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Proceedings of 25th International PhD Students Conference

7-8 November 2018, Brno, Czech Republic

Isolation of histamine using γ-Fe_2O_3 nanoparticles

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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 the amino acid histidine, and the ingestion of foods that contain high levels of histamine can lead to poisoning. Hence, the presence of this biogenic amine is considered as an indicator of food spoilage. Many different methods are available to detect the presence of histamine in food samples. The aim of this study was to design a fast and low-cost method for histamine identification employing magnetic isolation and subsequent reaction of desorbed histamine with ninhydrin for final ion exchange chromatography quantification.

Key Words: fish poisoning, biogenic amines, nanoparticles, food safety, rapid method

INTRODUCTION

Biogenic amines are non-volatile, heat stable, low molecular weight bases with biological activity and have aliphatic, aromatic or heterocyclic structure (Tapingkae et al. 2010). They can be formed and degraded as a result of normal metabolic activity in animals, plants, and humans and are usually produced by the decarboxylation of amino acids.

Histamine [2-(4-imidazolyl)-ethylamine] is short-acting biogenic amine synthesized from the amino acid histidine through the catalytic activity of enzyme histidine decarboxylase (Dy and Schneider 2004). It is synthesized and stored at high concentrations within granules in basophils and mast cells and effects of this biologically active chemical are usually seen when it is released in large amounts or ingested in unusually high quantity. Histamine exerts its effects through differential activation of four distinct subtypes of G-protein coupled receptors on cellular membranes (Lehane and Olley 2000).

Histamine in low concentrations is also produced during microbial decomposition of Scombroid fish flesh such as tuna and mackerel (Halasz et al. 1994). However, when fish is spoiled, the amount can increase to a toxic level (up to 50 mg per 100 g of the product), causing food poisoning. Histamine has been identified as a significant chemical hazard and formation of histamine can lead to adverse reactions, especially in allergic suffering consumers. To prevent potential risk caused by histamine its detection in foods is of great importance.

A plethora of techniques have been described to provide information about the levels of histamine in different sorts of food (Onal 2007). This study focuses on the design and optimization of the method based on the isolation of histamine using paramagnetic particles (PMPs) with consequent quantitative determination by ninhydrin colorimetric assay. Due to their unique physicochemical properties, PMPs (functionalized maghemite γ-Fe_2O_3 nanoparticles) have received considerable attention in the analysis

of residues in food samples. Application of magnetic particles for histamine analysis is a good alternative to traditional methods because histamine is typically present in very low concentration over a high concentration of background material. By using their magnetic properties particles are applied as adsorbents during isolation, separation, and preconcentration of the target analytes (Jimenez et al. 2016).

This study consists of the synthesis of particles with magnetic properties (maghemite Fe_2O_3) followed by a coating of the magnetic core with different organic compounds. The ability of synthesized particles to adsorb histamine on the surface was tested by employing ion-exchange liquid chromatography (IEC) with post-column ninhydrin derivatization together with visible light range (VIS) detection with an integrated two-channel photometer simultaneously working at 440 and 570 nm, respectively.

MATERIAL AND METHODS

Chemicals

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

Synthesis of paramagnetic nanoparticles

Maghemite nanoparticles were prepared by sodium borohydride reduction of iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) according to the already known procedure (Heger et al. 2015, Nejdl et al. 2014). 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 50mL of 3.5% NH_3. The obtained solution was heated at boiling temperature for 2h. After cooling, the dark product was separated by external magnetic field and washed several times with water. The nanoparticles, prepared in this way were used as a core for surface modification.

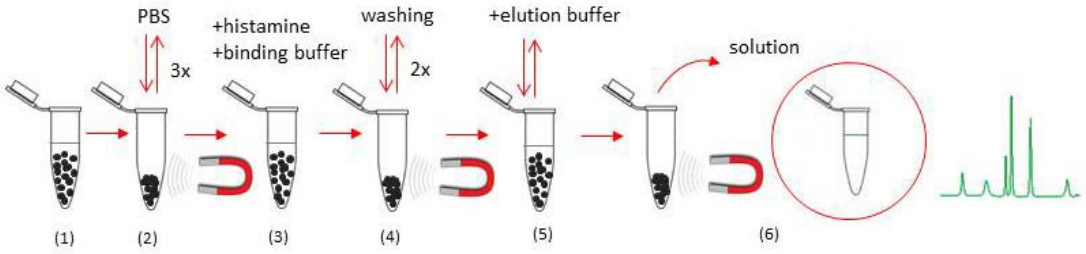
Seven different functionalized PMPs will be applied separately in order to obtain specific histamine binding activity, for each of them. A versatile and inexpensive method for the introduction of functional groups on the surface of maghemite particles will be established based on the particles modification with each of seven different organic molecules, as follow: N-(3-trimethoxysilylpropyl) diethylenetriamine, 3-(Triethoxysilyl)propyl isocyanate, (3-glycidyloxypropyl)trimethoxysilane, (3-aminopropyl)triethoxysilane, 2,6-Pyridinedicarboxylic acid, titanium(IV) butoxide and for the last surface functionalization, combination of tetraethyl orthosilicate (TEOS) and titanium(IV) butoxide will be used.

Procedure for histamine isolation for subsequent analysis using IEC-VIS

Initially, the paramagnetic particles are ultrasonicated and washed thrice with PBS (3x250 µl; pH 7.4). Consequently, the binding buffer is added (250 µl) and mixed with standard histamine of known concentration. Subsequently, an external magnetic field is applied to isolate the adsorbent with the adsorbed histamine on the surface of the magnetic particles. The liquid phase is decanted and the particles are washed twice with washing buffer. In the next step, histamine is eluted by the dispersion of the magnetic particle with elution buffer. Finally, the solution is collected and analyzed by IEC (see Figure 1).

For determination of histamine, 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 (Cernei et al. 2016). 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.

Figure 1 Experimental workflow



Legend: 1 – PMPs in storage solution, 2 – Washing of PMPs using PBS, 3 – Binding of histamine with PMPs and buffer, 4 – Washing of PMPs with bound histamine 5 – Elution of histamine 6 – IEC analysis of the obtained solution

Effect of pH

The interaction between the prepared PMPs and the histamine is significantly affected by the pH value of the buffers used in the experimental procedure. The pH value may alter the surface charge of particles and thus it may interact with the studied analyte. In order to determine the convenient pH value, the effect of pH on the adsorption and releasing of histamine was investigated.

Statistical analyses

The content of histamine was made using standard deviation from 3 determinations. The detection limits (3 signal/noise, S/N) were calculated according to Long and Winefordner (Long and Winefordner 1983).

Reusability of PMPs

We tested reusability of PMPs, to investigate whether PMPs could be totally released from the histamine and reused. Used PMPs were dissolved in hydrochloric acid (3 M). Particles were separated by using an external magnetic force field. Furthermore, the obtained solution was evaporated using a nitrogen evaporator Ultravap RC. The evaporated sample was resuspended in H_20 and the final product was quantified by IEC.

RESULTS AND DISCUSSION

The nanomaghemite synthesis was carried out by reduction of iron chloride and its subsequent modification. Seven different PMPs were prepared which differed from each other in their composition and functionalization procedure. To determine the binding specificity and effectiveness of the prepared PMPs toward histamine, IEC-Vis analysis was carried out. In the first step of the experiment, binding conditions were evaluated by mixing the histamine with Britton Robinson (BR) buffer at different pH values, eluting bound histamine with a high salt concentration buffer and analyzing fraction for the target analyte. According to the obtained results, BR pH 4 and 2M saturated BR pH 9 were selected for further experiments as binding and elution buffer, respectively. The protocol for the isolation and detection of histamine using functionalized PMPs is shown in Figure 1.

MAN_181 and MAN_183 were the most successful particles in the suggested assay. As shown in Table 1, high recovery percentages indicate that the aforementioned PMPs can be applied with high accuracy for the determination of the histamine. This was likely caused by the same surface coating of nanomaghemite core with titanium(IV) butoxide. To obtain further insight into beads morphology of these particles the SEM and XRF were employed. Due to the extremely low percentage recovery of the histamine, other particles were excluded from further characterization (Table 1).

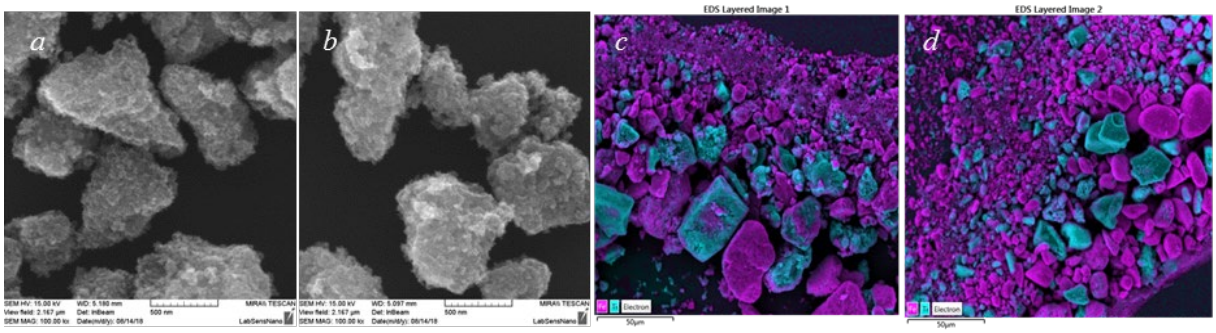
Results of the reusability test showed that particles could be recycled easily. Histamine was not retained on PMPs surface after elution step, very low analyte recoveries were detected after quantification by IEC analysis (data not shown). Reusability is one of the important properties of these particles and the possibility of full recovery after elution makes these particles highly desirable.

Table 1 Overview of synthesized PMPs, their modifications and extraction recoveries

Label	Modification	Recovery (%)
MAN_175	N-(3-trimethoxysilylpropyl)diethylenetriamine	0.5
MAN_176	3-(Triethoxysilyl)propyl isocyanate	17.12
MAN_177	3-glycidyloxypropyl trimethoxysilane	0.52
MAN_178	(3-Aminopropyl)triethoxysilane	0.06
MAN_179	2,6-Pyridinedicarboxylic acid	0.34
MAN_181	Titanium(IV) butoxide	94.5
MAN_183	TEOS + Titanium(IV) butoxide	80.82

Data from electron microscopy (Figure 2a–b) indicate that the magnetic particles were non-uniform clustered (agglomerated) polycharged particles, but it didn't have an influence on their magnetic properties. As is shown in Figure 2, the size of PMPs for MAN_181 and MAN_183 ranged in µm. Additionally, XRF was employed to reveal the elemental composition of PMPs. In both cases, iron and titanium were determined as the most abundant elements and that is direct confirmation of the functional groups provided by surface functionalization with titanium(IV) butoxide, a compound which is used for coating of PMP_181 and PMP_183 (see Figure 2c–d).

Figure 2 Characterization of MAN_181 and MAN_183. Micrographs of the surface of PMPs were obtained using SEM. The XRF elemental composition of the particles is shown in the third and fourth panel for MAN_181 and MAN_183 respectively.



Legend: a – SEM photography of MAN_181, b – SEM photography of MAN_183, c – XRF of MAN_181, d – XRF of MAN_183. Violet color corresponds to iron, blue color corresponds to titanium.

CONCLUSION

A major part of this study was to test binding affinity of seven different functionalized paramagnetic particles against histamine. Methodology optimized in this study employed the application of an external magnetic field in the isolation of the magnetic particles in the separation of histamine without additional steps that could cause loss of analyzed analyte. Two types of seven tested PMPs showed a certain potential for improving of efficiency of separation of histamine from the solution. Besides, specificity of these PMPs in complex matrices and interference from other compounds, commonly found in food samples, need to be tested. In order to address these challenges, other biogenic amines (tyramine, spermine, spermidine, putrescine, cadaverine) will be analyzed in parallel with histamine. This project will aim to ascertain the histamine level in different products as it can serve as a food quality marker and more research will be undertaken in our laboratory.

ACKNOWLEDGMENTS

The research was financially supported by grant no. AF-IGA-IP-2018/076.

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Name of publication:	MendelNet 2018 *Proceedings of 25th International PhD Students Conference*
Editors:	Assoc. Prof. Ing. Radim Cerkal, Ph.D. Ing. Natálie Březinová Belcredi, Ph.D. Ing. Lenka Prokešová
Publisher:	Mendel University in Brno Zemědělská 1665/1 613 00 Brno Czech Republic
Year of publication:	2018
Number of pages:	565
ISBN:	978-80-7509-597-8

Contributions are published in original version, without any language correction.