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A Novel Polymer-Based Monolithic Capillary with Sulphated B-Cyclodextrin Chiral for the Enantioselective Pharmaceutical Analysis by Nano-HPLC

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ABSTRACT

Here we report the first encapsulation of sulphated β-cyclodextrin in organic polymer monolith in capillary columns. The columns were prepared, characterized and investigated for the enantioselective nano-liquid chromatographic separation of 37 racemic pharmaceutical drugs, namely, α- and β-blockers, catecholamines, sedative hypnotics, anti-inflammatory drugs, antifungal drugs, norepinephrine-dopamine reuptake inhibitors, antihistaminics, anticancer drugs, antiarrhythmic drugs and miscellaneous drugs. Acceptable resolution was achieved for some drugs by using reversed phase chromatographic conditions while no separation observed by using normal phase conditions. The developed columns get more economical analysis under environmentally benign conditions.

Keywords: Sulphated β-cyclodextrin, Monolith, Organic polymer, Enantioseparation, Encapsulation, Capillary.

INTRODUCTION

The enantioselective separation of race mates by chromatography has been well reported [1,2]. the chiral resolution is at the upfront of other methods used to access pure enantiomers due to its unique features [3,4]. HPLC is the most widely technique used to access pure enantiomers. The enantioselective HPLC separations are done via direct resolution with a chiral stationary phase (CSP) attached, bound, adsorbed or immobilized to an appropriate support to make a CSP [5-7].

Because of their porous properties, monolith represents a good alternative to particle packed columns for both CEC and HPLC analysis [8-10]. Due to its diverse properties, monoliths can be used in both "conventional" HPLC columns and nano-HPLC capillaries [11]. One of the main classes of monoliths is Organic polymer-based monoliths prepared by an *in-situ* polymerization of monomer and cross-linker in a porogenic solvent, in presence of an initiator [12-14].

Natural carbohydrates play a very important role in chiral separation as useful CSPs. In general, the developments of chemically post-modified polysaccharides and oligosaccharides are the mainstream trend in the chiral stationary phases. Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven or eight D-glucopyranose units connected via α-1,4- linkage to give torch-like shape provides three points of interaction required for the chiral recognition complex formation [15]. The most known CDs are α-CD (cyclohexamylose), β-CD (cycloheptamylose) and γ-CD (cyclooctamylose) with different internal diameters.

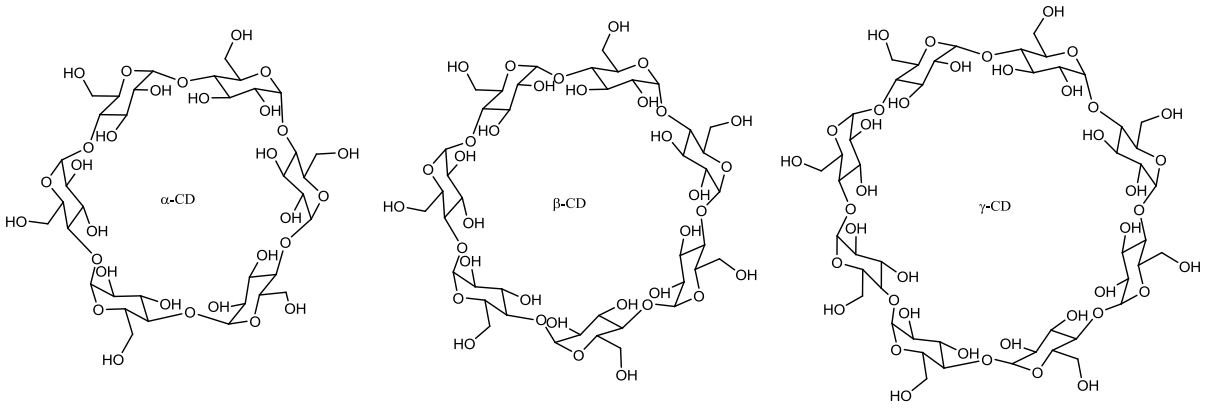

Figure 1: Schematic diagram showing the structures of the most widely characterized CDs.

The inner-side of the CD is relatively hydrophobic while the outside of the CD is hydrophilic, this enables CDs to form inclusion complexes with a variety of molecules 2, therefore, CDs have been widely used for the chiral separation of racemic compounds [16,17]. Derivatization of CD was performed to improve CD enantio-recognition properties, Derivatizing groups such as

phenylcarbamates produce CSPs with different enantiorecongnition abilities based on the nature and positioning of the groups on the phenyl ring [18,19].

Monolithic β-CD CSPs has previously been prepared via physical adsorption or chemical bonding [20-24]. Bonding of β-CD into the monoliths has been previously achieved via the *in situ* copolymerization of a β-CD functional monomeric precursors [25] which is simple one pot and less time consuming procedure than the post-modification of reactive monolithic groups [26-29]. The one pot strategy can be considered a potential alternative to simplify column preparation and improve reproducibility [30]. Few reports have achieved better enantioselectivities of the one pot procedure compared to the post-modification approach [28,30-32].

Sulfated β-CD (S-β-CD) represents a class of anionic derivatives of CDs, and has been used extensively in capillary electrophoresis [33] sulfated beta-cyclodextrin (S-beta-CD) was used before as a chiral selector in mobile phases with conventional reversed-phase ODS columns [34-36]. It also was used as attached chiral stationary phase (CSP) for chiral HPLC. The novel CSP was used to separate a number of enantiomers, including antidepressants, phenylhydantoins and antihistamines, using HPLC under a wide range of mobile phase conditions [37]. There are very few examples of the use of S-β-CD as an additive in chiral mobile phases for the enantiomeric separation of some selected chiral drugs [33,38,39].

In this study, Sulfated β-CD (S-β-CD) was used as a chiral selector for preparation of monolithic capillaries for nano-HPLC. Sulphated β-CD-based monolithic CSPs were prepared via encapsulation in organic polymer monolithic capillary for the enatioselective nano-liquid chromatographic separation of a set of racemic pharmaceuticals.

EXPERIMENTAL PROCEDURE

Reagents and materials

Sulphated β-CD (99%), Ethylene glycol dimethacrylate (EGDMA, 98%), glycidyl methacrylate (GMA, 98%), 1-propanol (99%), 1,4-butanediol (99%), trifluoroacetic acid (TFA, ≥ 99.5%), sodium hydroxide and hydrochloric acid were purchased from Aldrich (Milwaukee, WI, USA). Ethanol (HPLC grade) and acetone (AR grade) and were purchased from BDH (Kilsyth, Vic., Australia). Methanol (HPLC grade) was purchased from Scharlau (Sentmenat, Spain). All other reagents were of the highest available grade and used as received. The fused-silica capillaries (150 μm internal diameter and 365 μm outer diameter) were purchased from Polymicro Technologies (Phoenix, AZ, USA). 2, 2-Azobis (isobutyronitrile) (AIBN) was obtained from Wako

(Osaka, Japan). The racemic drugs were mostly purchased from Sigma Aldrich. Purified water used for experiments was purified by Nano-pure Infinity water system (NJ, USA).

Preparation and characterization of the monolithic columns

Activation of the fused silica capillaries

To get the polymer monolith into the fused silica capillary wall (150 µm ID), surface activation for the capillary wall was done by our procedure [29]. Briefly, the fused silica capillaries were washed using a Harvard syringe pump (Harvard Apparatus, Holliston, MA, USA) and a 250 µL gas-tight syringe (Hamilton Company, Reno, NE, USA) with acetone and water 2-3 times for each, activated with 0.2 mol/L sodium hydroxide (NaOH) for 6 h confirming the absence of any air bubbles, washed with water 3-4 times till neutral (pH:7), then washed with 0.2 mol/L hydrochloride (HCl) for 12 h, rinsed with water and ethanol 2-3 times for each. A 20% (w/w) solution of 3-(trimethoxysilyl) propyl methacrylate in 95% ethanol adjusted to pH 5 using acetic acid was pumped through the capillary at a flow rate of 0.25 µL/min for 6 h. The capillary was then washed with acetone one time and dried with a stream of nitrogen for 2 min., then left at room temperature for 24 h (Figure 2).

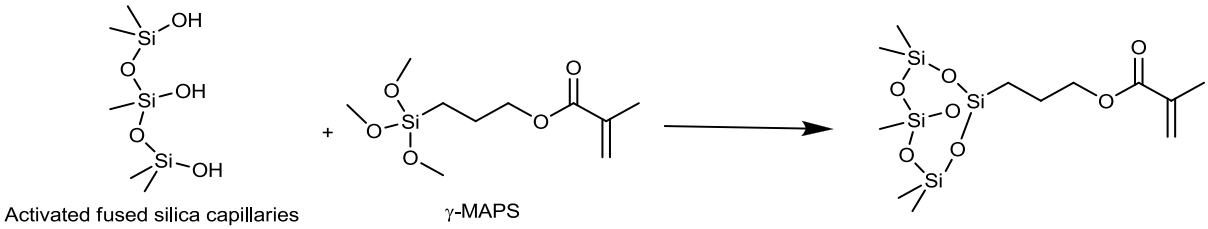

Figure 2: Vinylization of the inner capillary wall to enable polymer anchor, reproduced with permission from reference.

Preparation of Sulphated β-CD functionalized monomer

The short (~25 cm in length) surface modified capillary was filled by Harvard syringe pump with the degassed polymerization mixture at 0.25 µL/min using the syringe pump. Sulfated β-CD polymer-based monolithic capillaries S1 and S2 were prepared via *in situ* copolymerization of binary monomer mixtures consisted of GMA as a monomer and EGDMA as across linker along with different porogens namely; 1-propanol, 1,4-butanediol, ethanol and/or dodecanol in the presence of 1 wt% AIBN (with respect to monomers) and Sulfated β-CD as chiral selector. The filled capillaries were then sealed with a septum, placed in 70°C water bath for 18-20 h for the polymerization process to take place. The unreacted monomers were removed from the monolithic columns by pumping methanol at a flow rate of µL/min for 6 h before being investigated under light microscope to ensure its consistency and homogeneity of the polymerization mixture inside the capillary. The filled capillaries were conditioned with

mobile phase for 12 h at 1 µL/min. The ratios of the monomers to the porogens were kept 40% and 60%, respectively. The ratios of the porogens were fixed as 48% 1-propanol, 6% 1,4-butanediol and 6% chiral selector for S1 and48% 1-propanol, 6% ethanol and 6% chiral selector, all percentages are w/w. this mixture of porogens gave the best homogeneous polymerization mixture than other porogens tested.

Scanning electron microscopy (SEM) of the prepared monolithic capillaries

Scanning electron microscopy (SEM) was done to evaluate the morphological features of the prepared capillaries. The capillaries were cut into ~1 cm sections and put perpendicularly on 12.7 mm pin-type aluminum stub using double face epoxy resin tape. SEM was carried out and high resolution images were collected by sputter coating the capillaries sections with gold (Figure 3).

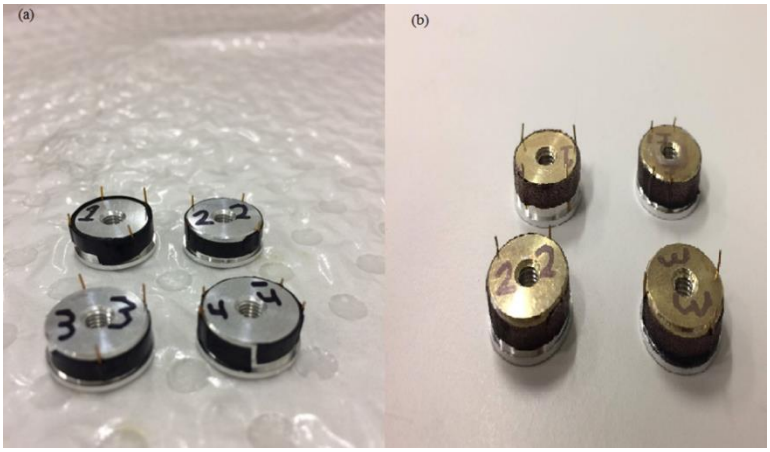

Figure 3: The capillary sections for SEM before (a) and after (b) staining with gold.

Preparation of standard solutions and samples

Stock solutions of 37 racemic drugs at concentrations of 1 mg/ml in filtered HPLC grade methanol were prepared. The prepared stock solutions were further diluted 10x and filtered through Sartorius Minisart RC 15 0.2 µm pore size filters (Goettingen, Germany) Prior to injection. The tested compounds include: β-blockers: alprenolol **1**, metoprolol **2**, propranolol **3**, acebutolol **4**, atenolol **5**, α-Blockers: naftopidil **6**; anti-inflammatory drugs: ibuprofen **7**, naproxen **8**, flurbiprofen **9**, indoprofen **10**, cizolirtine **11**, cizolirtine citrate **12**, carprofen **13**, glafenine **14**; antifungal drugs: hexaconazole **15**, miconazole **16**, diniconazole **17**, sulconazole **18**; norepinephrine-dopamine reuptake inhibitor: nomifensine **19**; catecholamines: arterenol **20**, normetanephrine **21;** sedative hypnotics: aminoglutethimide **22**; anti-histaminics: chlorpheneramine **23**; anticancer drugs: ifosfamide **24**; antiarrhythmic drugs: tocainide **25**, propafenone **26**; flavonoids: Flavanone **27**; amino acids: glutamic acid monohydrate **28**,

tyrosine **29**, phenylalanine **30**; anti-platelet agents: Clopidogrel **31**; immunomodulatory drugs: thalidomide **32**. Miscellaneous: 1-acenaphthenol **33**, o-methoxy mandelic acid **34**, 4-hydroxy-3-methoxymandelic acid **35**, 1-indanol **36** and ampicillin **37**. The chemical structures of tested drugs were illustrated in Figure 4.

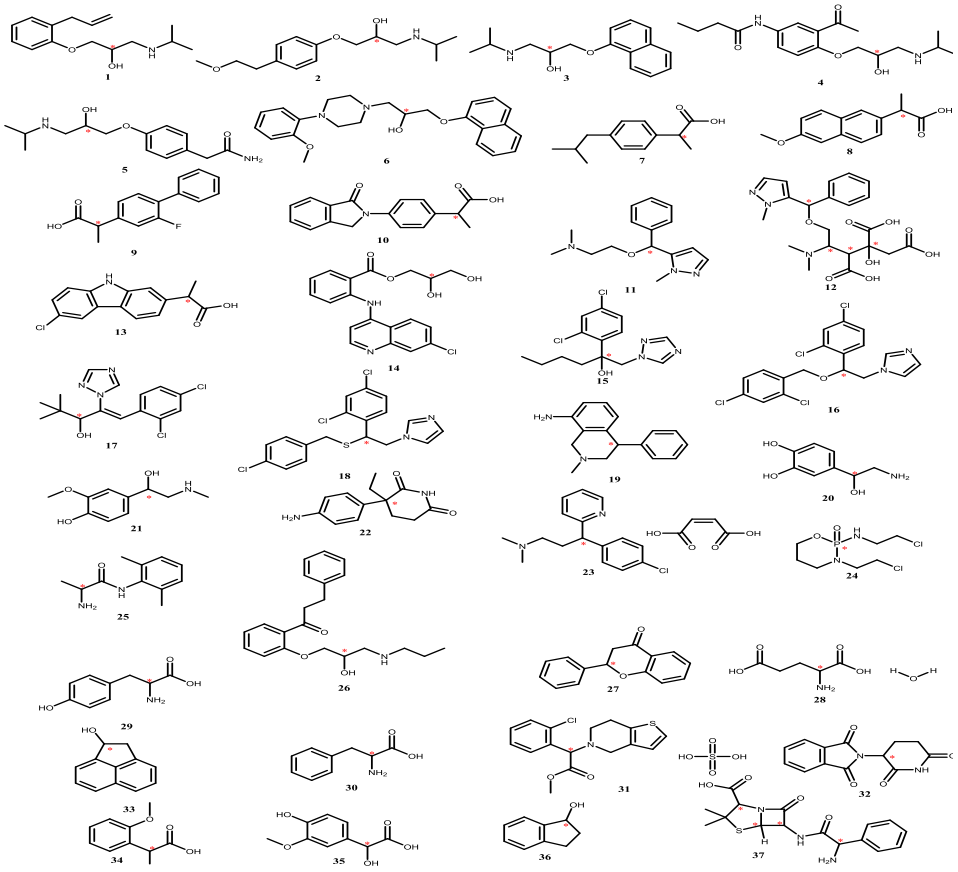

Figure 4: Chemical structures of the investigated racemates.

HPLC conditions

The mobile phase consisted of water/methanol (v/v) and acetonitrile/water (v/v) for the reversed phase HPLC and n-hexane/2-propanol for normal phase HPLC. For all samples, the injected volume was 1 µL. Preliminary UV analyses were performed at wavelength 219 nm.

RESULTS AND DISCUSSION

Polymer monoliths: Preparation and characterization

Preparation of sulfated β-CD- based polymer monoliths

Monolithic β-CD CSPs has previously been prepared via physical adsorption or chemical bonding [20-24,37] sulfated beta-cyclodextrin (S-beta-CD) was used before as an additive in mobile phases with conventional reversed-phase ODS columns [34-36]. It also was used as chiral stationary phase (CSP) for HPLC. However, sulfated beta-cyclodextrin (S-beta-CD) was never used as CSPs in organic polymer monolithic columns. This is may be due to the poor miscibility of sulfated beta-cyclodextrin (S-beta-CD) with the porogenic solvents used in monoliths' preparation. In an attempt to overcome this problem, the miscibility of sulfated beta-cyclodextrin (S-beta-CD) was tested in the porogenic solvents used in monoliths preparation namely 1,4-butandiaol, dodecanol, ethanol and 1-propanol. When homogeneous polymerization mixtures were obtained by using 1,4-butandiaol (**S1**) or ethanol (**S2**) with 1-propanol were used as porogenic solvents in the polymerization mixture.

Two polymer monoliths (**S1** and **S2**) were prepared via *in situ* copolymerization of GMA as a functional monomer and EDMA as a cross linker in the presence of a ternary porogenic system comprised of 1-propanol (48% w/w), 1,4-butanediol (6% w/w) (**S1**) or ethanol (**S2**) and Sulfated β-CD (S-β-CD) (6% w/w) (Figure 5). Blank column was prepared using the same polymerization mixture without chiral selector in order to compare the results with the prepared columns.

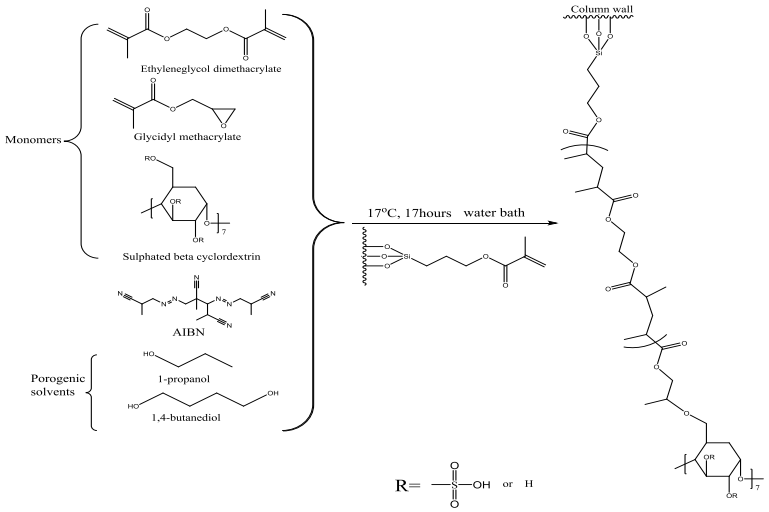

Figure 5: Schematic diagram showing the preparation of β-CD functionalized polymer monolith (S1)

Evaluation of column performance

The column total porosity (ε_T) and efficiency (in terms of separation and resolution factors) were performed to evaluate the quality of the prepared monolithic capillaries. It is reported that ε_T offers only partial characterization for the monolith while better characterization is determined by the average pores size [40]. ε_T was determined using the capillary HPLC flow method where the void volume of unretained uracil was measured using methanol as mobile phase [25]. The eluted volume of methanol was collected in a sealed 1.5 ml vial to avoid errors due to mobile phase evaporation and weighed out in a given time at 1 µL/min flow rate. The weight was then converted to volume using methanol density and the total porosity was calculated using the following equation:

$$\varepsilon_T = V/\pi r^2 u \times 100$$

Where ε_T is the total porosity, V (m^3/sec) is the mobile phase volume, r (m) is the inner radius of the empty capillary and u (m/sec) is the linear velocity of the mobile phase which is determined by the unretained uracil. The linear velocity is calculated by dividing the effective length of the column by the retention time of uracil. Measured ε_T values for the columns **S1** and **S2** are listed in Table 1.

Table 1: Measured εT values for the columns S1and S2.

Column	ε_T (%)
S1	28.9 ± 0.8
S2	20.7 ± 0.67

As illustrated in Table 1, it was observed that ε_T lower than the expected value to be equal or close to the porogens volume fraction (60%), however, the polymer monoliths porosity is strongly dependent on the polymerization conditions i.e., polymer composition, polymerization time and temperature [41]. Moreover, the polarity and size of the functional monomer, as well as its concentration in the polymerization, affect the early stages of phase separation where the formation of the early nuclei occurs and consequently have a major effect on the formed monolith [42]. It was observed that ε_T value for **S1** is higher than **S2**, this confirm that 1,4-butandiol was better to be used as porogenic solvent than ethanol.

Elemental analysis was performed to confirm the presence of chiral selector inside the prepared monolithic capillaries. Elemental analysis for a blank column (G column) was also conducted (Table 2).

Table 2: Measured nitrogen content in different columns.

Column	Sulphur (% w/w)
G	0
S1	0.4

Scanning Electron Microscopy (SEM)

SEM was used to study the effect of chiral selector on the morphology of the prepared monoliths. Columns **S1** and **S2** showed homogenous porous structure with interconnecting channels allowing the flow of the mobile phase under low column backpressure (Figures 6 and 7).

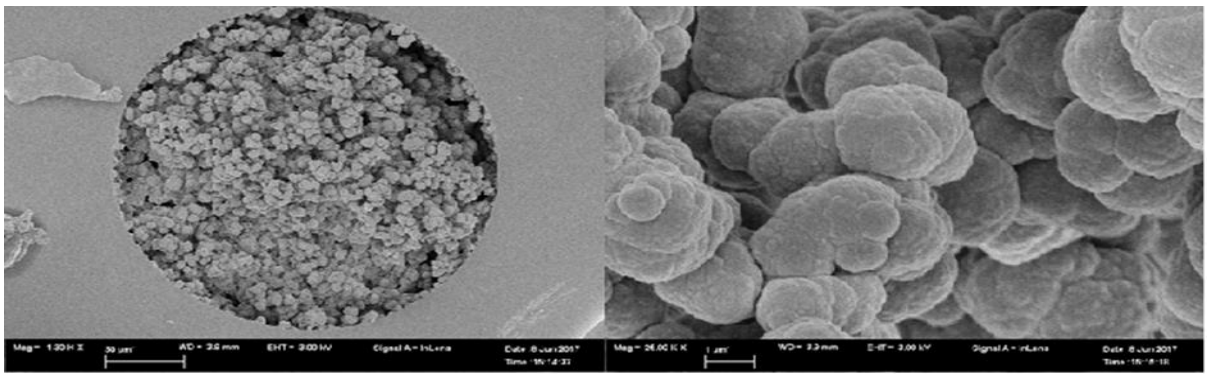

Figure 6: Scanning electron micrograph of S1 at 1300x (left) and 25,000x (right) showing a homogenous porous monolithic structure.

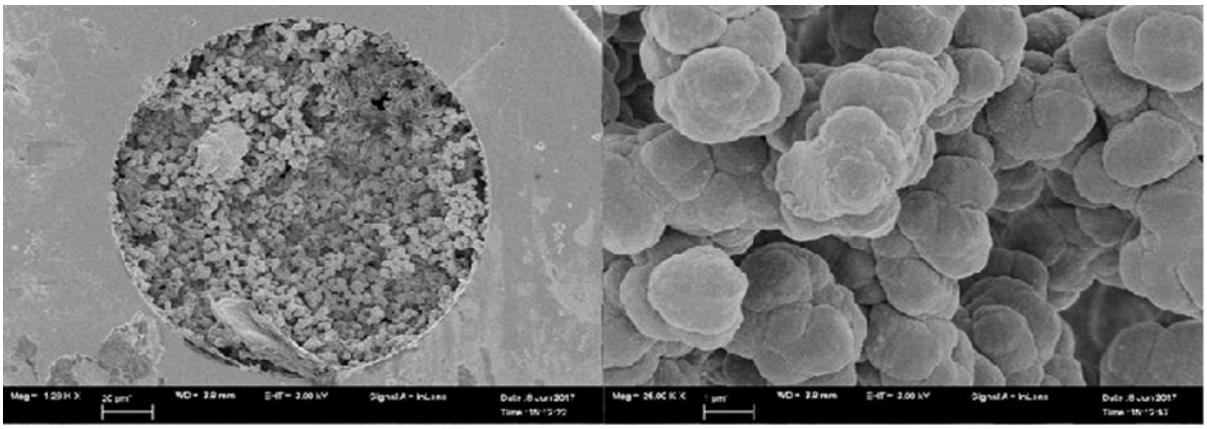

Figure 7: Scanning electron micrograph of s2 at 1300x (left) and 25,000x (right) showing a homogenous porous monolithic structure.

Column mechanical stability

The prepared polymer monolithic capillaries were tested for mechanical stability by pumping a mixture of methanol/water (80:20 v/v) at varying flow rates (0.1-1 µL/min). The pressure drop as a function of the mobile phase flow rate was linear indicating that the packing monolith maintained its integrity under high pressures. The overlaid plots of the backpressure versus the mobile phase flow rate are shown in Figure 8. **S1** and **S2** columns demonstrated good stability over wide range of pressure as confirmed by the excellent linear velocity at these pressures. However, linerarty was good for both **S1** and **S2** columns, this might help in predicting column performance at different porogenic solvents.

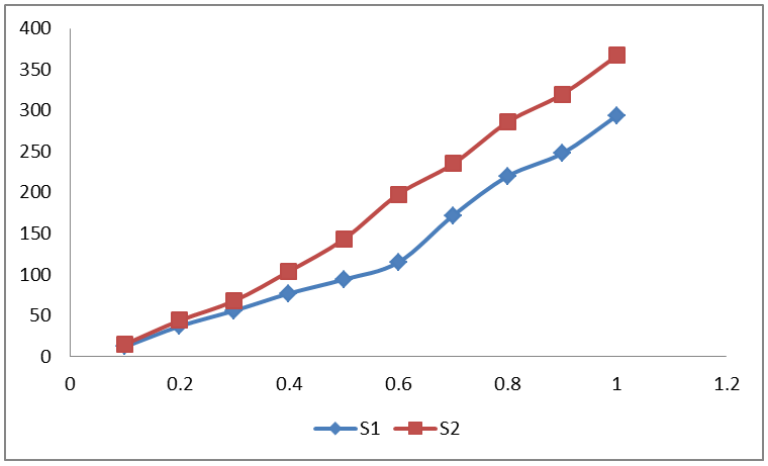

Figure 8: Overlay of S1 and S2 column backpressures versus flow rate.

Enantioselective separation of different classes of pharmaceutical racemates

Two monolithic capillary columns; **S1** and **S2** were investigated for the enantio selective nano-LC separation of a set of different classes of racemic pharmaceuticals namely: β-blockers, α-blockers, antiinflammatory drugs, antifungal drugs, dopamine antagonists, norepinephrine-dopamine reuptake inhibitors, catecholamines, sedative hypnotics, diuretics, antihistaminics, anticancer drugs, flavonoids, antiarrhythmic drugs and miscellaneous drugs. The choice of compounds was guided by preliminary investigations [31]. Initially, the enantioselective resolution was investigated using polar solvents, a mobile phase composed of acetonitrile and water mixture ranged from 10-90% (v/v) was tested. No enantioselective separation was observed under this condition for both columns **S1** and **S2**. When an aqueous methanol-based mobile phase was used, no specific separation was observed under this condition for column **S2**, while, acceptable separation ($Rs \geq 1$) was achieved for Ibuprofen 7, Indoprofen 10, Hexaconazole 15, Sulconazole 18 and Diniconazol 17 on the polymer-based monoliths **S1** (Figure 9). Also,

partial resolution (Rs <1) was achieved for Naftopidil 6, 1-acenaphthenol 33, Indoprofen 10 and Miconazole 16 on the polymer-based monoliths **S1** (Figure 9). It was observed that enantioseparation was achieved at high percentage of water in the mobile phase. It noted that the separation factor (α) is higher than 1.5 as in case of Sulconazole 18 and Ibuprofen 7 which mean that they are baseline separated, but the resolution is 1.5 or less due to the broadness of the second peak. Separation (α) and resolution (Rs) factors for the resolved compounds are listed in Table 3 below.

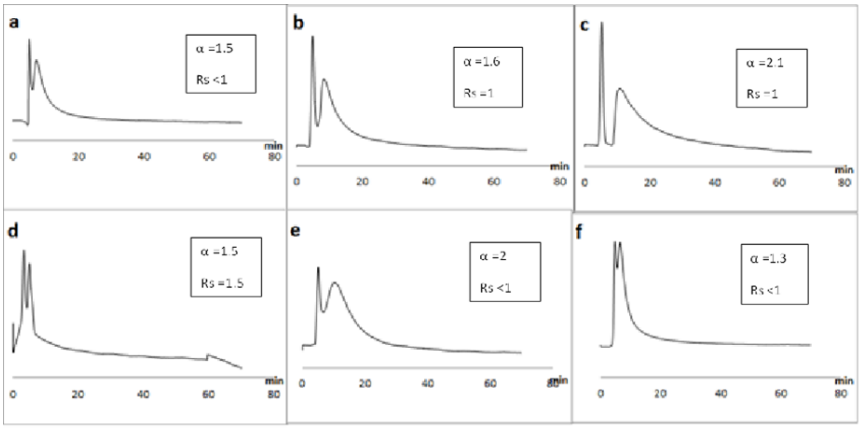

Figure 9: Enantioselective nano-lc separation of racemic Indoprofen **10 (a)**, Miconazole **16 (b),** and Sulconazole **18 (c) (**Mobile phase: methanol/water 3:97, 1%TFA v/v) and Ibuprofen **7**(e) **(**Mobile phase: methanol/water 3:97, 1%TFA v/v) Indoprofen **10 (f) and** Naftopidil **6(g) (**Mobile phase: methanol/water 20:80, v/v) on S1 capillary column (150 µm ID, 25 cm length). UV: 219 nm, flow rate: 1 µL/min.

Table 3: Chromatographic conditions, separation and resolution factors for the baseline/acceptably-resolved racemates.

Column S1				
Phase	**Mobile phase**	**Drug**	**Separation factor(α)**	**Resolution (Rs)**
Reversed phase	Methanol :Water 3:97, 1% TFA (v/v)	Naftopidil 6	1.3	<1
		Ibuprofen 7	1.5	1.5
		Indoprofen 10	1.4	1
		Hexaconazole 15	1.6	1
		Miconazole 16	1.5	<1
		Sulconazole 18	2.1	1
		Naftopidil 6	1.3	<1

	Methanol :Water 20:80% (v/v)	Indoprofen 10	2	<1
		Hexaconazole 15	2.4	1
		Miconazole 16	1.5	<1
		Diniconazol 17	2	1
		Sulconazole 18	1.4	<1
		1-acenaphthenol 33	1.2	<1

The blank column prepared by the same procedures without chiral selector was used to test the separated compounds and didn't give any separation under the same chromatographic conditions. These results confirm the presence of chiral selector inside the capillaries and also confirm its role in the enantioseparation process (Figure 10).

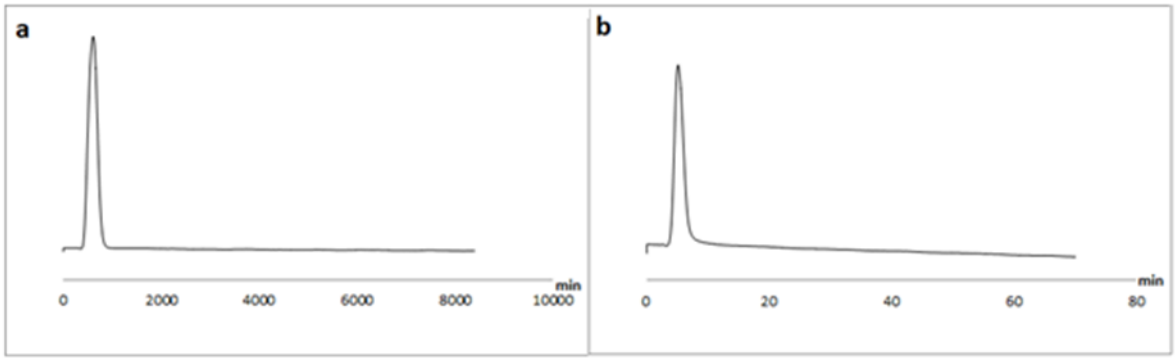

Figure 10: Enantioselective nano-lc single peak of racemic, Miconazole **16 (a),** and Sulconazole **18 (c) (**Mobile phase: methanol/water 3:97, 1%TFA v/v) on plain capillary column (G) (150 µm ID, 25 cm length). UV: 219 nm, flow rate: 1 µL/min.

Various carbohydrates have been applied on silica or polymer surface as a support either by encapsulation, immobilization, and coating or by covalent bonding which increases the chances for using wide range of mobile phases and creates a more robust CSP [43-44]. Sulfated beta-cyclodextrin (S-beta-CD) was reported as chiral selector in chiral chromatography by using in both mobile phases and stationary phase (CSP) for high-performance liquid chromatography [34-36]. Under reversed phase conditions, the formation of inclusion complexes within the oligosaccharide cavity is the most predominant mechanism of retention and enantioselectivity. When methanol-based mobile phase was used, enantioselective separation was observed for some analytes; this is due to diverse ways the mobile phases relate to the mutual interaction (adsorption) between the CSP surfaces and target racemates, this confirms the role of solvent polarity in enantioseparation mechanism in terms of the inclusion complex stability

[45]. It was also observed that the antioseparation was mostly occured at high water content of the mobile phase; this confirms that water improves the interaction between the CSP and the analytes.

Miniaturization of chiral separations, a future perspective

Various carbohydrates have been applied on silica or polymer surface as a support either by immobilization, encapsulation, and coating or by covalent bonding which increases the chances for using diverse mobile phases and creates a more robust CSP. The prediction of the enantioseparation mechanisms of CSPs has mainly been based on speculations based on the complexity of the enantioselective separation [46]. It is well established that CDs form transient diastereomeric inclusion complexes with enantiomers by means of the cavity under reversed phase chromatographic conditions. As demonstrated from testing 37 racemates from different chemical and pharmaceutical classes on sulfated β-cyclodextrin-based CSPs under reversed phase conditions, the formation of inclusion complexes within the oligosaccharide cavity is the most predominant mechanism of retention and enantioselectivity. Moreover, the presence of points of interactions between the enantiomers and the CSP via hydrogen bonds, π–π bonding, dipole–dipole stacking, etc. which can increase the selectivity towards some analytes. It was also observed that the antioseparation was mostly occured at high water content of the mobile phase; this confirms that water improves the interaction between the CSP and the racemates. On the other hand, when normal phase was used as mobile phase, no separation was observed for tested analytes. The resolved racemates didn't resolved on blank column, It was also demonstrated that the monolith backbone did not play any role in the chiral separation, It is worth noting that polymer monoliths are of smaller surface area this had affected the retention time of the racemates but not the enantioseparation.

From this study we can predict that sulfated β-cyclodextrin can be used as chiral selector for nano-HPLC. Although, the results obtained by using sulphated β-cyclodextrin as CSP were not satisfying, the future work can get better results by studying different conditions regarding preparation of the monolith, type of monomers, cross linkers and their percentages, type of progens and their percentages, the procedure of *in-situ* polymerization, different varieties of mobile phases,…..etc. Other strategies can be used to improve the recognition ability of sulfated β-cyclodextrin including immobilization or coating and using other supports as silica or zirconia.

CONCLUSION

Two sulfated β-cyclodextrin in organic polymer monolith was prepared via the *in situ* copolymerization of binary monomer mixtures consisted of GMA as a monomer and EGDMA as across linker along with different porogens namely; 1-propanol and 1,4-butanediol (**S1**) or ethanol (**S2**) in the presence of 1 wt% AIBN. The prepared capillary columns were characterized and investigated for the enantioselective separation of 37 pharmaceutical racemates using nano-HPLC. Under reversed phase

chromatographic conditions, acceptable separation was achieved for some racemates on S1 column only while by using normal phase, no separation was observed for tested analytes.

REFERENCES

1**.** Ali, I., and Aboul-Enein, HY., Enantioseparation of some clinically used drugs by HPLC using cellulose Tris (3, 5-dichlorophenylcarbamate) chiral stationary phase. *Biomedical Chromatography*. **2003.** 17(2-3): 113-117.

2. Aboul-Enein, HY., and Ali, I., Optimization strategies for HPLC enantioseparation of racemic drugs using polysaccharides and macrocyclic glycopeptide antibiotic chiral stationary phases. *Il Farmaco*. **2002.** 57(7): 513-529.

3. Younes, AA., et al. Chiral separations in reversed-phase liquid chromatography: evaluation of several polysaccharide-based chiral stationary phases for a separation strategy update. *Journal of Chromatography A*, **2012.** 1269:154-167.

4. Francotte, ER., Enantioselective chromatography as a powerful alternative for the preparation of drug enantiomers. *Journal of Chromatography A*, **2001.** 906(1–2): 379-97.

5. Glajch, J., et al. Effect of column degradation on the reversed-phase high-performance liquid chromatographic separation of peptides and proteins. *Journal of Chromatography A*, **1987.** 384: 81-90.

6. Wehrli, A., et al. Influence of organic bases on the stability and separation properties of reversed-phase chemically bonded silica gels. *Journal of Chromatography A* **1978.** 149:199-210.

7. McNeff, C., et al. Analytical advantages of highly stable stationary phases for reversed-phase LC. LC GC. **2000.** 18(5): 514-529.

8. Cabrera, K., Applications of silica-based monolithic HPLC columns. *Journal of Separation Science*. **2004.** 27(10-11): 843-852.

9. Nunez, O., et al. Preparation of monolithic silica columns for high-performance liquid chromatography. *Journal of Chromatography A*, **2008.** 1191(1-2): 231-252.

10. Kanateva, AY., et al. Monolithic stationary phases in liquid and gas chromatography, *Russian Chemical Reviews.* **2008.** 77(4): 373-379.

11. Nakanishi, K., and Soga, N., Phase separation in silica sol-gel system containing polyacrylic acid I. Gel formaation behavior and effect of solvent composition. *Journal of Non-Crystalline Solids*, **1992.** 139: 1-13.

12. Saunders, K.C., et al. Separation and sample pre-treatment in bioanalysis using monolithic phases: A review. *Analytica Chimica Acta.* **2009.** 652(1-2): 22-31.

13. Liao, J.L., et al. Continuous beds for standard and micro high-performance liquid chromatography. *Journal of Chromatography A*, **1991.** 586(1): 21-26.

14. Gibson, G.T., et al. Surface-mediated effects on porous polymer monolith formation within capillaries. *Polymer*, **2008.** 49(13-14): 3084-3090.

15. Xiao, Y., et al. Recent development of cyclodextrin chiral stationary phases and their applications in chromatography. *J Chromatogr A.* **2012.** 1269: 2-68.

16. Si-Ahmed, K., et al. Preparation and enantioseparation characteristics of three chiral stationary phases based on modified β-cyclodextrin for liquid chromatography. *Anal Bioanal Chem*, **2009.** 395(2): 507-518.

17. Lai, X.H., and Ng, S.C., Preparation and chiral recognition of a novel chiral stationary phase for high-performance liquid chromatography, based on mono(6A-N-allylamino-6A-deoxy)-perfunctionalized β-cyclodextrin and covalently bonded silica gel. *J Chromatogr A*, **2004.** 1031(1-2): 135-142.

18. Wikstrom, H., et al. Immobilisation and evaluation of a vancomycin chiral stationary phase for capillary electrochromatography. *Journal of Chromatography A*, **2000.** 869(1–2):395-409.

19. Karlsson, C., et al.. Evaluation of a Vancomycin Chiral Stationary Phase in Capillary Electrochromatography Using Polar Organic and Reversed-Phase Modes. *Analytical Chemistry*. **2000.** 72(18): 4394-4401.

20. Kang, J., et al. A silica monolithic column prepared by the sol-gel process for enantiomeric separation by capillary electrochromatography. *Electrophoresis*, **2002.** 23(7-8):1116-1120.

21. Wistuba, D., and Schurig, V., Enantiomer separation by capillary electrochromatography on a cyclodextrin-modified monolith. *Electrophoresis*, **2000.** 21(15): 3152-3159.

22. Wistuba, D., and Schurig, V., Enantiomer separation by pressure-supported electrochromatography using capillaries packed with Chirasil-Dex polymer-coated silica. *Electrophoresis*, **1999.** 20(13): 2779-2785.

23. Park, J.M., and Park, J.H., Enantiomer separations of basic chiral compounds by capillary electrochromatography on a phosphated β-cyclodextrin-modified zirconia monolith. *J Chromatogr A*, **2014.** 1339: 229-233.

24. Hong, J.S., and Park, J.H., Chiral separation of basic compounds on sulfated β-cyclodextrin-coated zirconia monolith by capillary electrochromatography. *Bull Korean Chem Soc*, **2013.** 34(6): 1809-1813.

25. Bragg, W., and Shamsi, S.A., A novel positively charged achiral co-monomer for β-cyclodextrin monolithic stationary phase: Improved chiral separation of acidic compounds using capillary electrochromatography coupled to mass spectrometry. *Journal of Chromatography A*, **2012.** 1267(0):144-155.

26. Lv, Y., et al. Preparation of novel β-cyclodextrin functionalized monolith and its application in chiral separation. *Journal of Chromatography B*, **2010.** 878(26): 2461-2464.

27. Ishii, H., et al. Preparation of highly efficient monolithic silica capillary columns for chiral separation. *Chromatography*, **2009.** 30(1): 87-88.

28. Zhang, Z., et al. Preparation of perphenylcarbamoylated β-cyclodextrin-silica hybrid monolithic column with "one-pot" approach for enantioseparation by capillary liquid chromatography. *Analytical Chemistry*, **2011.** 83(9): 3616-322.

29. Fouad, A., et al. Functionalized polymer monoliths with carbamylated amylose for the enantioselective reversed phase nano-liquid chromatographic separation of a set of racemic pharmaceuticals. *Journal of Chromatography A*, **2017.** 1515: 91-99.

30. Zhang, Q,, et al. Preparation of a β-cyclodextrin functionalized monolith via a novel and simple one-pot approach and application to enantioseparations. *Journal of Chromatography A*, **2014.** 1325(0): 147-154.

31. Ahmed M, Ghanem A. Enantioselective nano liquid chromatographic separation of racemic pharmaceuticals: a facile one-pot *in situ* preparation of lipase-based polymer monoliths in capillary format. *Chirality*. **2014.**

32. Wu, M., et al. "One-Pot" Process for Fabrication of Organic-Silica Hybrid Monolithic Capillary Columns Using Organic Monomer and Alkoxysilane. *Analytical Chemistry.* **2009.** 81(9): 3529-336.

33. Evans, CE., and Stalcup, AM., Comprehensive strategy for chiral separations using sulfated cyclodextrins in capillary electrophoresis. *Chirality*. **2003.** 15(8):709-723.

34. Sutton, KL., et al. A comparison of vancomycin and sulfated beta-cyclodextrin as chiral selectors for enantiomeric separations of selenoamino acids using capillary electrophoresis with UV absorbance detection. *Analyst*, **2000.** 125(2): 231-234.

35. McMurtrey, K., et al. HPLC resolution of the enantiomers of dihydroxyphenylalanine and selected salsolinol derivatives using sulfated beta-cyclodextrin. *Enantiomer*, **2000.** 5(3-4): 377-383.

36. Wang, J., et al. Enantioselective separation of epoxides by capillary electrophoresis employing sulfated beta-cyclodextrin as chiral selector. *The Analyst.* **2001.** 126(4): 438-440.

37. Stalcup, AM., and Gahm, KH., A sulfated cyclodextrin chiral stationary phase for high-performance liquid chromatography. *Analytical Chemistry*, **1996.** 68(8): 1369-1374.

38. Ameyibor, E., and Stewart, JT., Enantiomeric HPLC separation of selected chiral drugs using native and derivatized β-cyclodextrins as chiral mobile phase additives. *Journal of Liquid Chromatography & Related Technologies*, **1997.** 20(6): 855-869.

39. Ma, S., et al. Chromatographic and spectroscopic studies on the chiral recognition of sulfated β-cyclodextrin as chiral mobile phase additive: Enantiomeric separation of a chiral amine. *Journal of Chromatography A*. **2009.** 1216(8):1232-1240.

40. Gusev, I., et al. Capillary columns with *in situ* formed porous monolithic packing for micro high-performance liquid chromatography and capillary electrochromatography. *Journal of Chromatography A*, **1999.** 855(1): 273-290.

41. Urban, J., and Jandera, P., Recent advances in the design of organic polymer monoliths for reversed-phase and hydrophilic interaction chromatography separations of small molecules. *Anal Bioanal Chem*, **2013.** 405(7): 2123-2131.

42. Jandera, P., et al. Cross-linker effects on the separation efficiency on (poly)methacrylate capillary monolithic columns. Part I. Reversed-phase liquid chromatography. *J Chromatogr A*, **2013.** 1274:97-106.

43. Xiao, Y., et al. Recent development of cyclodextrin chiral stationary phases and their applications in chromatography, *Journal of Chromatography A*. **2012.** 1269: 52-68.

44. Chankvetadze B, et al. High-performance liquid chromatographic enantioseparations on capillary columns containing crosslinked polysaccharide phenylcarbamate derivatives attached to monolithic silica. *Journal of Separation Science,* **2006.** 29(13): 1988-1995.

45. Ahmed, M., et al. Single-walled carbon nanotube-based polymer monoliths for the enantioselective nano-liquid chromatographic separation of racemic pharmaceuticals. *Journal of Chromatography A,* **2014.** 1360:100-109.

46. Bao, X., et al. Insights into the complexity of chiral recognition by a three-point model. *Microporous and Mesoporous Materials,* **2013.** 172(0): 44-50.