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In-Tube Solid Phase Extraction with Graphitic-Based Polyurethane Sponge as a Superhydrophobic Sorbent and Determination of Drug Residues in Foodstuffs Using High-Performance Liquid Chromatography

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Abstract:

Veterinary drugs used in animal husbandry raise public health concerns due to their residues in the bodies of animals. This study employs a simple and quick sample preparation technique, in-tube solid phase extraction, to extract drug residues from foodstuffs, including eggs, honey, and water. This technique utilizes the synergy of graphitic-based materials and polyurethane sponges (PU) combined through dip coating method to make reusable sorbents for extracting drugs, including amoxicillin, paracetamol, ciprofloxacin, and cefixime. These prepared sorbents were characterized using FTIR, SEM, and XRD. HPLC analysis assessed the extraction efficiency, considering various parameters such as analyte concentration, sample solution pH, extraction time, type of

eluting solvent, and graphitic-based polyurethane sponge reusability and stability. The proposed method exhibited a linear repsonse for all three sorbents in the range of 0.03–1000 µg mL^{-1}, with LOD 0.03-1.60 µg mL^{-1} and LOQ 0.18-4.84 µg mL^{-1}. The % RSD ranged from 1.3–9.3 %, with recoveries of up to 98.42%.

Keywords: Graphitic-based sorbents; Polyurethane sponge; In-tube solid phase extraction; Drug residues, HPLC-UV

Highlights:

- Functionalization of polyurethane sponge with graphitic materials.
- Development of an In-tube SPE method.
- Extraction of drug residues from food samples.
- HPLC method development for the determination of drug residues.

1. Introduction:

Detailed analysis of food is crucial for minimizing the adverse consequences associated with drug residues. It safeguards animal-derived food contamination from drug residues, ultimately ensuring consumer health safety (Souza-Silva, Gionfriddo, & Pawliszyn, 2015). With a growing focus being given to food products, researchers have increasingly turned their attention to quantifying the presence of veterinary drug residues in food, making it a topic of interest in analytical fields (Amelin & Lavrukhina, 2017). The presence of drug residues, especially antibiotics, in animal-derived food products, can lead to harmful side effects on human health, including allergic reactions. Furthermore, the presence of drug residues is a significant and growing concern, as it can contribute to the development of drug-resistant pathogens (X. He et al., 2017). Antibiotics are frequently employed in animal husbandry to treat and prevent various diseases (Rodriguez, Moreno-Bondi, & Marazuela, 2011; Yu et al., 2015). Excessive antibiotic usage leads to the accumulation of drug residues in the tissues of animals, ultimately affecting the safety of food products derived from the animals (Sun, He, Lv, & Liang, 2007).

Furthermore, their controlled administration becomes necessary when significant quantities of drug residues are found in animal tissues. With the monitoring of residues in food products intended for human consumption mandatory to safeguard public health and food safety. Hence, it is standard practice to use target samples of food such as meat, eggs, and milk from animal sources to assess drug residues (Sajjad et al., 2023).

Different types of veterinary drugs boost animal growth and prevent diseases (S. Li, Zhang, Chen, Zhang, & Liu, 2020). Common veterinary drugs such as amoxicillin, paracetamol, ciprofloxacin, and cefixime are used in the treatment of different animal diseases like bacterial infections, liver damage, allergic reactions, inflammation, and gastrointestinal upsets (Gillies et al., 2015).

However, their excessive use is dangerous for both animals and humans. As a result, the European Union has set the range of MRL values for drug residues; 100–400 µg/kg (Mund, Khan, Tahir, Mustafa, & Fayyaz, 2017).

Therefore, developing selective and sensitive analytical methods is essential for extracting and quantifying drug residues from foodstuffs. Various analytical techniques have been developed for drug residue determination from food samples, including high-performance liquid chromatography (HPLC) (G. N. Wang, Yang, Liu, Feng, & Wang, 2016), mass spectrometry, capillary electrophoresis (Louppis, Kontominas, & Papastephanou, 2017), fluorescence detection (FLD) (Pochivalov, Timofeeva, Vakh, & Bulatov, 2017), microbial inhibition assays (Shahbazi, Ahmadi, & Karami, 2015), and enzyme-linked immunosorbent assays (ELISA) (Falowo & Akimoladun, 2019).

Reproducibile, standard approaches to sample preparation are essential to enable quantification of drug residues in samples (Ahmed et al., 2020). Food samples typically contain various ingredients, including trace amounts of drugs. As a result, it is essential to prioritize the sample pretreatment to purify and concentrate the analyte of interest. The purification and pre-concentration of samples prior to anaylsis are based on a number of different techniques, e.g. solid-phase microextraction (SPME) (L. Chen & Huang, 2016; X.-M. He et al., 2015), liquid-liquid microextraction (LLME) (Timofeeva, Shishov, Kanashina, Dzema, & Bulatov, 2017; Timofeeva, Timofeev, Moskvin, & Bulatov, 2017), magnetic solid-phase dispersion (MSPD) (Y. Li et al., 2015), and the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method (Frenich, del Mar Aguilera-Luiz, Vidal, & Romero-González, 2010).

The selectivity and specificity of the analytical methods depend directly on the performance of the sorbent materials in the case of solid phase extraction methods (Y. Li et al., 2015). As a result,

innovative sorbent materials have become useful tools in the study of complex matrices to improve the effectiveness of target analytes extraction (Yan, Sun, Liu, Row, & Song, 2014). Graphite powder has exceptional physical and chemical characteristics due to its two-dimensional structure, which is thermally and chemically stable, hydrophobic, and results in a high surface area. (Yan et al., 2014). Graphene oxide (GO) is prepared by the oxidation of graphite and is a single-atomic layered substance. However, graphene oxide has more hydroxyl and carboxyl groups than graphite powder (GP), which makes it more polar and facilitates bonding with other compounds (L. Wu et al., 2017). Because of the excellent sorption capacity and large surface area, reduced graphene oxide (RGO) is also attracting the attention of researchers (Petit & Bandosz, 2009). RGO forms strong π-π interactions with different bonds present in drugs because RGO contains a large, delocalsied π-electron system (Xuwei Chen, Hai, & Wang, 2016). Consequenlty, in comparison to other, commonly used, nanomaterial based adsorbent materials, graphene oxide has an exceptionally high adsorption capacity. It is most commonly used in the SPE procedure of organic compounds and for the separation of metal ions from different samples present in the environment (Liu, Shi, Sun, et al., 2011; Liu, Shi, Zeng, et al., 2011; X. Wang, Liu, Lu, & Qu, 2014). Graphene oxide is particualry suitable for use sample preparation via solid phase extraction because it addresses the issues with sample loading and elution (Guan et al., 2013).

Recently, a cheap and environment-friendly porous material, polyurethane (PU) sponges, were used for drug residue extraction from foodstuffs (Görmez, Görmez, Gözmen, & Kalderis, 2019). The porous structure of PU sponges has found signifiacnlty wider application in sorbents when compared with other porous spongy materials like melamine (Su, Yang, Song, Lu, & Chen, 2017; Q. Wu, Zhao, Feng, Wang, & Wang, 2011) or chitosan sponges (Su, Yang, Zhao, Liu, & Chen, 2017), and provides advantages like ease of use, flexibility, low cost, and good reusability (Meng,

Yan, Yu, & Jiao, 2018). Although PU sponges are hydrophilic, physical coatings like the functionalization of graphene-based materials are typically employed to achieve high separation efficiency and increased hydrophobicity (Meng et al., 2018). Different methods for functionalization, such as dip coating, in-situ chemical reactions, and chemical vapor deposition (CVD), are used to increase the hydrophobic nature of the sponge. Most commonly, dip coating is used because it is cheap, simple, and fast method for the preparation of hydrophobic sorbents that does not involve harmful and costly reagents. The cost-effective nature of dip coating means it is now widely used to prepare hydrophobic sponges on an industrial scale (Baig, Alghunaimi, Dossary, & Saleh, 2019; Xuemei Chen, Weibel, & Garimella, 2016), (Javadian, 2021).

The main goal of this study was to functionalize PU sponges with various derivatives of graphitic-based materials as the highly porous surface of PU sponges should allow for high levels of adsorption. With the resultant functionalized sponges explored as adsorbents for extracting drug residue from complex food matrices. It is noted that these PU-based sponges have not been utilized previous for in-tube, solid phase extraction of drug residues.

2. Materials and methods:

2.1: Chemicals and reagents:

All the chemicals were used to the following purities; graphite powder (99.99%), nitric acid (HNO_3, 99.9%), sulphuric acid (H_2SO_4, 98%), hydrogen peroxide (H_2O_2, 30%), potassium permagnate ($KMnO_4$, 99.0%), ethanol (EtOH, 99.5%), and hydrazine hydrate (80%). The solvents; acetonitrile (ACN), methanol (MeOH), acetone, and n-hexane, and the standard drug species; amoxicillin, paracetamol, ciprofloxacin, and cefixime, used in the research were all purchased from Sigma Aldrich, Germany and were of analytical grade.

2.2: HPLC conditions:

For the chromatographic analysis and separations, the HPLC system of Shimadzu was coupled to a UV detector to determine drugs from spiked standards. The separation column, a C18 column (4.6×250 nm, 5 µ Waters, USA), was sourced from Welchrom. The injection volume was 20 µL. Mobile phases were used with a 65:35 ratio of water and methanol with acetate buffer (pH = 3.60). Detection was performed at a fixed wavelength of 254 nm, with a mobile phase flow rate of 1.0 $mLmin^{-1}$. Peak identification was accomplished through a comparison of chromatograms and retention times of standard solutions.

2.3: Graphene oxide (GO) synthesis:

Graphene oxide was synthesized using a modified Hummers method. For this purpose, graphite powder (1 g) was mixed with H_2SO_4 (46 mL) and HNO_3 (18 mL) and stirred for 30 min in an ice bath at between 0-5°C. Then, $KMnO_4$ (6 g) was slowly added over 15 min to oxidise the graphite and stirred for 2 h until the solution turned dark green, maintaining a temperature of between 0-20°C. After removing it from the ice bath, the solution was stirred for an additional 1 h at 35°C. Gradually, deionized water (50 mL) was added, and the solution was heated to 95°C and stirred for 30 min until it turned deep brown. Excess $KMnO_4$ was eliminated by adding H_2O_2 (18 mL) stirring for 1 h. The resulting suspension was washed with deionized water until a neutral pH was reached and then the solid was filtered and dried in the oven at 90°C for 24 h to obtain pure GO powder.

2.4: Reduced graphene oxide (RGO) synthesis:

Reduced graphene oxide was synthesized from the graphene oxide obtained using the modified Hummers method described above. A mixture of GO (500mg) was mixed with a 50% solution of hydrazine hydrate (300 mL) and refluxed for 30 min. The resulting reduced graphene oxide was

washed several times with deionized water until the pH of the filtrate reached seven after cooling. The solid was then filtered and dried in an oven for 24 h at 60℃.

2.5: Fabrication of GP, GO, and RGO on Polyurethane (PU) sponge:

Commercial polyurethane (PU) sponge was cut into cubical blocks of equal size (1×1 cm). The PU sponge cubes were then washed with distilled water. The washed PU sponges were then dipped in ethanol and kept in an ultrasonic bath for 30 min. The sponges were then dried at 65°C in the oven overnight. The dried sponges were then dipped in an alcoholic suspension of either GP, GO, or RGO for 24 h at room temperature. The resultant functionalized sponges were finally dried in an oven for 24 h at 60°C. After drying, PU sponges appeared black showing the coating of graphite derivatives on the sponge.

Fig. 1: Photo of polyurethane sponges coated with graphitic materials.

2.6: Instruments and measurements:

The prepared materials were characterized using different characterization techniques; fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). Fourier transform infrared spectrum was performed on the samples as KBr pellets in the range of 4000-400 cm^{-1} using a Shimadzu FTIR-8400S instrument. X-ray diffraction was performed on a Powder D8 Advance, Bruker Germany, instrument at a current of 30 mA, a voltage of 40 kV for 2 (10-70°), with Cu K radiation = 0.154 nm. Scanning electron microscopy was performed using an FEI Nova 450 NanoSEM E-beam lithography instrument.

2.7: Stock solution preparation:

Stock solutions (1000 µg mL^{-1}) of amoxicillin, paracetamol, ciprofloxacin, and cefixime were prepared by dissolving each drug standard (10 mg) separately in water (10 mL). A combined mixture of the final drug solution was then prepared by mixing equal volume of each drug solution. Working solutions of different concentrations were then prepared by diluting the stock solution down appropriately for HPLC analysis.

2.8: Spiked samples preparation:

2.8.1: Spiked egg sample: A fresh egg purchased from a local supermarket was homogenized by magnetic stirring. Then, the egg sample (3 mL) was placed into an Eppendorf tube (15 mL), and methanol (5 mL) was added for deproteinization. Finally, the mixture was centrifuged for 10 minutes, and the upper supernatant layer was separated and spiked by adding the drug solution (5 mL).

2.8.2: Spiked honey sample*:* Honey samples were bought from the local supermarket. The honey was diluted with distilled water at a ratio of 2:10 (honey to water) before spiking. After dilution, the honey sample was spiked with the drug solution (5 mL). The drugs were extracted by in-tube SPE and analyzed by HPLC.

2.8.3: Spiked water sample: Water samples were collected from a nearby lake, and the drug solution (5 mL) was added directly to the water sample for spiking.

2.9: Optimization of in-tube SPE:

The in-tube SPE conditions were optimized to achieve maximum extraction by varying factors including; the analyte concentration, sample pH, extraction time, sample volume, and type of eluting solvent, and by exploring the reusability and stability of the sponge. The optimized conditions were subsequently applied for extracting drug residues from spiked food samples such

as water, eggs, and honey. Spiked food samples were passed through the functionalized sponges and left for 15 minutes. The analyte adsorbed on the functionalized sponges and was separated from the matrix solution at via centrifugation (167.7 g centrifugation force). Hexane (1 mL), as an eluting solvent, was added to extract the analyte from the functionalized sponges and left for 10 min. n-Hexane facilitated the complete extraction of drugs from sponges with the clear extractant containing the previously adsorbed drug residues obtained by centrifugation. The extraction was carried out at a low centrifugation speed for two reasons: (1) to facilitate easy and complete extraction of the analyte from the graphitic-based sponges and (2) to prevent graphitic-based materials from being removed from the structure of PU-based sponges. It was observed from initial experiments that the analyte concentration decreased when a high centrifugation rate was employed for extraction from the graphitic-based sponges. After centrifugation, the extractant was evaporated using N_2 atmosphere, mixed with the HPLC mobile phase (0.5 mL) and syringe-filtered before injection into the HPLC. Fig. 2 clearly illustrates the complete in-tube solid phase extraction method.

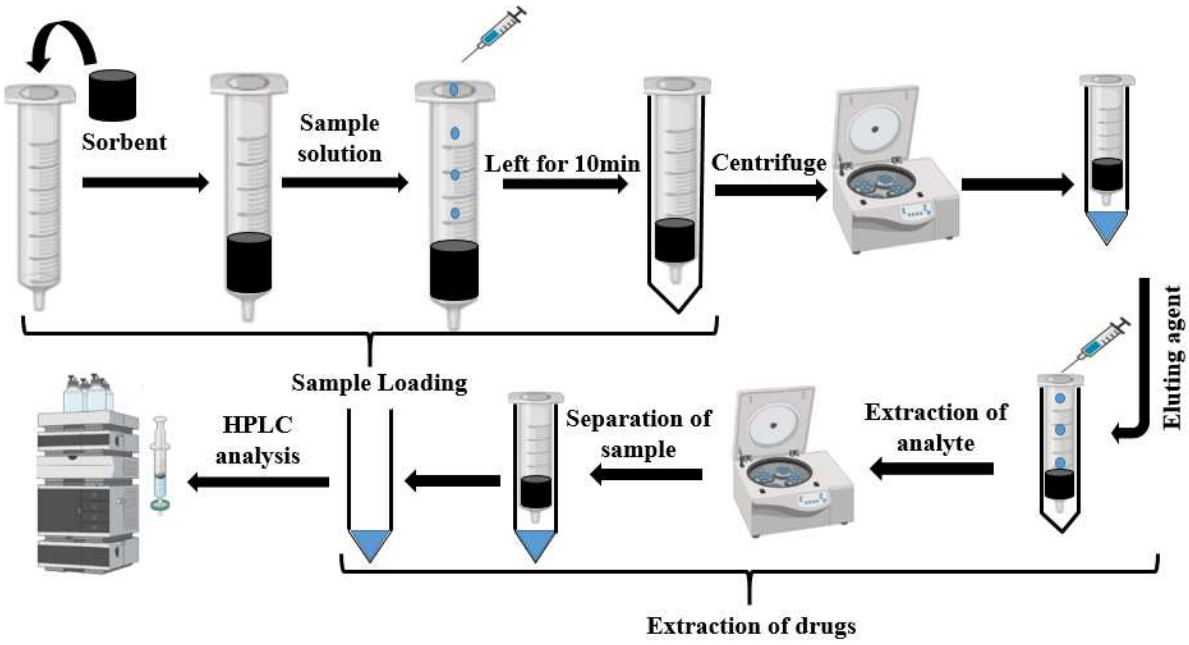


Fig.2: Schematic of the in-tube solid-phase extraction process using graphitic-based sponges.

3. Results and discussions:

3.1: Characterization:

The polyurethane sponges were functionalized with graphite powder, graphene oxide, and reduced graphene oxide, to form GP@PU, GO@PU and RGO@PU respectively, with the black colour of the resultant composite confirming successful functionalisation. PU based sponges contain pores in the structure, making them highly porous materials and rich surface chemistry due to functional groups (C=O and -N-H) in the skeleton of the sponge skeleton, in particular these surface groups, can interact with the functional groups of GO (epoxy groups).

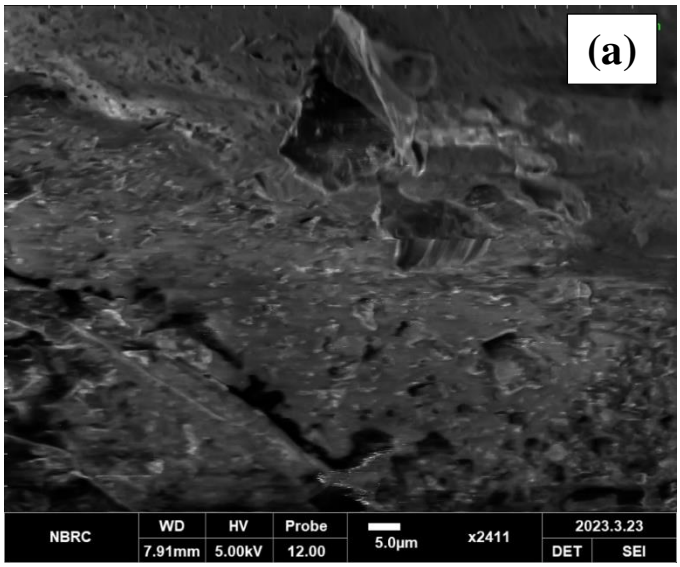
3.2: Fourier Transform Infrared Spectroscopy (FTIR):

Fourier transform infrared spectroscopy (FTIR) confirmed the presence of the functional groups in the sorbent materials. Graphite showed no significant peak, as shown in fig S3 (a), whereas graphene oxide showed more peaks containing more functional groups of oxygen after the

oxidation process, fig S3 (b). A new peak appeared between 3000–3500 cm^{-1} which was assigned to stretching vibrations of hydroxyl groups. A peak was observed at around 1696 cm^{-1} corresponding to the C=O stretching vibration of a carbonyl group and a peak at 1402 cm^{-1} was attributed to the stretching vibration of the epoxy C=C group. Polyurethane containing functional groups of C=O and N-H formed hydrogen bonds with graphene oxide. In fig S3 (c), as the reduction process occurred after the reaction with hydrazine hydrate, the intensity of the vibration atrriuted to the hydroxyl groups became lower, as hydrazine attacked OH groups, causing a difference in hydrogen band rearrangement. In Fig S3 (a-c), after the fabrication of polyurethane sponge with GP, GO, and RGO, respectively, similar peaks were observed in each sponge composite after coating with each graphene-based material.

3.3: Scanning electron microscopy (SEM):

The morphologies of graphite powder, graphene oxide, and reduced graphene oxide were examined using SEM. The morphology of graphite powder, as shown in Fig. 3 (a), contains well-defined edges with stacks that hold its layers together due to the presence of van der Waals forces. While Fig. 3 (b) showed that graphene oxide has a wrinkled structure with a visible arrangement of layers because of layer expansion. The laminated graphite structure was destroyed due to oxidation and subsequent delamination by the Hummers method. However, in Fig. 3 (c), the SEM image of RGO, where GO was chemically reduced to RGO by hydrazine hydrate, the surface of the RGO contained thin, wrinkled sheets that formed a disordered structure. PU shows uniform layers of sponge, see Fig.3 (d). The uniform coating of PU with GP, GO, and RGO can be seen in Fig.3 (e), (f) and (g), respectively, confirming the successful coating of the surface of PU sponges with graphitic material in the composites.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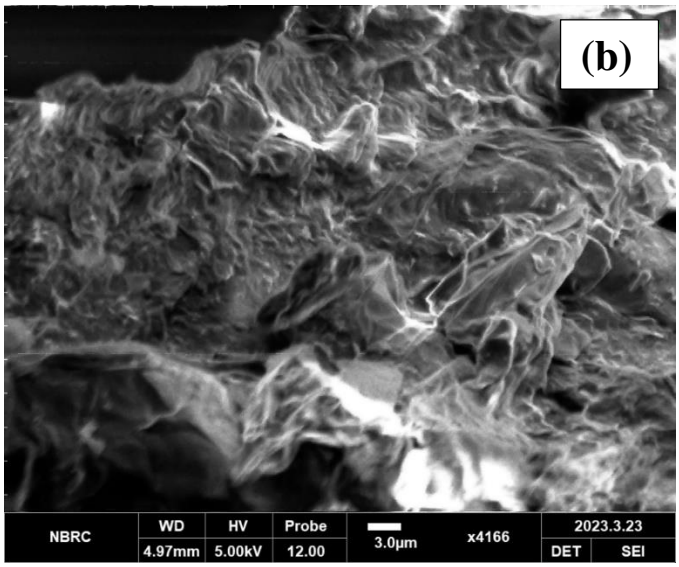
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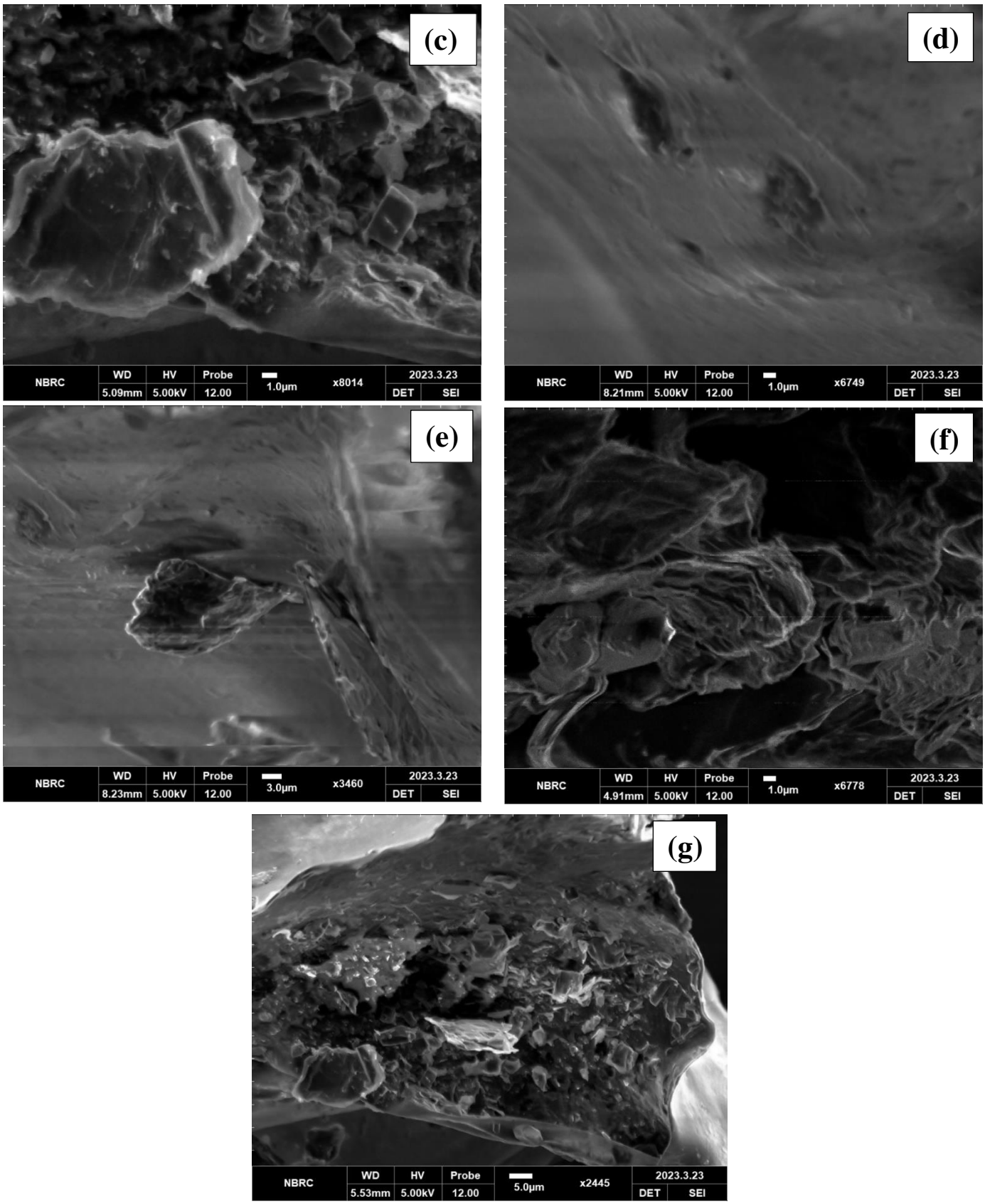

245 **Fig.3: SEM images: (a) GP, (b) GO, (c) RGO, (d) PU, (e) GP@PU, (f) GO@PU, and (g)**

RGO@PU.

3.4: X-ray diffraction (XRD):

The crystalline structure and interplanar spacing of sorbents were examined using XRD. Graphite powder exhibited a sharp diffraction peak at 26.4 Å, with the XRD patterns of GP and GP@PU sponge identical, indicating successful coating of GP onto the PU sponge. Graphene oxide displayed a sharp diffraction peak at 11.17 Å corresponding to the basal spacing of the GO because of oxygen-containing group intercalation. The same peaks were also observed on the GO@PU sponge, confirming the successful coating of GO on the surface of the PU sponge. In the RGO pattern, a broad peak at 21.43 Å indicated the presence of the carbon (002) plane. From Fig. S4, it can be concluded that the RGO pattern exhibits numerous defects in the carbon lattice and twisted ordering of the sheets in the plane direction, contributing to the prominent diffraction peak. Similarly, the prepared PU sponge displays the same diffraction peak, confirming the sucess introduction of RGO into the PU sponge.

3.5: HPLC analysis:

For the optimization of HPLC conditions, different factors including the effect of pH, concentration of sample solution, extraction time, eluting solvents, and the reusability of the sponges was analyzed.

3.5.1: Effect of pH:

The prepared mixture of the standard solutions of the indivudal drugs, with a pH in the range of 3 to 9, was adsorbed on the graphitic-based sponges to examine the effect pH has on the in-tube solid phase extraction. The results, presented in Fig. S1 (i), demonstrate that the pH of the sample solution significantly influences analyte extraction. Specifically, the extraction of all four drugs increased notably when the pH was raised from 3 to 5 and then decreased, as illustrated in Fig. S1

(i), as the pH increased further. Thus, maximum extraction was obtained at pH 5 and was thus employed for the remainder of the study. It was also concluded from Fig. S1 (i) that the maximum extraction was obtained with GO@PU among the studied graphitic-based sponges.

3.5.2: Effect of concentration:

The effect of concentration on extraction efficiency was investigated by changing the concentration of the analytes. As the analyte concentration increases, extraction efficiency is expected to improve. However, this is limited by the saturation of active sites on the adsorbent. Once all active adsorbent sites are occupied, a further increase in analyte concentration does not affect the extraction process (Negarestani et al., 2023). Solutions of 5, 10, 20, and 50 ppm concentrations were used to investigate the extraction process. Based on the results in Fig. S1 (ii), it was concluded that a 10 ppm solution exhibited maximum extraction efficiency. Increasing the analyte concentration from 20 to 50 ppm did not result in further increased extraction efficiency. It was illustrated from Fig. S1 (ii) that at a concentration higher than 5 ppm, all four analytes show maximum extraction efficiency for graphitic-based sponges. Among graphitic-based sponges, GO@PU demonstrates higher extraction than GP@PU and RGO@PU.

3.5.3: Effect of extraction time:

The effect of time on the extraction efficiency of graphitic-based sponges was observed by analyzing the extraction at intervals of 5, 10, 15, and 20 min. Sufficient interaction time allows analytes to adsorb onto the sorbent surface. Based on the results in Fig. S1 (iii), analyte extraction from sponges increased from 5 to 10 min, after which it stabilized. Here, GO@PU exhibited its highest extraction efficiency at 10 min; thus, a 10 min extraction time was chosen for subsequent studies.

3.5.4: Effect of eluting solvent:

The choice of eluting solvent in the solid-phase extraction determines the efficiency of the analyte extraction from the sorbent surface. The choice of eluting solvent depends on the type of analytes that need to be extracted. To investigate this, we employed various eluting solvents such as acetonitrile, methanol, acetone, and n-hexane, and the results are depicted in Fig. S1 (iv). The results indicated that n-hexane was the most effective eluting solvent, providing the highest extraction efficiency compared to methanol, acetonitrile, and acetone. Among the graphitic-based sponges, as depicted in Fig. S1 (iv), GO@PU demonstrated the highest extraction efficiency compared to GP@PU and RGO@PU.

3.5.5: Reusability and stability of sponge:

The efficiency of the graphitic-based sponges was assessed further by exploring the reusability in multiple extraction cycles. In this study, we examined the reusability and stability of the sponges by conducting extraction using the same sponge for up to 5 cycles. After each extraction cycle, the sponge was washed with MeOH and distilled water and then dried in an oven prior to use in the next cycle. The results in Fig. S1 (v) revealed a minor or negligible difference in extraction efficiency in the first three cycles, but a decrease in extraction efficiency was observed in the last two regeneration cycles. GO@PU exhibited the highest stability among the other graphitic-based sponges.

3.6: Interaction of analyte with graphitic-based sponges:

There are a number of different types of interactions possible responsible for driving the sorption of amoxicillin, paracetamol, ciprofloxacin, and cefixime onto GP@PU, GO@PU, and RGO@PU sponges. These include, hydrogen bonding and π–π interactions between the graphene materials and the benzene rings present in the analytes, as illustrated in Fig. S2. The hydrogen atoms and

oxygen groups in both the analytes and graphene oxide can also be involved in hydrogen bonding, representing another possible interaction.

3.7: Method validation:

In-tube SPE method under optimized conditions and HPLC determination of drug residues was confirmed using statistical analysis. The values of statistical analysis for R^2, % relative standard deviation (%RSD, 1.3–9.3 %), the limit of quantification (LOQ, 0.18-4.84 µg mL^{-1}), and the limit of detection (LOD, 0.03-1.60 µg mL^{-1}) are given in Table 1. As listed in Table 2, the obtained recoveries were up to 98.42%, with RSD values of less than 9.3%. The detection limits, accuracy and precision of the proposed method are well aligned with the guidelines of codex alimentarius (CAC/GL 71-2009) (Alimentarius, 2009). The accuracy and precision of the method was check by making triplicate meaurements. Furthermore, the reliability of this developed in-tube solid phase extraction method was confirmed by applying the method for drug residues extraction of four drugs (amoxicillin, paracetamol, ciprofloxacin, and cefixime) from food samples of egg, water, and honey. The results have shown that the proposed in-tube SPE method is suitable with excellent reproducibility, good accuracy, and high precision for extracting drugs from food samples.

Table 1: Validation of developed in-tube SPE method for the extraction of drug residues.

Sorbent	Analyte	R^2	Linear Range (µg mL^{-1})	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
GP@PU	Amoxicillin	0.999	1.60-1000	1.60	4.84
	Paracetamol	0.964	1.15-1000	1.15	3.48
	Ciprofloxacin	0.997	1.35-1000	1.35	4.09
	Cefixime	0.987	1.40-1000	1.40	4.24
GO@PU	Amoxicillin	0.889	0.09-1000	0.09	0.27

	Paracetamol	0.998	0.03-1000	0.03	0.99
	Ciprofloxacin	0.996	0.05-1000	0.05	0.18
	Cefixime	0.998	0.06-1000	0.06	0.18
RGO@PU	Amoxicillin	0.997	0.50-1000	0.50	1.51
	Paracetamol	0.979	0.15-1000	0.15	0.45
	Ciprofloxacin	0.961	0.30-1000	0.30	0.90
	Cefixime	0.998	0.35-1000	0.35	1.06

331

332 **Table 2:** Intraday and interday precision of method and % recovery.

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Sorbent	**Analyte**	Intraday (Spiking µg mL^{-1}) **%RSD**			Interday (Spiking µg mL^{-1}) **%RSD**			%Recovery **Spiked Food Sample**		
		20	**50**	**100**	**20**	**50**	**100**	**Egg**	**Honey**	**Water**
GP@PU	Amoxicillin	3.7	3.7	3.6	4.4	8.8	5.4	64.84	66.12	65.82
	Paracetamol	3.2	4.3	8.7	5.4	6.3	4.9	73.62	71.57	77.02
	Ciprofloxacin	3.7	6.9	6.3	6.3	2.2	4.2	67.54	69.16	73.68
	Cefixime	2.6	3.8	2.1	9.5	8.5	5.6	64.34	67.86	70.59
GO@PU	Amoxicillin	1.8	2.5	3.8	1.5	1.3	3.9	96.78	97.97	95.87
	Paracetamol	2.1	3.3	1.8	3.8	2.5	2.0	97.25	96.51	97.58
	Ciprofloxacin	1.4	1.6	2.9	3.5	2.1	4.2	95.63	96.29	98.42
	Cefixime	2.3	2.3	2.8	1.9	3.8	3.7	96.36	95.24	97.80
RGO@PU	Amoxicillin	4.1	3.5	3.0	7.6	4.9	9.3	74.95	72.76	73.34
	Paracetamol	6.0	5.0	2.4	4.9	6.4	5.0	78.09	77.98	80.20
	Ciprofloxacin	5.1	6.8	7.4	4.3	7.9	8.0	74.39	74.48	76.31

	Cefixime	5.8	7.7	6.2	5.8	7.0	7.4	72.96	73.83	74.12

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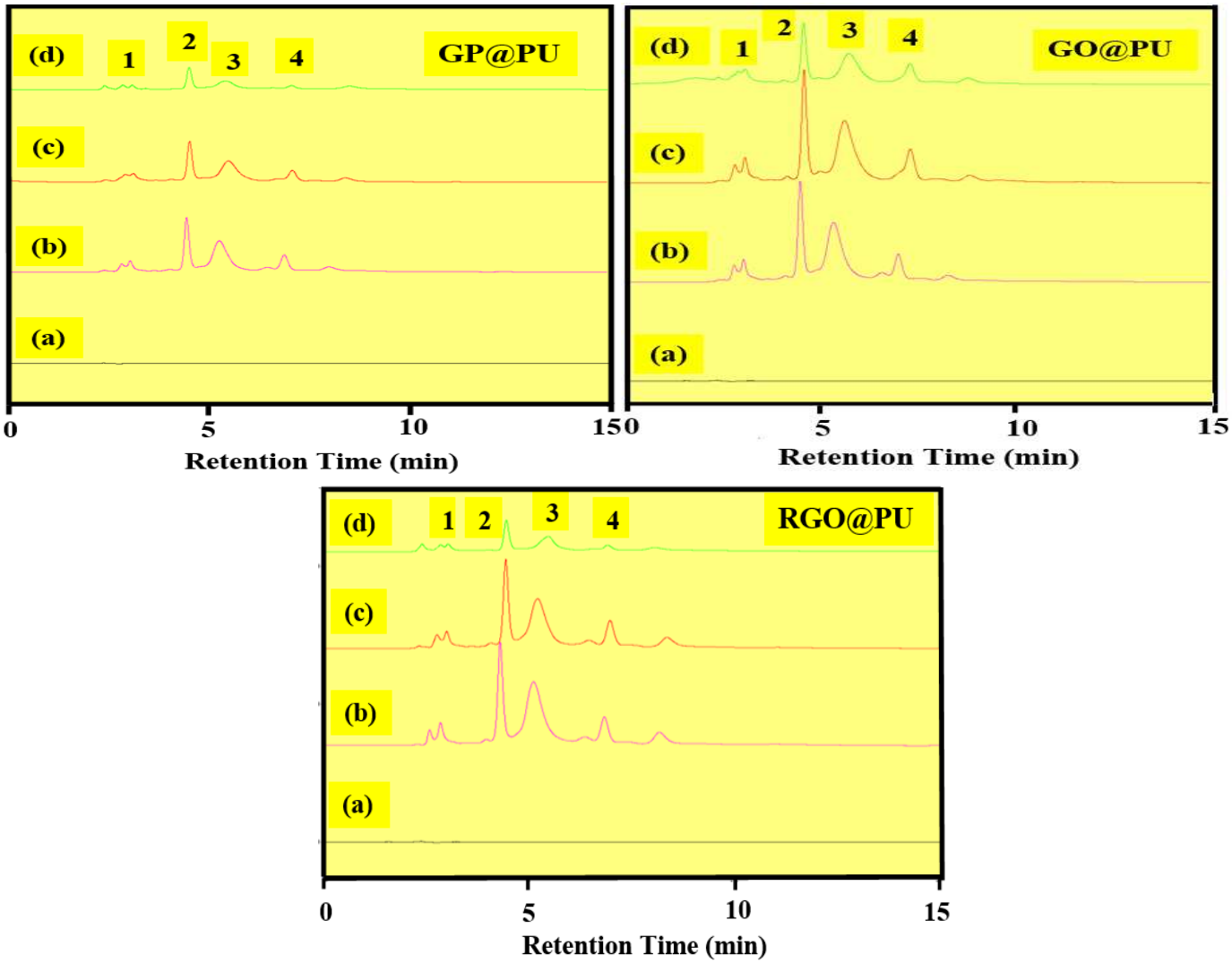

335 **Fig. 4: HPLC chromatograms of spiked food samples showing; (a) Blank, (b) Egg, (c)**
336 **Water, and (d) Honey.**

3.8: Comparison study:

A comparison study was conducted between the developed analytical method and other published methods, as summarized in Table S1. Table 1 presents various parameters, including the; nature of the sorbent, analytical samples, techniques, linear range, and LOD. The results indicate that in-tube solid-phase extraction using GP@PU, GO@PU, and RGO@PU as sorbent materials is highly effective for the extraction of amoxicillin, paracetamol, ciprofloxacin, and cefixime.

4: Conclusions:

In this research we have developed efficient and reusable graphitic-based polyurethane sponges as sorbents to extract drug residues from spiked food samples, enabling reproducible and accurate HPLC analysis. The graphitic-based polyurethane sponges were formed by functionalising the polyurethane sponge with GP, GO, and RGO using a dip coating method. These sponges were then utilized as sorbents to extract drug residues from spiked food samples with the extracted samples analysed by HPLC. The simple sponge preparation and quick sample extraction method made this in-tube solid phase extraction ideal for analyzing complex matrices like eggs, water, and honey. These sponges exhibited outstanding sorbent capacity, exceptional selectivity, and impressive recovery rates and could be reused multiple times. Our findings highlighted that GO@PU demonstrated superior sorption capacity, excellent recoveries, and reusability among the graphitic-based sponges, making it a valuable tool for extracting various drug residues from food samples. Moreover, GO@PU demonstrated strong π- π interactions and hydrogen bonding with the analytes, which played a pivotal role in extracting drug residues from food products. This research streamlines graphitic-based sponges for in-tube solid phase extraction of drug residues from real food samples.

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