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Research Article

# Novel Molecularly Imprinted Nanofiber Mats for the Selective Removal of Interfering Cholic Acid Prior to Drug Residue Analysis

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## Abstract

Monitoring the efficacy of an administered drug in a patient is a key step in patient management. It usually involves collecting a biological sample such as bile from the patient and analysing it for drug residues that may have remained after a certain drug was administered to patient and expended. Detection instruments employed for the accurate analysis of drug residues that are usually present in trace quantities are often challenged by the complex, 'dirty' matrix that commonly characterize biological samples from which the residues are sampled. This work considered bile, whose main constituent is cholic acid, as the biological sample. High concentration of cholic acid contributes to ion suppression during the ionization step when performing the mass spectrometry detection of the drug residues. It also masks the residues from easily being separated and detected as they exist in trace quantities in the complex matrix. Furthermore, it lowers the sensitivity of the analysing instruments, gets co-eluted with the targeted analytes, resulting in imprecise and inaccurate results. Consequently, in this work, we describe an optimal sample clean-up strategy that involved the fabrication of cholic acid (CA)-molecularly imprinted (MI) electrospun nanofiber mats that prior to instrumental analysis, selectively removed the CA that interfered with the accurate analysis of the trace drug residues. We successfully fabricated the CA-MI electrospun nanofiber mats as was demonstrated by the estimated nano sized fibrous structures of magnitude 660 nm from the SEM images. At macro level mat like structures were observed and harvested from the aluminium collector. The performance of the prepared CA-MI electrospun nanofiber mats was evaluated and was found to selectively remove 100.1% cholic acid from equimolar standard solutions consisting of cholic acid and analogous compounds, owing to the their calculated high selectivity created by the molecular imprinting technology that was employed during the synthesis and fabrication of the nanofibers that created reaction sites at molecular level, that could only fit CA as it was included as a print reagent in the spinning solution together with all other general molecular imprinting reactants and later extracted by solvent extraction leaving behind a memory for CA only in the newly prepared nanofiber mat structure. The higher CA % removal of 100.1% by the CA-MI electrospun nanofiber mats compared to the slightly lower CA %removal, 79.1%

by the molecularly imprinted powder materials that were prepared for comparison, was attributed to the high surface area to volume ratio of the nano sized fiber mats that were produced by electro-spinning technology. Both, the CA-MI nanofiber mats and the CA MIP powder exhibited high selectivity since the same molecular imprinting reactants, including their quantities were used during the synthesis of both thus the same number of created selective sites but more accessibility on the nanofiber mats was realized due to their one dimensionality (thinness) compared to the thick structure of the MIP powder materials that had to be maneuverered in order to access the selective sites. This together with the large surface area to volume ratio of the nanofibers gave the CA-MI electrospun nanofiber mats the advantage in selectively removing the interfering CA in drug residue analysis from the 'dirty' biological samples such as bile samples prior to instrumental analysis better the MI powder.

**Keywords:** Molecularly Imprinted Electrospun Nanofibers; Drug Residue Analysis; Optimal Sample Clean-up; Cholic Acid; Bile

## Introduction

Administration of drugs for most illnesses has proven vital but may expose potential harm to the patients receiving medication [1]. On the other hand, drug residue analysis (DRA) has become necessary and is normally performed to monitor the level of accumulation of these drug residues and to know their toxicity levels [2]. The toxic level of these drug residue impose health problems through tissue damaging, more especially the liver [3]. Pharmaceutical authorities also depend on drug residue analysis in order to certify the half-life of drugs and to know the rate of their metabolism, hence acquiring the right pharmacokinetics information about the efficacy of the administered drugs [4]. Cholic Acid is known to exist in high concentrations, in salt forms known as cholates [6]. These salts would then interferer by blocking analytical parts like chromatographic columns, masking the drug residues that exist in lower concentrations from being detected, even when using sensitive analyzing instruments like GC-MS/MS or LC-MS/MS [7]. This would then affect the accuracy, reproducibility and the sensitivity of the instruments, hence giving wrong efficacy information [8]. Prior to DRA, sample preparation strategies, relying on extraction procedures have been employed in order to reduce interfering cholic acid but these were found to lack selectivity and were inefficiently removing interfering cholic acid from the bile matrix, hence drug residues would not be pre-concentrated in a clean state [9]. Solid phase extraction (SPE) has been widely used to extract target analyte, usually through simultaneous sample clean-up and pre-concentration. SPE employs different sorbents to extract analytes of interest or to directly remove interfering matrix components. These sorbents like C-18, polymeric resins and other silica based sorbents are known for their lack of selectivity, hence encouraging co-elution of drug residues with cholic acid as the unwanted interferent [10]. For optimal sample clean-up achievement in drug residue analysis, effective and efficient clean-up strategies relying on selective, sensitive, robust, cheap and durable recognition materials are greatly needed towards bile clean-up prior to its analysis for drug residues. One of those strategies is the employment of molecular imprinting and electro-spinning technology to prepare these recognition materials, towards sample preparation, hence this paper reports output results on synthesis of sorbents in form of molecularly imprinted polymer (MIP) and an electrospun nanofiber-based MIP, possessing greater affinity and selectivity towards the target analyte [11-13]. Optimization and comparison of the prepared sorbents were performed towards optimal removal of cholic acid as the interferent by batch approach in a selective manner, prior to drug residue analysis.

### Materials and Methods Reagents

Methacrylic acid (MAA) (99.99%), Ethylene glycol dimethacrylate (EGDMA) (99.99%), Dichloromethane (DCM) (99%), Cholic acid (CA) (99.99%), Chenodeoxycholic acid (CCA) (99.99%), Deoxycholic acid (DCA) (99.99%), Cholesterol (99.99%), Propranolol, Asprin, Polyethylene terephthalate (PET), Trifluoroacetic acid (TFA) (99.99%), Ethanol, Azobisisobutyronitrile (AIBN), all of analytical grade were supplied by Sigma- Aldrich (Johannesburg, South Africa).

### Instruments and equipment

A custom-made electrospinning set-up consisting of a power supply, a 10mL glasssyringe with stainless still needle from Poulten GmbH (Berlin, Germany), mounted on a new Era, NE-1000 programmable syringe pump (New York, USA). was employed to fabricate molecularly imprinted electrospunnanofibers. A Custom-made

pressurized hot water extraction (PHWE) system was employed for the removal of cholic acid (template) from the MIP materials. Bruker Tensor 27 FTIR spectrophotometer from(Ettlingen, Germany) was employed to obtain the spectra of the MIP materials. JSM 840 field emission scanning electron microscope (FE-SEM) from JEOL, (Tokyo,Japan) was employed for morphological micrographs. A lambda 25 Perkin-Elmerspectrophotometer, from (Santa Clara, CA,USA) was employed to monitor the absorbance values during rebindingexperiments.

### Preparation of MIP materials

### Cholic acid MIP particles

To prepare cholic acid imprinted particles, CA, MAA, EGDMA and AIBN as template, functional monomer, cross linking monomer and initiator in a ratio of 1:5:30:0.5, respectively, were all dissolved in DCM and the mixture was refluxed at 75ºC for 4.5 hr, resulting in a polymer monolith. The monolith was ground to powder and sieved to obtain particles of ≤45 μm in diameter, which were then introduced to the PHWE set up for template (cholic acid) removal. Thereafter the particles were left to dry in open air overnight, prior to their employment for the batch rebinding experiments. The control polymer referred to as non-imprinted polymer (NIP), without the template (cholic acid) was also prepared following the same procedure.

Cholic acid electrospun MIP nanofibers

Polyethylene terephthalate (PET) (6.4491 g) and all the MIP reagents mentioned earlier, in the same ratio were mixed with TFA (16mL) and DCM (4mL) to form an optimized homogenous spinnable solution, which was stirred overnight until complete dissolution. Cholic acid imprinted nanofibers were fabricated by electrospinning following the optimized procedure. The solution was pumped at a rate of 0.005 mL/h through a steel needle of 0.84 mm internal diameter. The distance between the needle tip and the collector was kept at 15 cm, while the needle tip and the collector were held at optimized voltages of +20 and -5 kV, respectively. Continuous PET-MIP nanofibers were collected on a hot plate at 80 ºC covered with a grounded aluminium foil in form of fibrous mats.

### Template removal

### Pressurized hot water extraction (PHWE)

800 mg of cholic acid MIP particles and the electrospun MIP nanofibers were extracted in a 34mL PHWE extraction cell with water as the solvent. All extraction procedures were carried out at a flow rate of 2 mL/min, employing optimized temperatures of 195 and 180 °C for MIP particles and MIP nanofibers, respectively. Aliquots of the washings from the PHWE system were then collected at 10 min intervals, analysed in triplicates until the detected absorbance of the templates in subsequent washings was constant.

### Batch rebinding experiments

### Optimization of the quantity and the time needed for maximum removal of cholic acid by the prepared MIP materials

Increasing quantities of MIP materials of cholic acid in the form of particles/nanofibers were added to 5mL aliquots of concentrated 10% (w/v) cholic acid standards solutions to determine the optimal quantity needed to remove cholic acid from the standard solutions. The absorbance of cholic acid in the supernatant was measured with a UV spectrophotometer until there was a constant reading with further addition of the MIP materials. The experiments were carried out in triplicates, percentage removed were calculated and plotted against quantity of MIP materials for the cholic acid. Following the procedure for optimization of quantity, absorbance of cholic acid in the supernatant after addition of the optimized quantities of cholic acid MIP materials were monitored at 5 min intervals until a constant value of absorbance was reached. This marked the optimal time needed by each MIP material to maximally remove the interfering cholic acid.

## Results and Discussions

### Template removal curve

The following graph in figure 1 is a representation of cholic acid template removal from the MIP particles, which was observed to be the same as that of cholic acid removal from the MIP nanofiber particles. According to the graph, cholic acid was successfully removed from the MIP particles under 60 minutes for all the MIP materials using the HWPE system. A constant behaviour in relation to the measured absorbance was observed in the graph from 60 minutes onwards, hence that indicated a complete template removal from the MIP materials, prior to their use in rebinding experiments.

### Conformation of Template Removal from IR Spectra

The removal of cholic acid from the imprinted polymer materials was confirmed by FT-IR spectroscopy after PHWE procedure. Before template removal, the MIP particles and the nanofiber exhibited identical FT IR spectrum in figure 2A with three charac-

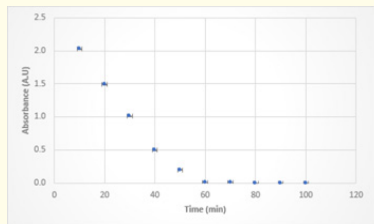


**Figure 1:** Template removal plot from MIP particles monitored at 10 minutes intervals.

teristic stretching bands at 3333.53, 2973.19 and 2928.56 cm-1 indicating the presence of OH and CH bonds within the polymers. The band at 3333.53 cm-1 disappeared on washing the MIP particles/nanofibers and the bands around 2973 and 2928 cm-1 were reduced and shifting was observed figure2B. The FT-IR confirmed template removal hence creation of binding sites within the polymer matrix.

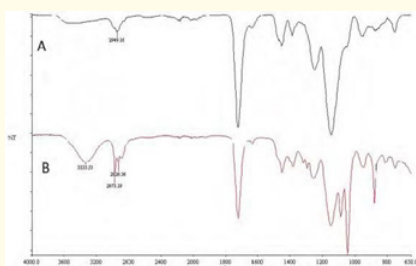


**Figure 2:** FT IR spectra of the washed (A) and unwashed (B) cholic acid MIP particles/nanofiber.

## Morphological characterization of the prepared MIP electrospun nanofibers

The SEM image of the prepared MIP nanofibers in figure 3 was obtained to represent the MIP or NIP nanofibers as they both showed similar morphological characteristics. According to the SEM image, both the nanofibers displayed smooth thin and long continuous nanofiber threads. The nanofibers had an average diameter of 660 nm as determined by the scandium 4.0 software. All these are excellent physical properties for the nanofiber material that was to be employed as a sorbent material for SPE. The nano dimensions contribute to the high surface area to volume ratio that nanofibrous material are well known for, hence improved sensitivity for the prepared nanofibers.

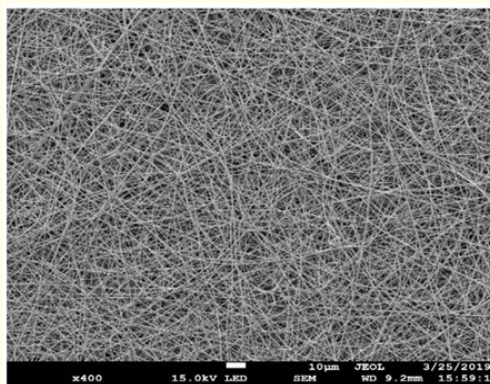


**Figure 3:** A typical SEM image of the cholic acid MIP or NIP electrospun nanofibers.

## Results on Optimization of both the quantity and the time needed for maximum removal of cholic acid by the prepared MIP materials

Figure 4 shows that 100 mg of both the MIP particles and the MIP nanofibers was obtained to be the optimal quantity for maximal binding of cholic acid. This value was reached for the nanofiber extracting 100% of cholic acid from the standard solutions while only 79% was bound to the MIP particles. For optimization of time figure 5, It was observed that 4 min was the optimal time needed by either the ordinary MIP particles or the MIP nanofibers for maximum extraction before a constant trend occurred. 100% of cholic acid was bound by the nanofiber while only less than 70% was bound by the MIP particles for the same duration. From the percentage bounds, comparisons were made. It was observed that the electrospun MIP-based nanofiber was more effective with a 100% binding than the ordinary MIP particle which exhibited only less than 80%. This could be attributed to the large surface to volume ratio that the nanofiber material possessed, in comparison to the MIP particle.

Cholic acid nanofiber/MIP selectivity Cholic acid MIP nanofibers and the MIP particles exhibited greater affinity for the target analyte (cholic acid) in selectivity studies (Table 1). The nanofiber showed greater affinity than the MIP particles in binding with cholic acid endorsing the excellent performance that nanomaterials possess. The NIP as the non-imprinted material adsorbed negligible quantities of cholic acid hence this could be attributed to the presence of non-selective binding sites possessed by the NIP as the reference material.

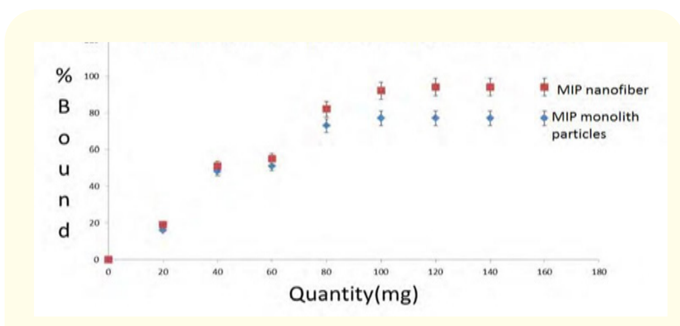


**Figure 4:** Optimization of the quantity of MIP nanofiber or MIP particles needed for maximum extraction of cholic acid from 10% (w/v) standard solution.

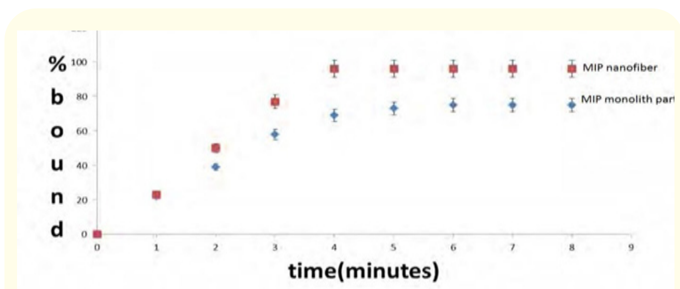


**Figure 5:** Optimization of the time needed by 100 mg (optimal quantity) of MIP nanofiber or MIP particles for maximum extraction of cholic acid from 10% (w/v) standard solution.

| Compound | % Bound by MIP monolith particles | %Bound by MIP nanofiber |
|---|---|---|
| Cholic acid | 79.7(0.1) | 100.1(0.7) |
| Cholesterol | Nq | Nq |
| Deoxycholic acid | Nq | Nq |
| Chenodeoxycholic acid | Nq | Nq |
| Propranolol | Nq | Nq |
| Aspirin | Nq | Nq |

**Table 1:** Percentage of analyte bound to (%Removed by) MIP, Nanofiber and NIP at optimized conditions.
Note: Nq stands for non-quantifiable.

## Effectiveness of the MIP/MIP nanofiber on removing cholic acid

The prepared cholic acid MIP and MIP nanofiber materials were applied to standard solutions, hence the results indicated potential removal of interfering cholic acid during drug residue analysis in human biological fluids especially bile. On the other hand, the MIP nanofiber entirely removed cholic acid from the concentrated standard solution of cholic acid of 10% (w/v), hence, the nanofiber-based material with large surface to volume ratio, high reactivity and enhanced absorbability exhibited high efficiency and selectivity than the MIP and the NIP materials.

## Conclusions

Both cholic acid nanofiber and MIP powder presented themselves as selective, sensitive, and cheaper materials with potential to replace the existing extraction materials that are expensive, non-selective and exhibit poorer sample clean-up in removing interfering species during drug residue analysis resulting in detection systems clogging with interferents after a few runs of analysis, followed by frequent detection instruments downtime, reduced throughput and high maintenance costs. The custom-made CA-MI electrospun nanofiber mats with their higher removal efficiency of interferents than the newly prepared MIP powder and conventional sample clean-up materials/methods, achieved more cleaner samples from which more precise and accurate results could be realised. The CA-MI nanofiber mat offer an alternative synthesis route through which more efficient sample clean-up extraction sorbent materials with high selectivity and high % interferent removal could be fabricated in a cheaper way and with ease. The work herein is an endeavour towards optimal sample clean-up in molecular diagnostics assaying involving biological samples which would improve accuracy, precision and reliability of the results as well as reduce the maintenance cost and detection instruments downtime which in turn will result- in improved throughput. In future, this work will be performed *in vivo* to further authenticate it for applicability in real samples.

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