ISOLATION OF FLAVONOIDS USING MAGHEMITE NANOPARTICLES

KOPEL Pavel[1,2], CERNEI Natalia[1,2], KLEJDUS Borivoj[1], ADAM Vojtech[1,2]

[1]Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic, EU
[2]Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic, EU

Abstract

In our study, we aimed to synthesize superparamagnetic nanoparticles (PMPs) able to isolate and immobilize and thus preconcentrate flavonoids for subsequent analysis. We report the optimal conditions such as different pH of Britton Robinson buffer, temperature and time of incubation on the isolation of different types of flavonoids, such as Genkwanin and Pratol from extracts of freshwater algae. For the determination and their characterization was used an Agilent 1200 Series Rapid Resolution LC system coupled on-line Agilent Technologies 6460 Triple quadruple MS detector with Agilent Jet Stream (all from Agilent Technologies, Waldbronn, Germany). We modified surface of nanomaghemite (γ-Fe_2O_3) by chitosan and amine groups. One type of PMPs was able to bind Genkwanin and Pratol with 34.49 % and 27.65 % recoveries, respectively. In future, the nanoparticles can serve for application and as a platform of biosensors.

Keywords: Flavonoids, Maghemite nanoparticles, genkwanin, pratol

1. INTRODUCTION

Flavonoids are widely distributed secondary metabolites with different metabolic functions in plants. More than the 4000 various types of flavonoid were described [1]. Flavonoids are also responsible for the display of fall color in many plants, which may protect leaf cells from photooxidative damage, enhancing the efficiency of nutrient retrieval during senescence. Flavonoids protect plants against pathogen and herbivores [2]. The biological functions of flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation. Some flavonoids provide stress protection, for example, acting as scavengers of free radicals such as reactive oxygen species (ROS), as well as chelating metals that generate ROS via the Fenton reaction. On the other hand flavonoids have antioxidant properties and may protect tissues against oxygen free radicals and lipid peroxidation [3]. Flavonoids play an mayor role in the cancer and cardiovascular diseases prevention [4]. Genkwanin is an O-methylated flavone, with molar mass 284.27 g/mol, firstly was isolated from seeds of *Alnus glutinosa.* Usually for detection of flavonoids different types of methods such as rapid resolution liquid chromatographic-tandem mass spectrometric determination [5], ultrahigh-pressure liquid chromatography with UV-Vis diode array detector [6], high performance liquid chromatography with spectrometric/electrochemical detection [7] and spectrophotometry [8] were used.

2. EXPERIMENTAL PART

2.1. Chemicals

All the chemicals were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

2.2. Chromatography

An Agilent 1200 Series Rapid Resolution LC system consisted of an on-line degassed, a binary pump, a high performance SL auto-sampler, a thermostated column compartment, and UV-Vis photodiode array detector. The system was coupled on-line to an Agilent Technologies 6460 Triple quadruple MS detector with Agilent Jet Stream (all from Agilent Technologies, Waldbronn, Germany).

2.3. SEM and SECM characterization

PMPs were characterised by electron microscope Tescan, a.s., Brno, Czech Republic. 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. An accelerating voltage of 15 kV and beam currents about 1 nA gives satisfactory results regarding maximum throughput. Scanning electrochemical microscope consisted of 10 mm measuring platinum disc probe electrode with potential of 0.2 V. Another platinum disc electrode with O-ring as conducting substrate used potential of 0.3 V. During scanning, the particles were attached on the substrate platinum electrode by magnetic force from neodyme magnet, situated below the electrode. Platinum measuring electrode was moving from 150 µm above the surface. The mixture consisted of 5 % ferrocene in methanol mixed in ratio 1:1 with 0.05 % KCl in water (*v/v*). Measuring was performed in Teflon cell with volume of 1.5 mL according to the following parameters: amperometric mode, vertical scan was carried out in area 500 × 500 µm with rate 30 $\mu m.s^{-1}$.

2.4. X-ray fluorescence analysis (XRF)

XRF of PMPs was carried out on Xepos (SPECTRO analytical instruments GmbH, Kleve, Germany). , Analyses were conducted in Turbo Quant cuvette method.

2.5. Preparation of nanoparticles

MAN-161

Maghemite was prepared by sodium borohydride reduction of iron nitrate. $Fe(NO_3)_3$ (6 g) was dissolved in 800 ml of water followed by addition of $NaBH_4$ (2 g) in 100 ml of 3.5% NH_3. Maghemite was separated by a magnet and washed several times with water. Final volume was 100 ml. To 20 ml of this solution was added methanol (100 ml) and 3-[2-(2-Aminoethylamino)ethylamino]propyltrimethoxysilane (200 µl) with stirring (Biosan OS-10) overnight. The product was separated by a magnet, washed with water and suspended in water.

MAN 170

The particles were prepared in a similar way, only 10 ml of 1% chitosan was added instead of 3-[2-(2-Aminoethylamino)ethylamino]propyltrimethoxysilane .

3. RESULTS AND DISCUSSION

Two kinds of PMPs were prepared. The particles were modified with biopolymer chitosan and 3-[2-(2-Aminoethylamino)ethylamino]propyltrimethoxysilane. The aim was the introduction of amine groups on the surface and possible interaction of the groups with oxygen atoms of flavonoids. Large scale of flavonoids was used for interaction with particles but it was found that only two flavonoids were bound in high yields, namely Genkwanin and Pratol.

PMPs were firstly characterized by XRF providing information about element composition of particles. The results of MAN-170 are not shown. In the **Figure 1A** can be seen that as the most abundant elements were iron (Fe 47.87 %) and silicium (Si 0.61 %). It confirms that the core is formed from iron oxide and surface contains silicone groups. In **Figure 1B** can be seen a micrograph expressing particles surface and morphology in resolution of 100 µm for MAN-161 and in **Figure 1C** is SEM scan of paramagnetic particle surface in resolution of 2 µm.

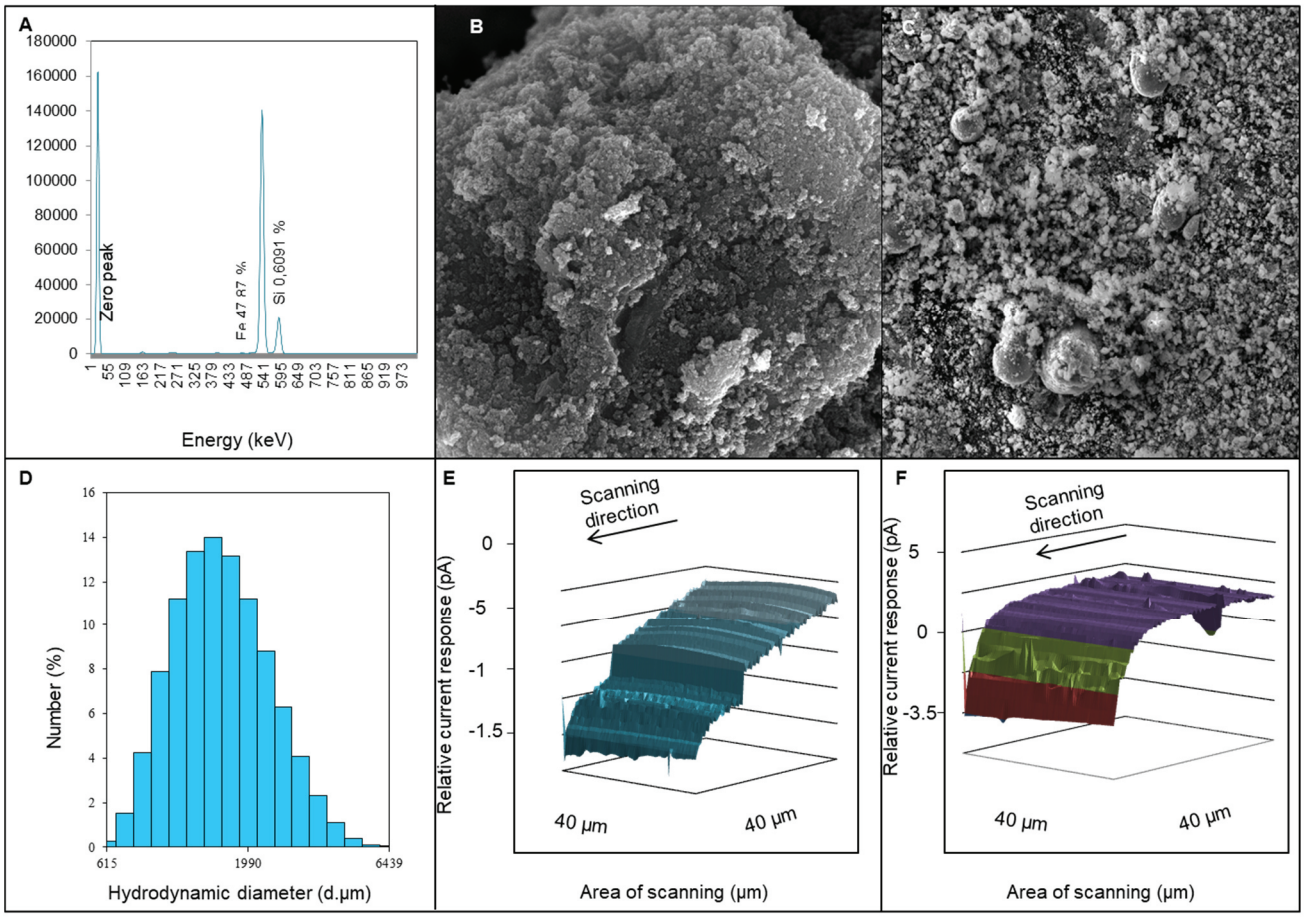

Figure 1 Characterization of PMPs MAN161. (**A**) XRF, showing elemental composition. (**B**) SEM scan of MAN161 in resolution 100 µm. (**C**) SEM scan in resolution of 2 µm. (**D**) 2 Percentage representation of PMPs measured by Zetasizer. (**E**) SECM scan showing relative current response of PMPs without Genkwanin. (**F**) SECM scan of PMPs with Genkwanin bound showing increased relative current response.

In addition, for further particles characterization we carried out scanning electrochemical microscope analysis for recognition of PMPs surface relative current response changes in dependence on Genkwanin binding. 3D image, expressing a relative current response of PMPs surface without Genkwanin bound. Comparing with **Figure 1E** considerable changes of surface current response can be observed. Relative current response after establishing of Genkwanin binding is higher than before binding. This obvious difference (approximately 20 pA) is indicating that the Genkwanin binding leads to a change of PMPs attributes. As was mentioned above, flavonoids are protonated under influence of acidic pH maintained by Britton-Robinson buffer used during workflow process. Positively charged molecule increases current response of PMPs. Hence, we received a confirmation that our PMPs bind properly and are usable for its isolation and preconcentration from various types of matrices.

Our PMPs showed also relatively good selectivity, which is probably based on principles of workflow process used for deprivation of undesired impurities from beads. Britton-Robinson buffer with pH 6 causes protonation, which leads into a positive charging of its molecules.

4. CONCLUSION

Superparamagnetic particles able to bind Genkwanin and Pratol. were prepared. Especially, superparamagnetic particles modified by 3-[2-(2-Aminoethylamino)ethylamino]propyltrimethoxysilane is able

to bind the two flavonoids with good yields. The particles have potential for better isolation in the samples of freshwater algae and can be used as biosensors.

ACKNOWLEDGEMENTS

This research was financially supported by the Grant Agency of Czech Republic (project no. GA14-28933S).

REFERENCES

[1] HOLLMAN, P.C.H. and M.B. KATAN, *Dietary flavonoids: Intake, health effects and bioavailability.* Food and Chemical Toxicology, 1999. **37**(9-10): p. 937-942.

[2] MIADOKOVÁ, E., *Isoflavonoids - an overview of their biological activities and potential health benefits.* Interdisciplinary Toxicology, 2009. **2**(4): p. 211-218.

[3] KANDASWAMI, C. and E. MIDDLETON Jr, *Free radical scavenging and antioxidant activity of plant flavonoids*, in *Free radicals in diagnostic medicine*1994, Springer. p. 351-376.

[4] HERTOG, M.G., et al., *Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study.* Archives of internal medicine, 1995. **155**(4): p. 381-386.

[5] KLEJDUS, B., et al., *Development of new efficient method for isolation of phenolics from sea algae prior to their rapid resolution liquid chromatographic-tandem mass spectrometric determination.* Journal of Pharmaceutical and Biomedical Analysis, 2017. **135**: p. 87-96.

[6] KLEJDUS, B., et al., *Ultrahigh-pressure liquid chromatography of isoflavones and phenolic acids on different stationary phases.* Journal of Chromatography A, 2008. **1195**(1-2): p. 52-59.

[7] SOCHOR, J., et al., *Mathematical Evaluation of the Amino Acid and Polyphenol Content and Antioxidant Activities of Fruits from Different Apricot Cultivars.* Molecules, 2011. **16**(9): p. 7428-7457.

[8] POHANKA, M., et al., *Automated assay of the potency of natural antioxidants using pipetting robot and spectrophotometry.* Journal of Applied Biomedicine, 2012. **10**(3): p. 155-167.