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Imprinted nanospheres based on precipitation polymerization for the simultaneous extraction of six urinary benzene metabolites from urine followed by injector port silylation and gas chromatography-tandem mass spectrometric analysis

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ABSTRACT

In the present communication, uniformly sized molecularly imprinted polymer (MIP) as nanospheres were synthesized based on precipitation polymerization using dual-template imprinting approach and used it as sorbent for solid phase extraction of six urinary benzene metabolites (UBMs). This approach in combination with injector port silylation (IPS) has been used for the quantitative determination of these UBMs by gas chromatography-tandem mass spectrometry. The MIP was synthesized by using *t,t*-muconic acid (*t,t*-MA) and 1,2,4-trihydroxybenzene (THB) as templates, methacrylic acid (MAA) as a monomer, ethyleneglycoldimethacrylate (EGDMA) as crosslinker, acetonitrile and dimethylsulphoxide as a porogen and azobisisobutyronitrile (AIBN) as an initiator. The factors affecting the performance of polymer and IPS were investigated and optimized for the simultaneous determination of UBMs in urine. Binding study of imprinted and non-imprinted polymer (NIP) shows that, MIP possesses higher affinity in comparison to NIP for these analytes. Under the optimum conditions, the method developed was found to be linear with regression coefficients falls in the range of 0.9721–0.9988 for all the analyzed metabolites. The percent recovery of the metabolites analyzed in urine was found to be in the range of 76–89%, while the limit of detection and limit of quantification were found to be in the range of 0.9–9.1 ng mL^{-1} and 2.8–27 ng mL^{-1} respectively. The validated method was successfully applied to the real urine samples collected from different groups (kitchen workers, smokers and petroleum workers) and found that the developed method has been promising applications in the routine analysis of urine samples of benzene exposed population.

1. Introduction

Benzene, known to be a ubiquitous pollutant is a common industrial chemical and a prominent constituent of gasoline, tobacco smoke and engine emission [1,2]. Population exposes to benzene includes petrochemical workers, petrol station attendants, smokers [1,3]. International Agency for Research on Cancer (IARC) classified the benzene as a group-I carcinogen [4]. Exposure to benzene results in aplastic anaemia, dysfunction of the immune system and leukemia [5]. Permissible exposure limit to benzene, introduced by the United States Occupational Safety and Health Administration (OSHA), was set to be 10 ppm, which later changed to 1 ppm by taking the consideration of benzene risk associated with neoplasia [6], while as per the National Institute of Occupational Safety and Health (NIOSH), the time weighted average concentration is 0.1 ppm and short-term exposure limit (STEL) value is 1 ppm (http://www.cdc.gov/niosh/npg/npgd0049.html), (http://www.cdc.gov/niosh/idlh/7782505.html). As per the American Conference of Governmental Industrial Hygienist (ACGIH), benzene is considered as a carcinogen with an eight hour time weighted average threshold limit value (TLV) of 0–1 ppm [7,8].

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Previous studies shown that, the metabolism of benzene to its active intermediates is an imperative for its toxic and carcinogenic effects and many of these metabolites plays a vital role in imparting the toxicity to benzene [9,10]. Benzene gets metabolized into phenol, hydroquinone (HQ), S-phenylmercapturic acid (SPMA) and catechol, which are excreted in urine after conjugation or further get metabolized to 1, 4-benzoquinone and 1,2,4-trihydroxybenzene (THB). Another transformed product of benzene is *t,t*-muconic acid (*t,t*-MA), which is a potential biomarker for benzene exposure [11–13]. According to researchers, HQ, Catechol, *t,t*-MA and SPMA are the sensitive markers for the illustration of the benzene exposure in humans [14].

Several analytical methods have been reported on the determination of benzene metabolites through various analytical instruments, such as, high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS) [11,15–18], gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) [19,20]. All the methods reported earlier either uses liquid–liquid extraction (LLE) or solid-phase extraction (SPE) for sample extraction before instrumental analysis. However, these methods consume a large amount of toxic and hazardous solvents and extract interfering substances along with the desired analytes through non-specific interactions [21,22].

Molecularly imprinted polymers (MIP) are the materials which possess artificial receptor like-binding sites and show to exhibit selectivity to the analytes of interest [23,24]. These selective sites were generated in polymeric material by copolymerization of the functional monomer in the presence of template (analyte of interest) and crosslinker. On removing the template from the polymeric matrix, artificial binding sites were intact in the polymer and were complimentary to the template's shape and size [25,26]. MIPs are shown promising applications to various fields and used them as sensors, solid-phase extraction sorbents, drug delivery systems, etc. due to their high binding affinity and high stability at harsh conditions. These materials has reusability of 30–80 extractions without losing recognition abilities and found to be thermally stable [27]. Various methods like bulk polymerization, precipitation polymerization, suspension polymerization, multi-step polymerization were used during the synthesis of MIPs [28]. Among these methods, precipitation polymerization is a simple to use method for the synthesis of uniformly sized micro and nanosphere MIPs (nMIP) [29]. Other methods suffer from many complications, for example; suspension polymerization method requires stabilizers that make the method costly and bulk polymerization requires mechanical grinding and sieving, which leads to the formation of irregular size and shape of MIP particles and loss of material. Advantages of precipitation polymerization include (a) synthesis of a uniform size particles (b) increase in surface area and (c) receptiveness by the template for the recognition sites and hence improve the performance for sensing and separation applications [30,31].

In recent years, the use of gas chromatography-mass spectrometry (GC–MS) has increased in the analysis of environmental contaminants due to its advantage of simultaneous identification and quantification, better resolution, low ion suppression, higher precision and provision for library of the reference spectra's for several compounds and low cost of analysis in comparison to liquid chromatography-tandem mass spectrometry (LC–MS/MS). Analysis of polar analytes using GC–MS cannot achieve without their conversion into non-polar and more volatile compounds through derivatization. The most preferred derivatization for polar analytes is silylation as it can react with various polar fucntional groups such as $-NH_2$, $-OH$, $-COOH$ etc., which enhances the volatility and improves peak shape and makes these analytes amenable for GC–MS analysis [32–34]. However, the in-vial silylation requires external moisture-free conditions, need of a large amount of reagent and takes longer time for the completion of reactions. To aovid these limitations, Rasmussen has proposed injector port derivatization which takes place inside the injector port of GC or GC–MS [35]. This will reduces the time required for derivatization (30 min to 1–2 sec) and amount of silylating reagent (100 μL to 1–2 μL) and also eliminates the requirement of external anhydrous conditions for silylation [36–39].

The combination of molecularly imprinted solid-phase extraction (MISPE) with injector port silylation (IPS) helps in the selective, rapid and sensitive analysis of urinary benzene metabolites (UBMs) by GC–MS/MS. Only few reports were available for UBMs analysis based on MIP approach. One such report deals with the analysis of *t,t*-MA in smokers' urine through MISPE followed by HPLC analysis while others combine the MISPE with dispersive liquid–liquid microextraction (DLLME) for the analysis of *t,t*-MA followed by GC–MS analysis [40,41]. Reports for the analysis of phenol, HQ, catechol based on MIP were also available, but they deal with their individual analysis using individual MIPs [42–45]. As per our knowledge, no report is available based upon the synthesis of uniformly sized MIP nanospheres using dual-template imprinting approach through precipitation polymerization for the simultaneous extraction of six UBMs through MISPE followed by IPS and GC–MS/MS analysis. Approach of dual-template imprinting helps in eliminating the requirement of synthesis of individual MIPs for each metabolite, which could not only save the time but also makes analysis rapid and cost effective [46,47]. Present communication deals with dual-template imprinting approach in the synthesis of nMIP for UBMs based on precipitation polymerization method, which was successfully applied to the selective preconcentration and analysis of six UBMs from urine samples of smokers, kitchen workers and petroleum workers.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents used for this study were of analytical grade, unless otherwise stated. *t,t*-MA, catechol, HQ, phenol, THB, SPMA, methacrylic acid (MAA), 4-vinylpyridine (4-VP), acrylamide (AM), ethylene glycol dimethacrylate (EGDMA), divinylbenzene (DVB) and trimethylolpropane dimethacrylate (TRIM), 2,2′-azobisisobutyronitrile (AIBN) and N,O-Bis(trimethylsilyl) trifluoroacetamide + trimethylchlorosilane (BSTFA + TMCS) (99:1, v/v) were procured from Sigma–Aldrich (St. Louis, MO, USA). All the solvents used for this study were procured from Sd. Fine Pvt. Limited (Mumbai, India) and Merck (Darmstadt, Germany), whilst dimethyl sulphoxide (DMSO) was procured from Laboratory Reagent (Baroda, India). Empty SPE cartridges were purchased from Supelco (Bellefonte, PA, USA). A stock solution of catechol, HQ, phenol, THB and SPMA was prepared in acetonitrile while the stock solution of *t,t*-MA was prepared in ethanol. The working standard solution was prepared by appropriate dilution of the stock solution.

2.2. Preparation of MIP

Precipitation polymerization method was used to prepare nMIP for UBMs. *t,t*-MA and THB (0.25 mmol of each) were taken as templates and dissolved in 2 mL of acetonitrile and 3 mL of DMSO in a round bottom flask. To this, the functional monomer MAA (5 mmol) was added and ultrasonicated for 2 min, then kept it in an ice bath for 30 min with constant stirring. Add 35 mL of acetonitrile, EGDMA (5 mmol) and 500 μL of AIBN to the solution and sonicate for another 20 min. After sonication, the pre-polymerization mixture was purged with nitrogen for 5 min. Now, the reaction mixture was placed in an oil bath at 75 °C for 24 h. After completion of the polymerization, the flask was taken out from the oil bath and cooled