*Highlights (for review)

- One-pot, low-cost and reproducible g-C_3N_4 grafting onto silica microparticles
- g-C_3N_4@silica novel application for SPE of Fluoroquinolones from natural water
- Quantitative recovery in tap and raw river waters (low ng L^{-1})
- Good precision and sensitivity for g-C_3N_4@silica SPE coupled to HPLC-FD
- g-C_3N_4@silica advantageous in terms of preparation, cost, efficiency, reusability

Facile and fast preparation of low-cost silica-supported g-C_3N_4 for solid-phase extraction of fluoroquinolone drugs from environmental waters

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Abstract

The analytical application of silica-supported graphitic carbon nitride (g-C_3N_4) for solid-phase extraction (SPE) of fluoroquinolone (FQ) pollutants from water is presented for the first time. g-C_3N_4 was easily and quickly prepared by one-pot thermal condensation of dicyandiamide and characterized by powder X-ray diffraction, thermogravimetric analysis, scanning electron microscopy, Fourier transform infrared spectroscopy and surface area measurements. The novel composite was applied as sorbent for SPE of FQs from water prior high-performance liquid chromatography with fluorescence detection. The extraction efficiency of g-C_3N_4 was tested in tap and surface waters at actual concentrations (10-100 ng L^{-1}). Quantitative adsorption was achieved using 100 mg sorbent (20 wt% g-C_3N_4) for pre-concentration of 50-500 mL sample, at the native pH (~ 7.5-8). Elution was performed with 25 mM H_3PO_4 aqueous solution-acetonitrile (80:20), obtaining recoveries in the range 70-101%, enrichment factors up to 500 and inter-day RSDs ≤ 12 %. The batch-to-batch reproducibility was assessed on 3 independently synthesized g-C_3N_4@silica preparations (RSD < 6-12 %). g-C_3N_4 supported on silica microparticles proved to be of easy preparation, costless, reusable for at least 4 extractions of raw surface waters and suitable for determination in real matrices.

Keywords: Antibiotics; Determination; Graphitic carbon nitride; Pollutants; Sample preparation; Sample enrichment

1. Introduction

Graphitic carbon nitride (g-C_3N_4), the most stable allotrope of carbon nitride mainly made of carbon and nitrogen, is presently considered the most promising candidate to complement carbon materials. g-C_3N_4 is the newest carbon-based 2D material and shows a "graphene-like" structure of layered sheets of tri-s-triazine connected via tertiary amines. These form a defected-rich and N-bridged macromolecular structure that can be easily swelled and exfoliated by aqueous sonication into nearly transparent ultrathin nanosheets, stable in both acidic and alkaline media [1]. The synthesis, entailing use of simple precursors under ambient conditions [2,3], is easy and inexpensive and, for instance, can be carried out by thermally induced bulk condensation or pyrolysis of melamine or urea [4-9], also with no need for additives such organic solvents [8].

In the last years, g-C_3N_4 proved to have great potentiality in many fields, including sensing [10], bioimaging [11] and photocatalysis [12,13], and in principle could be a very efficient sorbent in analytical sample preparation. Because of its similarity to graphene, good compatibility with aqueous phase and expected great affinity for a wide range of compounds, g-C_3N_4 is a very promising sorbent for solid-phase extraction (SPE) [14,15]. Indeed, its double-sided polyaromatic scaffold shows both sides of the planar sheets available for molecules adsorption, and the large π-electron system also endows a strong affinity for carbon-based ring structures, typical of many drugs, pollutants, and biomolecules [5].

Few papers are currently available on the application of g-C_3N_4 for pre-concentration purposes, specifically in batch (magnetic) extraction [5-8,16,17] and solid-phase microextraction (SPME) [4], but none of them deals with application to column-SPE. Nonetheless, the use of g-C_3N_4 for SPE of pharmaceuticals, fluoroquinolones (FQ) included, from water is not documented at present.

FQs, antibiotics for human and veterinary medicine endowed with broad activity spectrum and good oral absorption, are an important class of emerging contaminants. Their occurrence, assessed in various environmental compartments [18,19], is essentially due to the partial metabolization and partial abatement by wastewater treatment plants (WWTPs) [20]. This causes the release of pharmaceutically active drugs into water basins, affecting their transformation/removal rates. Moreover, the common practice of recycling manure from livestock farming and sewage sludge from WWTPs as fertilizers, and recycling of sludge for

the production of compost, widely employed as soil conditioner/fertilizer, are other major contamination routes [21,22]. Despite FQs photosensitivity, both in water [23,24] and soil systems [25] their presence in the environment implicates serious threats to the ecosystem and humans. Mainly, toxicity [24], bacterial resistance stimulation [26] and formation of pharmacologically active photoproducts that contribute to the overall impact [24,27]. It is evident that monitoring FQs in the environment is a priority task that needs accurate, robust and sensitive analytical methods. In particular, the low concentrations detected in surface waters (typically tens ng L^{-1}) require a SPE pre-concentration step prior instrumental analysis [28-32]. SPE can be carried out by reversed-phase or mixed-mode polymer-based sorbents [33]. Recently, graphene – a 2D carbon material capable of multi-type interaction – grafted onto silica microparticles, has been proposed for FQs pre-concentration [34].

Prompted by the performance of mixed-mode sorbents, herein we report the preparation and characterization of a composite consisting of silica-supported g-C_3N_4, and its original application as SPE sorbent. g-C_3N_4 was grafted onto silica in order to have a sorbent to be easily used in conventional SPE columns, thus avoiding the excessive packaging that would otherwise occur using the bulk material [7]. The preparation of the material, simple and costless, is carried out by mixing the g-C_3N_4 precursor (in the present case dicyandiamide) and silica followed by a thermal treatment under nitrogen environment. g-C_3N_4@silica has been characterized by X-ray diffraction (XRD), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and surface area measurements (BET), and tested as SPE sorbent for pre-concentration of Ciprofloxacin (CIP), Danofloxacin (DAN), Enrofloxacin (ENR), Levofloxacin (LEV) and Marbofloxacin (MAR) from environmental waters. After SPE on g-C_3N_4@silica, separation and quantification were performed by high performance liquid chromatography coupled with fluorescence detection (HPLC-FD).

2. Experimental

2.1 Chemicals and materials

All chemicals were reagent grade or higher in quality. Silica (< 63 μm/230 mesh) from Merck (Darmstadt, Germany) was used. Analytical grade FQs standards (CIP, DAN, ENR, LEV, MAR), tetrabutylammonium hydroxide (TBAH, 1 M in H_2O), ethanol (≥ 99.8 %), dicyandiamide (99 %), polypropylene tubes and

polyethylene frits were supplied by Sigma-Aldrich (Milan, Italy). HPLC gradient grade acetonitrile (ACN) and methanol (MeOH) were purchased by VWR (Milan, Italy). H_3PO_4 (85 %, w/w), HCl (37 %, w/w), NH_3 (30 %, w/w) and anhydrous NaOH (97 %) were obtained from Carlo Erba Reagents (Milan, Italy). Ultrapure water (resistivity 18.2 MΩ cm^{-1} at 25 °C) was produced by a Millipore Milli-Q system. pH was measured by an Orion 420A pH meter (Thermo Electron, Rodano, Italy). FQs stock solutions of 200 μg mL^{-1} were prepared in MeOH containing 0.1 % (v/v) 1 M NaOH and stored in the dark (4 °C). FQs working solutions of 600 μg L^{-1} in 25 mM H_3PO_4 aqueous solution were renewed weekly. Laboratory operations were conducted under red light.

2.2 Synthesis of g-C_3N_4@silica

Dicyandiamide was dissolved in ethanol-water (4:1) by heating to 80 °C and kept at this temperature for 5 min to ensure all the reagent was dissolved. To this solution, proper amounts of silica to obtain 5, 20 and 50 wt% g-C_3N_4@silica composites, were added and the mixture stirred vigorously. After that, the mixture was heated at 100 °C until the ethanol and water were evaporated and a white solid was obtained. The resulting material was then dried at 100 °C in an air oven overnight and thermally treated in N_2 atmosphere at 550 °C for 4 h with a heating ramp of 1 °C min^{-1} and a cooling ramp of 10 °C min^{-1} [35].

2.3 Characterization of g-C_3N_4@silica

XRD patterns have been collected on a Bruker D8 Advance diffractometer using a Cu Kα radiation between 5 and 35° with step size 0.02°. TGA has been carried out with a Q5000 thermobalance (TA Instrument) under N_2 atmosphere between room temperature and 750 °C. Microstructural characterization of the samples was made using a high-resolution scanning electron microscope (SEM, TES- CAN Mira 3) operated at 15 kV. FTIR spectra were recorded using a Nicolet FT-IR iS10 spectrometer (Nicolet, Madison, WI) equipped with ATR (attenuated total reflectance) sampling accessory (Smart iTR with ZnSe plate) by coadding 256 scans in the 4000-650 cm^{-1} range at 4 cm^{-1} resolution. Specific surface area measurements were performed by a Sorptomatic 1990 instrument by Thermo Fisher Scientific. About 350 mg of powder was charged in the glass sample holder and degassed at 100 °C for 60 h. Subsequently, samples were cooled down at -196 °C

and 2 adsorption–desorption cycles followed by a last adsorption run were performed (BET method, analyzing gas N_2, 50 points for run; blank done in He before the first adsorption run).

2.4 Water samples

Tap water was from the Pavia municipal waterworks. Northern Italy surface waters were collected at 30-50 cm depth, directly in amber glass bottles, from Ticino River, Adda River, Naviglio Pavese River, Garda Lake and from a ditch downstream swine farms near Pavia (physical-chemical parameters reported in Supplementary Data). Samples were stored in the dark (4 °C) and analyzed within 24 h.

2.5 g-C_3N_4@silica SPE procedure

The cartridges (6 mL polypropylene tubes, 12 mm inner diameter, 65 mm length) were prepared by placing 100 mg g-C_3N_4@silica between two polyethylene frits, and were washed under vacuum with 30 mL MeOH followed by 50 mL ultrapure water to remove impurities and minimize void/channeling effect. For the extraction procedure, performed by means of a vacuum system (Resprep manifold, Restek Corporation, Bellefonte, USA), the cartridge was conditioned with 5 mL MeOH and 5 mL ultrapure water; the water sample (50-500 mL) was fed to the column at a flow rate of ca. 2 mL min^{-1}, and then the cartridge was dried under vacuum for 5 min. The analytes were simultaneously eluted (1 mL min^{-1}) with 6 mL 25 mM H_3PO_4 aqueous solution-ACN (80:20) and submitted to HPLC-FD analysis. For sample concentrations lower than 50 ng L^{-1}, the extracts were evaporated to 1 mL by XpressVap™ microwave accessory (radiation power 800 W, 13 min, CEM MarsXpress). For reusability tests, the cartridge was washed with 20 mL extracting solution to avoid potential carry over.

2.6 Liquid chromatography with fluorescence detection

The HPLC-FD system consisted of a pump Series 200 equipped with vacuum degasser and interfaced with a programmable fluorescence detector (Perkin Elmer, Monza, Italy). A Thermo Scientific Accucore™ Polar Advantage II C18 column (4.6 × 150 mm, 3 μm, 120 Å), purchased from Microcolumn (Lissone, Italy), was used coupled to a similar guard-column. After 5 min equilibration, 50 μL of each sample was injected into the HPLC system. The FD excitation/emission wavelengths selected were 280/450 nm for CIP, DAN and

ENR, 280/500 nm for LEV, and 297/507 nm for MAR. Elution was performed in 25 mM H_3PO_4-ACN (87:13) for 6 min, followed by a 1 min linear gradient to 25 mM H_3PO_4-ACN (90:10), then isocratic elution for 18 min (1 mL min^{-1}).

2.7 Analytical evaluation

Since the aim was applying g-C_3N_4@silica for pre-concentration of FQs from real samples, the whole analytical procedure was evaluated. In the absence of certified reference materials (CRMs), trueness was evaluated by recovery tests [36,37] in tap and river waters fortified in the range 10-100 ng L^{-1} of each FQ. The background FQs concentrations were checked by an independent method [34]. The inter-day precision (within-laboratory reproducibility) was explicated as relative standard deviation (RSD%). Selectivity of detection was checked from the HPLC-FD chromatograms of the SPE extracts obtained by pre-concentration of raw river water [34,38] showing a negligible FQ native content (experimentally verified according to ref. [34]). To calculate recovery at the concentrations expected after SPE, three independent calibration curves (1-50 μg L^{-1}) were generated for each analyte in the SPE eluting solution, i.e. 25 mM H_3PO_4 aqueous solution-ACN (80:20), as previously reported [39]. Method detection and quantification limits (MDL, MQL) were experimentally determined. MDL was the minimum concentration that provided a FD signal significantly different from zero, and MQL was the lowest concentration that provided acceptable recovery (≥ 70 %) and precision (RSD < 20 %) [38]. The batch-to-batch reproducibility was assessed by recovery tests on tap water spiked with 1 μg L^{-1} of each drug, using 3 g-C_3N_4@silica powders independently synthesized.

3. Results and discussion

3.1 Characterization of g-C_3N_4@silica

As reported in the Experimental part, three different composites have been synthesised, namely with 5, 20 and 50 wt% of g-C_3N_4. However, as highlighted later in the text, after a preliminary evaluation, the SPE study has been carried out on the 20 wt% composite only, and for this reason we are reporting here the characterization limited to this composition.

Fig. 1a shows the XRD patterns of pure silica, pure g-C_3N_4 and the g-C_3N_4@silica composite (20 wt% in g-C_3N_4). As can be seen, silica is fully amorphous while g-C_3N_4 presents the common XRD pattern characterized by two intense peaks around 12.8°, due to the intralayer distances, and around 27.5° related to the distances between graphitic layers. The g-C_3N_4@silica composite presents an XRD pattern characteristic of an amorphous sample as well. However, the absence of the features related to g-C_3N_4 is not due to the formation of amorphous carbon nitride but to the relatively low amount of this phase (which is a bad scatterer) in the composite. As a matter of fact, by increasing the amount of g-C_3N_4 in the composites it is possible to observe the contribution to the XRD pattern of the graphitic phase as well (see for example the 50 wt% g-C_3N_4@silica composite in the Supplementary Data).

Thermogravimetry has been used to assess the actual amount of g-C_3N_4 present in the composite. As can be inferred from Fig. 1b, above ca. 700 °C g-C_3N_4 is completely decomposed (see the green curve) while SiO_2 is stable up to that temperature. The weight loss for the g-C_3N_4@silica sample is around 17 % thus indicating the successful loading of g-C_3N_4 on silica which is also close to the nominal value of 20 %. For this reason, throughout this paper we are going to keep the nominal stoichiometry as reference by using, however, for the discussion the real value determined by TGA.

Representative SEM images of the g-C_3N_4@silica 20 wt% composite are reported in Fig. 2. For comparison, the SEM data for pure SiO_2 are as well shown. Pure silica is made of micron-size particles with flat and clean surfaces, while the incorporation of g-C_3N_4 leads to the appearance of layered structures on the SiO_2 particles surface.

FTIR measurements have been further used to confirm the successful loading of g-C_3N_4 on silica. Fig. 3 shows the spectra for pure silica, pure g-C_3N_4 and the g-C_3N_4@silica composite (20 wt% in g-C_3N_4). The FTIR for pure g-C_3N_4 is in fully agreement with previous reported data on samples prepared starting from dicyandiamide [40] and characterized by a sharp peak around 800 cm^{-1} assigned to out-of-plane bending of six-membered rings common to either triazine or eptazine structures [41]. Bands in the 1200-1600 cm^{-1} range are instead typical of C-N stretching and bending vibrations of nitrogen heterocycles. The FTIR spectrum of amorphous silica (black line in Fig. 3) shows an intense peak around 1100 cm^{-1} assigned to the Si–O–Si asymmetric stretching vibration, while the peak at about 850 cm^{-1} is characteristic of the Si–OH bending from silica itself [42]. No hydroxyl groups are detected on the sample (expected to be present around 3500

194 cm^{-1}). The spectrum of g-C_3N_4@silica (blue line in Fig. 3) presents characteristics feature of both pure silica
195 and g-C_3N_4. In particular, the g-C_3N_4 representative peaks are clearly visible in the 1200-1600 cm^{-1} region
196 (highlighted with a red ellipse) while the sharp peak around 800 cm^{-1} is superimposed to the Si-OH bending
197 peak of pure silica.
198 Finally, the surface area of the g-C_3N_4@silica 20 wt% composite has been measured by means of gas
199 porosimetry method and resulted to be 210 ± 5 m^2 g^{-1}, to be compared to those of pristine silica (450 ± 5 m^2
200 g^{-1}) and pure g-C_3N_4 (4 ± 2 m^2 g^{-1}).
201
202 **3.2 Solid-phase extraction on g-C_3N_4@silica**
203 Due to its aromatic nitrogen-rich 2D carbon structure, g-C_3N_4 is endowed with good polarity, water
204 compatibility and potential affinity for a wide range of molecules. Thus, it has great potentiality to be used as
205 mixed-mode sorbent for extraction of polar organics from water samples [14,15]. This is the case of FQs,
206 amphoteric compounds consisting of an aromatic quinolone core bearing polar ionisable groups, that in
207 principle are prone to interact with the g-C_3N_4 layers grafted onto silica.
208 The feasibility of using g-C_3N_4@silica for SPE of FQs was first investigated in tap water, considering the
209 effect of various parameters, i.e. g-C_3N_4 silica loading, sorbent amount, sample volume and type/volume of
210 eluting solvent. Experiments in tap water samples – 50 mL, native pH, 5 μg L^{-1} MAR and ENR, 200 mg
211 sorbent, elution with 3 × 5 mL 10^{-3} M HCl aqueous solution-ACN (80:20) – were initially undertaken to
212 investigate the affinity of FQ species for the material. The g-C_3N_4 loading (nominal 5-20-50 wt%) affected
213 both adsorption and desorption. Specifically, at the highest loading considerable amounts of FQs were not
214 retained (11-42 %), and 61-82 % of the sorbed fraction was eluted. At the lowest loading, despite better
215 adsorption (2-11 % not retained), elution was lower (58-64 %). This behaviour can be explained in terms of
216 g-C_3N_4 sheets aggregation, which is reasonably caused by increasing the loading. Accordingly, more active
217 sites available for adsorption should be present in g-C_3N_4@silica 5 wt%, resulting in a stronger retention
218 capability. It was observed that the intermediate amount of g-C_3N_4 (i.e. 20 wt%) provided quantitative
219 removal of FQs from water (residual drugs < IDL) and mean recovery around 78 % (RSD < 14 %, n=3).
220 Elution was improved using 25 mM H_3PO_4 aqueous solution-ACN (80:20), requiring only 10 mL to elute the
221 analytes.

Since quantitative FQ adsorption from the sample was gained at the native pH, no pH adjustment was done. This indicates good affinity of the analytes zwitterionic (globally uncharged) form for carbon nitride, clearly due to the combination of Van der Waals, π stacking, dipole-dipole, hydrophobic and hydrogen bond interactions [15]. This behaviour was in line with other mixed-mode phases [28,34]. Moreover, considering that carbon nitride isoelectric point is around 4.5-5 [15,43] and that no significant variation in the recovery was observed also in high-salinity samples (5-15 % w/v NaCl), π stacking seems to be the driving force for FQ molecular adsorption.

Additional work was addressed to further investigate the elution step. Considering both the g-C_3N_4 isoelectric point and the zwitterionic nature of FQs (CIP pK_a1 5.90, pK_a2 8.89; DAN pK_a1 6.07-6.32, pK_a2 8.56-8.73; ENR pK_a1 6.27, pK_a2 8.3; LEV pK_a1 5.70-6.05, pK_a2 7.90-8.22; MAR pK_a1 5.51-5.69, pK_a2 8.02-8.58) [33] both acidic and alkaline solutions were tested. Fig. 4a gathers the responses obtained by SPE of 250 mL tap water (100 ng L^{-1} FQs) as function of the type of eluting phase.

The best elution was provided by 25 mM H_3PO_4 aqueous solution-ACN (80:20), successfully used also for the commercial mixed-mode hydrophilic-lipophilic HLB polymer [44]. Inferior or higher ACN percentages gave poorer recovery (data not shown). Lower recoveries were observed under alkaline conditions, i.e. 4 % NH_3-MeOH (85:15), suggested for FQs elution using a variety of commercial cartridges [33]. Moreover, differently from graphene [34], FQs were better desorbed in acidic solution rather than in 25mM TBAH aqueous solution-ACN (70:30), hence use of ion pairing agents was not necessary. These findings could be explained by the different polarity of the two 2D materials, and suggest that FQs desorption from g-C_3N_4 is easier. This is justified by the lower aromaticity of g-C_3N_4 compared to graphene, involved by the C-N polar bonds that decrease π-delocalization [15].

The effects of sorbent amount and elution volume are shown in Fig. 4b and 4c, respectively. These indicate that FQs recovery is lowered in going from 100 to 300 mg g-C_3N_4@silica, and that volumes below 6 mL were not convenient. Conversely, using higher sorbent amounts would require higher elution volumes, thus lowering enrichment factor.

Under best conditions, i.e. 100 mg sorbent and 6 mL 25 mM H_3PO_4 aqueous solution-ACN (80:20) elution, recovery in the range 70-101 % was obtained for the five drugs (Table 1). A typical chromatogram is reported in Fig. 5.

The procedure has been tested also in raw river water, obtaining good extraction efficiency. The mean recoveries, shown in Table 1, indicated that FQs adsorption is not hampered by other matrix constituents. As displayed in Fig. 6, FD allowed selective detection of FQs in raw surface water, as no interfering signals were noticed in "blank" river water (i.e. with negligible FQ native content) at the retention times of the 5 compounds.

It was found that 100 mg g-C_3N_4@silica 20 wt% (≡ 20 mg g-C_3N_4) allowed SPE of 500 mL raw river water spiked with FQs at 10-100 ng L^{-1}; moreover, clean extracts were obtained from actual matrices (see Fig. 6), with no need for additional anion-exchange sample cleanup, that instead is necessary for FQs pre-concentration using HLB SPE [44-46].

The cartridge proved to be reusable for more than one SPE, as that the extraction efficiency was maintained also in raw river water for at least 4 runs (recovery > 70 %), accounting for good chemical/mechanical stability of the composite.

The batch-to-batch reproducibility was assessed by recovery tests (100 mL tap water, 1 μg L^{-1} FQs) on 3 independent preparations of g-C_3N_4@silica. RSD values in the range 6-12 % accounted for good overall reproducibility, from synthesis to instrumental analysis.

3.3 Analytical performance of g-C_3N_4@silica SPE coupled to HPLC-FD

The key features of the whole analytical procedure are hereafter summarized. The mean recoveries (%) obtained in water samples spiked at environmentally significant concentrations are reported in Table 1. Recovery was calculated as the ratio between the concentration determined in the g-C_3N_4@silica extract and that expected after pre-concentration (calculated considering the initial amount of analyte and the enrichment factor). As apparent, recovery was generally between 70 % and 101 % in tap water; quantitative extraction (70-101 %) was also obtained in untreated river water spiked with 20-100 ng L^{-1}, and for ENR, LEV and MAR recovery was good also at 10 ng L^{-1} spike. Precision was evaluated by calculating the RSDs associated to the mean recovery obtained for each concentration. As shown in Table 1, the inter-day precision showed RSDs ≤ 11 % (n=4) in tap water; suitable reproducibility was also observed in the surface water, obtaining RSDs lower than 12 % (n=3).

277 Selectivity of detection was confirmed comparing the HPLC-FD profile of a "blank" river water SPE extract
278 and the one of the same river water sample spiked with 20 ng L^{-1} of each FQ prior pre-concentration (Fig. 6).
279 Moreover, as FQs are detected as native compounds, information provided by fluorescence detection is
280 valuable in terms of selectivity [32,34,44].
281 Good linearity ($r^2 > 0.9991$) was found for the 5 drugs in the concentration range 1-50 μg L^{-1}, in agreement
282 with literature [39]. Regarding method sensitivity, MDL and MQL experimentally determined in raw river
283 water were respectively 3 ng L^{-1} and 10 ng L^{-1}, except for CIP and DAN that were quantitatively determined
284 at concentrations ≥ 20 ng L^{-1} (see Table 1). The sensitivity achieved is comparable with or better than that
285 reported for FQs in natural waters (see Table 2), thus g-C_3N_4@silica appears as a suitable sorbent for
286 determination at the low nanograms per litre.
287 Compared to silica derivatized with graphene [34] or pyrolyzed Kraft lignin [32], this preparation is easier
288 (one-pot, just need for ethanol and dicyandiamide); moreover, great advantage is gained in terms of costs in
289 comparison with graphene (dicyandiamide 0.025 €/g, graphene oxide 272.44 €/g, April 2016 prices).
290
291 **3.4 Application to real surface water samples**
292 g-C_3N_4@silica was applied to SPE of environmental samples, i.e. lake, river and ditch water, collected in the
293 Lombardy plain (Northern Italy). As it can be seen in Table 3, FQs were detected in all samples, attesting
294 their environmental diffusion in surface waters.
295 In particular, while in these lake and river water samples FQs amounts were below MDLs and/or MQLs, the
296 veterinary MAR and ENR were quantified at tens nanograms per litre in the ditch directly receiving swine
297 farms effluents, thus underlining the environmental impact of livestock farming.
298
299 ## 4. Conclusions
300 Graphitic carbon nitride obtained from dicyandiamide has been supported onto silica microparticles,
301 characterized and tested for solid-phase extraction of FQ emerging contaminants from natural waters. g-
302 C_3N_4@silica was successfully employed for FQs determination in actual surface water samples prior HPLC-
303 FD, obtaining sensitivity better than or similar to that achieved using current carbon-based sorbents,
304 including graphene, but at remarkably lower costs. The material preparation is environmentally benign,

simple (one-pot), inexpensive and reproducible. g-C_3N_4@silica is efficient for enrichment of FQs from aqueous complex matrices, and can be employed for more than one pre-concentration. The results here presented contribute to enlarge the application of g-C_3N_4 for analytical purposes. It is believable that use of supported g-C_3N_4 for column-extractions could be extended to other contaminants.

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440
441

Figure captions

Fig. 1 XRD patterns (a) and TGA curves (b) for SiO_2, g-C_3N_4@silica 20 wt%, and g-C_3N_4.

Fig. 2 SEM images at 10.000X and 25.000X for silica (a, b), and g-C_3N_4@silica 20 wt% (c, d).

Fig. 3 FTIR spectra for SiO_2, g-C_3N_4@silica 20 wt%, and g-C_3N_4.

Fig. 4 Effect of type eluting solution (a), sorbent amount (b) and eluting volume (c). Samples: 250 mL tap water spiked with 100 ng L^{-1} of each FQ (n=4).

Fig. 5 HPLC-FD chromatogram obtained by SPE of tap water (500 mL) spiked with 20 ng L^{-1} FQs.

Fig. 6 HPLC-FD chromatograms overlay of raw river water (500 mL) SPE extract and the SPE extract of the same river water sample spiked with 20 ng L^{-1} of each FQ prior pre-concentration.

Table titles

Table 1 Mean recoveries (%) and inter-day precision (RSD%) obtained by g-C_3N_4@silica SPE of tap and river water samples spiked with each compound. Conditions: 100 mg sorbent, 6 mL 25 mM H_3PO_4 aqueous solution-ACN (80:20) elution, sample volume 50-500 mL (native pH).

Table 2 Comparison with recent analytical methods for SPE of FQs from natural waters.

Table 3 FQs amounts determined in actual surface water samples by SPE on g-C_3N_4@silica followed by HPLC-FD.

Figure 1

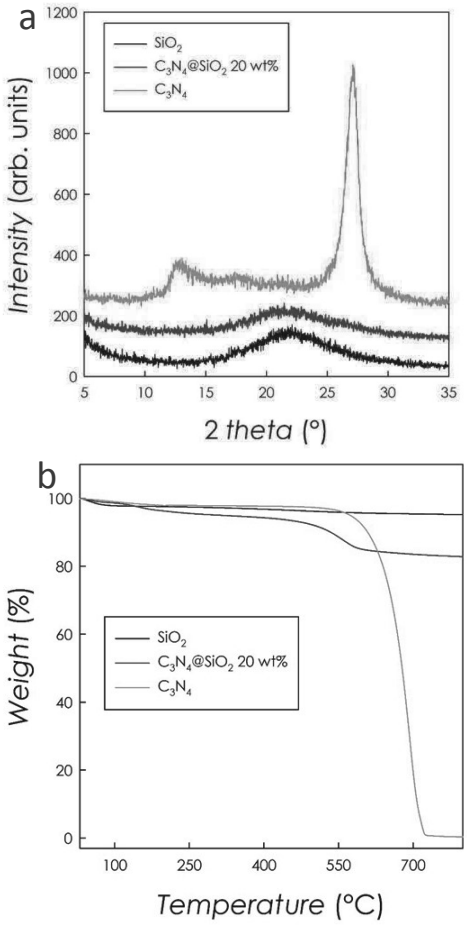
a
Intensity (arb. units)
2 theta (°)
SiO2
C3N4@SiO2 20 wt%
C3N4
b
Weight (%)
Temperature (°C)
SiO2
C3N4@SiO2 20 wt%
C3N4

Figure 2

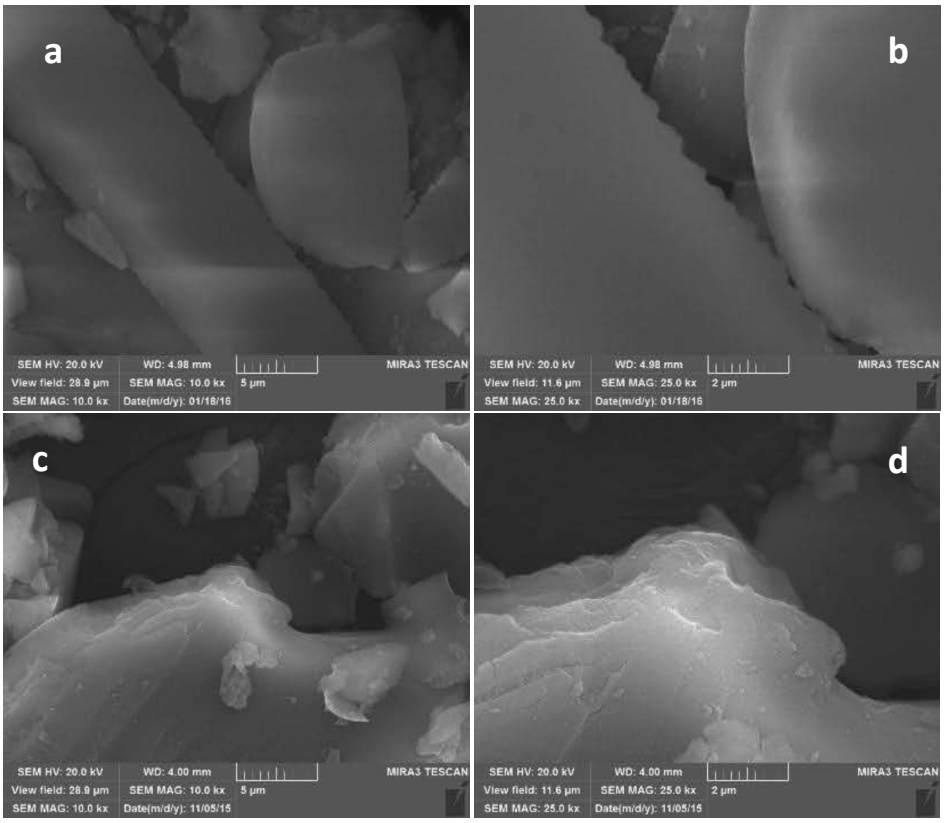
a
SEM HV: 20.0 kV
WD: 4.98 mm
MIRA3 TESCAN
View field: 28.9 µm
SEM MAG: 10.0 kx
5 µm
SEM MAG: 10.0 kx
Date(m/d/y): 01/18/16
b
SEM HV: 20.0 kV
WD: 4.98 mm
MIRA3 TESCAN
View field: 11.6 µm
SEM MAG: 25.0 kx
2 µm
SEM MAG: 25.0 kx
Date(m/d/y): 01/18/16
c
SEM HV: 20.0 kV
WD: 4.00 mm
MIRA3 TESCAN
View field: 28.9 µm
SEM MAG: 10.0 kx
5 µm
SEM MAG: 10.0 kx
Date(m/d/y): 11/05/15
d
SEM HV: 20.0 kV
WD: 4.00 mm
MIRA3 TESCAN
View field: 11.6 µm
SEM MAG: 25.0 kx
2 µm
SEM MAG: 25.0 kx
Date(m/d/y): 11/05/15

Figure 3

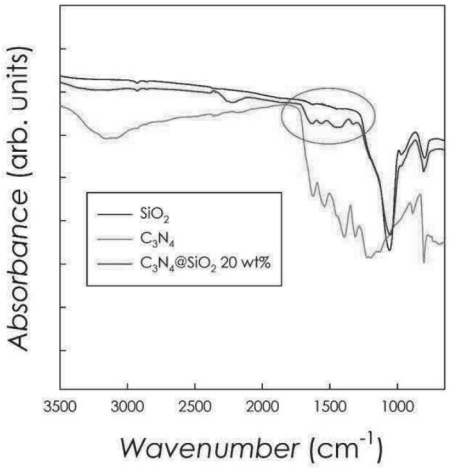
Absorbance (arb. units)
SiO2
C3N4
C3N4@SiO2 20 wt%
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

Figure 4

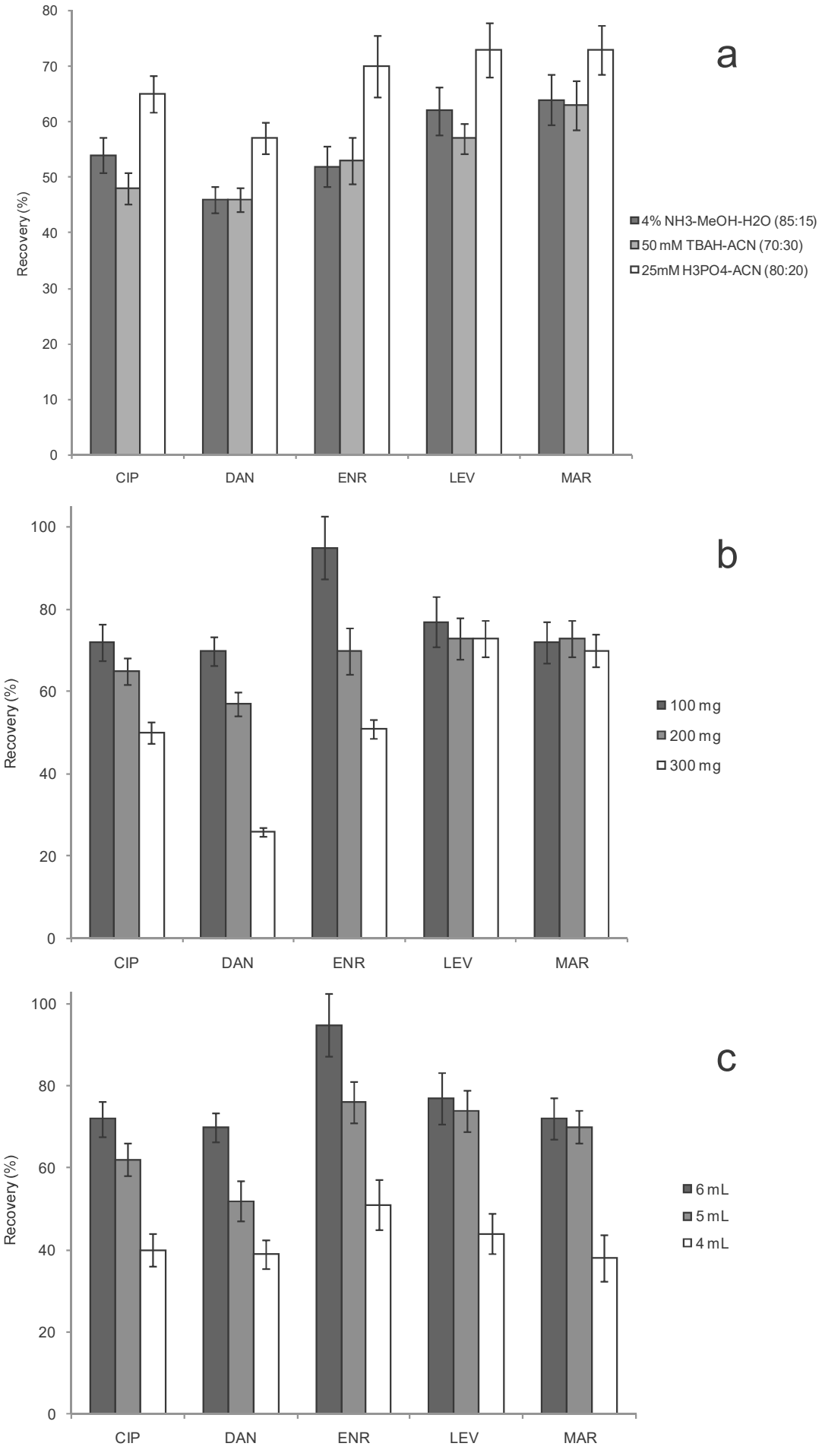
a
Recovery (%)
80
70
60
50
40
30
20
10
0
CIP
DAN
ENR
LEV
MAR
4% NH3-MeOH-H2O (85:15)
50 mM TBAH-ACN (70:30)
25mM H3PO4-ACN (80:20)
b
Recovery (%)
100
80
60
40
20
0
CIP
DAN
ENR
LEV
MAR
100 mg
200 mg
300 mg
c
Recovery (%)
100
80
60
40
20
0
CIP
DAN
ENR
LEV
MAR
6 mL
5 mL
4 mL

Figure 5

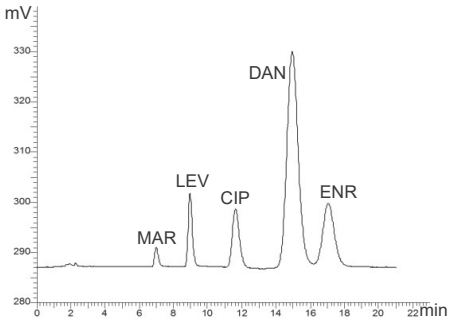
mV
330
320
310
300
290
280
0
2
4
6
8
10
12
14
16
18
20
22
min
MAR
LEV
CIP
DAN
ENR

Figure 6

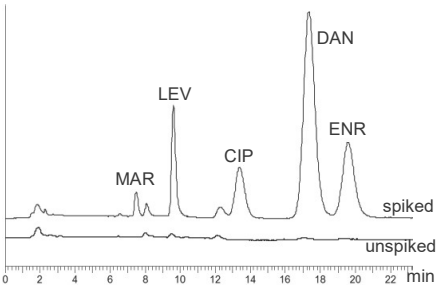
DAN
LEV
ENR
CIP
MAR
spiked
unspiked
0 2 4 6 8 10 12 14 16 18 20 22 min

Table 1

	Mean recovery (%) and inter-day precision [a]							
	Tap water (*n*=4)				River water (*n*=3)			
Spike (ng L^{-1})	*100*	*50*	*20*	*10*	*100*	*50*	*20*	*10*
CIP	72 (6)	70 (6)	73 (8)	89 (8)	85 (8)	70 (8)	90 (10)	66 (12)
DAN	70 (5)	71 (7)	82 (11)	88 (9)	74 (8)	71 (9)	70 (11)	55 (14)
ENR	95 (8)	70 (5)	76 (8)	100 (11)	101 (9)	80 (9)	99 (10)	76 (12)
LEV	77 (8)	80 (7)	92 (10)	101 (9)	91 (9)	80 (9)	82 (10)	84 (11)
MAR	72 (7)	73 (8)	70 (9)	92 (10)	80 (9)	70 (9)	75 (10)	114 (12)

[a] reported in parentheses as RSD%

Table 2

Sorbent material	MQL (ng L^{-1})	Determination	Ref.
HLB[a]	4-26	UHPLC-MS	28
HLB	1.81-45.17	UHPLC-MS	31
Cigarette filters	2-5	HPLC-UV	29
HyperSep Retain AX[b]	10-260	HPLC-FD	30
GN-silica[c]	5	HPLC-FD	34
LG-silica[d]	20	HPLC-FD	32
g-C_3N_4@silica	10-20	HPLC-FD	This work

[a] hydrophilic-lipophilic balanced polymer
[b] anion exchange polymer
[c] silica-supported graphene
[d] silica-supported pyrolized Kraft lignin

Table 3

Sample	FQs concentrations (ng L^{-1})				
	CIP	DAN	ENR	LEV	MAR
Adda River	n.d.	< 20	< 10	< 10	< 10
Naviglio Pavese River	< 20	< 20	< 10	< 10	< 10
Garda Lake	< 20	< 20	< 10	< 10	< 10
Farm ditch 1	n.d.	n.d.	31[a]	n.d.	27[a]

[a] RSD < 12% (*n*=3)
n.d. not detected

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