# ISOLATION OF ARGININE USING MAGHEMITE PARTICLES

Natalia CERNEI[1,2], Pavel KOPEL[1,2], Pavel HORKY[3], Ondrej ZITKA[1,2], Vojtech ADAM[1,2]

*[1]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic, EU, vojtech.adam@mendelu.cz*
*[2]Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic, EU*
*[3]Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic, EU*

**Abstract**

In this paper, we report the optimal conditions for the isolation of amino acid arginine from different types of matrix, such as blood, plasma or tissue and their characterization using ion-exchange liquid chromatography with VIS detector. Paramagnetic microparticles able to isolate and immobilize amino acid arginine were prepared and used to preconcentrate arginine for subsequent analysis on ion-exchange liquid chromatography with VIS detector. Commercially available Dowex was covered by maghemite nanoparticles and the composite showed excellent binding and superparamagnetic properties. It was found that the paramagnetic microparticles were able to bind specifically amino acid arginine. Therefore, these paramagnetic microparticles have potential for better isolation of arginine and in future can use for application as a platform of delivery system.

**Keywords:** Ion exchange chromatography, maghemite microparticles, amino acids, arginine

## 1. INTRODUCTION

Arginine is semiessential amino acid and plays a various roles in biosynthetic pathways in tumor biology [1]. Major cellular functions of arginine were nitric oxide production [2], creatine production and polyamine synthesis [3]. Arginine influences in tumor cells has been shown to inhibit metastatic tumor growth [4]. Long-term and significant of arginine deprivation induces free radical formation and peroxynitrite stress especially in connection to arginine-dependent cancer cells [5]. Based on intensive study, it was shown the effect of arginine is to reduce nitric oxide (NO) production and the significant increase of oxidative stress [6]. On the other hand, L-arginine regulates blood flow, is the main cause of blood pressure and increased of the oxidative stress conditions of patients suffering from cardiomyopathy [7]. L-arginine, shows various functions in human health and may play a major role in age-related degenerative diseases such as Alzheimer's disease (AD) [8]. Nowadays, multiple methods are developed for semiessential amino acid arginine determination. Most of them are based on chromatography (GC, LC, UHPLC) [9] with tandem of mass spectrometry (MS) [10], IEC [11] or CE [12]. In our study we decided to use ion exchange chromatography with post-column ninhydrin derivatization and VIS detector for arginine determination [13].

## 2. METHODOLOGICAL BASES AND EXPERIMENTAL PART

### 2.1. Chemicals

Arginine of purity 99 % was obtained from Sigma Aldrich (St. Louis, Missouri, USA). Solution of arginine for preparing of calibration curve was prepared in the dilution buffer Na: TDG ($NaN_3$ - 0.10 g, NaCl -11.5g, $C_6H_8O_7$-14 per 1L $H_2O$). For experiment were used Citric acid, NaCl, $NaN_3$, TDG, HCl 35 % from Sigma - Aldrich, Ninhydrine from Sigma - Aldrich, Methylcellosolve (Ingos, Prague, Czech Republic), $SnCl_2$ (Ingos).

### 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•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. SECM characterization of DOWEX modified maghemite particles

Structure and elemental composition of paramagnetic microparticles were characterised by electron microscope. For documentation of the selected nanomaterials a FEG-SEM MIRA XMU (Tescan, a.s., Brno, Czech Republic) was used. This model is equipped with a high brightness Schottky field emitter for low noise imaging at fast scanning rates. The SEM was fitted with Everhart-Thronley type of SE detector, high speed YAG scintillator based BSE detector, panchromatic CL Detector and EDX spectrometer. The MIRA 3 XMU system is based on a large specimen chamber with motorized stage movements 130×130 mm. Samples were coated by 10 nm of carbon to prevent sample charging. A carbon coater K950X (Quorum Technologies, Grinstead, United Kingdom) was used. For automated acquisition of selected areas a TESCAN proprietary software tool called Image Snapper was used. The Accepted Article software enables automatic acquisition of selected areas with defined resolution. Different conditions were optimized in order to reach either minimum analysis time or maximum detail during overnight automated analysis. An accelerating voltage of 15 kV and beam currents about 1 nA gives satisfactory results regarding maximum throughput.

### 2.4. X-ray fluorescence analysis of PMPs

XRF elemental analysis of PMPs was carried out on Xepos (SPECTRO analytical instruments GmbH, Kleve, Germany) fitted with three detectors: Barkla scatter - aluminium oxide, Barkla scatter - HOPG and Compton/secondary molybdenum respectively. Analyses were conducted in Turbo Quant cuvette method of measuring. Analysis parameters were set to - measurement duration: 300 seconds, tube voltage from 24.81 to 47.72 kV, tube current from 0.55 to 1.0 mA, with zero peak at 5000 cps and vacuum switched off.

### 2.5. Preparation of microparticles

Maghemite nanoparticles were prepared by sodium borohydride ($NaBH_4$) reduction of iron chloride ($FeCl_3.6H_2O$). The product was separated by a magnet, washed with water and dried at 40 °C. Product was heated at 400 ºC for approximately 1 h in the muffle furnace (Verkon, Prague, Czech Republic). Product has a red color and was dispersed in water. To this solution was added Dowex with stirring on Biosan OS-10 overnight. Then the product was separated by a magnet, washed with water and dried at 40 °C.

## 3. RESULTS AND DISCUSSION

Aim of our study was an mainly isolation and 2D separation of the semiessential amino acid arginine, able to serve as a possible marker of metastatic tumor growth inhibition, based on the adsorption on the paramagnetic microparticles on commercially available Dowex and subsequent determination using ion-exchange liquid chromatography (IEC). We modified Dowex with nanomaghemite and $NH_2$ functional groups, and these PMPs showed excellent properties for binding of arginine (recovery 42.48 %) (**Figure 1.A**). Britton-Robinson buffer with pH 2 causes semiessential amino acid arginine protonation which leads to a positive charging of molecules due to its pI = 10.76. Interaction between surface of our magnetic microparticles and positively charged molecule provides the binding between them. These interactions depend on isoelectric point of amino

acid, which are in this mode behaving as the ion-exchangers. Other amino acids and tripeptides such as GSH and GSSG, showed also relatively good recoveries (serine recovery 1.1 %, leucine 10 %, sarcosine 1.3 %, GSSG 0.5 %,GSH 0.45 %, glutamic acid 6.7 %, glycine 0.8 %, valine 4.35 % and arginine 42.48 %, respectively); however, their quantity in real sample of blood are not so high to interfere during arginine binding to PMPs (**Figure 1D**). Furthermore, our second separation step - analysis using Ion Exchange Chromatography (IEC) - eliminates the influence of interferents to a minimum.

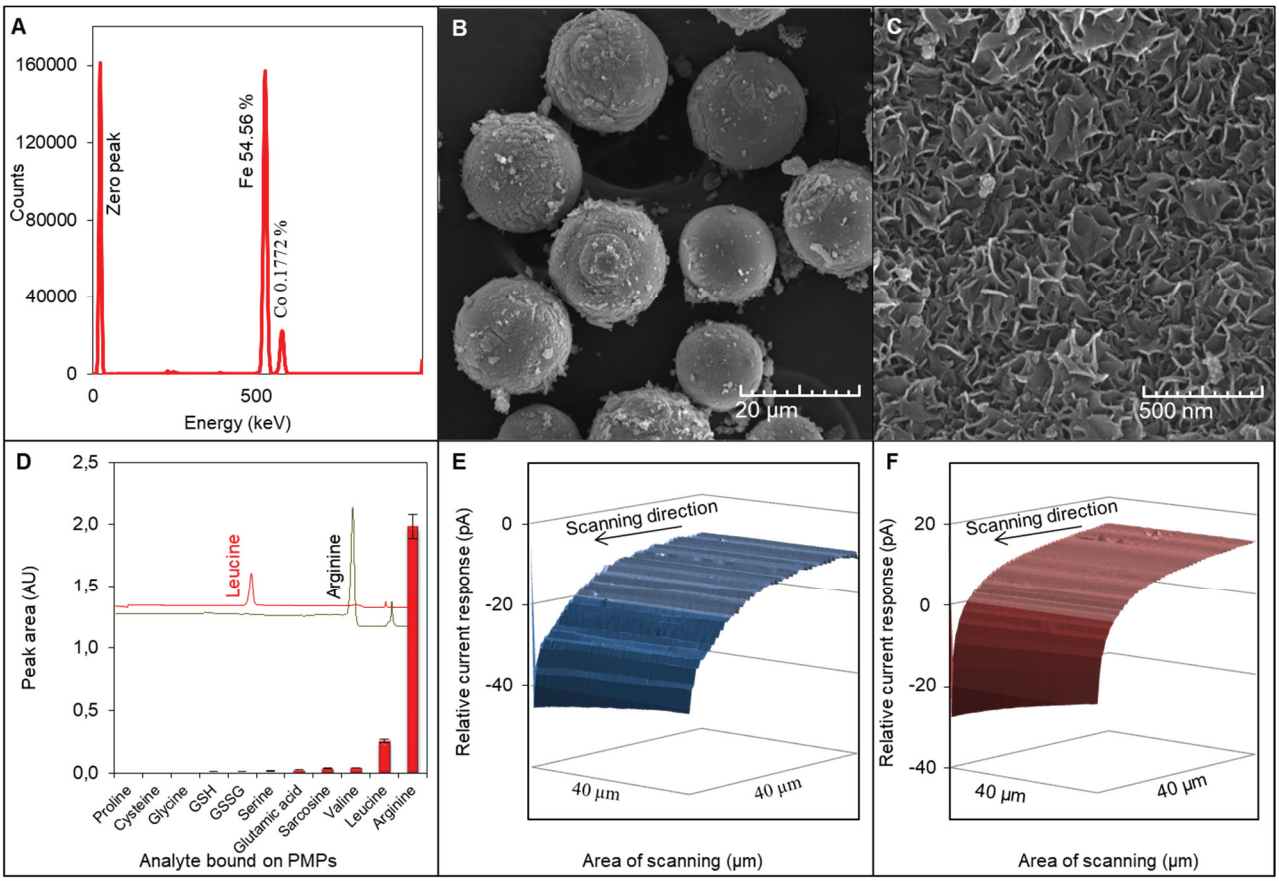


**Figure 1** Characterization of PMPs. (**A**) XRF showing elemental composition of paramagnetic microparticles. (**B**) SEM image in resolution of 20 µm. (**C**) SEM scan in resolution of 500 nm. (**D**) IEC results showing ability of PMPs bound required substances specifically. (**E**) SECM scan showing relative current response of PMPs without arginine bound. (**F**) SECM scan of PMPs with arginine bound showing increased relative current response.

After confirmation of capability to bind amino acid arginine, we carried out a various characterization of PMPs. Primarily characterization was XRF analysis providing information about elemental composition of microparticles. In the **Figure 1A** can be seen that iron was determined to be the most abundant element (Fe represented in 54.56 %) and cobalt (Co represented 0.1772 %). This information was not surprising, because we carried out SECM analysis for recognition of PMPs surface relative current response changes in dependence on arginine binding. In Fig 1B can be seen a micrograph expressing microparticles surface and morphology in resolution of 20 µm for PMPs and in **Figure 1C** is SEM micrograph of PMP surface in resolution of 500 nm. In **Figure 1E, F** can be seen 3D images, expressing a relative current response of PMP surface without arginine and with arginine bound.

## 4. CONCLUSION

In this study, we synthesized paramagnetic microparticles able to bind semiessential amino acid arginine - as a possible marker of metastatic tumor growth inhibition. The paramagnetic microparticles have potential to better isolation of arginine from the samples of plasma, cells or tissue and in future can serve for application as a platform for delivery system.

## ACKNOWLEDGEMENTS

***The financial support from NAZV QK1720349 is highly acknowledged.***

## REFERENCES

[1] ASSELIN, B.L. The three asparaginases - comparative pharmacology and optimal use in childhood leukemia. In Drug resistance in leukemia and lymphoma iii, Kaspers, G.J.L.; Pieters, R.; Veerman, A.J.P., Eds. Kluwer Academic / Plenum Publ: New York, 1999; Vol. 457, pp 621-629.

[2] WU, G.Y.; MORRIS, S.M. Arginine metabolism: Nitric oxide and beyond. Biochem. J. 1998, 336, 1-17.

[3] MORRIS, S.M. Arginine metabolism revisited. J. Nutr. 2016, 146, 2579-2586.

[4] LIND, D.S. Arginine and cancer. J Nutr 2004, 134.

[5] XIA, Y.; ZWEIER, J.L. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. Proc Natl Acad Sci U S A 1997, 94, 6954-6958.

[6] DASGUPTA, T.; HEBBEL, R.P.; KAUL, D.K. Protective effect of arginine on oxidative stress in transgenic sickle mouse models. Free Radical Biology and Medicine 2006, 41, 1771-1780.

[7] TRIPATHI, P.; PANDEY, S. L-arginine attenuates oxidative stress condition during cardiomyopathy. Indian J Biochem Biophys 2013, 50, 99-104.

[8] YI, J.; HORKY, L.L.; FRIEDLICH, A.L.; SHI, Y.; ROGERS, J.T.; HUANG, X. L-arginine and alzheimer's disease. International Journal of Clinical and Experimental Pathology 2009, 2, 211-238.

[9] AL-TANNAK, N.F. Uhplc-uv method for simultaneous determination of perindopril arginine and indapamide hemihydrate in combined dosage form: A stability-indicating assay method. Sci. Pharm. 2018, 86.

[10] MESONZHNIK, N.V.; POSTNIKOV, P.V.; APPOLONOVA, S.A.; KROTOV, G.I. Characterization and detection of erythropoietin fc fusion proteins using liquid chromatography-mass spectrometry. J. Proteome Res. 2018, 17, 689-697.

[11] DUSKOVA, K.; VESELY, S.; SILVA, J.D.; CERNEI, N.; ZITKA, O.; HEGER, Z.; ADAM, V.; HAVLOVA, K.; BABJUK, M. Differences in urinary amino acid patterns in individuals with different types of urological tumor urinary amino acid patterns as markers of urological tumors. In Vivo 2018, 32, 425-429.

[12] DE CARVALHO, R.C.; MATHIAS, T.R.D.; NETTO, A.D.P.; MARQUES, F.F.D. Direct determination of amino acids in brewery worts produced by different processes by capillary zone electrophoresis. Electrophoresis 2018, 39, 1613-1620.

[13] CERNEI, N.; ZITKA, O.; RYVOLOVA, M.; ADAM, V.; MASARIK, M.; HUBALEK, J.; KIZEK, R. Spectrometric and electrochemical analysis of sarcosine as a potential prostate carcinoma marker. Int. J. Electrochem. Sci. 2012, 7, 4286-4301.