γ-Fe_2O_3 NANOPARTICLES AS A POTENTIAL SENSOR FOR HISTAMINE ISOLATION

[1,2]Milica GAGIC, [1,2]Pavel KOPEL, [1,2]Natalia CERNEI, [1,2]Ondrej ZITKA, [1]Pavel SVEC, [3]Ewelina JAMROZ, [1,2]Vojtech ADAM

[1]Department of Chemistry and Biochemistry, Mendel University in Brno, Faculty of AgriSciences, Brno, Czech Republic, EU, gagic.milica@gmail.com
[2]Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic, EU
[3]Insitute of Chemistry, University of Agriculture, Cracow, Poland, EU

Abstract

Histamine, biologically active amine, is normally present in the body and it is involved in a local regulation of physiological processes. It occurs in food as a product of microbial decarboxylation of amino acid histidine, and consumption of histamine-rich food can lead to intoxication. Hence, its identification, quantification, and awareness of this foodborne toxin are important for food safety as it can serve as an indicator of food spoilage. This study presents a synthesis of various γ-Fe_2O_3 nanoparticles, which differ in surface functionalization, application of particles for histamine detection and isolation, and subsequent reaction of desorbed histamine with ninhydrin for final ion exchange chromatography quantification. The aim of this study was to develop easy to use, cost-effective, and error-free procedure for quantification of histamine.

Keywords: Histamine, nanoparticles, food safety

1. INTRODUCTION

Histamine is normal constituent of many foods, such as fish, meat, cheese, and wines and is described as low molecular weight organic base with a heterocyclic structure. The formation of histamine in food is mainly derived from enzymatic decarboxylation of histidine and can have a toxicological effect especially in histamine sensitive consumers. Usually, histamine does not represent any health hazard but due to ingestion of large quantities, when food is spoiled, it causes anaphylactic reactions [1]. Therefore, the presence of histamine has been used as an indicator for quality control during food production [2].

Histamine poisoning also referred as scombroid poisoning, is the most common seafood intoxication in the world. It occurs after the consumption of food containing histamine at concentration higher than 500ppm [3]. To prevent potential risk, the European Legislation set a limit for histamine levels in fishery products, up to maximum of 10 mg/100 g [4].

Merits of the conventional methods for histamine quantifications are high sensitivity and accuracy and most of them are based on liquid chromatography where histamine must be converted to a derivative capable to be detected in the visible range of the spectrum [5]. Despite sensitivity and reproducibility, chromatographic methods are complex, expensive, and time-consuming. Therefore, there is an urgent need to develop simple, rapid and reliable method for the routine analysis of this potentially hazardous food compound.

The strategy described in this article used nanotechnology for separation of histamine, based on the adsorption of histamine on the paramagnetic particles (PMPs) with subsequent ion exchange chromatography (IEC) determination. The IEC based method used to determine histamine employed chemical derivatization of histamine using ninhydrin. A simple, sensitive and rapid method is described for histamine determination.

2. EXPERIMENTAL PART AND METHODOLOGY

2.1. Chemicals

The chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity unless noted otherwise.

2.2. Synthesis of paramagnetic particles

Maghemite nanoparticles were prepared by sodium borohydride reduction of iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) according to following procedure. 7.48g of $Fe(NO_3)_3 \cdot 9H_2O$ was dissolved in 400 mL of water. Under stirring 1g of $NaBH_4$ was added, which was previously dissolved in 50 mL of 3.5 % NH_3. The obtained solution was heated at boiling temperature for 2h. After cooling, dark product was separated by external magnetic field and washed several times with water. 100 mL of prepared nanoparticles were used as a core for surface modification.

2.3. Synthesis of modified paramagnetic particles

MAN181: 50 mL of methanol was added to a maghemite and mixture was shaken. Titanium(IV) butoxide was applied to modify the nanomaghemite surface Firstly 0.7 mL of titanium(IV) butoxide was applied to modify the nanomaghemite surface and subsequently 1 mL of 28 % ammonium (*w/v*) was mixed. The resulting mixture was stirred at Biosan OS-10 (Biosan, Riga, Latvia) for 2 h at room temperature. Resulting product was separated using magnet and washed with water.

MAN183: 50 mL of methanol was added to nanomaghemite and the mixture was shaken. Tetraethyl orthosilicate (TEOS) and titanium(IV) butoxide was applied to modify the nanomaghemite surface. Firsly 0.7 mL of TEOS was mixed with nanomaghemite, after 1oh 0.7 mL of titanium(IV) butoxide was added. After 1 h of constantly stirring 1 mL of 28 % ammonium (*w/v*) was mixed. The resulting mixture was stirred at Biosan OS-10 for 2 h at room temperature. Resulting product was separated using magnet and washed with water.

2.4. Ion exchange chromatography with visible light range detector

For the identification of paramagnetic particles binding capacity an IEC Model AAA - 400 (Ingos, Prague, Czech Republic) with post-column derivatization by ninhydrin and an absorbance detector in visible light range was used. Experimental conditions were applied according to our preliminary study [6]. A glass column with an inner diameter of 3.7 mm and length of 350 mm was filled manually with strong cation exchanger Ostion LG ANB (Ingos, Prague, Czech Republic) in sodium cycle with ~12 µm particles and 8 % porosity. The column was thermostated at 60 °C. Double channel VIS detector with an inner cell of 5 µL was set to two wavelengths: 440 and 570 nm. Prepared solution of ninhydrin was stored under a nitrogen atmosphere in the dark at 4 °C. Elution of histamine was carried out by a buffer containing 10.0 g of citric acid, 5.6 g of sodium citrate, and 8.4 g of sodium chloride per liter of solution (pH 2.7). The flow rate was 0.25 mL/min. The reactor temperature was set to 120 °C.

2.5. Scanning electron microscopy

Morphology of paramagnetic particles was revealed using scanning electron microscopy (SEM) FEG-SEM MIRA (Tescan, Brno, Czech Republic). The SEM was fitted with Everhart-Thronley type of SE detector, high speed YAG scintillator based BSE detector, panchromatic CL Detector, and EDX spectrometer.

3. RESULTS AND DISCUSSION

Purpose of our study was to synthesize the paramagnetic particles able to establish a binding with histamine, and thus to isolate and preconcentrate this analyte from complicate matrices for its subsequent determination using IEC. Nanomaghemite acts as an excellent superparamagnetic carrier due to its unique physic-chemical properties and ability to be simply functionalized with chemically active groups. We reported controlled procedure for preparation of stable PMPs which are responsive to magnetic field and can be used for histamine isolation. For particles coating, substances with potential to cover the nanomaghemite surface and to provide active binding sites for histamine were selected as it is mentioned in the previous chapter where the entire

synthesis procedure is described. The surface coating is crucial for enhancement of the nanoparticles properties because it can regulate their stability and their selectivity.

3.1. Workflow process resulting in binding of histamine onto MAN181 and MAN183 surface

First, binding capacity of synthesized paramagnetic particles was tested by the described IEC method. Overall workflow process scheme can be seen in **Figure 1A**. Before PMPs application, it was necessary to include three washing steps with phosphate buffered saline (PBS) to remove undesired impurities, contained in the solid PMPs after their synthesis. Further, to achieve to highest yields of histamine, binding and elution conditions for histamine were optimized. The recoveries of histamine were 94.5 and 80.82 % for MAN181 and MAN183, respectively (**Figure 1C**). Synthesized PMPs showed high selectivity for binding of histamine which is probably based on the underlying principles of the proposed workflow process. Two pKa values of 5.8 and 9.4 have been reported for histamine [7]. Therefore, in strongly basic environments histamine will be predominantly present in its neutral form and upon lowering the pH below 5.8, histamine exists as a dication. In this experiment, pH of the sample played an important role in the adsorption of histamine and Briton-Robinson (BR) buffer pH 4 was used, causing histamine protonation and its interaction with the surface of PMPs (**Figure 1B**).

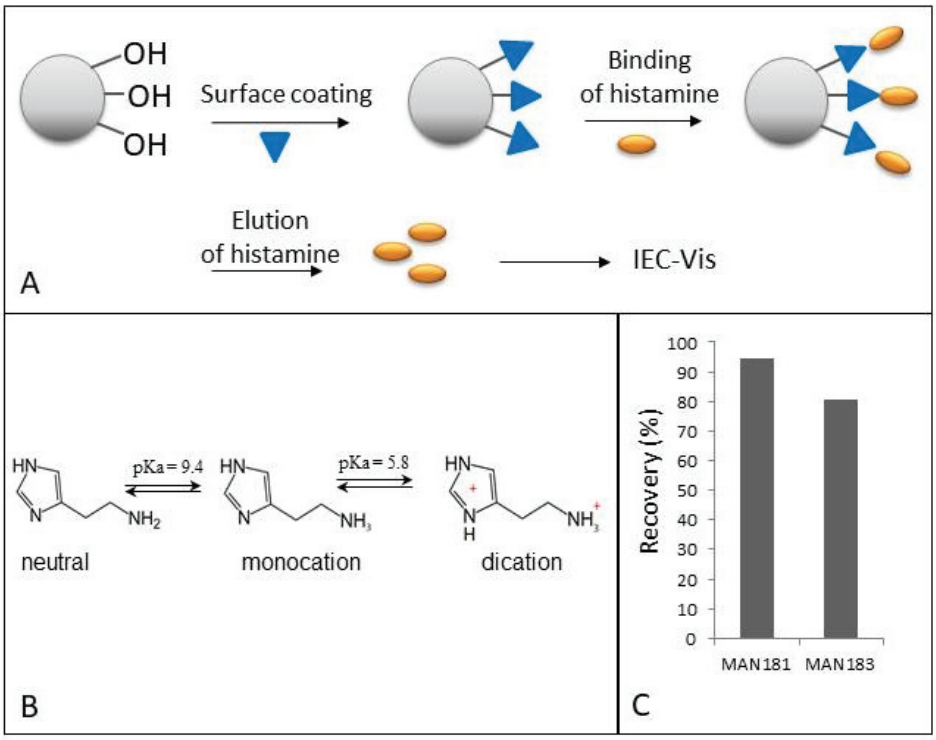

Figure 1 (A) The workflow process, providing the binding between paramagnetic particles and histamine molecules. Firstly, the paramagnetic beads are synthesized. Secondly, the particles are functionalized. Thirdly, beads are mixed with histamine. As a fourth step incubation is included, following elution step. Sample was prepared to be analyzed using IEC. (B) Effect of pH on the protonation of histamine. (C) Histamine recoveries, in percentage, for MAN181 and MAN183.

3.2. Morphological characterization

After confirmation of ability to bind histamine combination of complementary characterization techniques for comprehensive morphological and chemical characterization of PMPs were carried out. To reveal the particles morphology scanning electron microscopy (SEM) was employed. As it shown in **Figure 2**, paramagnetic

particles are irregularly shaped, amorphic structures and form clusters very willingly, but it did not influence on their superparamagnetic ability.

3.3. Characterization of elemental composition of PMPs

The initially formed maghemite nanostructure changed into titanium modified particles evidenced by energy dispersive X-ray (EDX) spectra. EDX showed that the MAN181 and MAN183 are composed primarily of iron (Fe) and titanium (Ti) (**Figure 2**).

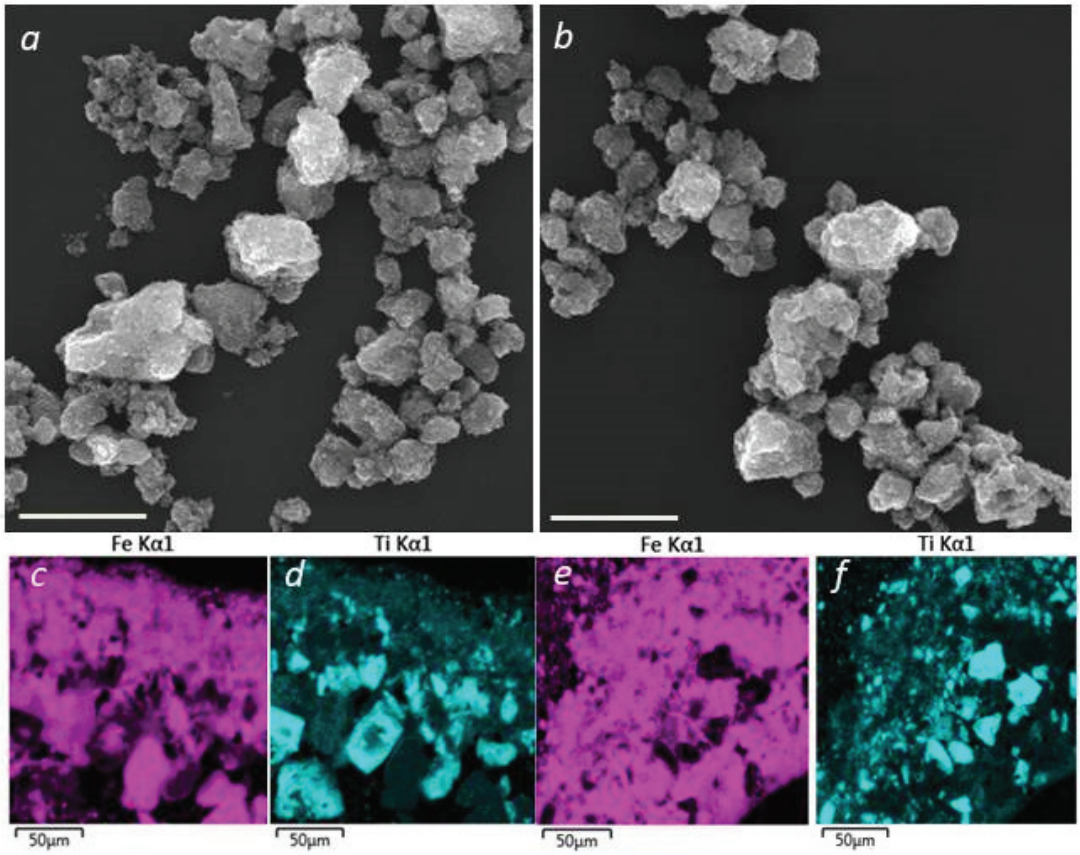

Figure 2 (a)(b) Micrographs of PMPs showing their morphology and size (Scale 2 µm) were obtained using SEM for MAN181 and MAN183, respectively. (c)(d) EDX results providing information about elemental composition of MAN181, Fe - iron, Ti - titanium (e)(f) EDX results for MAN183 Fe - iron, Ti - titanium.

4. CONCLUSION

In this study, we synthesized titanium(IV) oxide functionalized paramagnetic particles with high affinity and specificity towards histamine. The development of these potential sensors for histamine analysis appears to provide alternative methods to separation techniques. The main advantage of proposed protocol is simple and fast PMPs based isolation which makes it especially suitable for routine determination of histamine in order to control potential toxic effects. Further application of this method is quite possible and more research will be undertaken in our laboratory.

ACKNOWLEDGEMENTS

The research was financially supported by Internal Grant Agency of the Faculty of Agronomy, Mendel University in Brno no. AF-IGA-IP-2018/076.

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