The $\Delta\Delta H^\circ$ values largely favor the micellar phase, whereas the $\Delta\Delta S^\circ$ and ultimately $\Delta\Delta G^\circ$ values are evenly split between the two phases. In contrast, while the magnitude of ΔS° was lower in all cases for the microemulsion results, the microemulsion was still able to provide a large enough difference in entropy ($\Delta\Delta S^\circ$) between enantiomers to elicit separation.

4. Conclusion

van't Hoff data analysis provides a valuable insight into both chiral and achiral analyte–selectand interaction mechanisms. While a linear van't Hoff relationship was not observed with the 4% DDCV microemulsion, the 1% DDCV microemulsion exhibited linearity over the range of 15–30 °C. For the 1% DDCV microemulsion, the enthalpic contribution to retention was consistently favorable ($\Delta H < 0$), whereas the entropic contribution varied from compound to compound. Specifically, this work highlights that there are important differences to consider when using a micellar or microemulsion phase. While the achiral attraction of the analytes was greater for the micellar phase, the microemulsion provided a suitable difference in entropy (and Gibb's free energy) between enantiomers to achieve chiral discrimination.

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