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Simultaneous quantification of three alkylated-purine adducts in human urine using sulfonic acid poly(glycidyl methacrylate-divinylbenzene)-based microspheres as sorbent combined with LC-MS/MS

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ABSTRACT

Three alkylated DNA adducts, N^3-methyladenine, N^3-ethyladenine and N^7-ethylguanine, have been proved to be potential biomarkers for DNA injury caused by exposure to cigarette smoke. In this study, a highly specific and sensitive method using a new mixed-mode sulfonate-functionalized poly(glycidyl methacrylate-divinylbenzene) as a solid-phase extraction sorbent was developed for the analysis of these three alkylated-purine adducts in human urine. Under optimized conditions, the prepared sorbent interacts strongly with these urinary adducts, demonstrating high clean-up efficiency and extraction recovery. The method detection limits (S/N ≥ 3) of N^3-MeA, N^3-EtA and N^7-EtG were 1.75, 0.20, and 0.15 pg mL^{-1}, respectively, while the method quantitation limits were found to be 5.78, 0.66, and 0.49 pg mL^{-1} for N^3-MeA, N^3-EtA and N^7-EtG, respectively. The intra-day and inter-day precisions were investigated, of which were in the range of 1.6–3.8% and 3.2–5.6%, respectively. The recovery values of the alkylated DNA adducts in spiked urine sample were ranged 89.7–104.5%. Their concentrations were statistically significantly higher in smokers than in nonsmokers. These results show that the proposed method is suitable for the analysis of alkylated DNA adducts.

1. Introduction

Tobacco smoke is a complex mixture with over 5000 chemical constituents. More than seventy of these are carcinogenic agents and causally related to human cancers, such as lung, oral and bladder cancer [1]. Prolonged exposure to these carcinogens may cause the formation of DNA adducts in human body, and consequently, the concentration of DNA adducts in human body is regarded as the earliest indicator of biological effects of exposure to carcinogens. Alkylation DNA adducts which are formed by the reaction of DNA with alkylating agents or other carcinogens, are considered to be positively associated with smoking [2–5]. Furthermore, the level of alkylation DNA adducts is positively correlated with carcinogenesis and cancers, has already been confirmed by animal experiments [6,7]. Higher levels of O^4-ethylthymidine were detected in the normal lung tissue adjacent to tumors in smokers than in nonsmokers suffering from lung cancer [8,9].

To date, many alkylated DNA adducts have been identified in humans, including those methylated or ethylated at the *O*-6, *N*-2, *N*-3 and *N*-7 sites of guanine; the *N*-7 and *N*-3 sites of adenine; the *O*-2 site of cytosine; and the *O*-2, *O*-4 and *N*-3 sites of thymine [5,10,11]. Due to the activity of *N*-7 position of guanine and of the *N*-3 position of adenine, the adducts N^3-methyladenine (N^3-MeA), N^3-ethyladenine (N^3-EtA) and N^7-ethylguanine (N^7-EtG) are formed following depurination, and can be detected in human urine [12]. The N^3-MeA is a promutagenic and cytotoxic compound, while N^3-EtA and N^7-EtG are not regarded as promutagenic DNA lesions because the position of apurinic sites can be repaired by glycosylases of the base excision repair system [13,14]. Nevertheless, in the absence of efficient repair of the apurinic sites, a break in the DNA strand and opening of the imidazole ring could occur. Furthermore, higher levels of alkylated-purine adducts have been reported [2,3] in urine from smokers as opposed to that of nonsmokers'. These alkylated DNA adducts could therefore be considered to be potential biomarkers for DNA lesions caused by cigarette smoke.

The levels of human urinary DNA adducts could be used as a signal of the balance between the body repair system and the levels of adducts

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induced by exposure to carcinogens at a certain time. Thus, a highly specific, sensitive and quantitative analytical method for alkylated DNA adducts in urine would be useful. So far, many analytical techniques, including an enzyme-linked immunosorbent assay [15], HPLC [16], ICP-MS [17], electrochemical biosensor [18] and GC–MS [19,20], have been employed for the determination of urinary alkylated DNA adducts. Although these methods have been proved to be successful, they still have drawbacks, including low sensitivity, laborious sample preparation and the need for chemical derivatization procedures. Recently, due to its high specificity and sensitivity, LC-MS/MS has emerged as a powerful technology for the determination of DNA adducts [21–25]. Sample pretreatment methods are however essential as a consequence of the trace level of DNA adducts and the complex matrix effect of urine samples. With its simplicity and low solvent consumption, solid-phase extraction (SPE) has proven to be an attractive alternative means of sample preparation [26–28]. Because traditional SPE sorbents usually employing only ion-exchange or hydrophobic interaction technology, these indiscriminate adsorption effects may result in inaccurate determination, a large amount of interfering matrix material may be extracted along with the target analytes. It is important to develop an effective sample preparation method for the analysis of DNA adducts in urinary samples.

Due to their large specific surface area and high chemical stability in solution at extreme pH, microspheres of organic polymers, such as poly (methacrylates), poly(acrylates) and poly(styrene-divinylbenzene) microspheres have revealed their advantages as separation materials [29–33]. Zhang [33] reported the synthesis of glycidylmethacrylate-divinylbenzene microspheres by a dispersion polymerization method. These microspheres are functionalized with propyl sulfonic acid and packed into fused-silica capillaries to separate proteins. The polymerization method can seriously affect the uniformity, surface area, particle and pore size, as well as the adsorption capacity of the polymer. Currently, mono-dispersed porous microspheres, obtained by the copolymerization of glycidylmethacrylate (GMA) and divinylbenzene (DVB) using the swelling and polymerization method, have been successfully applied as chromatographic separation materials [34–36]. Owing to the peculiar structural characteristics of the monomers, poly (glycidyl methacrylate-divinylbenzene) (poly(GMA-DVB)) microspheres can offer a variety of properties, such as hydrophobic, π–π, stereoselective and polar interactions [34–36] that are relevant to their separation capability. Compared with other organic polymer microspheres, poly(GMA-DVB) microspheres can be easily modified by direct covalent immobilization of functional groups. In this case, a novel material capable of cationic exchange interactions was prepared by incorporating a modified sulfonic acid group into poly(GMA-DVB). An attempt was made to utilize the functional microsphere as an SPE sorbent in the cleanup and enrichment of alkylated DNA adducts in urinary samples.

In the present work, a novel sulfonic acid poly(glycidylmethacrylate-divinylbenzene) (SA-poly(GMA-DVB)) sorbent with multiple interaction was used to develop a reliable sample preparation procedure for the analysis of three alkylated-purine adducts in human urine. All the main factors that affect extraction efficiency were investigated, and the results from the proposed LC-MS/MS method based on SA-poly (GMA-DVB) sorbent suggested that it is an effective method for the determination of alkylated-purine adducts in complex biological samples.

2. Experimental

2.1. Chemicals, materials and apparatus

HPLC-grade acetonitrile and methanol used for chromatographic analysis were obtained from J&T Baker Chemicals (USA). HPLC-grade ammonium formate and formic acid were supplied by TEDIA Company Inc. (Fairfield, CT, USA). N^3-MeA, N^3-EtA, N^3-MeA-d_3, and N^3-EtA-d_5 were supplied by Toronto Research Chemicals (North York, Canada). Standards of N^7-EtG and N^7-EtG-d_5 with purity of > 98.5% by LC using a UV detector, were synthesized according to previously reported methods [4]. The deuterated ratio was above 99%. Ultrapurified water was produced by a Millipore Milli-Q purification system (Billerica, MA, USA). The sorbents of C18, HLB, and MCX were obtained from Waters Corporation (Ann Arbor, MI, USA).

All samples were analyzed on an Agilent 1290 Infinity LC Series (Agilent Technologies, Santa Clara, CA, USA) coupled with an API 5500 triplequadrupole MS equipped with a TurboIonSpray™ source from Applied Biosystems SCIEX (Foster City, CA, USA). A Waters Atlantis T3 liquid chromatography column (3.0 mm × 100 mm, 3 μm) was used for LC separation. A scanning electron microscope (SEM, Auriga FIB, Zeiss Company, Germany) was used to characterize the surface morphology. A surface area and porosity analyzer, ASAP 2020 HD88 (Micromeritics, Atlanta, GA, USA) was used to measure the surface characteristics. The infrared (IR) spectra were recorded on a Bruker Vector 22 Fourier-transform spectrometer (Ettlingen, Germany). Elemental analyses were performed on a Flash EA 1112 elemental analyzer (Thermo Electron Corporation).

2.2. Preparation of SA-poly(GMA-DVB)

SA-poly(GMA-DVB) was prepared by a two-step modification process [33,34]. First, poly(GMA-DVB) microspheres were synthesized by a swelling and polymerization method reported previously [34], and used as a precursor in the following reaction. Second, SA-poly(GMA-DVB) was obtained by means of the sulfonation reaction described as follows: poly(GMA-DVB) microspheres (3.0 g) were dispersed into 0.1 mol L^{-1} H_2SO_4 solution, and reacted at 60 °C for a further 24 h. After having been washed and dried, the microspheres were added to an alkaline solution of allyl glycidyl ether, and stirred at 50 °C for 24 h. The allyl substituted poly(GMA-DVB) was obtained after washed and dried.

The allyl substituted poly(GMA-DVB) was dispersed into sodium metabisulfite (3 mol L^{-1}, pH 6.5), and reacted at room temperature for 24 h under a gentle stream of N_2. After the reaction was finished, the microspheres were filtered, and washed in sequence with methanol and distilled water. Then the SA-poly(GMA-DVB) was dried under vacuum at 60 °C for 24 h. The detailed synthetic procedure for SA-poly(GMA-DVB) is illustrated in Fig. 1.

2.3. Calibration solutions

Standard stock solutions of the three alkylated-purine adducts were prepared in methanol-water solution (v/v, 1:1), and stored at −20 °C in a refrigerator. Standard working solutions with concentrations of 2.5, 5, 10, 20, 40, 60, 80, 120, 160, 240 and 320 ng mL^{-1} for N^3-MeA, and 0.05, 0.1, 0.2, 0.5, 1, 1.5, 2, 2.5, 5, 10 ng mL^{-1} for N^3-EtA and N^7-EtG, were prepared by serial dilution of the stock solution with water. Working solution of internal standards N^3-MeA-d_3, N^3-EtA-d_5 and N^7-EtG-d_5 at concentrations of 50, 2.5 and 2.5 ng mL^{-1} respectively were prepared by dilution of the stock solution.

2.4. Urine sample collection

In studies approved by the Ethics Committee of Zhengzhou University, forty healthy adult male volunteers aged > 21 years old (including 20 smokers and 20 non-smokers) were recruited. The smokers typically smoked 10–20 cigarettes/day. All volunteers refrained from eating cooked food for two days before the urine collection. There were no smoking restrictions for the smokers during the experimental period. For all the volunteers, 24 h urine samples were collected and stored at −80 °C prior to analysis.