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Problems and solutions in chiral thin-layer chromatography: a two-phase "Pirkle" modified amino-bonded plate

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ABSTRACT

The preparation of a two-phase "Pirkle" modified amino-bonded thin-layer chromatography (TLC) plate for enantiomer separation is described. The plate was prepared by partially immersing a commercially prepared aminopropyl high-performance TLC plate in a solution of the chiral selector N-(3,5-dinitrobenzoyl)-L-leucine. The portion of the plate modified with the chiral selector was used to separate the enantiomers of the model compounds 2,2,2-trifluoro-(9-anthryl)ethanol and 1,1'-binaphthol. The separated enantiomers were then eluted, using continuous development onto the unmodified portion of the plate. The absence of the chiral selector on this segment of the phase allowed the detection of the separated enantiomers by fluorescence quenching.

INTRODUCTION

The availability of cheap, reliable, robust and efficient methods for the resolution of enantiomers by thin-layer chromatography (TLC) would represent a valuable addition to the high-performance liquid and gas–liquid chromatographic methods currently available. However, at present enantiomer resolution by TLC is still poorly developed. Indeed the only type of separation to be commercialised is that based on ligand exchange [1,2] and whilst these plates give excellent results [3,4] they are limited in the range of compounds for which they can be used. Some success has also been achieved using cyclodextrins, and their derivatives, as mobile phase additives [5,6] or cyclodextrins chemically bonded to silica gel [7]. Ion-pair reagents such as N-benzoxycarbonylglycyl-L-proline and related materials [8,9] and champhor sulphonic acid [9] have also been employed as mobile phases additives in order to effect chiral separations.

The use of "Pirkle"-type stationary phases in TLC represents another obvious method for enantiomer separation, and indeed such plates have been described by several groups [10,13]. A potential disadvantage of this type of phases is the very high

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UV background due to the chiral selector (*e.g.*, dinitrobenzoylphenylglycine or dinitrobenzoylleucine) which limits their use to fluorescent or coloured materials.

As we describe here, this limitation can be overcome by the use of two-phase plates where one portion of the plate is modified with the chiral selector to obtain the separation and the remainder left untreated to enable detection.

EXPERIMENTAL

Test compounds and reagents

2,2,2-Trifluoro-(9-anthryl)ethanol, (*R*)-(+)-1,1′-bi-2-naphthol and (*S*)-(−)-1,1′-bi-2-naphthol were obtained from Aldrich (Gillingham, U.K.). The chiral selector, N-(3,5-dinitrobenzoyl)-L-leucine, was purchased from Sigma (Poole, U.K.) Solvent were of HPLC grade and were obtained from BDH (Poole, U.K.).

TLC plates and treatments

Amino-bonded high-performance (HP) TLC plates (10 × 10 cm, glass backed, E. Merck, Cat. No. 15647) were purchased from BDH. Plates were prepared by immersing the portion of the plate to be modified (5–6 cm) into a 0.05 *M* solution of N-(3,5-dinitrobenzyl)-L-leucine in tetrahydrofuran (THF) for a few seconds. The plate was then immersed in THF to remove any unbound chiral selector and left to dry in a fume cupboard at ambient temperature.

Chromatography

Samples (0.5–20 μg) of the test compounds were applied to the treated plates as solution (~1 mg/ml) in methanol either as spots using 1-μl glass capillaries or as 1-cm streaks using a Camag Linomat IV TLC sample applicator (Camag, Switzerland). The plates were then subjected to continuous chromatography, with the solvent allowed to migrate up to approximately 8 cm before evaporation, using mixtures of 2-propanol–hexane as solvents (see text for details).

Following chromatography compounds present on the plate were detected visually as yellow spots if present on the Pirkle phase or by fluorescence quenching at 254 mm if present on the untreated portion of the plate. Scanning densitometry was performed using a Shimadzu CS 9000 scanning densitometer. For 2,2,2-trifluoro-(9-anthryl)ethanol the wavelengths used for scanning densitometery were 380 and 230 nm for the Pirkle and untreated portions of the plate, respectively. For bi-2-naphthol the wavelengths employed for scanning densitrometry on the Pirkle phase and the untreated portion of the plate were 328 and 230 nm, respectively. These wavelengths were chosen based on *in situ* UV absorbance spectra obtained on the compounds of interest.

RESULTS AND DISCUSSION

In order to demonstrate the principle of these two-phase plates for the separation and subsequent detection of enantiomers the model compounds 2,2,2-trifluoro-(9-anthryl)ethanol and 1,1′-binaphthol were chosen. A particular advantage of these compounds is that they can be visally detected on the Pirkle modified portion of the plate [4,10–13] (albeit at lower sensitivity than on the untreated segment of the

plate). This enabled enantiomer separation to be optimised prior to elution onto the untreated "detection zone". Clearly, with compounds which are not visible on the Pirkle phase method development will be more protracted.

We have used both N-(3,5-dinitrobenzoyl)-*R*-(−)-α-phenylglycine and L-leucine for the preparation of ionically coated Pirkle plates [4,13]. In our hands the L-leucine analogue, when used to modify commercially prepared aminopropyl-bonded TLC plates, gave the best results for the separation of 2,2,2-trifluoro-(9-anthryl) ethanol. In the studies described here Pirkle plates were prepared very simply by dipping aminopropyl-bonded HPTLC plates into a solution of (*R*)-N-(3,5-dinitrobenzoyl)-L-leucine (0.05 *M*) in THF. In Fig. 1A a typical separation of (±)-2,2,2-trifluoro-(9-anthryl)ethanol enantiomers on such a plate is shown. This separation was obtained using *n*-hexane–2-propanol (80:20, v/v) as solvent. The separated enantiomers could be detected visually as yellow spots when present on the Pirkle phase. Scanning densitometry gave the relative proportions of the separated enantiomers to be 47 and 53% for the (−) and (+) forms, respectively.

In Fig. 1B a similar separation of (*R*)-(+)-1,1′-bi-2-naphthol and (*S*)-(−)-1,1′-bi-2-naphthol is illustrated, These substances were also readily detected as yellow spots on the Pirkle phase. This separation was achieved using *n*-hexane-2-propanol (20:80, v/v) as solvent. Once again scanning densitometry allowed the relative proportions of the two components to be determined as 50.2 and 49.8% for the (+) and (−) enantiomers, respectively.

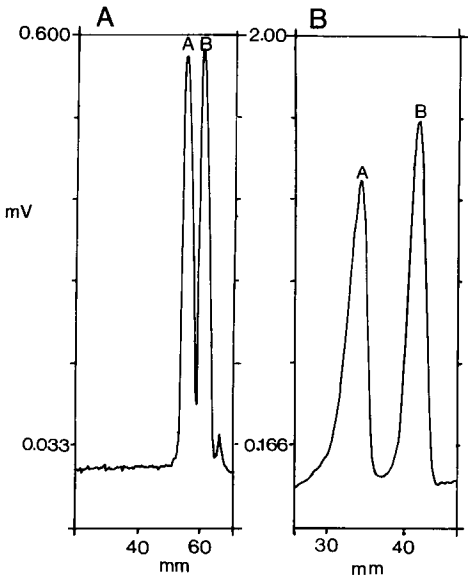

Fig. 1. (A) Separation of the enantiomers of 2,2,2-trifluoro-(9-anthryl)ethanol (peaks A and B) on N-(3,5-dinitrobenzoyl)-L-leucine ionically bonded to aminopropyl-bonded silica gel HPTLC plates using *n*-hexane–2-propanol (80:20, v/v) as solvent. Scanning densitometry was performed at 380 nm. Spots appeared yellow on a pink background. (B) Separation of (*R*)-(+)-1,1′-bi-2-naphthol (peak A) and (*S*)-(−)-1,1′-bi-2-naphthol (peak B) on N-(3,5-dinitrobenzoyl)-L-leucine ionically bonded to aminopropyl-bonded silica gel HPTLC plates using *n*-hexane–2-propanol (20:80, v/v) as solvent. Scanning densitometry was performed at 328 nm. Spots appeared yellow on a pink background.

Having obtained these separations, plates were then prepared with only the lower portion of phase modified with the N-(3,5-dinitrobenzoyl)-L-leucine. The enantiomers of both 2,2,2-trifluoro-1-(9-anthryl)ethanol and 1,1′-bi-2-naphthol were separated on the lower, Pirkle portion of the plate and then eluted into the untreated region using continuous development.

Multiple development techniques were also investigated but were not as effective as continuous development.

The results of these experiments are shown in Fig. 2A and B. These chromatograms show only results for the untreated portion of the plates.

The separated enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol are shown in Fig. 2A. Excellent resolution was obtained, with scanning densitometry giving the relative proportions of the separated components to be 48.5 and 51.4 for the (−) and (+) enantiomers, respectively. As can been seen from the chromatogram the "solvent front" (*i.e.* the point at which the solvent was allowed to evaporate) was marked by the presence of significant UV absorbance. This interference appears to have been due to the leaching out of the chiral selector (or a degradation product) from the Pirkle portion of the plate.

The separation of the binaphthol enantiomers on the Pirkle phase followed by migration onto the untreated detection zone of these two-phases plates is shown in Fig. 2B. Once again excellent separation of the two-enantiomers was achieved. However, in the particular example illustrated in the figure the continuous development

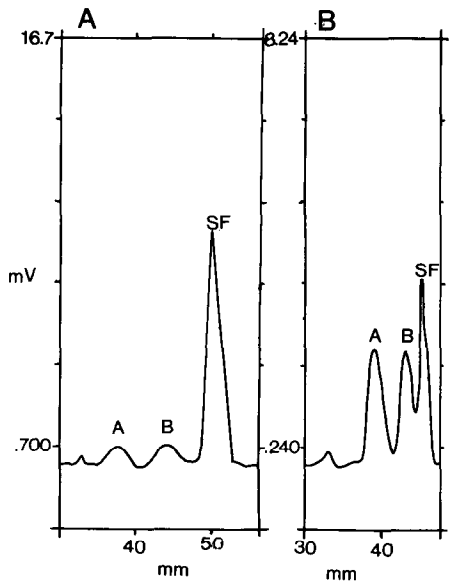

Fig. 2 (A) Separation of the enantiomers of 2,2,2-trifluoro-(9-anthryl)ethanol (peaks A and B) on aminopropyl-bonded HPTLC plates partially coated with N-(3,5-dinitrobenzoyl)-L-leucine (see Fig. 1A for solvents). Scanning densitometry was performed at 230 nm. Only the uncoated portion of the plate is shown. SF-solvent front. (B) Separation of (*R*)-(+)-1,1′-bi-2-naphthol (peak A) and (*S*)-(−)-1,1′-bi-2-naphthol (peak B) on aminopropyl-bonded HPTLC plates partially coated with N-(3,5-dinitrobenzoyl)-L-leucine (see Fig. 1B for solvents). Scanning densitometry was performed at 230 nm. Only the uncoated portion of the plate is shown. SF = solvent front.

was continued for too long with the result that the fastest moving (−) enantiomer had begun to merge with the interfering Pirkle phase-related material running at the solvent front. This example is shown to emphasise the importance of careful optimisation of (a) the relative proportions of the Pirkle and detection zones of the plate and (b) of the length of time employed for continuous development once the enantiomers have been eluted from the Pirkle phase.

Subsequent separations (not shown) with a larger proportion of the plate left unmodified with the selector, giving a larger detection zone, eliminated the problem highlighted by Fig. 2B.

In the configuration described here the lower portion of the plate was modified with the chiral selector and the upper portion left untreated for detection. An alternative configuration, where the separation of the enantiomers requires more of the plate to be modified with the chiral selector, or where the mixture to be separated is more complex, is where only a thin strip (1–3 cm) of the plate is coated with the Pirkle reagent. The enantiomers are separated on this strip of Pirkle phase using the whole length of the plate (with or without continuous development) and the plate is then rotated through 90° and subjected to two-dimensional development to elute the separated components onto the detection zone. Clearly, only one sample can be loaded onto a plate of this configuration.

In order to obtain good results spots rather than streaks must be used reducing the amount of material which can be applied. This type of approach has also been implemented by other workers with some success [14].

CONCLUSIONS

The use of two-phase TLC plates to first separate and then detect enantiomers is a practical and readily implemented solution to the problems posed by the fluorescence quenching of Pirkle chiral selectors. We have found such plates to be robust and reliable, giving good separations of suitable compounds over the range 0.5–20 μg (applied as 1 cm bands). Continuous development gave the best results with this type of plate.

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