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Nanomaghemite core functionalized with ion-exchange resins for isolation of biogennic amines

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Paramagnetic particles composed of nanomaghemite core suitable for isolation of biogennic amines

Herein, we describe a synthesis of nanomaghemite core, functionalized with two types of ion-exchange resins - Dowex and sulfoxyethyl cellulose, and their utilization for evaluation of isolation potential towards a chemical group of biogenic amines (BAs). Isolation was carried out after charging of resins with Britton-Robinson buffer (pH 2) and the binding attributes were characterized by using ion-exchange chromatography with Vis detection (440 nm) of complexes, resulting from post-column derivatization with ninhydrin. Based on recovery calculations, Dowex-based particles were able to bind most of BAs with relatively good recovery (36% for tyramine; 33% for cadaverine), while sulfoxyethyl cellulose particles were more specific towards spermidine (21%), but not to other BAs. Our suggested particles are suitable for BAs isolation, and thus may serve as a first isolation step of BAs prior analysis. Moreover; due to their perfect paramagnetic properties may be applied in development of sensors.

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1. Introduction

Biogenic amines (BAs) are basic nitrogenous low mass compounds with aliphatic (spermine, spermidine, putrescine, cadaverine), heterocyclic (e.g. tryptamine, histamine) or aromatic (e.g. tyramine) structure derived mainly from the decarboxylation of amino acids[1]. They may be formed by the action of yeast, lactic acid bacteria or other microorganisms during alcoholic and malolactic fermentation[2].

Most of BAs have strong physiological effects and play important biological role as source of nitrogen and precursors for synthesis of broad spectrum of biomolecules, such as hormones or nucleic acids[3]. On the other hand BAs have been widely studied as potentially toxic substances, since excessive intake of BAs manifests as food poisoning[4]. Moreover, BAs are potential precursors for the formation of carcinogenic N-nitroso compounds[5].

In order to determine the concentrations of biogenic amines in biological matrices, techniques providing high resolution and sensitivity are demanded. To determine BAs is challenging because of strong polarity and no natural UV absorption nor fluorescence. Thus BAs must be pre or post-column derivatized before detection[6]. Magnetic separation may be employed for isolation of a sample from complicated biological matrixes (food, body fluids) and may thus form the first separation and pre-concetration step prior to analysis to enhance an applied methodological approach[7].

The main aim of the present study is synthesis of nanomaghemite core and its functionalization with ion-exchange resins (Dowex and sulfoxyethyl cellulose), which can provide binding sites for chosen BAs (Tyramine-Tyr; Putrescine-Put; Histamine-His; Cadaverine-Cad, Spermine-Spm and Spermidine-Spd respectively).

Synthetic particles were finally employed for isolation and subsequent analysis by using ion-exchange chromatography.

2. Materials and Methods

2.1 Chemicals and pH measurements

Standards of biogenic amines (Tyr, Put, His, Cad, Spm, Spd) in purity of 99 % were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solutions of biogenic amines were prepared with dilution buffer called sodium cycle composed of 1.5 mM N_3Na, 197 mM NaCl and 73 mM $C_6H_8O_7$ in MilliQ H_2O. Further, we used citric acid, sodium citrate, isopropanol, potassium hydroxide, potassium bromide, hydrochloric acid and ninhydrine all purchased from Sigma-Aldrich. Methyl cellosolve was purchased from Ingos (Prague, Czech Republic), as well as tin chloride. All buffer solutions were prepared with deionized water obtained using a reverse osmosis equipment Aqual 25 (Aqual s.r.o., Brno, Czech Republic).

2.2 Synthesis of Paramagnetic Microparticles

Two types of paramagnetic microparticles were employed in this study. Both were based on nanometric maghemite core, synthesized according to protocol[8].

Nanomaghemite obtained in this way was further modified. (I) In the case of paramagnetic microparticles MAN1, 20 mL of nanomaghemite solution was mixed with 0.5 g sulfoxyethyl cellulose (Sigma Aldrich) (m/v) or (II) with 0.5 g of Dowex 50WX4-400 (Sigma Aldrich) (m/v). Mixtures were stirred at Biosan OS-10 (Biosan, Riga, Latvia) overnight. Resulting products were separated using external magnet and washed with water. Finally the product was dried at 40 °C.

2.3 Sample Preparation

To obtain information about behaviour of our own prepared PMPs, biogenic amines (volume 250 µL) in concentrations of 100 $\mu g.mL^{-1}$ were bound to them according to isolation conditions optimized in our preliminary study dealing with amino acid[9]. For isolation 250 µL of suspension of paramagnetic microparticles in PBS (10 $mg.mL^{-1}$) was employed. After isolation, sample was dissolved in 3 M hydrochloric acid (250 µL) and evaporated using nitrogen evaporator Ultravap RC (Porvair Sciences, Leatherhead, UK). Finally evaporated sample was resuspended with dilution buffer (250 µL) and analysed using ion-exchange liquid chromatography. In addition, real urinary samples (250 µL) were prepared similarly to be ready to be analysed by paramagnetic microparticles.

2.4 Ion-Exchange Liquid Chromatography

As a second part of our 2D separation approach an AAA 400 (Ingos, Prague, Czech Republic) ion-exchange liquid chromatography (IEC) apparatus was used. The system consisted of a glassy filling chromatographic column and steel precolumn, two chromatographic pumps for transport of elution buffers and derivatization reagent, cooled carousel for 25 eppendorf tubes of, dosing valve, heat reactor, Vis detector, and cooled chamber for derivatization reagent according to[9].

2.5 Descriptive Statistics

Mathematical analysis of the data and their graphical interpretation were realized by Microsoft Excel®, Microsoft Word® and Microsoft PowerPoint®. Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise. The detection limits (3 signal/noise, S/N) were calculated according to Long and Winefordner[10], whereas N was expressed as standard deviation of noise determined in the signal domain unless stated otherwise.

3. Results and Discussion

BAs analyses are commonly challenging due to nature of target analytes and the undesired effects of matrix which complicates the accuracy and sensitivity of analyses. Since magnetic separation offers few advantageous attributes such as simple operation, ability to pre-concentrate a sample for further analyses or low production costs, we attempted to combine the magnetic separation with subsequent off-line analysis by using IEC.

IEC separation is commonly employed for separation of BAs, based on their pI[11] and we uti-

lized this technique for monitoring the binding capacity/recovery of paramagnetic particles. The conditions for separation and detection of BAs were optimized in our previous study[12]. The separation and elution was based on gradient elution using buffers with different ionic strength and pH together with temperature gradient. By using this method, we were able to discriminate all of six chosen biogenic amines (Tyr, Put, His, Cad, Spm, Spd) as is shown in **Fig. 1** with LoD between 59 - 110 ng.mL^{-1}.

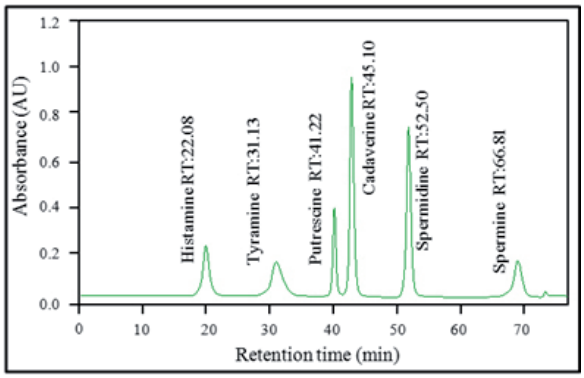

Figure 1. Chromatogram of mixture of six biogenic amines (His, Tyr, Put, Cad, Spm, Spd) in final concentration of 100 µg.mL^{-1}, obtained by using ion-exchange liquid chromatography with Vis detection in wavelength of 570 nm.

Magnetic separation by using nanomaghemite-based materials can be easily used for isolation and preconcentration of many different molecules, such as sarcosine[13], viral particles [14] and many others. Nanomaghemite can be modified with various chemical moieties, forming both - sites for covalent or non-covalent bonds. Thus we employed nanomaghemite to constitute a core for further modification with shell formed of ion-exchange resins: *i*) Dowex in case of paramagnetic particles, hereinafter named MAN21 and *ii*) sulfoxyethyl cellulose in paramagnetic particles, hereinafter named MAN1. Both resins are insoluble support structures, providing high surface area, where the trapping of ions occurs with concomitant releasing of other ions[15]. To remove the undesired impurities, paramagnetic particles were washed with Britton-Robinson buffer (pH 2), which simultaneously introduction of SO_3^- of both ion-exchange resins. After washing, standards of BAs (100 µg.mL^{-1}) were isolated following optimized incubation protocol[9] and finally BAs immobilized by using external magnet were dissolved in HCl (3 M). The evaporated samples were then diluted in dilution buffer for subsequent IEC analyses. Chromatograms of determined biogenic amines with corresponding formula are shown in **Fig. 2A-F**. It is obvious that paramagnetic particles MAN21 shows bigger affinity towards most of BAs (Spm, Tyr, Put, His and Cad), whereas MAN1 were evaluated as more suitable only for isolation of Spd. To reveal a real binding attributes of both types of paramagnetic particles, recoveries of isolation were calculated as (1):

$$cBAdetermined / cBAapplied * 100 \quad (1)$$

Recovery describes the real binding capacity of certain material and thus it is highly important for its characterization.

In **Fig. 3** there are summarized recoveries for both particles

From **Fig. 3A** it is obvious that Dowex functionalization (MAN21) lead to much higher binding of BAs (Tyr - 36%; Cad – 33%; His - 30%; Put - 22%; Spm - 12% and Spd - 5%, respectively), when compared to sulfoxyethyl cellulose (MAN1) (Spd - 21%; Cad - 11%; Tyr - 8%; Spm - 6%; Put - 5%; His - 3%). Thus, it can be concluded that MAN21 offer lower specifity towards chosen BAs, however they are able to isolate practically all of them instead of Spd with relatively good yields. On the other hand MAN1 exhibit lower isolation yields; nevertheless they bind more specifically Spd. This phenomenon is tightly connected with core functionalization and the principle of increased/decreased specifity is hard-to-describe, although it is probably linked with steric arrangement of functional moieties and their charge-charge interaction with target BA.

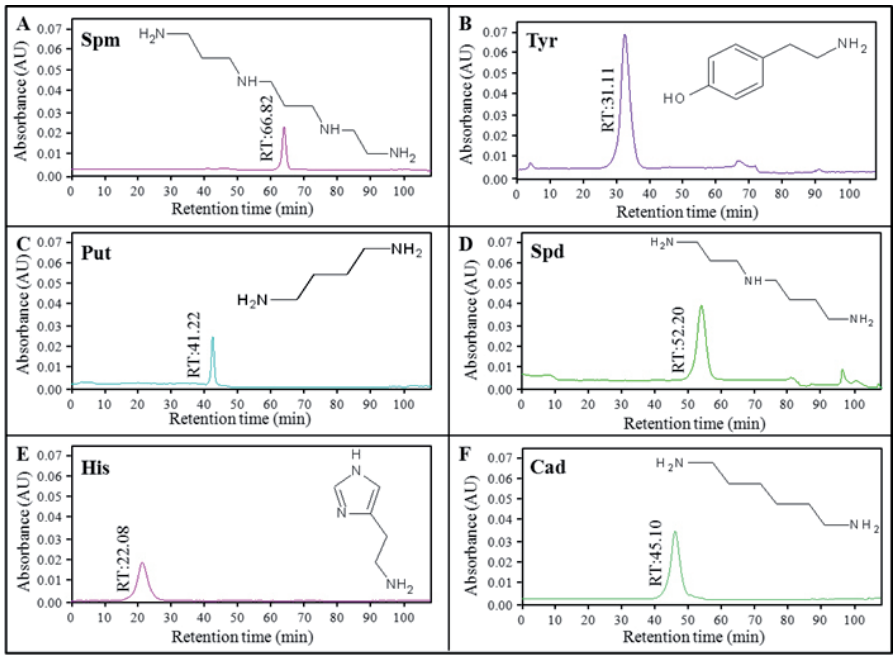

Figure 2. The chromatogramms of biogenic amines (100 μg.mL^{-1}) corresponding to (**A**) spermine isolated by using MAN21, (**B**) tyramine isolated by using MAN21, (**C**) putrescine isolated by using MAN21, (**D**) spermidine isolated by using MAN1, (**E**) histamine isolated by using MAN21 and (**F**) cadaverine isolated by using MAN21, with expression of their retention times. All records were obtained by using ion-exchange liquid chromatography with Vis detector (440 nm).

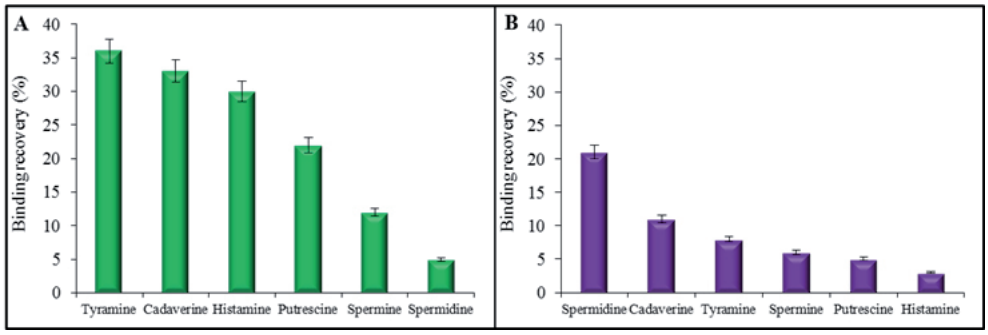

Figure 3. Expression of binding recovery of all of six biogenic amines (His, Tyr, Put, Cad, Spm, Spd) after isolation on paramagnetic particles (**A**) MAN21 and (**B**) MAN1. Biogenic amines (100 μg.mL^{-1}) were isolated following optimized conditions and recovery was calculated from quantification of biogenic amines carried out on ion-exchange liquid chromatography.

4. Conclusion

We succesfully suggested and synthesized paramagnetic particles composed of nanomaghemite core functionalized with two types of ion-exchange resins - Dowex and sulfoxyethyl cellulose. Both paramagnetic particles were able to bind biogenic amines, particularly spermidine, tyramine, cadaverine and histamine, and thus may be employed as a first isolation step of these compounds from various biological matrixes with subsequent separation on ion-exchange liquid chromatography or other analytical approaches. Moreover, paramagnetic particles are well suitable for utilization in fluidic devices or biosensors combined with the power of magnetic field.

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The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study.

The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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