MAGHEMITE PARTICLES FOR SPERMIDINE SEPARATION

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Abstract

We report the optimal conditions for the separation of spermidine from different types of samples, for example blood or cancer cells and its subsequent determination by ion-exchange liquid chromatography (IEC) with UV-VIS detector. Here in, we synthesized paramagnetic particles able to isolate and immobilize spermidine from blood of patients with cancer or cancer cells and thus preconcentrate it for analysis. Dowex surface was covered by nanomaghemite (γ-Fe_2O_3) or maghemite particles surface was modified by chitosan and sulfoxyethyl cellulose. The best separation properties showed Dowex microparticles. The paramagnetic particles can be used in the future for isolation of spermidine from real samples and diagnosis of cancer.

Keywords: Ion exchange chromatography, maghemite, spermidine, chitosan and sulfoxyethyl cellulose

1. INTRODUCTION

Spermidine is a polyamine, usually isolated from sperm, located in the living cells, tissues and ribosomes [1]. Spermidine plays important role in various biological processes, such as regulation of plant growth, assisting *in vitro* process of transcribing RNA, and/or inhibition of nitric oxide synthase (NOS) [2]. Spermidine and spermine, are promising biomarkers of Parkinson disease (PD). Each polyamine enhance longevity *via* autophagy induction, under physiological conditions demonstrated ability of their metabolite to act as an diagnostic, age-related and severity of Parkinson disease [3]. On the other hand, increased levels of polyamines are toxic to cells, and can facilitate cell death based on the oxidative stress [4]. Polyamines, such as spermidine, spermine and putrescine have antioxidant properties, play major roles in the prevention of chronic diseases for example cardiovascular diseases and in the differentiation and development of immune system [5]. Currently, different methods are developed for spermidine detection. Most of them are based on chromatography (GC, HPLC) [6,7] with tandem of mass spectrometry (MS) [8], IEC [9] or CE [10]. In our study, we decided to use ion exchange chromatography with post-column ninhydrin derivatization and VIS detector for spermidine determination [10].

2. METHODOLOGICAL BASES AND EXPERIMENTAL PART

2.1. Chemicals

Spermidine of purity 99 % was obtained from Sigma Aldrich (St. Louis, Missouri, USA). Solution of spermidine for preparing of calibration curve was prepared in the dilution buffer Na: TDG (N_3Na - 0.10 g, NaCl -11.5 g, $C_6H_8O_7$ - 14 g per 1L H_2O). For experiment were used citric acid, NaCl, N_3Na, TDG, HCl 35 %, ninhydrine,

sulfoxyethyl cellulose, chitosan and Dowex from Sigma - Aldrich, Methylocelosolve (Ingos, Prague, Czech Republic), $SnCl_2$ (Ingos, Prague, Czech Republic).

2.2. Ionex chromatography

An AAA 400 (Ingos, Czech Republic) liquid chromatography apparatus was used for determination of amino acids. The system consists of a glassy filling chromatographic column and steel precolumn, two chromatographic pumps for transport of elution buffers and derivatization reagent, cooled carousel for 25 test tubes of 1.5 - 2.0 mL volume, dosing valve, heat reactor, VIS detector and cooled chamber for derivatization reagent. Chromatographic columns for transfer of elution buffers and derivatization reagent are able to work at flow 0.01-10 $mL \bullet min^{-1}$ under a maximum pressure of 40 MPa. Volume of injected sample was 100 µL with an accuracy of application RSD of about 1%. A two-channel VIS detector with a 5 µL flow volume cuvette was operated at wavelengths of 440 and 570 nm.

2.3. SEM characterization of PMPs

Structures of particles were characterized by electron microscope MIRA 3 XMU (Tescan, a.s., Brno, Czech Republic). The SEM was fitted with Everhart-Thronley type of SE detector, high speed YAG scintillator based BSE detector, panchromatic CL Detector. For automated acquisition of selected areas, a TESCAN proprietary software tool called Image Snapper was used.

2.4. Preparation of microparticles

MAN 1

Iron chloride(III) hexahydrate (2g) was dissolved in water (160 mL) and a solution of $NaBH_4$ (0.4 g) in 3.5 % NH_3 (20 mL) was added with stirring. After hydrogen evolution, the mixture was heated to boiling for 2 h and left overnight. Nanomaghemite was separated on magnet and washed several times with water. Sulfoxyethyl cellulose (0.5 g) was poured into nanomaghemite suspension, agitated overnight, separated on external magnet and dried at 40 ºC.

MAN 16

Nanomaghemite was prepared as described for MAN 1. Dowex (1 g) was poured to nanomaghemite suspension instead of cellulose.

MAN 25

In the preparation chitosan solution (1 ml, 1 %) was added with stirring to the suspension of nanomaghemite. Product was treated as in previous cases.

3. RESULTS AND DISCUSSION

Goal of our paper was primarily isolation and separation of spermidine, based on the adsorption of analyte on the paramagnetic microparticles and subsequent determination using ion-exchange liquid chromatography (IELC). We modified nanomaghemite with sulfoxyethyl cellulose (MAN 1), DOWEX (MAN 16) and chitosan (MAN 25). SEM characterization of the prepared microparticles is visualized of **Figures 1** and **2**. Paramagnetic particles on the surface of Dowex sphere are well seen on **Figure 1A** (MAN 16). The fibres of sulfoxyethyl cellulose covered with magnetic particles are visualized on **Figure 1B** and C for MAN 1, whereas the structure

of MAN 15 is seen on **Figure 2** at three different magnifications. All the three kinds of microparticles are stable in solution and are perfectly separated from the solution by external magnetic field. In our experiments of amines binding to paramagnetic particles, we have tested six amines. The results can be seen on graphs in **Figure 3**. These PMPs showed excellent properties for binding of spermidine (recovery 37.25 %) for MAN 16, 15.6 % for MAN 25 and 14.32 % for MAN1 (**Figure 3D**). We used Britton-Robinson buffer with pH 2 which causes spermidine protonation that leads to positive charging of molecules due to its pI = 5.3 for spermidine. Interaction between surface of magnetic microparticles and positively charged molecules provides the binding between them. These interactions depend on isoelectric points of polyamines, which are in this mode behaving as the ion-exchangers.

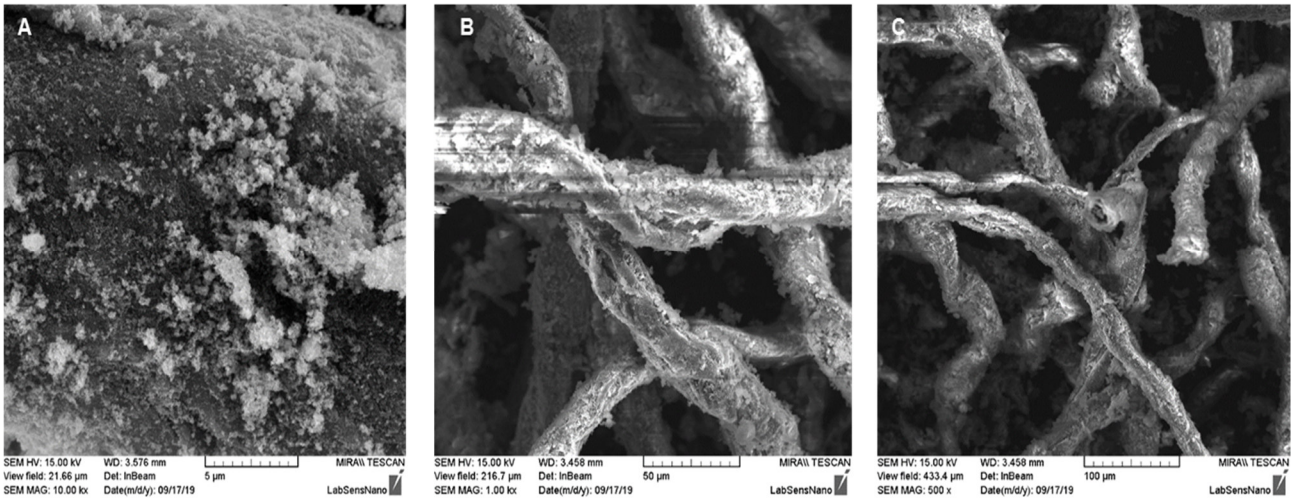

Figure 1 Characterization of paramagnetic particles. (**A**) SEM scan of paramagnetic microparticle MAN 16 in resolution of 5 µm, showing the particle size, (**B**) SEM scan of paramagnetic microparticle MAN 1 in resolution of 50 µm and (**C**) SEM of MAN 1, scale 500 µm.

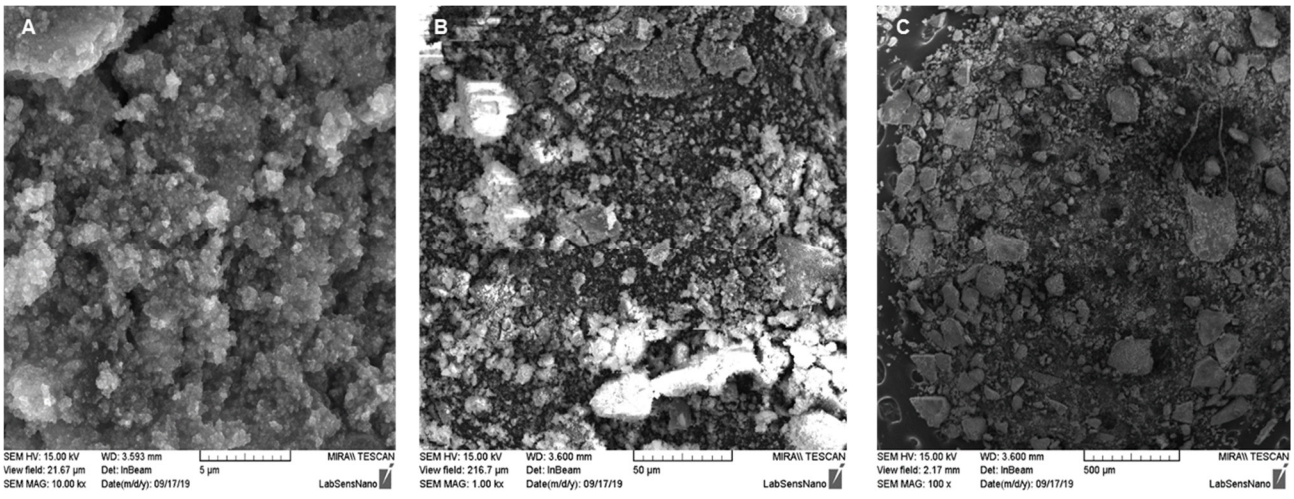

Figure 2 Characterization of paramagnetic particles MAN 25. (**A**) SEM scan of paramagnetic microparticles in resolution of 5 µm, showing the particle size. (**B**) SEM scan in resolution of 50 µm. (**C**) 500 µm.

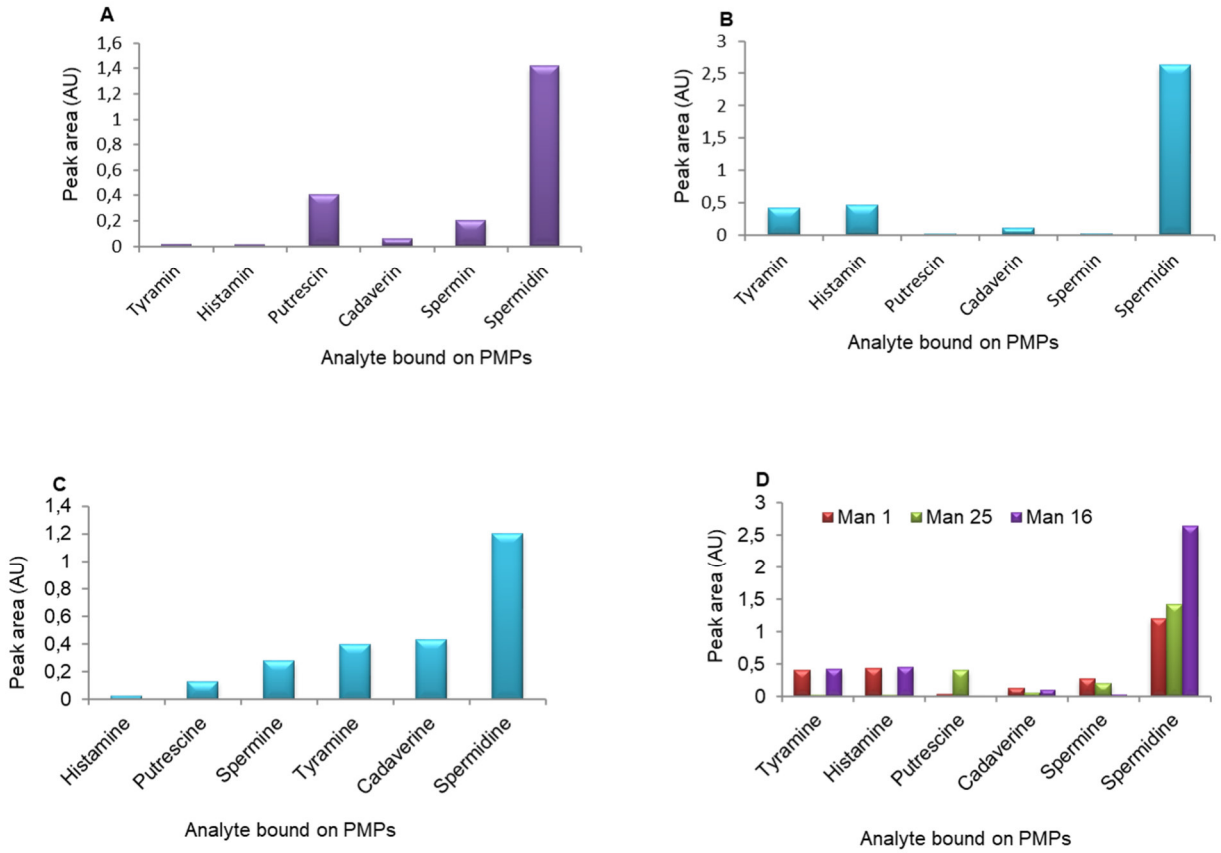

Figure 3 IELC characterization of paramagnetic particles (**A)** MAN 25, (**B**) MAN 16, (**C**) MAN 1, (**D**) IELC results showing ability of paramagnetic microparticles bound required substances specifically

3. CONCLUSION

In our study, we synthesized new paramagnetic microparticles able to bind spermidine that can be considered as promising biomarker of Parkinson disease. The paramagnetic microparticles (MAN 16) have potential to better isolation from the samples of plasma, cells and/or or tissue and in future can serve for application as a biosensor.

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REFERENCES

[1] MINOIS, Nadege. Molecular basis of the 'anti-aging' effect of spermidine and other natural polyamines - a mini-review. *Gerontology.* 2014. vol. 60, pp. 319-326.

[2] ANDERSEN, S.C., BASTOLA, D.R. and MINOCHA, S.C. Metabolism of polyamines in transgenic cells of carrot expressing a mouse ornithine decarboxylase cdna. *Plant Physiol.* 1998. vol. 116, pp. 299-307.

[3] SAIKI, Shinji, SASAZAWA, Yukiko, FUJIMAKI, Motoki, KAMAGATA, Koji, KAGA, Naoko, TAKA, Hikari, LI, Yuanzhe, Z., SOUMA, Sanae, HATANO, Taku, IMAMICHI, Yoko *et al.* A metabolic profile of polyamines in parkinson disease: A promising biomarker. *Ann. Neurol.* 2019. vol. 86, pp. 251-263.

[4] TIL, H.P., FALKE, H.E., PRINSEN, M.K. and WILEMS, M.I. Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. *Food Chem Toxicol.* 1997. vol. 35, pp. 337-348.

[5] MUNOZ-ESPARZA, Nelly C., LATORRE-MORATALLA, Luz M., COMAS-BASTE, Oriol, TORO-FUNES, Natalia, VECIANA-NOGUES, Teresa M. and VIDAL-CAROU, Carmen M. Polyamines in food. *Front. Nutr.* 2019. vol. 6, 108.

[6] PETRARCA, Mateus H., FERNANDES, Jose O., GODOY, Helena T., CUNHA and Sara C. Determination of polyamines in baby food by gas chromatography-mass spectrometry: Optimization of extraction and microwave-assisted derivatization using response surface methodology. *Food Anal. Meth.*2017. vol. 10, pp. 3548-3557.

[7] BRANDSTETEROVA, E., HATRIK, S., BLANARIK, I. and MARCINCINOVA, K. HPLC determination of polyamines in urine. *Neoplasma.* 1991. vol. 38, pp. 165-174.

[8] ONAL, Armagan, TEKKELI, Serife E.K. and ONAL, Cem. A review of the liquid chromatographic methods for the determination of biogenic amines in foods. *Food Chem.* 2013, vol, 138, pp. 509-515.

[9] CERNEI, Natalia, HEGER, Zbynek, KOPEL, Pavel, SKLADANKA, Jiri, ZITKA, Ondrej, ADAM, Vojtech and KIZEK, Rene. Isolation of biogenic amines using paramagnetic microparticles off-line coupled with ion exchange liquid chromatography. *Chromatographia.* 2014. vol. 77, pp. 1451-1459.

[10] DANIEL, Daniela, dos SANTOS, Vagner B., VIDAL, Rajh D.T. and do LAGO, Claudimir L. Determination of biogenic amines in beer and wine by capillary electrophoresis-tandem mass spectrometry. *J. Chromatogr. A.* 2015. vol. 1416, pp. 121-128.