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ARTICLE

A Smart Method for the Fast and Low-Cost Removal of Biogenic Amines from Beverages by Means of Iron Oxide Nanoparticles

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Paramagnetic iron oxide nanoparticles have been synthesized and covered by a silica shell for a dual function: SiO₂ capping improves the stability of the nanoparticles and at the same time promotes the bonding between the paramagnetic nanoparticles@SiO₂ (MNPs@SiO₂) and biogenic amines. The constituents of the paramagnetic nanoparticles have been identified to be magnetite and maghemite by Infrared and Raman spectroscopy; these optical investigations allow also to confirm the key role of the capping layer in the interaction with the amines. The magnetic adducts with the biogenic amines can be removed simply and rapidly through the application of weak magnetic fields. The observation of the quickness and ease of the biogenic amines elimination have prompted us to check the application of this new approach to real commercial wine samples containing these toxic fermentation products: their complete removal has been observed by absorption spectra, thus confirming the potentiality of this novel approach in agroindustrial area and agribusiness.

Introduction

Biogenic amines (BAs) are chemical compounds that are spontaneously generated in foodstuff and drink products where microbial fermentation occurs.^{1,2} Such substances are mainly produced as decarboxylation of amino acids or by amination and transamination of aldehydes and ketones.³ The well-known toxicity of biogenic amines for the human health⁴ is still promoting an increasing interest towards the sensing and the removal of such compounds. Biogenic amines can be classified according to their chemical structures: aliphatic BAs (putrescine also known as 1,4-diaminobutane, cadaverine also known as 1,5-diaminopentane, spermine and spermidine), aromatic BAs (tyramine and phenylethylamine), heterocyclic BAs (histamine and tryptamine).⁵ Polyamines play a fundamental role for the growth, regeneration and the normal metabolic activity, even though biogenic amines have been indicated as precursor species in the formation of carcinogenic N-nitrosous compounds.⁶ Furthermore, remarkable and rapidly developing attention has been recently paid to some biogenic amines which can strongly affect the taste and the flavour of many foodstuff and drinks; consequently, a novel challenge in agroindustrial research and business is emerging. Biogenic amines detection has been widely investigated. Determinations of biogenic amines are often accomplished by reversed-phase HPLC followed by UV or fluorescence detection.^{7, 8, 9, 10, 11} To the best of our knowledge, in the literature other removal methods for biogenic amines from beverages or food are not reported. In this connection, a rapid and drastic innovative removal method of biogenic amines from aqueous matrices is

proposed. As the active substrate able to bind the biogenic amines, paramagnetic iron oxide nanoparticles (MNPs) capped by a silica shell are proposed (MNPs@SiO₂). Nanoparticles have been widely used in many different fields, such as for example energy production,¹² medical applications¹³ and just in a few cases for the treatment of foodstuff and drinks.^{14, 15} The paramagnetic nature of the nanoparticles allows the removal of the adduct MNPs@SiO₂/BAs by means of a weak magnetic field. In this paper, in order to cover the whole variety of Bas, we studied aqueous solutions contaminated by phenylethylamine (belonging to aromatic BAs class), histamine and tryptamine (class of the heterocyclic BAs) and putrescine (aliphatic BA).

In addition, nanosystems very similar to the MNPs proposed in this paper are completely biocompatible¹⁶ and already used for biomedical applications, such as drug delivery¹⁷ or magnetic resonance imaging.^{18,19} Nevertheless, it is worth to highlight that a crucial role is played by the shell used to cap the MNPs. SiO₂ capping has been largely used to stabilize magnetite nanoparticles that quickly oxidize in presence of oxygen with dramatic consequences on magnetic properties and dimensions of the nanoparticles.²⁰

Materials and methods

The iron oxide (γ -Fe₂O₃ and Fe₃O₄) paramagnetic nanoparticles (MNPs) were obtained by the modification of a conventional co-precipitation method.^{16, 17, 18} 0.4 g of FeCl₃·6H₂O and 0.3 g of FeSO₄ were dissolved in 20 ml of ultrapure MilliQ water under nitrogen gas and vigorous stirring. 20 ml of a water solution containing 0.3 g of NaOH was added drop by drop.

The colour solution turned from orange to dark black immediately. The co-precipitation reaction was carried out for 2h at RT under continuous stirring. The naked paramagnetic nanoparticles were separated by means of a neodymium permanent magnet able to attract up to 12 kg at a distance of 0.5 cm and twice washed with MilliQ water. The MNPs surface was functionalized with a SiO₂ shell by the typical sol-gel method with some modifications.²¹ The prepared γ -Fe₂O₃ and Fe₃O₄ MNPs and 0.5 g of SiO₂ were dissolved in 19.5 ml of MilliQ water under vigorous stirring. The pH value was adjusted to around 8.5 by adding 0.5 ml of a 30% ammonia solution. The reaction was performed under stirring for 4 h at R.T. The silica coated MNPs (MNPs@SiO₂) were separated by means of a neodymium permanent magnet and five times washed with MilliQ water. The final pH of MNPs@SiO₂ water dispersion is 7.5.

A Horiba MicroRaman Xplora (laser at 532 nm, power 0.7 mW) and a FTIR Spectrum One (Perkin Elmer) were used to characterized the synthesized MNPs and the MNPs@SiO₂, all UV-Visible spectra were acquired by means of a Cary 5000 (Agilent).

FeCl₃, 6H₂O, FeSO₄, NaOH, ammonium hydroxide and silica dioxide were purchased from Sigma Aldrich® and used as received without further purification. Biogenic amines were purchased by Alfa Aesar® and used as received.

Results and discussion

A mixture of maghemite and magnetite nanoparticles was obtained by means of a slight modification of an already proposed simple co-precipitation method from ferrous and ferric ion solutions in the presence of NaOH in the molar ratio of 1:2:8, respectively.^{22,23,24} The iron oxides precipitation was performed at a pH value of 12, according to the thermodynamics of the reaction: complete precipitation of Fe₃O₄, for instance, is expected at a pH value between 8 and 14, with a stoichiometric ratio Fe³⁺/Fe²⁺ of 2:1 in a non-oxidizing environment.^{18,25} Bubbling nitrogen gas allowed to avoid further oxidation and aggregation events, also reducing the particle size if compared to other synthesis methods.^{26,27}

A silica layer was set down to the MNPs surface in order to obtain well-dispersed and stable MNPs, thus avoiding both aggregation and oxidation. The pointed out easy procedure was performed starting from an aqueous dispersion of MNPs, SiO₂ and ammonium hydroxide at room temperature.

The silica coated MNPs (MNPs@SiO₂) suspended in water were washed five times in order to reach a pH value of 7.5. It is worth to highlight that the obtained pH value is suitable for a direct application in foodstuff and drinks.

Transmission Electron Microscopy (TEM) investigations were carried out in order to better characterize the obtained MNPs@SiO₂ in this paper. In particular they showed a spherical shape and an average diameter of about 10.8 nm (see ESI file, figure S1), in complete accordance with the dimensions already reported for analogous synthesis methods.²⁸ Infrared spectroscopy was used to verify the presence of silica capping on the paramagnetic core. In figure 1, FTIR spectra in the range 1800-600 cm⁻¹ of MNPs@SiO₂ after the capping process and of SiO₂ are reported.

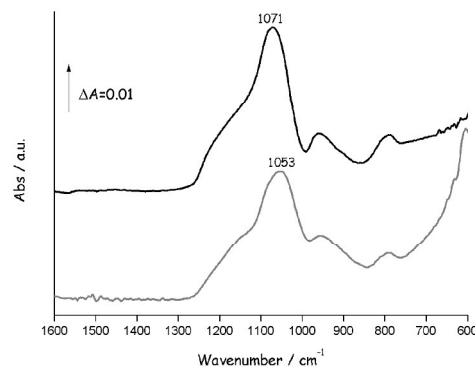


Figure 1. Infrared spectra of a cast film of SiO₂ suspended in water (black line) and of MNPs@SiO₂ (grey line). An absorption shift toward lower energy was observed when SiO₂ capped the MNPs.

The black spectrum shows the absorption of SiO₂ with typical peaks mainly due to stretching vibrations modes. In particular, the signal centered at 1071 cm⁻¹ corresponds to the asymmetrical stretching vibration of the Si-O-Si bond.²³ The spectrum of a cast film of MNP@SiO₂ (grey line) is characterized by the presence of similar signals, confirming the presence of SiO₂. Furthermore, the asymmetric stretching of Si-O-Si appears 18 cm⁻¹ shifted to lower wavenumbers suggesting the MNPs surface functionalization.

A cast film of the MNPs@SiO₂ was characterized by means of Raman spectroscopy, which allows to easily and unambiguously identify the precise composition of the iron oxide. Magnetite, maghemite, wüstite and hematite show different vibrational bands in the Raman spectrum.²⁹ In particular, the Raman spectrum reported in figure 2 evidences a medium absorption centered at 306 cm⁻¹ and a strong asymmetric band located at 668 cm⁻¹. This peculiar pattern suggests that the MNPs@SiO₂ are mainly composed of magnetite²⁹ even though the intense shoulder at 710 cm⁻¹ indicates that a mixture of magnetite and maghemite has been obtained.¹⁹ Another rationale, as suggested by the studies of Pourroy and collaborators,²⁸ is that the particles with diameters below 20 nm are nonstoichiometric magnetite in their volume. Anyway, both magnetite and maghemite are good paramagnetic materials, and this peculiarity is of paramount importance to remove easily the MNPs@SiO₂/BAs adduct from the aqueous solutions.

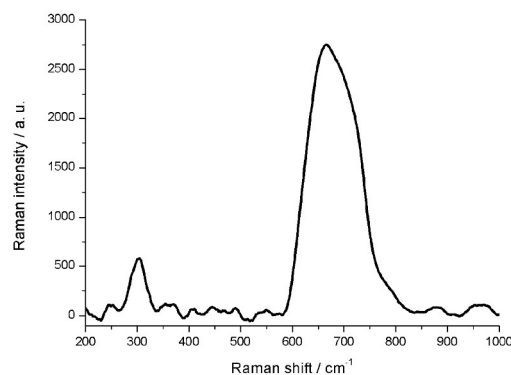


Figure 2. Raman spectrum of the MNPs@SiO₂ adduct.

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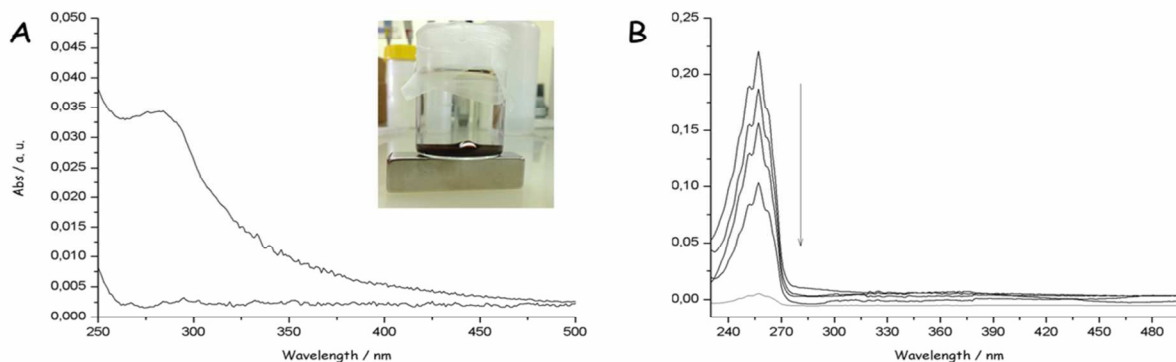


Figure 3. Effect of MNPs@SiO₂ treatment on BA aqueous solutions. In the box A, the removal of putrescine is evident from the bleaching of absorption peak at 280 nm. In the box B, the effect of the MNPs@SiO₂ further treatment on phenylethylamine concentration is evident.

The advantage of the silica coating resides in its the surface chemistry. The outer layer can be chemically modified with different coupling agents in order to bind specific ligands and to modify the chemical-physical properties of the nanostructures.^{30, 31} More in particular, the SiO₂ can be further functionalized with the NH groups of the biogenic amines, using the MNPs@SiO₂ as a removal vector of these toxic compounds.

The presence of biogenic amines in aqueous solution was monitored by means of UV-Vis spectroscopy. Solutions containing 10⁻³ M putrescine, 10⁻³ M phenylethylamine, 10⁻² M histamine and 10⁻⁴ M tryptamine were prepared and 1 ml of MNPs@SiO₂ aqueous suspension was injected in the solutions. Amine concentrations were chosen in order to be clearly detected with a UV-Vis spectrophotometer (see ESI file, figure S2). The content of each vial was stirred for 5 min at room temperature, then the MNPs@SiO₂ nanostructure was separated from the aqueous solution by means of a magnet (see inset in figure 3). The UV-Visible spectra acquired after the removal of the paramagnetic nanoparticles do not show any signal imputable to MNPs@SiO₂ and the absorption peak is strongly reduced. More in detail, as reported in figures 3A, the putrescine absorption peak at 280 nm is drastically reduced after the treatment.

Figure 3B illustrates the spectra recorded for an aqueous solution of phenylethylamine treated with the silica coated MNPs. Probably, chemical affinity between the MNPs@SiO₂ and phenylethylamine is lower than that one observed for putrescine. It results in a less efficient removal of BA. It is worth to observe that, as reported in figure 3B, further treatments of the aqueous solution by fresh MNPs@SiO₂ suspension allowed to remarkably reduce step by step the phenylethylamine concentration.

UV-Vis spectra of aqueous solution of histamine (figure S3A) and of tryptamine (figure S3B), the two heterocyclic BAs considered in this contribution, recorded after MNPs treatment, confirmed that BAs should anchor the MNPs silica surface. The

intensity of the absorption peaks is reduced also in these cases. In order to investigate the interaction between the MNPs@SiO₂ and the biogenic amines, FTIR spectroscopy was carried out on the MNPs@SiO₂/BAs adducts.

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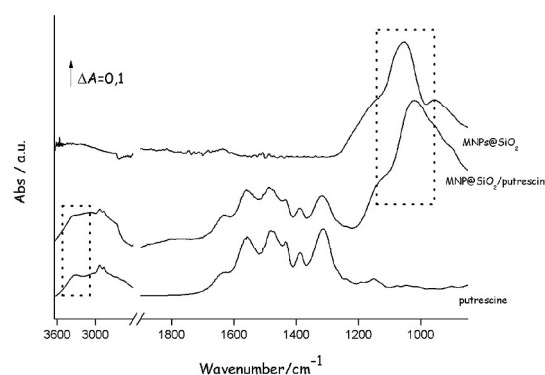


Figure 4. FTIR spectra of cast films of MNP@SiO₂, of MNP@SiO₂/putrescine adduct and of putrescine.

The signals in the 1650-1200 cm⁻¹ region in the MNP@SiO₂/putrescine adduct spectrum confirmed the presence of the BA if overlapped with BA vibration modes. A further 20 cm⁻¹ shift of the asymmetrical stretching band of the Si-O-Si bond, if compared with the MNPs@SiO₂ signal, indicated that the silica surface is directly involved in the

binding process. The broad peak at around 3310 cm^{-1} is due to the contribution of both asymmetrical and symmetrical stretching vibrations of the primary amine groups of putrescine. After the MNPs treatment, the signal is centered at around 3365 cm^{-1} , confirming the interaction between NH groups and silica.

FT-IR spectra of a cast film of phenylethylamine and of its adduct with MNPs@SiO₂ are reported in figure S4. Also in this case, the $1750\div 1200\text{ cm}^{-1}$ region shows the presence of the IR signals of the amine in both spectra, even if the adduct exhibits some differences. In particular the scissoring band of the NH group results shifted to higher frequencies and strongly reduced, as a consequence of the binding with the silica coating. Also in this case, the Si-O-Si asymmetrical stretching band is shifted to 1020 cm^{-1} and the $3700\text{-}2400\text{ cm}^{-1}$ region shows drastic differences in the 3255 cm^{-1} signals (NH stretching mode) when phenylethylamine is linked to the MNPs surface. Figure S5 shows the FTIR spectra of a cast film of histamine and of MNPs@SiO₂/histamine adduct. The results confirm also in this case the presence of the amine in the final complex. BAs spectra, so, typically showed a broad asymmetric band due to the asymmetrical and symmetrical primary NH group stretching modes. Otherwise, adducts spectra are characterized by the presence of a single NH stretching band. This could remind to stretching vibration of a secondary amine, suggesting the generation of robust interaction among silica shell and NH groups. Moreover, as clearly shown in figure S4, the NH bending mode of the primary amine of the phenylethylamine, for instance, was strongly affected by the presence of the MNPs@SiO₂. The band is strongly reduced. It is already known that the NH bending in secondary amines is seldom detectable, suggesting once more the interaction NH-silica shell.

Finally, the effect of the MNPs@SiO₂ capped nanoparticles on the biogenic amines content was directly tested on a white wine, easily available on market shelves, in order to check and demonstrate the potentiality of the proposed approach on a real sample.³² The UV-Vis spectrum of the white wine was recorded and is reported in Figure 5 (black spectrum). The signal at 270 nm highlights the presence of biogenic amines, mainly phenylethylamine. After the treatment with MNPs@SiO₂, the peak is strongly reduced and no other new signals appeared in the UV-Vis spectrum of the sample (grey line, figure 5).

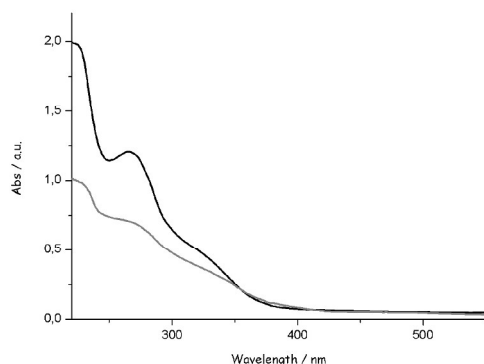


Figure 5. Effect of MNPs@SiO₂ treatment on a commercial white wine. The removal of BAs is evident from the bleaching of absorption peak at 267 nm.

In order to evaluate that MNPs treatment did not affect the total content of polyphenols in wine samples, we compared their

amount in MNPs treated and not-treated wine samples by means of the well-known Folin-Ciocalteu method. As reported in figure S6 the two obtained visible spectra did not evidence significant differences.³³

The reported experimental data demonstrate that the proposed method appears to be cheap, quick and extremely efficient to remove completely the biogenic amines from aqueous drink and foodstuff; moreover it can be also recommended for large scale applications in agribusiness in order to improve with low costs the quality of agroindustrial products.

Conclusions

Iron oxides nanoparticles have been synthesized in water by means of a co-precipitation procedure. The nanostructures have been covered by a silica shell for a double objective: first, as largely reported in the literature, SiO₂ capping induces strong stability to the nanoparticles; second, the capping has been used to promote a stable bonding between the paramagnetic nanoparticles@SiO₂ (MNPs@SiO₂) and biogenic amines. Infrared and Raman spectroscopy have been used in order to characterize the MNPs@SiO₂ confirming the formation of SiO₂ shell and the nature of the paramagnetic nanoparticles, i.e. magnetite and maghemite.

The paramagnetic nature of the synthesized nanostructures has been employed to remove quickly biogenic amines (BAs) from water solutions, as demonstrated by UV-Visible spectroscopy. The interaction between MNPs@SiO₂ and biogenic amines has been monitored by means of FTIR spectroscopy, confirming that the surface silica coating played a crucial role in the binding of the BAs. Si-O-Si asymmetric stretching mode resulted in each case at least 20 cm^{-1} shifted to lower frequencies confirming its role in the binding. Furthermore, the typical stretching and bending modes of the NH group resulted drastically changed as a consequence of the adduct formation.

Finally, the biogenic amine removal efficiency of the proposed method has been tested on a real wine sample containing biogenic amines and a complete disappearance of the BA absorption band has been observed. This demonstrates the well-defined and promising potentiality of the suggested procedure in agribusiness.

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Notes and references

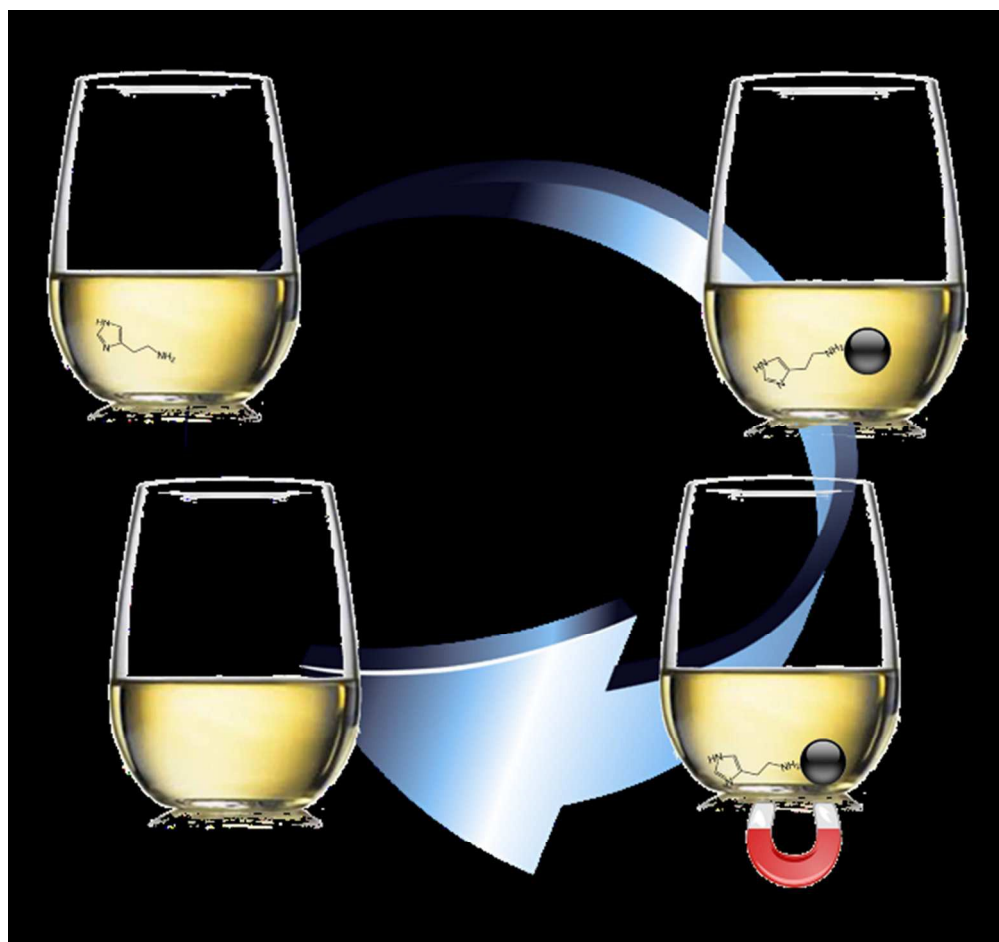
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Iron oxide nanoparticles capped with a silica shell are used to bind biogenic amines dissolved in a commercial wine sample. The adduct formed by capped paramagnetic nanoparticles and amines is separated by means of a weak magnetic field without affecting wine taste.
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