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A Molecularly Imprinted Polymer Stationary Phase for Chiral Separation of Ofloxacin by Thin Layer Chromatography

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A molecular imprinting procedure was adopted to prepare a highly crosslinked polymer for the racemic resolution of ofloxacin, a wide spectrum quinolone anti infective agent. A combination of acidic and basic functional monomers, acrylic acid and 4-vinyl pyridine at different proportions, were copolymerized with ethylene glycol dimethacrylate by thermal initiation. These polymers, after drying, sizing, and removal of print molecule, were used in a batch binding experiment and made into thin layer chromatography (TLC) plates for thin layer chromatographic procedure to examine their enantioselectivity for levofloxacin or S-ofloxacin. The results showed that polymer P33 has comparatively higher binding capacity and selectivity than the other polymers as far as S-ofloxacin is concerned. This was confirmed by CD-ORD results. This preliminary report demonstrates a novel and promising family of stationary phase (based on predetermined selectivity) for use not only for solid phase extraction but also for liquid chromatography and capillary electrophoresis.

Keywords: ofloxacin; levofloxacin; molecular imprinting; thin layer chromatography; chiral separation

INTRODUCTION

Numerous drugs have been produced so far and approximately 25% of these drugs are marketed as racemates or mixtures of diastereoisomers. Such stereoisomers frequently differ in terms of biological activity and pharmacokinetic profiles and the use of these mixtures may lead to the adverse effects of the drug usually associated with the inactive or less active isomer [1]. At present, stricter regulations regarding the use of such drugs have been imposed. For this purpose, excellent chiral resolution techniques are necessary for the analysis as well as separation and purification purposes. One such therapeutic drug is ofloxacin. Ofloxacin, sold as a racemic mixture, is a broadspectrum antibacterial agent that is highly in demand among the middle class group. Studies have shown that the S-ofloxacin is 8-128 times more active than the R and two times more active than the racemate [2].

Several methods have been reported for the enantiomeric resolution of this drug such as capillary electrophoresis using vancomycin [3] and bovine serum albumin [4] as chiral selector, or gamma cyclodextrin-Zn(II)-D-phenylalanine solution as running solution [5]. Enantiomeric resolution was also achieved by high performance liquid chromatography with L-isoleucine as chiral selector [6]. However, their applicability is often limited. This paper describes the preparation of chiral stationary phase tailor-made for the enantioseparation of ofloxacin by means of molecular imprinting.

Molecular imprinting is a technique for preparing synthetic polymers containing specific recognition sites and is particularly useful for enantiomeric separations. In principle the method involves the polymerization of a selected monomer and a crosslinker in the presence of the print molecule. Extraction of the template molecule results in the formation of a molecularly imprinted polymer (MIP) which selectively binds the template molecule [7]. The technique may be used in preparing polymers tailor-made for a specific isomer of a substance and used as chiral stationary phase instead of scanning a wide range of systems to serve the purpose. The order of elution may even be predicted since the imprinted polymer is always retained in the column the longest [8]. Since the first MIP for a pharmaceutical drug, *B*-adrenergic, was first synthesized for its enantiomeric resolution, no MIP was prepared for ofloxacin. For this study it was attempted to prepare a molecularly imprinted polymer which is selective for the more potent isomer, levofloxacin. Based on literature search no MIP, which has affinity for levofloxacin has been prepared.

EXPERIMENTAL

Reagents. Ofloxacin (the racemic mixture) and levofloxacin (the S form) were donated by United Laboratories (Mandaluyong City, Philippines), and were used for polymer preparation and as standard. The crosslinker ethylene glycol dimethacrylate (EDMA), acrylic acid (AA), 4-vinyl pyridine (VP), and the initiator 2,2'-azobisisobutyronitrile (AIBN) were all purchased from Sigma (USA) and used without further purification. Chloroform, methanol, ethanol, acetonitrile, and the other solvents used for polymer preparation, extraction, chromatographic evaluation and other tests were HPLC grade or of analytical grade and were used as received.

Equipment. A UV-Vis 3101PC Spectrophotometer (Shimadzu) was used to determine the amount of ofloxacin and levofloxacin in solutions and in the samples. A Jasco J-715 Spectropolarimeter instrument was used in the CD-ORD analysis of different sample and solutions.

Preparation of the polymers. For this study, thermal polymerization was used (40°C) since the drug is light sensitive. The polymers were prepared using the monomer compositions and solvents indicated in Table 1. Levofloxacin (0.25 mmol) was dissolved in chloroform. The required amount of crosslinker and monomers were added. The initiator (AIBN) dissolved in chloroform (2 mL) was added, and the solution was sonicated for 10 min and then purged with nitrogen for 3 min. The solution was sealed in scintillation vials and left in an oven at 40°C for 24 h. Once polymerized, the bulk polymers were washed with chloroform. The bulk polymers were vacuum dried, crushed and ground using an agate mortar and the particles was dry-sieved through a 38*u* sieve. Blanks were prepared under the same conditions and amounts of reagents but minus the levofloxacin. The polymers were extracted free of levofloxacin using ethanol water (90:10) mixture while removing the fine particles by suspension, sedimentation, and decantation. The washings and extract were assayed for levofloxacin by UV-Vis spectroscopy to determine percentage efficiency of imprinting and percentage levofloxacin released by the extraction process. The MIP was then washed four times with ethanol then twice with acetone then dried while loosening the particles.

Batch-binding experiment. Each of the dry polymers was subjected to a batch-binding experiment. A 0.1 g of the polymer was placed in a vial with 5 mL of 0.025 mM of levofloxacin and it was equilibrated overnight. After incubation, an aliquot of the liquid was assayed for levofloxacin using UV-Vis spectrometer. The difference in the concentrations before and after incubation gives the binding capacity of the polymers. In another experiment, 5 mL of 0.025 mM of ofloxacin was used and the results were compared with the initial results.

Thin layer chromatography. A mixture of the dry polymer (0.1 g) and plaster of Paris (0.1 g, $CaSO_4$ 1/2H_2O) was slurried in a solution of 1.4 mL distilled water and 10 *u*L ethanol and sonicated for 5 min. The slurry was then poured and spread evenly on a clean microslide glass and allowed to dry overnight before use [9]. Blank TLC plates were also prepared

Table 1. Polymer Preparation and Characterization

Mole Ratio Monomers[a]			*Gravimetric Yield (%)*	*Efficiency of Printing*		*Release*	
Polymer	**AA**	**4VP**		**%**	**mg/g Dry Polymer**	**%**	**mg/g Dry Polymer**
P31	1.0	0	102.9	97.7	90.1	78.9	58.8
P32	0.75	0.25	99.9	98.3	90.6	61.1	46.8
P33	0.50	0.50	100.8	98.4	90.7	62.2	46.8
P34	0.25	0.75	99.6	96.0	88.5	66.7	49.2
P35	0	1.0	98.9	96.4	88.9	60.0	44.5

[a] amount of monomers in mmole
See experimental section for details on the preparation of polymer.
Levofloxacin: 0.25 mmole or 92.2 mg
EGDMA: 5 mmoles or 1.0112 g
AIBN: 0.4 mmole or 55.8 mg
$CHCl_3$: 10 mL

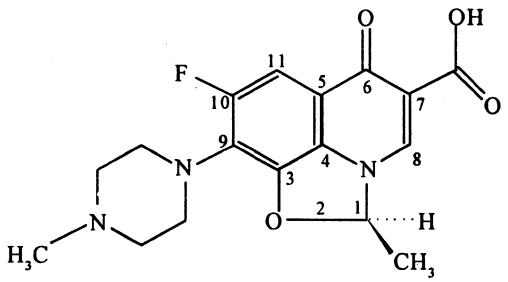

Fig. 1. Molecular structure of ofloxacin.

using non-imprinted polymer. A small spot of a solution of levofloxacin in acetonitrile (0.2 mg/mL) and another spot for ofloxacin were made on prepared TLC plates. The development, which took approximately 5 to 10 min, was done using a mobile phase consisting of methanol, acetonitrile, 10% acetic acid (7:2:1). Visual detection of the substance on the plate was done using UV light (366 nm and 254 nm). The Rf values, the ratio of the distance migrated by the sample to the distance migrated by solvent were calculated. Some of the spots were scraped where separations were seen, suspended in 90% ethanol, filtered and then subjected to CD-ORD analysis.

RESULTS AND DISCUSSION

A molecular imprinting procedure was adopted to prepare highly crosslinked polymers for the racemic resolution of ofloxacin (Fig. 1), a synthetic quinolone anti infective agent. The procedure involves noncovalent interaction of the S-ofloxacin, simultaneously, with two chemically distinct functional monomers, namely the weakly basic 4-vinyl pyridine (4VP) and the acidic and hydrogen bonding acrylic acid (AA) which were copolymerized with ethyleneglycol dimethacrylate (EGDMA). Five MIP's were prepared which vary in the relative proportions of AA to 4VP while keeping the mole ratio of template to monomer/s constant (1:4). These polymers, after drying, sizing, and removal of print molecule, were used in a batch binding experiment and thin layer chromatographic procedure to examine their enantio selectivity for levofloxacin. All the prepared bulk polymers were translucent yellow solid except for polymer P33 which is somewhat greenish.

After washing the bulk with CH_3Cl, the percentage efficiency of imprinting was determined by UV-Vis. The computed percentage efficiency of imprinting ranges from 96.0 to 98.4% or 88.5–90.7 mg ofloxacin was imprinted per gram of polymer formed. After drying under vacuum the bulk polymer cracked and turned opaque, probably due to shrinkage upon porogen evaporation. The gravimetric yield obtained was high (99–103%). Detailed result is shown in Table 1.

Table 2. Result of batch binding experiment.

Polymer	*Binding Capacity* (mg/g Dry Polymer)	
	Levofloxacin	**Ofloxacin**
P31	36.2	44.1
P32	41.3	45.4
P33	46.6	37.6
P34	45.8	47.3
P35	43.6	44.0

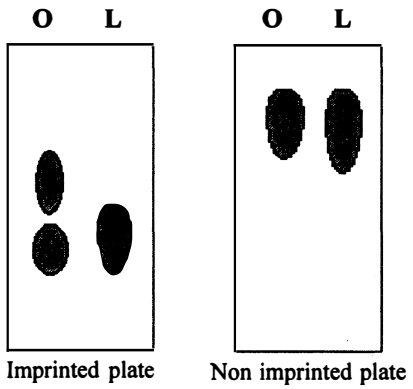

Fig. 2. TLC plate for P33.

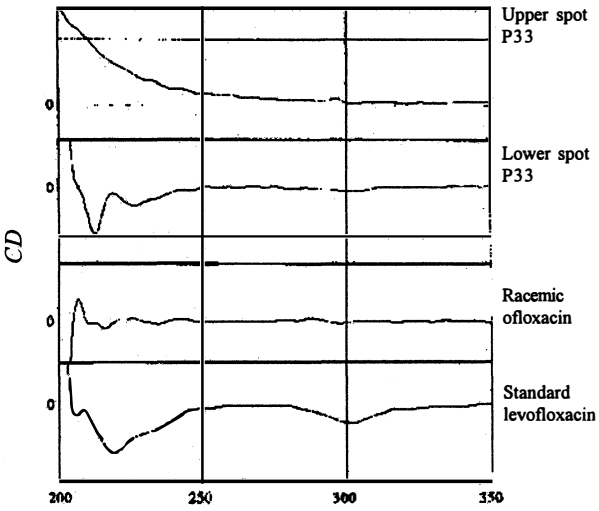

Fig. 3: CD-ORD spectra for levofloxacin and ofloxacin compared with that obtained from upper and lower spots of P33 TLC plate. Conditions: Range- 350 to 200 nm; Bandwidth – 2.0 nm; Sensitivity – 100 mdeg; Resolution – 0.5 nm; Response – 1 sec; Speed -100 nm/min; Accumulation- 1.

The recovery of template after repeated extraction of a known weight of the ground and sized polymer was determined by UV-Vis spectrometry. % Recovery was rather low at a range of 60–79%. Based on the results, the site available for rebinding in mg levofloxacin/g polymer was computed to be equal to 44.5–58.8 mg/g polymer (See Table 1).

For the batch binding experiment, the result showed that polymers P33 and P34 have comparatively higher binding capacity for S-ofloxacin than the other three polymers (Table 2). But a marked decreased in this binding capacity was observed for polymer P33 when equilibrated with the solution of the racemic drug. This may well indicate selectivity of this polymer for levofloxacin.

The result of this batch binding experiment was confirmed by thin layer chromatographic study in which only polymer P33 showed separation of the racemic mixture on TLC plate with a separation factor equal to 2.5 (see Fig. 2). Thin layer chromatography plates were made from levofloxacin imprinted polymer and non imprinted polymer. S-ofloxacin was much more retained on the imprinted plate compared to the non imprinted plate due to the imprinting effect. CD-ORD spectra obtained further confirmed enrichment of S-ofloxacin using P33 imprinted TLC plate as shown in Fig. 3.

This report demonstrates a novel and promising family of stationary phase (based on predetermined selectivity) for use not only for thin layer chromatography but also for liquid chromatography, capillary electrophoresis, and solid phase extraction.

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