Thin Layer Chromatography using Synthetic Polymers Imprinted with Quinine as Chiral Stationary Phases

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Summary

We describe the use of the synthetic polymers imprinted with quinine as chiral stationary phases (CSPs) in thin-layer chromatography (TLC). The stereoselectivity of MIPs obtained was investigated for resolution both of the diastereomers, including quinine/quinidine and cinchonine/ cinchonidine and of the enantiomers, including pseudoephedrine, ephedrine, norephedrine and epinephrine. The polymers exhibited to be good chromatographic stationary phase in TLC using $CaSO_4$ as a binder. We found that during imprinting process the some residual print molecules existed in polymer, giving rise a difference of UV absorption between polymer and all compounds, particularly at 366 nm which this was the advantage for spot detection. Further, MIPs showed the resolving ability with the diastereomer pair of its own print molecule and other stereoisomers structurally related to print molecule.

1 Introduction

Many chiral drugs are currently administered as racemates (50:50 mixtures of two enantiomers). The efficient, accurate and reliable methods are very important for separation of racemates and optical isomers since the stereoisomers display different pharmacological activity and can even cause side effects and toxicity [1]. In general, administering stereochemically pure drug would entail bulk scale processes, such as chromatographic separation [2] or asymmetric synthesis [3,4]. Stereospecific chromatography, including high-performance liquid chromatography (HPLC) [5,6] and thin-layer chromatography (TLC) [7] have been developed using either chiral stationary phases (CSPs) and chiral mobile phase additives. The stationary phases containing cyano, amino, diol, cyclodextrin and derivatized cyclodextrin bonded to silica gel have been successfully employed in chiral TLC [8-10]. The use of unbonded-phases, including molecularly imprinted polymer [11], and cellulose and its derivatives [12] as CSP in TLC have been also reported. With respect to TLC chiral mobile phase additives involve the adding the chiral selector, such as cyclodextrin and its derivatives [11,12] and chiral ligand exchange [13,14] to mobile phase.

Compared to the high level of research into chiral HPLC, the more basic yet potentially very important area of chiral TLC has received little attention. Even though, TLC can be considered to be advantageous technique for the simple, rapid and economical separation of compound mixtures and purity monitoring [7]. The problems of development in chiral TLC include the lack of adhesive

property of CSPs and also many potential CSPs absorb UV light which limit the method of detection. Subsequently, commercially availability of CSPs are limited in range of compounds for which they can be used.

This paper describes the use of TLC chiral stationary phases based on molecularly imprinted polymer (MIP) which have been prepared by using quinine (**Figure 1**) as print molecule. Molecular imprinting is a technique for preparing specific recognition site in polymer [17-19]. Polymerization is allowed and print molecule is subsequently extracted from the polymer matrix, producing recognition sites within polymer network with affinity for the original print molecule. In during imprinting process, the print molecule reversibly binds to functional monomer by covalent or non-covalent interaction and should be completely stripped from rigid polymer. However, in practice, some residual portion of print molecule always incorporates within polymer. The portion remaining with the polymer must be minimized to avoid interference in spot detection on plate. In this work, MIPs were prepared as stationary layer on plate and resolution of the diastereomer pairs and other stereoisomers; enantiomers or racemates, which structurally relate to print molecule was investigated on these CSPs. In addition, the elution strength of mobile phases was adjusted to gain the suitable separation using various concentrations of acetic acid. A non-imprinted polymer was included as control experiment.

2 Experimental

2.1 Materials

(+)-(1S:2S)- and (-)-(1R:2R)-Pseudoephedrine, (±)-norephedrine, (-)-(1R:2S)-norephedrine, (±)-epinephrine, (-)-(R)-epinephrine, cinchonine, cinchonidine, methacrylic acid (MAA), itaconic acid (ITA), and ethylene glycol dimethacrylate (EDMA) were obtained from Aldrich Chemical Co. (USA). 2,2'-Azobis(2-methylpropionitrile) (AIBN) was purchased from Janssen Chemica (Geels, Belgium). (+)-(1R:2S)-Ephedrine, quinine and quinidine were obtained from Sigma (St. Louise, MO, USA). (±)-Ephedrine HCl was obtained from Fluka (Poole, Dorset, UK) and the free base was prepared by neutralizing with 1N NaOH. Anhydrous $CaSO_4$ was supplied from BDH (Poole, Dorset, UK). Other reagents were analytical grade or equivalent. All chemicals were used without further purification.

2.2 Preparation of molecularly imprinted polymers

MIPs were prepared by using a previously described method [20]. Two different monomers; methacrylic acid and itaconic acid were employed as functional monomer such that carboxyl group of the monomer ionically interacts with the amine group in quinine. Quinine (0.5 mmol) was dissolved in tetrahydrofuran. Methacylic acid or itaconic acid (18 mmol), EDMA (0.54 mol) and the initiator AIBN (0.25 mmol) were added. The mixture was then degassed under vacuum in sonicating water bath and sparged with nitrogen for 5 min before polymerization under UV light (366 nm) at 4°C for 24 h. Polymer was ground and sieved through a 100 μm sieve. To remove print

followed by washing with acetonitrile and dried overnight in vacuum. Non-imprinted polymers were prepared in the absence of print molecule using same mean as preparation of imprinted polymer. A scanning electron microscope (JSM 5800 LV) was used to examine the features of polymers prepared.

2.3 Chromatographic procedures

Each polymer and anhydrous $CaSO_4$ were gradually mixed with distilled water and a small amount of ethanol as wetting agent using a pestle and mortar. Polymers were used to prepare TLC plates on glass microscope slide (size 76x26 mm) and dried at least 24 h at room temperature. Control experiment were performed with the plates based on imprinted polymer. The compounds tested were the diastereomers pairs of quinine/quinidine and cinchonine/ cinchonidine, the racemates of ephedrine, norephedrine and epinephrine, and both pure enantiomers of pseudoephedrine. The chromatograms were developed with mobile phases containing various concentrations of acetic acid (0,1,5,10% v/v) in either methanol or acetonitrile. UV light (366 nm) was used for spot detection. All the cases, the spots were visible under 366 nm UV light. Then, the spots were identified by measuring and comparing the R_f values of those with co-spots to obtain separation factor (α), when either diastereomer pair or individual enantiomer was applied separately. In the use of racemate, the separation factor of two separated spots (α) was calculated as the ratio of the higher R_f value and the lower R_f value.

3 RESULTS AND DISCUSSION

The amount of the remained print molecule was determined by FT-IR (Perkin Elmer 1600) difference spectra between imprinted polymer and non-imprinted polymer, resulting no IR bands originating from quinine structure could be detected (not shown). **Figure 2A, B** show the electron micrographs of polymers prepared from itaconic acid in the presence and absence of print molecule, respectively. From these images, it can be seen that the MIP was agglomerates of randomly packed micropheres and between these micropheres comprise numerous voids, and non-imprinted polymer existed smooth surface with no pore incorporated. This confirms that the imprinted polymer was achieved under condition used.

In order to ensure the MIPs with identical properties were used, the MIPs of the same batch were used throughout all experiments. Some residual quinine remained in the polymer binding to methacrylic or itaconic acid was attributed to UV absorption of polymer at 366 nm whereas quinine and other diatereomers absorbed only shortwave UV light (254 nm). Moreover, the enantiomers or racemates tested did not absorb UV light at 366 nm and also had very low sensitivity for UV absorption at 254 nm. Hence, all compounds tested were clearly visualized under UV light at 366 nm. The residual quinine in polymer is therefore proven not to cause any problem in spot detection step as it usually does but turned to be advantage for detection of spots of the substances.

Table 1 shows resolution of various compounds on MIPs imprinted with quinine. In all the cases, increasing concentration of acetic acid in mobile phase increased in R_f values of compounds. MIPs prepared from MAA efficiently resolved quinine/quinidine, giving the R_f value of quinine higher than that of quinidine in mobile phase containing 10% acetic acid in acetonitrile (α = 1.6, **Figure 3a**). Also, these diastereomers were well separated on MIP of ITA using 1% acetic acid in acetonitrile as mobile phase (α = 3.0, **Figure 3a**). These results indicate a stereospecificity of the MIP with its own print molecule. Cinchonine/cinchonidine showed remarkably different R_f values on only MIP of ITA, although some separations of these compounds gave spots with tail. In those separations, cinchonine demonstrated higher affinity than cinchonidine. This is not surprising because chemical structure of cinchonine closely relates to that of quinine (see **Figure 1**). No resolution was observed on the plates containing non-imprinted polymers of either MAA or ITA (data not shown), indicating that recognition sites did not display within polymers.

It can be seen in **Table 1** that the polymer of MAA enable to separate ephedrine recemate into enantiomers rather than other compounds, producing great separation factor for this racemate (α = 2.4, **Figure 3b**) in mobile phase acetonitrile. The enantiomers of ephedrine, epinephrine and pseudoephedrine were poorly resolved on MIP of ITA ($\alpha \approx 1$) but enantiomeric separation of norephedrine racemate was occurred on this CSP, giving the best separation of this compound in mobile phase acetonitrile (α = 2.8, **Figure 3c**). It is noteworthy that the structures of enantiomers are

partly similar to the structure of quinine, particularly in alkyl alcohol side chain on aromatic moiety (see **Figure 1**). This implies that the shape and the functionality of the molecule have important role in the recognition of the MIP, although only some related compounds were resolved on these CSPs. In enantiomeric separations, (-)-enantiomer was always retarded more than (+)-enantiomer, considering that the MIP are selective with enantiomers contian geometry of chiral carbon (hydroxyl substituted carbon) altered from that of print molecule. However, it is difficult to give explanation about the influence of the geometry around chiral carbon of print molecule on chiral separation because, in overall, the structural difference between the print molecule and the enantiomers were considerably large.

4 Conclusions

The results demonstrate the potential of MIP imprinted with quinine as chiral stationary phases in TLC. The TLC resolution of such compounds will be potentially useful in the analytical control of enantiomeric purity. Although the results were determined only qualitative, application of scanning densitometry to the thin-layer chromatograms should provide quantitative information. We believe that further ramification of this work is that the separations obtained may be scaled up to provide a preparative or semi-preparative for isolating gram of pure enantiomers.

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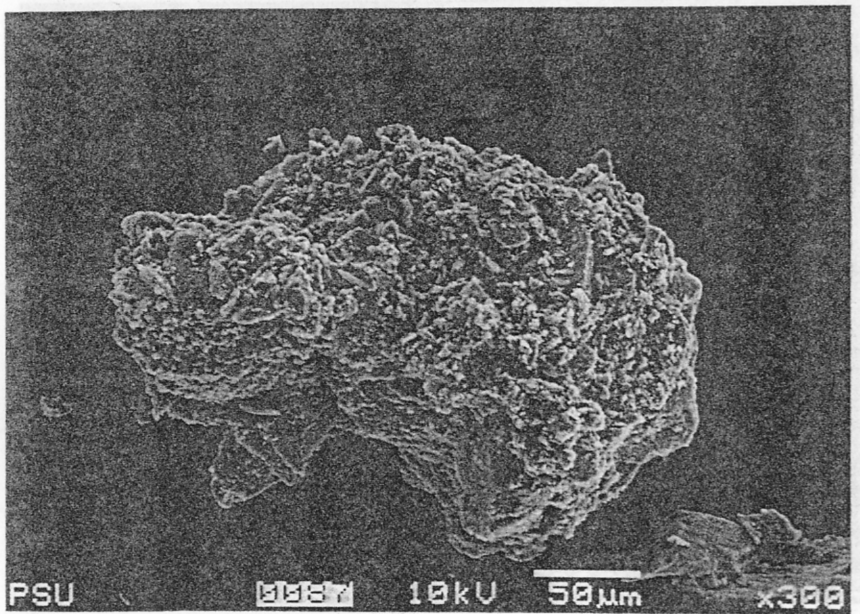
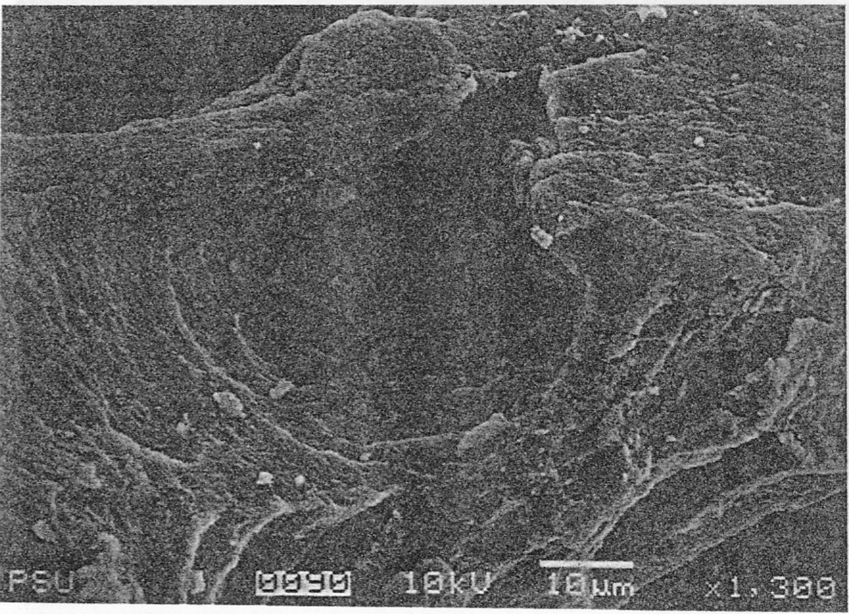
A





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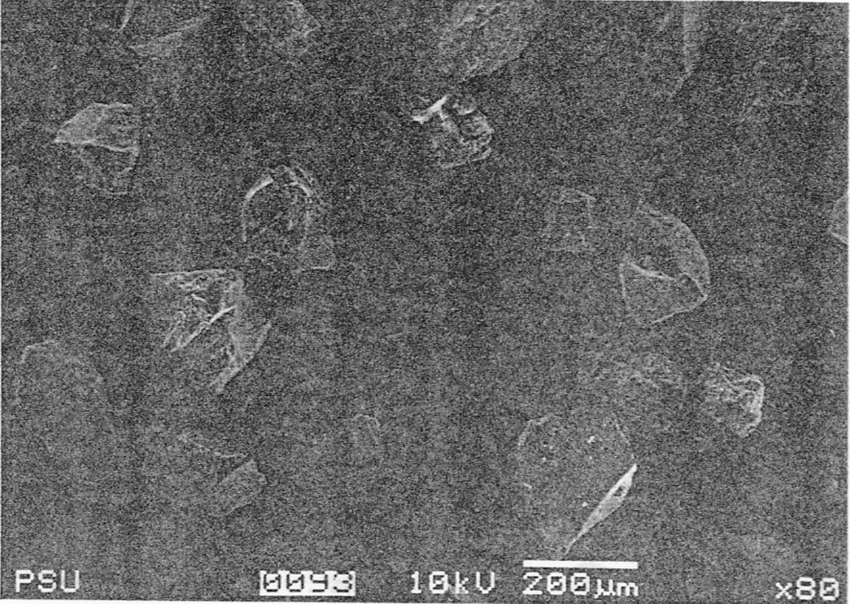
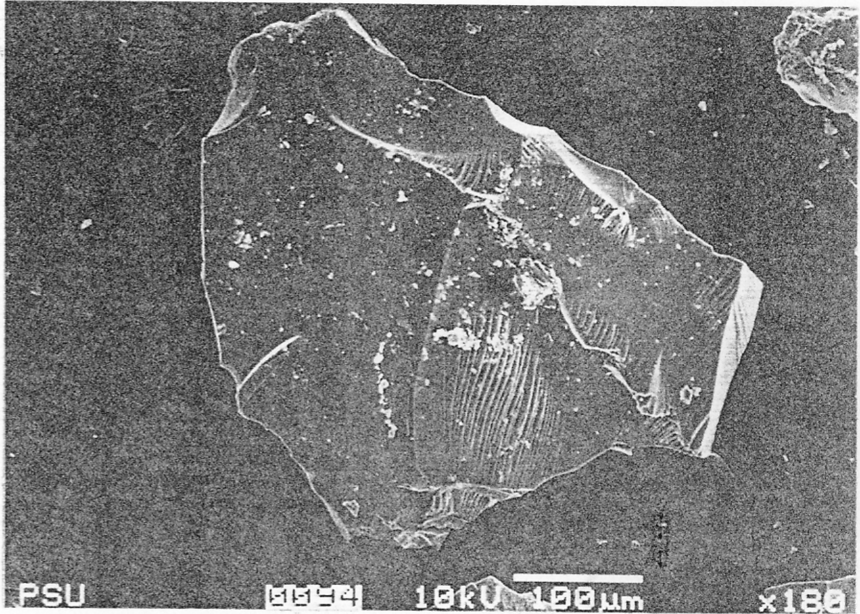
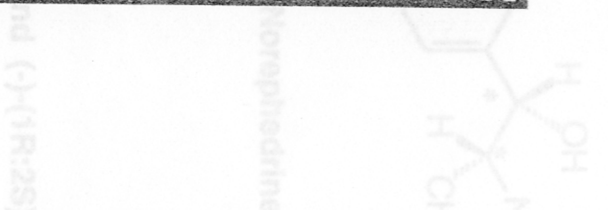
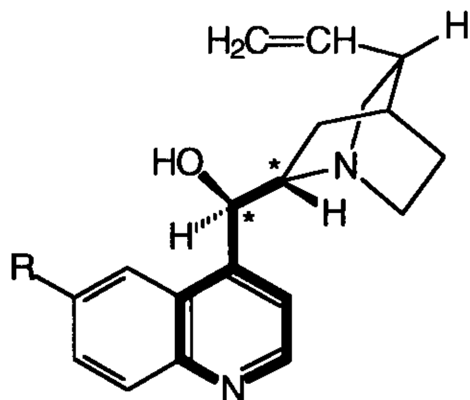


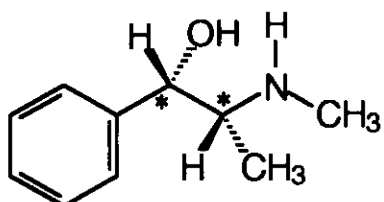


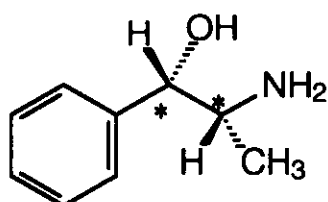




Quinine; R = OCH_3

Cinchonine; R = H





(-)-Ephedrine **(-)-Norephedrine**

Figure 1

Chemical structures of quinine, cinchonine and (-)-(1R:2S)-ephedrine and (-)-(1R:2S)-norephedrine

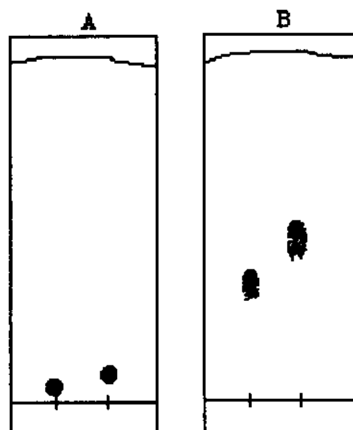


Figure 2a

Thin-layer chromatograms of quinine **(left)** and quinidine **(right)**: **(A)** on MIP of methacrylic acid, mobile phase: 10% acetic acid in acetonitrile; and **(B)** on MIP of itaconic acid, mobile phase: acetonitrile.

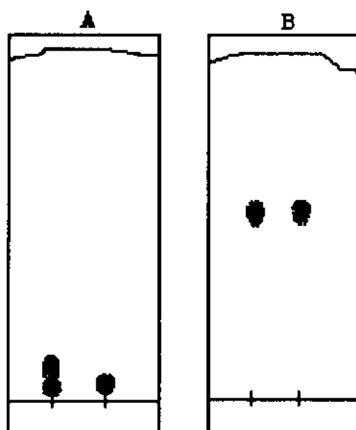


Figure 2b

Thin-layer chromatograms showing resolution of ephedrine racemate on MIP of itaconic acid: **(A)** developed with mobile phase: methanol; and **(B)** developed with mobile phase: acetonitrile. **Left,** (±)-ephedrine and **Right,** (-)-(1R:2S)- ephedrine.

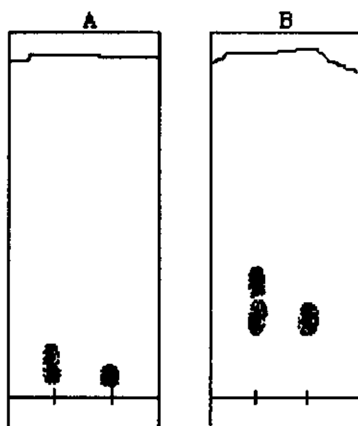


Figure 2c

Thin-layer chromatograms showing resolution of norephedrine racemate on MIP of itaconic acid: **(A)** developed with mobile phase: methanol; and **(B)** developed with mobile phase: acetonitrile. **Left,** (±)-norephedrine and **Right,** (-)-(1R:2S)-norephedrine.

TABLE 1
Compounds separated using MIPs imprinted with quinine.

Compound	Monomer	Acetic acid concentration in mobile phase (%)	Methanol			Acetonitrile		
			$R_f(1)$	$R_f(2)$	α	$R_f(1)$	$R_f(2)$	α
Diastereomers								
1. Quinine/quinidine	MAA	1	0.44	0.50	1.2	0.08	0.08	1.0
		5	0.64	0.68	1.1	0.10	0.10	1.0
		10	0.62	0.73	1.1	0.36	0.56	1.6
	ITA	1	0.08	0.10	1.3	0.02	0.06	3.0
		5	0.46	0.58	1.3	0.10	0.12	1.2
		10	0.52	0.64	1.2	0.14	0.13	1.1
2.cinchonine/cinchonidine	ITA	1	0.10	0.22	1.2	0.04	0.08	2.0
		5	0.24	0.36	1.5	0.14	0.20	1.4
		10	0.36	0.40	1.1	0.18	0.24	1.3
Enantiomers								
Ephedrine	MAA	0	0.06	0.06	1.0	0.05	0.12	2.4
		1	0.56	0.60	1.1	0.36	0.36	1.0
		5	-	-	-	0.62	0.62	1.0
Norephedrine	ITA	0	0.24	0.40	1.7	0.05	0.14	2.8
		1	0.50	0.58	1.1	0.14	0.20	1.4
		5	0.54	0.60	1.1	0.44	0.60	1.4

$R_f(1)$ = R_f value corresponds to quinine, cinchonine and (-)-enantiomer of compounds studied.
R_f (2)= R_f value presents for the another antipodes and relates to $R_f(1)$.