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Separation of 13-*cis* and all-*trans* retinoic acid and their photodegradation products using capillary zone electrophoresis and micellar electrokinetic chromatography (MEC)*

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Abstract: Two retinoic acid isomers; 13-*cis* retinoic acid and all-*trans* retinoic acid and their photodegradation products were resolved with capillary electrophoresis (CE) (UV detector, 345 nm) using three different mobile phases: method 1 — an acetonitrile modified borate buffer (pH 8.5); method 2 — borate buffer (pH 8.5) modified with acetonitrile and α-cyclodextrin; and method 3 — borate buffer (pH 8.5) modified with SDS (MEC). Concentration of acetonitrile in the buffer was varied from 10 to 50% in method 1 and resolutions of 0–1.9 were obtained for the two retinoic acid isomers. Similarly in method 2, concentration of α-cyclodextrin in the buffer (with 10% acetonitrile) was varied from 0 to 40 mM, giving resolutions of 0–3.8. In method 3, concentration of SDS in the buffer was varied from 5 to 60 mM resulting in resolutions of 1.3–4.1. Optimum separation conditions for the three methods were applied to the separation of photodegradation products of the two retinoids after exposure to fluorescent light for 36 h. A buffer modified with 45% acetonitrile and the same buffer modified with 10 mM SDS gave incompletely resolved electropherograms with a 72 cm × 50 μm capillary (50 cm to the detector). A buffer containing 20 mM α-cyclodextrin 10% acetonitrile gave completely resolved peaks for each isomer. The buffer containing 10 mM SDS gave completely resolved peaks for the photodegradation products when a 122 cm × 50 μm capillary (100 cm to detector) was used.

Keywords: *Retinoic acid; capillary zone electrophoresis; micellar electrokinetic chromatography; sodium dodecyl sulphate; organic modifier; cyclodextrins.*

Introduction

Capillary electrophoresis (CE) as described in the early 1980s [1] is recognised as a powerful analytical separation technique. Capillary zone electrophoresis (CZE) may be the method of choice for separation of charged compounds. Micellar electrokinetic chromatography (MEC), another separation method developed in 1984, permits electrically neutral or nonionic compounds to be separated via their differential distribution (between an aqueous mobile phase and a pseudostationary micellar phase) [2, 3] and the differential migration of the micellar phase [4, 5]. MEC is also effective for the separation of charged compounds [6, 7].

Many parameters can be used to effect separation and manipulate selectivity in CE. These include the capillary dimensions, applied voltage, buffer composition, ionic strength and pH. Buffer additives such as surfactants (MEC), organic solvents and cyclodextrins (CDs) may be used to achieve this. Under the appropriate conditions cyclodextrins are capable of inclusion of selective guest neutral molecules or ions with the appropriate structural features. There are several reports in the literature which show the use of CE to resolve isomers. These include separation of enantiomers [8–10], positional substitution isomers [11], and *cis–trans* isomers [12].

Retinoic acid (RA) and its analogues are involved in a variety of biochemical functions. Retinoids stimulate bone growth and epithelial differentiation [13]. Retinoids have again found therapeutic use in dermatology and oncology [14]. As an example, isotretinoin (13-*cis* RA) produces both dramatic clearing and prolonged remission of acne lesions [15]. 13-*cis* RA is also effective against photo-damaged skin [16]. The existence of four unsaturated

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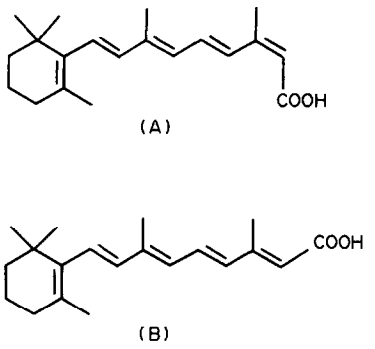

Figure 1
Structures of 13-*cis* retinoic acid (A) and all-*trans* retinoic acid (B).

bonds (Fig. 1) in RA gives rise to *Z–E* isomerism [17]. Retinoids isomerize by the action of light and heat and isomerization is also known to occur under physiological conditions [18]. The *cis* and *trans* isomers of a particular retinoid may have differing biological activity [19–21], therefore an analytical method capable of separating RA isomers is essential.

An LC method has been developed to achieve this separation [22]. CE has several advantages which makes it an analytical method of choice for such a separation. These advantages include high resolution, high mass sensitivity, small sample volumes (1–50 nl), extraordinary small mobile phase requirement and rapid separation. A CZE method for the baseline separation of all-*trans* RA and 13-*cis* RA has been reported [12]. This paper reports an optimized CE method for the separation of the two retinoids and their photodegradation products. The effects of buffer concentration and pH, modifier type and concentration, and capillary length on the resolution of the two retinoid isomers are also reported.

Experimental

Chemicals and reagents

All-*trans* retinoic acid (all-*trans* RA) and 13-*cis* retinoic acid (13-*cis* RA) were supplied by Hoffmann-La Roche (Nutley, NJ, USA). Boric acid, sodium borate decahydrate, naphthalene and acetonitrile (HPLC grade) were purchased from Baker (Phillipsburg, NJ, USA). Sodium dodecyl sulphate (SDS), α-, β- and γ-cyclodextrins (CDs) were obtained from Sigma (St Louis, MO, USA). Benzyl alcohol was from Fisher (Fair Lawn, NJ, USA).

Capillary electrophoresis system

Analytical Capillary Electrophoresis system model 270A (ABI, San Jose, CA, USA) was used. Fused silica capillaries (50 μm i.d.) were used and the lengths were either 72 cm (50 cm to the detector) or 122 cm (100 cm to the detector). On-column UV detection at 345 nm was used for all analysis. Samples were injected hydrodynamically and a constant voltage of 30 kV and temperature of 30°C were used throughout this work. Migration times and peak area were measured by a Hewlett–Packard 3392A integrator.

Methods

Borate buffers were prepared by adjusting the pH of sodium borate decahydrate solution with boric acid solution of the same concentration to the desired pH. The modified buffer solutions were made by adding the required amount of the modifier to the prepared buffer solution. Modified buffers were vacuum-filtered using 0.2 μm membrane filter to remove particles and to degas the solutions. Analyte solutions were made in acetonitrile. For the photodegradation studies 4.0×10^{-5} g ml^{-1} analyte solution in acetonitrile was exposed to fluorescent light for 36 h.

The capillary was washed with 0.1 M NaOH for 3 min followed by another 3-min wash with the modified buffer solution before starting each run.

Results and Discussion

Effect of modifier concentration on resolution

The effect of modifier (ACN, α-CD and SDS) concentration on the resolution of 13-*cis* RA and all-*trans* RA is shown in Fig. 3. Values for resolution (R_s) of the two isomers using CZE (ACN modified buffer and α-CD–ACN modified buffer) were calculated by

$$R_s = 0.177\ (\mu_{eff1} - \mu_{eff2})[V(\mu_{av} + \mu_{eo})/D]^{1/2}, \quad (1)$$

where V is the applied voltage, D is the diffusion coefficient and μ_{av} is the average mobility of the two components. μ_{eff1}, μ_{eff2} and μ_{eo} are the effective mobilities of the two components and the electroendosmotic mobility (measured with benzyl alcohol), respectively [23].

Resolution (R_s) from the MEC were calculated by

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{1 + k'} \cdot \frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'}, \quad (2)$$

where α is the selectivity factor and N is the number of theoretical plates. The capacity factor (k') of a weak acid is calculated by

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})}, \quad (3)$$

where t_r, t_0 and t_{mc} are the migration times of the solubilized solute, the solute in absence of micelles and in the presence of micelles, respectively [7]. Figure 3 shows that high concentrations of SDS are less effective for resolving 13-*cis* RA and all-*trans* RA under the electrophoretic conditions (30 mM borate buffer, pH 8.2). It was found that 13-*cis* RA migrates faster than the all-*trans* RA in MEC with SDS under the specified conditions [Fig. 2(i)]. This suggests that the all-*trans* isomer has a greater affinity for the micellar interior than the 13-*cis* isomer even though at the buffer pH 8.5, the two exist largely in the anionic form. The selectivity decreases with increasing micelle concentration, as shown by the diminishing resolution. On the contrary, increasing concentration of α-CD in 30 mM borate buffer pH 8.2 containing 10% acetonitrile improved the resolution of the two retinoids. The mechanism is believed to be similar to that explained above — the all-*trans* RA fits better into the α-CD cavity than the 13-*cis* isomer, and in this case since the α-CD carries no charge, the all-*trans* RA migrates faster to the negative electrode than the 13-*cis* RA [Fig. 2(ii)]. The cavities of β- and γ-CD may be large enough to non-selectively include the two retinoic acid isomers hence both failed to resolve the two isomers.

Figure 3 also shows that unlike the MEC separation with SDS, the resolution of the two isomers improved with increasing concentration of acetonitrile in 30 mM borate buffer pH 8.2. However as in the MEC with SDS, the migration times of the two isomers increased rapidly with increase in acetonitrile concentration. The difference in the migration times in acetonitrile modified buffer has been attributed to the difference in the hydrodynamic radii of the two isomers [18]. Very poor resolution was obtained with this method compared with the other two.

Effect of pH on resolution

Figure 4 shows the effect of pH of a 30 mM borate buffer solution containing 10 mM SDS, the same buffer modified with 20 mM α-CD–10% acetonitrile or 45% acetonitrile on the separation of the two isomers. MEC separation showed a great improvement in resolution above pH 7 but generally good resolution and sharp peaks devoid of any tailing were obtained at buffer pH above 8 and this is probably due to the fact that at these high pHs retinoic acid exist mainly in the anionic state. pH 8.5 was chosen for subsequent analysis.

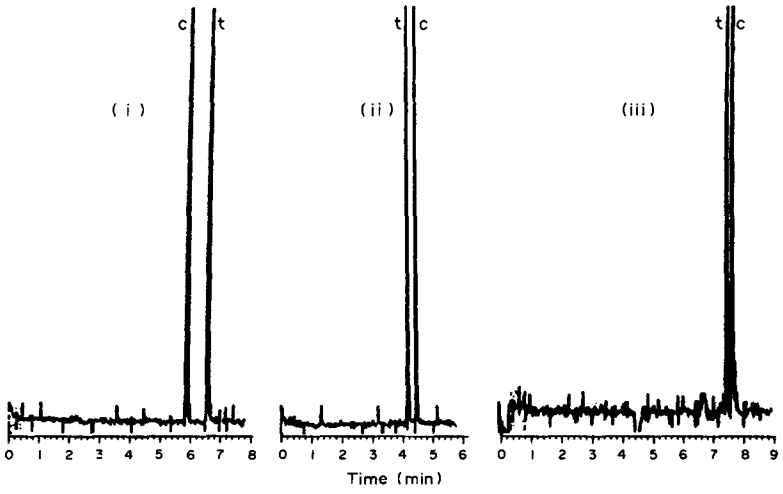

Figure 2
Electropherograms of 13-*cis* (c) and all-*trans* (t) retinoic acid (6 μg ml^{-1} each). Conditions: 30 mM borate buffer, pH 8.5 modified with (i) 10 mM SDS, (ii) 20 mM α-CD–10% ACN and (iii) 45% ACN. Other conditions; injection: vacuum, 2.5 s; capillary: 72 cm × 50 μm i.d. (50 cm to the detector); UV detection, 345 nm; applied voltage: + 30 kV; temperature: 30°C.

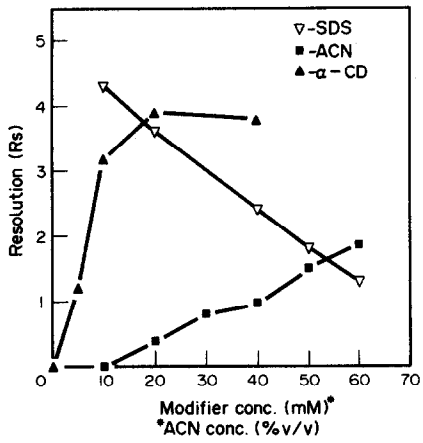

Figure 3
Effect of modifier (SDS, ACN and α-CD) concentration on the resolution of 13-*cis* and all-*trans* retinoic acid. Buffer: 30 mM borate buffer solution, pH 8.2; other conditions as in Fig. 2.

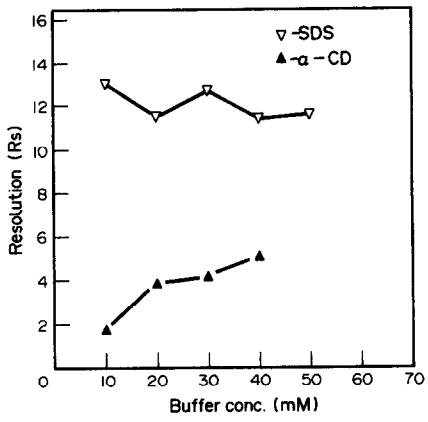

Figure 5
Effect of borate buffer ionic concentration on the resolution of 13-*cis* and all-*trans* retinoic acid. Conditions: borate buffer solutions (pH 8.5) modified with (a) 10 mM SDS and (b) 20 mM α-CD–10% ACN; capillary dimensions: 122 cm × 50 μm (100 cm to the detector). Other conditions as in Fig. 2.

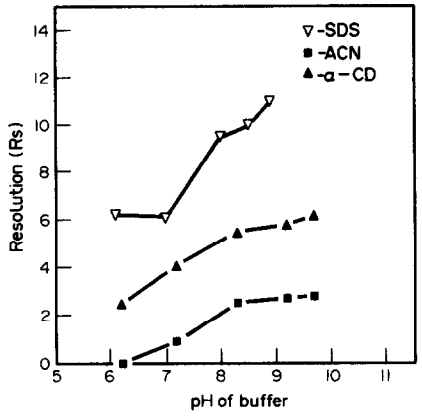

Figure 4
Effect of buffer pH on the resolution of 13-*cis* and all-*trans* retinoic acid. Conditions: 30 mM borate buffer modified with (a) 10 mM SDS, (b) 20 mM α-CD–10% ACN and (c) 45% ACN; capillary: 122 cm × 50 μm (100 cm to the detector); applied voltage: +30 kV; other conditions as in Fig. 2.

Table 1
Effect of capillary length on migration time and resolution of 13-*cis* retinoic acid and all-*trans* RA

		Migration time (min) 13-*cis* RA	All-*trans* RA	Resolution (R_s)
SDS	L_1	7.5	8.3	4.3
	L_2	20.7	23.0	10.0
α-CD	L_1	5.0	4.6	4.0
	L_2	16.2	14.6	5.5
ACN	L_1	8.5	8.3	1.5
	L_2	29.0	28.2	2.5

L_1, L_2 — 72, 122 cm length capillary, respectively.
SDS — 10 mM SDS in 30 mM borate buffer, pH 8.5.
α-CD — 20 mM α-CD–10% ACN in 30 mM borate buffer, pH 8.5.
ACN — 45% ACN in 30 mM borate buffer, pH 8.5.
Other electrophoretic conditions as in Fig. 2.

Effect of buffer concentration on resolution

Increasing the borate buffer concentration from 10 to 50 mM at pH 8.5 appears not to have much effect on resolution of the retinoic acid isomers (Fig. 5).

Effect of capillary length on resolution/ migration time

Table 1 shows the effect of doubling the capillary length on migration times and resolution of the two retinoic acid isomers. Resolution was more than doubled for the MEC separation while only slight increases were obtained for the other two methods. Analysis time, however, increased approximately threefold for all the three methods. From Figs 6 and 7 it is realised that while α-CD–10% acetonitrile modified buffer solution gave baseline separation of the photodegradation products when a 72 cm long capillary (50 cm to detector) was used, a 122 cm long capillary (100 cm to the detector) of the same diameter was required to separate the degradation products with SDS modified buffer solution.

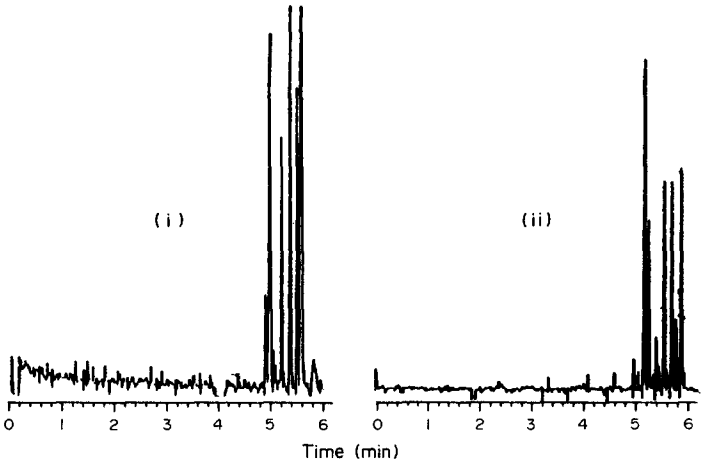

Figure 6
Electropherograms of (i) 20 μg ml^{-1} all-*trans* retinoic acid and (ii) 20 μg ml^{-1} 13-*cis* retinoic acid solution exposed to light for 36 h. Conditions: 20 mM α-CD–10% ACN in 30 mM borate buffer, pH 8.5; capillary: 72 cm × 50 μm (50 cm to the detector). Other conditions as in Fig. 2.

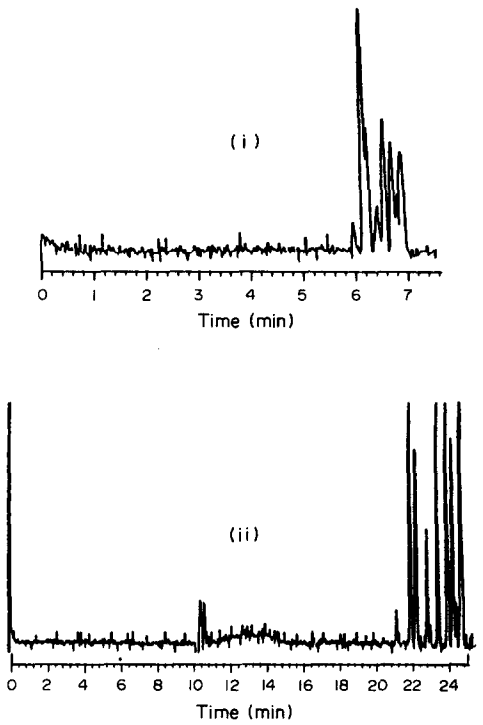

Figure 7
Electropherograms of 20 μg ml^{-1} all-*trans* retinoic acid solution exposed to light for 36 h. Conditions: 10 mM SDS modified 30 mM borate buffer, pH 8.5; capillary: (i) 72 cm × 50 μm (50 cm to the detector); (ii) 122 cm × 50 μm (100 cm to the detector). Other conditions as in Fig. 2.

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