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Chiral separation of amino acids by capillary electrophoresis with octyl-β-thioglucopyranoside as chiral selector

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Abstract

1-*S*-Octyl-β-D-thioglucopyranoside (OTG) was evaluated as a chiral selector for the separation of dansyl-amino acids by capillary electrophoresis. Enantiomeric separations of the amino acids can be accomplished by judiciously adjusting the pH of the solution and the concentration of OTG. Better separation can be achieved, however, when OTG is used, not as a sole chiral selector but rather together with sodium dodecyl sulfate (SDS) and cyclodextrin (CD). Interestingly, not only can this OTG–SDS–CD system provide better separation than OTG alone but also it can separate compounds which cannot be separated when only CD or CD and SDS are used. All amino acids were baseline separated with this OTG–SDS–CD system at optimal conditions.

Keywords: Enantiomer separation; Buffer composition; Amino acids; Octylthioglucopyranoside

1. Introduction

Enantiomeric separation is an important subject in science and technology. The popularity stems from the fact that very often only one form of enantiomer is chemically and/or biologically active. The other or others can reverse or otherwise limit the effect of the desired enantiomer [1–6]. It is thus hardly surprising that the pharmaceutical industry needs effective chiral separation methods. High-performance liquid chromatography (HPLC) and gas chromatography (GC) are the two most widely used methods for chiral separations [1–6].

The recent advances in capillary electrophoresis (CE) have provided an alternative means for chiral separations, especially for cases where samples are available in limited amounts, and when short analysis time is needed [7,8]. However, the number of chiral selectors which are known to be effective in CE is considerably less than the chiral stationary phases available in HPLC (i.e. only about 40 chiral selectors compared to more than 400 different chiral stationary phases) [1–8]. As a consequence, the search for new chiral selectors has become an important issue in CE separation.

A variety of compounds including cyclodextrins (CDs), crown ethers, proteins, polysaccharides, macrocyclic antibiotics, chiral micelles and metal–chiral ligand complexes can be effectively used as chiral selectors for CE [7,8]. Chiral surfactants derived from amino acids are relatively popular since they

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are either available or can be synthesized readily [9,10].

However, it has been known that when these surfactants are used alone, they usually lead to poor selectivity and low resolution. Substantial increase in the efficiency and resolution can be obtained when SDS is used together with the chiral surfactants to form mixed micelles [11–14]. The selectivity of the system can be manipulated by changing either the type and/or the concentration of the surfactants, or by adding additional additives (e.g. CD) to the buffer. In fact, the use of additional pseudophase additives has been shown to be an effective approach for resolving enantiomers.

The objective of this work is to search for a new chiral selector. A novel, naturally occurring chiral surfactant, octyl-β-thioglucopyranoside (OTG) will be investigated for possible use as a chiral selector for CE. The effect of the concentration of this chiral surfactant, together with the effect of another additive, including SDS and cyclodextrin, on the separation will be investigated in order to gain insight into the mechanism of chiral separation.

2. Experimental

2.1. Chemicals

The reagents used in this study were of analytical grade. Eight dansyl (or Dns-) DL-amino acids, i.e. glutamic acid (Glu), leucine (Leu), methionine (Met), norleucine (Nle), norvaline (Nva), phenylalanine (Phe), tryptophan (Trp), and valine (Val) were purchased from Sigma (St. Louis, MO, USA) and used as received. Octyl-β-thioglucopyranoside (OTG) was purchased from Pierce (Rockford, IL, USA). Dibasic sodium phosphate, sodium dodecyl sulphate (SDS) and β-CD were from Aldrich (Milwaukee, WI, USA). Water was deionized and distilled.

Stock buffer solutions were prepared with 250 m*M* $Na_2HPO_4 \cdot 7H_2O$ and 1 *M* H_3BO_3, and the solutions were adjusted to pH 6.5 with 0.1 *M* HCl or 0.1 *M* NaOH. Final buffered solutions used in this study had 25 m*M* Na_2HPO_4 and 100 m*M* boric acid. All micellar solutions were made by weighing appropriate amounts of SDS and OTG and diluting with the stock buffer solution and distilled water in a 100-ml volumetric flask to obtain the desired concentrations. The SDS concentrations ranged from 25, 50, 75 to 100 m*M*, and those of OTG were from 12, 36, 60, 90 to 120 m*M*. All micellar buffered solutions were filtered through 0.45 μm membrane filters (Gelman Sciences, Ann Arbor, MI, USA) and degassed in an ultrasonic bath before use. All dansyl-DL-amino acid solutions were made by dissolving the appropriate amount of the solid samples in distilled water to obtain concentrations of 0.75 mg/ml.

The migration times of the electroosmotic flow and micellar phase, t_0 and t_{mc}, were determined by adding methanol (1%, v/v) and Sudan III to the sample solutions and following their elution time. This is because methanol is reported to behave as an unretained solute, namely, it moves at the same velocity as electroosmotic flow and elutes at time t_0. Sudan III [1-(*p*-phenylazophenylazo)-2-naphthol] is known to be completely solubilized in the micellar phase and elutes at time t_{mc} [15].

2.2. Apparatus

An ISCO 3850 capillary electrophoresis system (ISCO, Lincoln, NE) equipped with an on-column UV absorption detector set at a fixed wavelength of 246 nm was used for all separations. The operation voltage was 15 kV. A fused-silica capillary column (ISCO) of 66 cm (48 cm from injection to detector)×50 μm I.D. was used for all experiments. Dns-DL-amino acids were injected by vacuum for 3 s. A CID-AD 08 converter board (Computer Boards, Mansfield, MA, USA), installed in a Gateway 2000 microcomputer (North Sioux City, SD, USA) and controlled by a data acquisition program written in C++ was used to collect, to analyze data and to real-time display electropherographs. All experiments were performed at ambient temperature (ca. 25 °C).

2.3. Methods

The new capillary column was conditioned as follows: the capillary was filled with 1.0 *M* NaOH solution and allowed to equilibrate for 1.0 h. It was then filled with 0.1 *M* NaOH overnight. Subsequently, it was flushed with about 5.0 ml of distilled

water, 5.0 ml of 0.1 *M* HCl and 5.0 ml of distilled water in sequence for 0.5 h.

At the beginning of every day, the capillary was flushed with 0.1 *M* NaOH for 10 min, then with distilled water for 10 min. Then, it was filled with operating buffer and allowed to equilibrate for 0.5 h. Between each run, the capillary was purged with the operating buffer for 5 min. It was stored overnight filled with 0.1 *M* NaOH.

It was reported that some surfactants may be adsorbed onto the inner wall of the capillary. This may lead to poor reproducibility [16,17]. In the present study, each separation was performed at least three to four times in order to determine the reproducibility.

Methanol and Sudan III were added to the buffer to determine the migration times of the electroosmotic flow and the micellar phase, t_0 and t_{mc}, respectively. These values were then used to calculate the electroosmotic velocity, ν_{eo}, and the apparent velocity of the micelle, ν_{mc}.

Retention factors (k) for all analytes studied were calculated using Eq. (1):

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

where t_r is the migration time of the solutes. In case peaks of enantiomers were overlapped at or above the half height, a deconvolution program (GRAM-386, Galactic Industries, Salem, NH, USA) was employed to deconvolute them into individual components.

3. Results and discussion

1-*S*-Octyl-β-D-thioglucopyranoside (OTG) used in this study is similar to the natural surfactant octylglucopyranoside (OG). The difference between OTG and OG is that in the OTG, the glucopyranoside head group is substituted with the thioglucopyranoside group. Since OG has been used for chiral separation with some success, it is expected that OTG could also be used as a chiral selector. OTG is known to form micelles in solution with a CMC value of 9 m*M* [18].

3.1. Effect of pH on separations

pH 5.0 and 6.5 were used for the separations of Dns-DL-amino acids. The electropherograms of DL-Nle at both pH values are shown in Fig. 1. As illustrated, the resolution at pH 5.0 is 1.48 which is about 1.1-fold higher than that at pH 6.5 (1.30). However, the migration time at pH 5.0 is 1.3 times longer than that at pH 6.5. It seems that lower pH would give better resolution, but longer retention time.

pH is known to influence the electroosmotic flow (EOF) as well as the ionization state of the solutes. This, in effect, would result in a higher EOF or shorter analysis time [19]. However, at pH higher than the isoelectric points, the amino acids will be negatively charged. This will lead to the repulsion

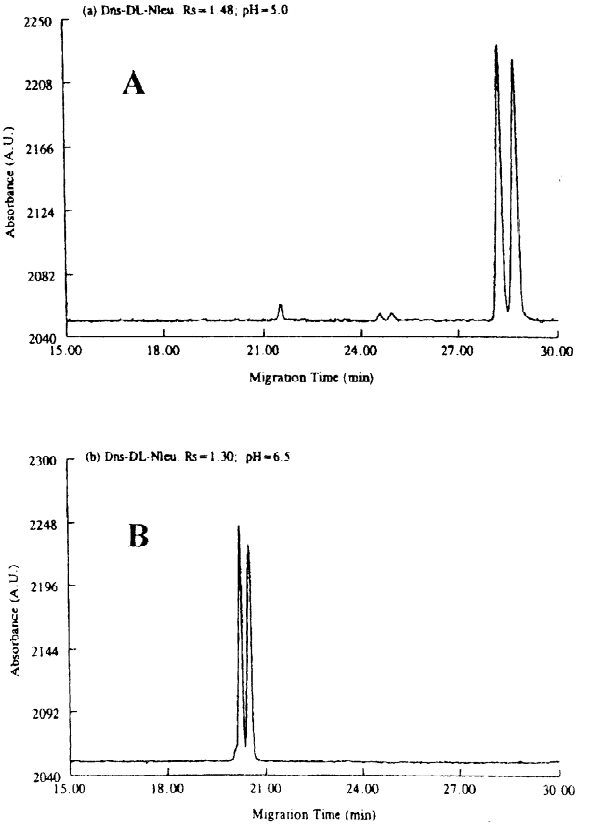

Fig. 1. Electropherograms of Dns-DL-Nle at pH 5.0 (A) and 6.5 (B). Separation buffer contains 25 m*M* Na_2HPO_4, 100 m*M* H_3BO_3, 10 m*M* β-CD, 36 m*M* OTG and 50 m*M* SDS.

between the solute and the anionic micelles which, in turn, would hinder the separation. Conversely, the EOF will be slower and analysis time will be longer when lower pH is selected. This allows the enantiomers to have longer time to distribute between the micellar phase and the aqueous phase which, in turn, would lead to a better resolution. pH 6.5 was selected for subsequent study because it is close to the isoelectric points of all amino acids used in this study except Glu. Therefore, at this pH, all amino acids (except glutamic acid) exist in the zwitterionic form.

3.2. Effect of OTG concentration

3.2.1. Resolution

The effect of OTG concentration on resolutions of all amino acids is shown in Fig. 2. As illustrated, in all cases, as OTG concentration increases, the resolution increases initially, goes through a peak and then decreases. The optimal OTG concentration for the separations of Phe and Trp was found to be 36 m*M*. Met and Nva were optimally resolved at 60 m*M* of OTG, whereas Val, Leu, and Nle need up to 90 m*M* of OTG to achieve maximum resolution. At optimal OTG concentrations, all resolution values are higher than 1.2. Leu, Phe and Leu were baseline resolved. Glu was not resolved at this pH.

In micellar electrokinetic chromatography, the resolution R_s is known to be dependent on t_0/t_{mc}, k_1, k_2 and α, namely, higher resolution is attained when

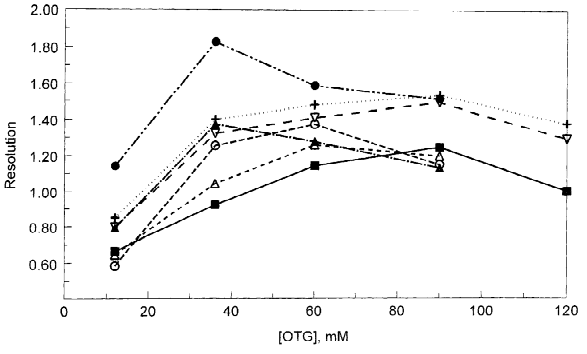

Fig. 2. Plot of resolution as a function of OTG concentration for different Dns-DL-amino acids: Val (■), Nva (△), Met (○), Leu (+), Trp (▲), Phe (●), Nle (▽). Buffer contains 25 m*M* Na_2HPO_4, 100 m*M* H_3BO_3, 10 m*M* β-CD and 50 m*M* SDS.

t_0/t_{mc} and k_1 are small, k_2 and α are large [20]. To investigate the effect of the mixed micelles on the separations, these terms were individually investigated in detail, and results obtained are presented in the following sections.

3.2.2. Retention factor

The effect of OTG concentration on the retention factor (k) was also investigated. It was found that k increased concomitantly with the concentration of OTG (figure not shown). Interestingly, the differences between the retention factors of each pair of enantiomers were also increased with the OTG concentration. The increase in k values could be explained by the following equation:

$$k = KV\left[\frac{[S] - \mathrm{CMC}}{n}\right] \quad (2)$$

where K is the distribution coefficient of a solute between micellar phase and aqueous phase, V is the partial molar volume of the surfactant, $[S]$ is the surfactant concentration, n is the surfactant aggregation number and $([S] - \mathrm{CMC})/n$ is the micellar concentration (CMC is the critical micellar concentration). The increase in the retention factor is obviously attributed to the increased concentration of OTG which resulted in the increase in the micellar concentration. However, because in all cases, they are not exactly straight lines, but rather curves, the results seem to suggest that the increase in k is also caused by the increase in the distribution coefficient. This may be due to the fact that micelles are composed of OTG and SDS. Because OTG has a relatively bulkier head group and shorter tail than SDS, the relative size of the micelles may be different at different OTG/SDS ratio (i.e. as the concentration of OTG increased because SDS concentration was fixed). Accordingly, it is expected that the distribution coefficients of the solutes will be different at different OTG concentrations. Additionally, the chiral binding sites are expected to be increased as the concentration of OTG increases. This will lead to larger differences in the k values of each pair of enantiomers.

3.2.3. Selectivity

As shown in Fig. 3, increasing OTG concentration

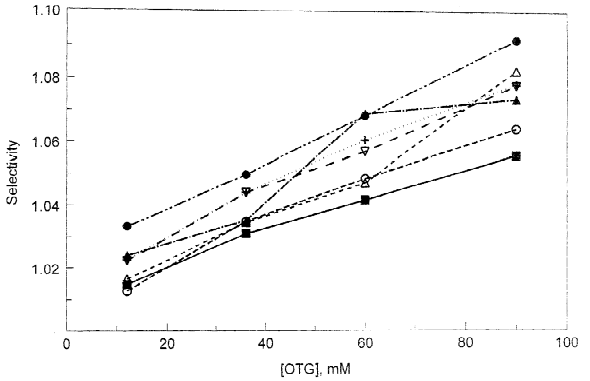

Fig. 3. Plot of selectivity as a function of OTG concentration for different Dns-DL-amino acids: Val (■), Nva (△), Met (○), Leu (+), Trp (▲), Phe (●) and Nle (▽). Buffer contains 25 m*M* Na_2HPO_4, 100 m*M* H_3BO_3, 10 m*M* β-CD and 50 m*M* SDS.

from 12 m*M* to 90 m*M* leads to an increase in the selectivity for all amino acids.

3.2.4. Efficiency

As listed in Table 1, the efficiencies for all amino acids at several different concentrations of OTG are higher than 10^5. The effect of OTG concentration on the efficiency is rather complicated. It was found that depending on the types of amino acids, increasing concentration of OTG may produce several different effects including (i) increasing the efficiency (Phe and Nle); (ii) producing a bell shape relationship (Val, Met and Trp) and (iii) maintaining constant values (Nva and Leu).

3.2.5. Elution range

Because t_0 and t_{mc} values for different amino acids are identical, they were averaged to give the ratio t_{mc}/t_0 or the elution range. The change in the elution range with OTG concentration is listed in Table 2. As illustrated, increasing OTG concentration leads to the decrease in the t_{mc}/t_0 value.

The ratio of t_{mc}/t_0 is important because a wider elution range gives a longer time for the solutes to distribute between pseudostationary phase and aqueous phase. As a consequence, efforts have been made to bring the electrophoretic velocity of the micellar phase close to that of the EOF so that the pseudostationary phase is almost immobilized thereby behaving like the real stationary phase in chromatography. This, in turn, would improve the resolution. However, it should be realized that such improvement is accomplished at the expense of very long analysis time [22].

Table 2 lists averaged values of t_0 and t_{mc} for all amino acids at different concentration of OTG. t_0 increased by only 6%, whereas t_{mc} decreased by more than 100% as the concentration of OTG increased from 12 to 90 m*M*. It seems that there may be slight changes in t_0 and ν_{eo}. The slight change in ν_{eo} might be caused by the slight increase in the viscosity of the buffer when the concentration of

Table 1
Efficiencies of chiral separation of Dns-DL-amino acids at different concentrations of OTG chiral selector

OTG (m*M*)	Val	Nva$\times10^{-4}$	Met$\times10^{-4}$	Leu$\times10^{-4}$	Trp$\times10^{-4}$	Phe$\times10^{-4}$	Nle$\times10^{-4}$
12	9.9	14.1	9.4	21.8	22.8	13.0	24.1
36	12.0	14.3	19.9	20.2	24.4	16.9	26.3
60	17.0	15.0	20.2	20.2	22.5	25.9	25.4
90	15.3	14.7	14.8	20.7	23.3	31.6	37.4

Table 2
Values of t_0, t_{mc}, ν_{eo}, ν_{mc} and average values of t_{mc}/t_0 for chiral separations of Dns-DL-amino acids by CE at different concentrations of OTG

OTG (m*M*)	t_0 (min)	ν_{eo} (mm/min)	t_{mc} (min)	ν_{mc} (mm/min)	Average t_{mc}/t_0
12	7.43	64.62	39.45	12.17	5.38
36	7.45	64.47	28.24	17.00	3.75
60	7.58	63.37	23.80	20.17	3.13
90	7.86	61.07	21.27	22.57	2.69

OTG increased. Also, as the concentration of OTG increased, the migration time for all amino acids became shorter, namely, it decreased by 1–15% when the concentration of OTG is increased from 12 to 90 m*M*.

3.3. Effect of SDS concentration on separations

The plot of the resolution as a function of the concentration of SDS is shown in Fig. 4. Bell-shape curves were obtained for all amino acids. The maximum resolution was obtained at 50 m*M* of SDS for Phe, Leu, Trp and Met. For Nva, the optimum concentration of SDS was 75 m*M*. Nle was resolved optimally at the lowest concentration of SDS, 25 m*M*, while the Val could not be resolved optimally until the concentration of SDS was 100 m*M* or higher.

As stated previously, SDS was selected because it is an anionic surfactant and it forms mixed micelles with OTG. The mixed micelles lag behind EOF, and therefore, function as a ''pseudostationary phase''.

To date, the effect of SDS concentration on chiral separations has usually been neglected in all previous studies. Usually, 20 m*M* was the typical value used in most studies [21]. The present results indicate that 50 m*M* SDS is a relatively better concentration. Furthermore, it was found that the operating current increased from 24 to 41 μA when the concentration of SDS increased from 25 to 100 m*M*. Higher current is not desirable since it leads to lower resolution. The results seem to suggest that for each study, there is a specific SDS concentration at which optimal resolution is achieved. It is, therefore, important that rather than arbitrarily deciding on a fixed concentration, the concentration of SDS should be optimized for each study in order to achieve optimal separation.

3.4. Effect of β-CD on separations

To investigate the effect of β-CD on the separations, the chiral separations were performed under three different conditions: (i) without β-CD, i.e. only 36 m*M* OTG was used as chiral selector; (ii) without OTG but with 10 m*M* of β-CD as chiral selector and (iii) with 10 m*M* of β-CD and 36 m*M* of OTG (36 m*M* of OTG was selected because all the solutes except Val were resolved with R_s values higher than 1.0 at this concentration). All other reagents were kept the same for all three systems, i.e. 50 m*M* of SDS, 25 m*M* of Na_2HPO_4 and 100 m*M* of H_3BO_3. Results obtained for these three systems are shown in Fig. 5. It is evidently clear that the system with both β-CD and OTG provided the best resolution for all amino acids. In all cases, the resolution values were 88.8% to 168.3% higher than those with only β-CD as chiral selector. When only β-CD was employed as the chiral selector, Nva, Met, and Leu were not resolved at all even though Glu was resolved.

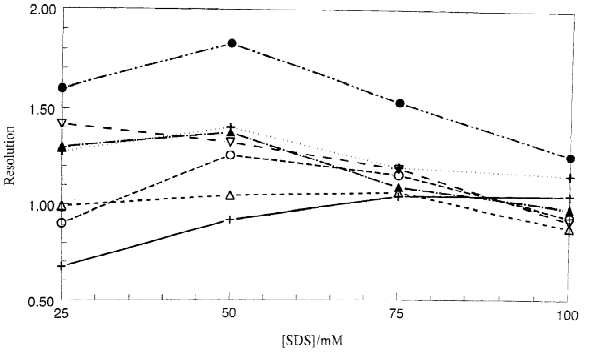

Fig. 4. Plot of resolution as a function of SDS concentration for different Dns-DL-amino acids: Val (+), Nva (△), Met (○), Leu (+), Trp (▲), Phe (●) and Nle (▽). Buffer contains 25 m*M* Na_2HPO_4, 100 m*M* H_3BO_3, 10 m*M* β-CD and 36 m*M* OTG.

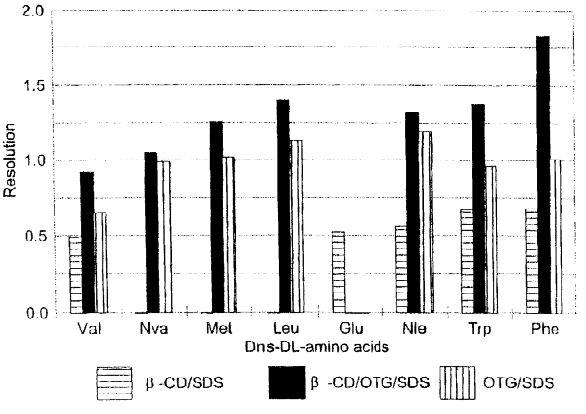

Fig. 5. Resolution of chiral separation of different Dns-DL-amino acids with three different buffer solutions: ▤, 10 m*M* β-CD and 50 m*M* SDS; ■, 10 m*M* β-CD, 50 m*M* SDS and 36 m*M* OTG; ▥, 36 m*M* OTG and 50 m*M* SDS. All three buffers contain 25 m*M* Na_2HPO_4 and 100 m*M* H_3BO_3.

Furthermore, as seen in Fig. 4, the resolutions of the system with both OTG and β-CD were increased by 5.7% to 81.4% compared to those with only OTG as chiral selector. The result suggests that β-CD and OTG micelles have a synergistic effect on the chiral separations.

3.5. Reproducibility

In this study, each CE separation was repeated three times to test the repeatability and also to monitor the possible adsorption of the micelles on the inner wall of the capillary. This is because it was reported that Brij 35 was adsorbed onto the inner wall of the capillary [16]. The result shows that equilibration of the capillary is the key to maintaining good reproducibility, i.e. the capillary must be equilibrated with the separation buffer for half an hour before the new buffer was used. In the experiments, the reproducibility was good as long as the capillary was equilibrated and conditioned sufficiently. Specifically, for all amino acids studied, the RSD of the migration times of the three repeated trials were always less than 1%.

3.6. Separations at optimized conditions

The separation results of all amino acids at the optimized conditions are given in Fig. 6A–H. All amino acids eluted in 20 min. Except for Glu, the enantiomeric resolutions of all amino acids are larger than 1.2. Phe, Trp and Nle were baseline resolved.

There are numerous reports on the chiral separations of Dns-DL-amino acids [23–28]. Although a comprehensive review of all these is beyond the scope of this paper, a comparison of our results and some literature may be useful. Essentially, there are two types of chiral selectors: CDs and micelles. α-, β-CD, and their derivatives have been tried as chiral selectors for chiral separations of Dns-DL-amino acids. However, the results obtained were not so encouraging [23–28]. Recently, a system with 60 m*M* γ-CD–150 m*M* SDS in 10% acetonitrile or methanol buffer was found to provide good chiral separations of Dns-amino acids with resolutions >2.0 [26]. As chiral selector, micelles such as sodium taurocholate and sodium taurodeoxycholate were studied but in both cases, only partial separations of Dns-amino acids were obtained [27]. Glycyrrhizic acid and β-escin were reported to provide good separation for Leu and Val, but the resolutions were poor for other amino acids [28].

4. Conclusions

In summary, it has been demonstrated that 1-*S*-octyl-β-D-thioglucopyranoside (OTG) can be used as an effective chiral selector for the separation of Dns-amino acids by CE. Enantiomeric separations of the amino acids can be accomplished by judiciously adjusting the pH of the solution and the concentration of OTG. For example, it was found that pH 6.5 is suited for the separation of all amino acids except Glu because at this pH, all amino acids exist in the zwitterionic forms. The resolution was found to be dependent on the type of amino acids as well as on OTG concentration. Optimal resolution was found to be at 36 m*M* of OTG for Phe and Trp, 60 m*M* for Met and Nva, and 90 m*M* for Val, Leu and Nle. It is possible to enhance the capacity factors by increasing concentration of OTG; however, in order to achieve better separation it is necessary to use not only the OTG as a sole chiral selector but rather the OTG together with SDS and cyclodextrin (CD). Interestingly, this OTG–SDS–CD system provides enantiomeric separations which are not possible when only individuals or two of these three components are used. Furthermore, the separation was found to be dependent on the concentration of SDS in the three-component system, namely maximum separation was obtained at 50 m*M* of SDS for Phe, Leu, Trp and Met, and 75 m*M* for Nva. Nle can be resolved optimally at 25 m*M* whereas Val cannot be resolved optimally until SDS concentration reaches 100 m*M*. It was also found that the selectivity can be manipulated by changing the ratio of concentration of OTG vs. the concentration of SDS.

Using the OTG–SDS–CD system and operating at optimal conditions for each amino acid, it was possible to enantiomerically resolve all amino acids with baseline separation (with resolution larger than 1.2) and elution time less than 20 min.

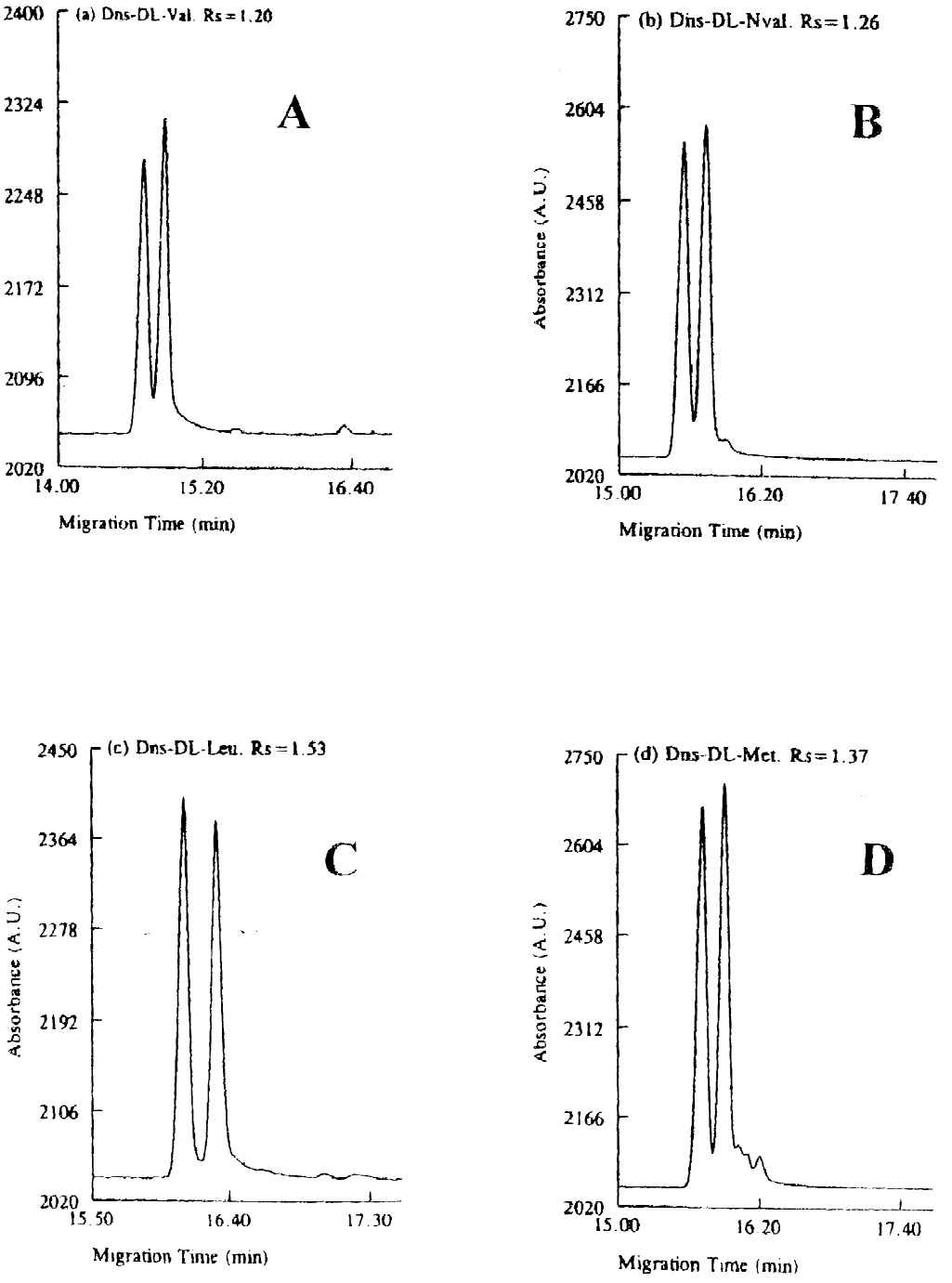

Fig. 6. Separations of Dns-DL-amino acids with optimal buffer solutions: (A) Dns-DL-Val with buffer contains 90 m*M* OTG, 100 m*M* SDS and 10 m*M* β-CD; (B) Dns-DL-Nva with buffer contains 60 m*M* OTG, 75 m*M* SDS and 10 m*M* β-CD; (C) Dns-DL-Leu with buffer contains 90 m*M* OTG, 50 m*M* SDS and 10 m*M* β-CD; (D) Dns-DL-Met with buffer contains 60 m*M* OTG, 50 m*M* SDS and 10 m*M* β-CD; (E) Dns-DL-Trp with buffer contains 36 m*M* OTG, 25 m*M* SDS and 10 m*M* β-CD; (F) Dns-DL-Phe with buffer contains 36 m*M* OTG, 25 m*M* SDS and 10 m*M* β-CD; (G) Dns-DL-Nle with buffer contains 90 m*M* OTG, 25 m*M* SDS and 10 m*M* β-CD; (H) Dns-DL-Glu with buffer contains 60 m*M* OTG, 50 m*M* SDS and 10 m*M* β-CD. All other reagents and conditions were the same.

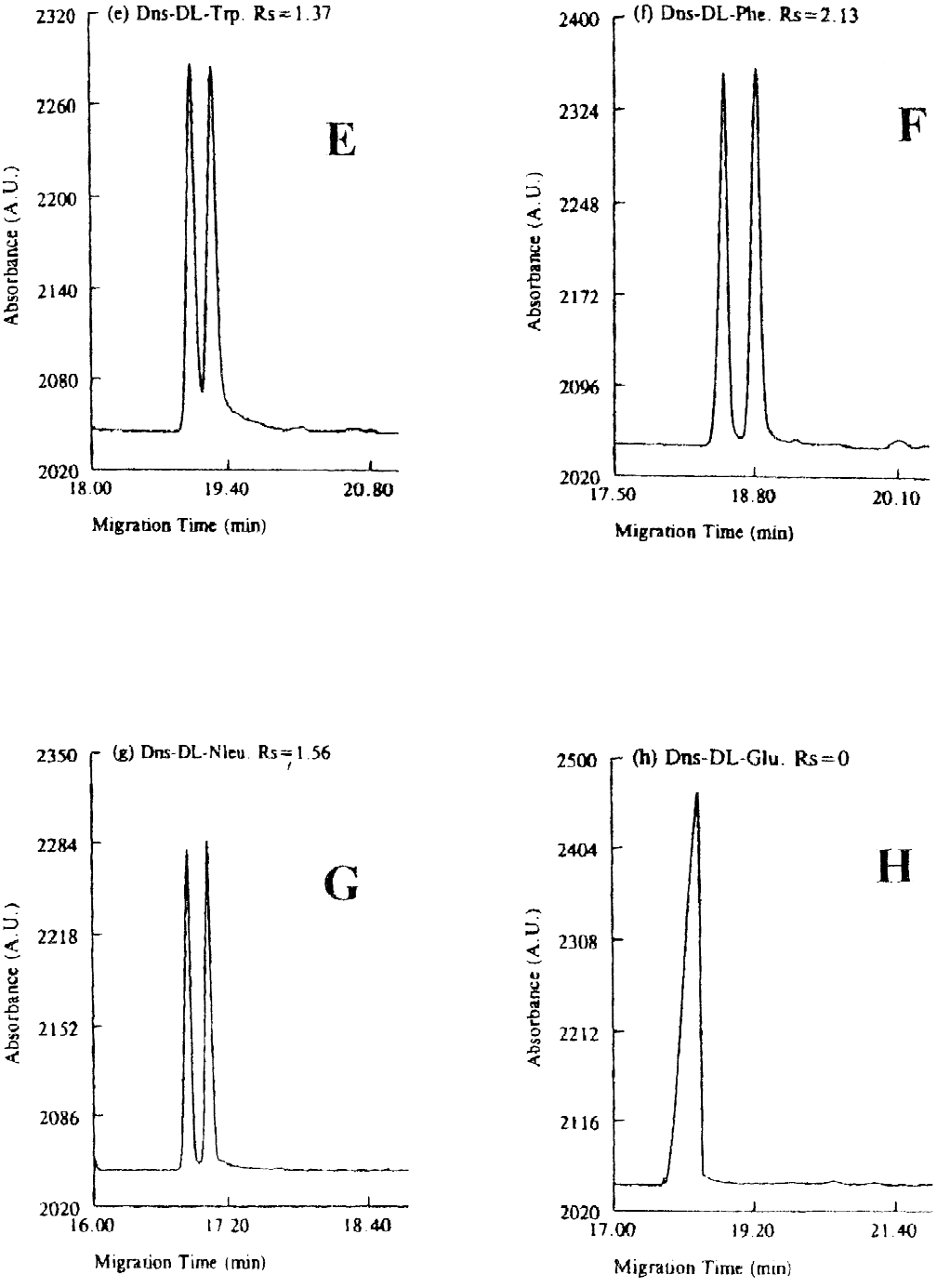

Fig. 6. (*continued*)

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