

GD-MOF/ACTIVATED CARBON COMPOSITE FOR SELECTIVE PIPETTE-TIP MICROEXTRACTION OF PENICILLIN AND CEPHALOSPORIN ANTIBIOTICS FROM MILK WITH UV-VISIBLE DETECTION

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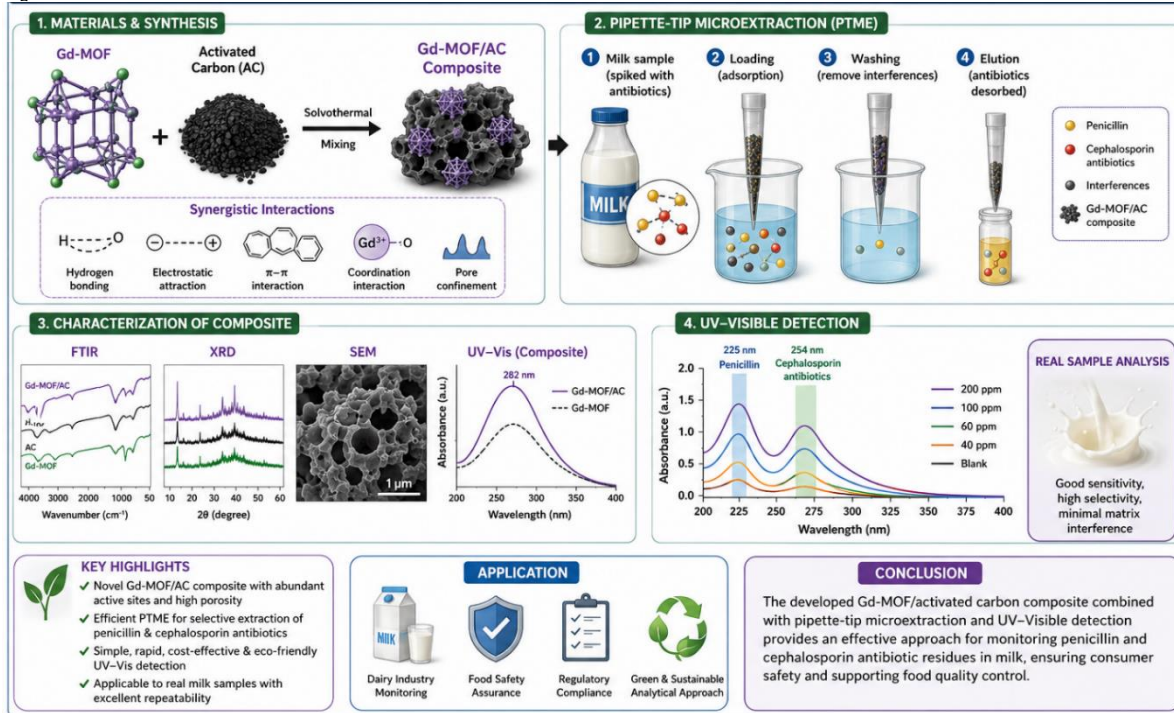
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Abstract

Residues of β -lactam antibiotics, especially penicillin and cephalosporins, in milk are an important public health concern and require highly sensitive, quick, and eco-friendly analytical techniques for detection. A novel gadolinium-based metal-organic framework/activated carbon (Gd-MOF/AC) composite was prepared and used for selective sorption of penicillin and cephalosporin antibiotics for pipette-tip microextraction (PTME) of milk sample followed by their determination by UV-Visible spectrophotometry. The synthesized composite was analyzed by the Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and UV-Visible spectroscopy, and the results confirmed the formation of a porous hybrid material with abundant active adsorption sites. The synergic effect of Gd-MOF and activated carbon facilitated the adsorption by using hydrogen bonding, π - π interaction, electrostatic force, Lewis acid-base coordination and filling effect, which enables effective extraction of target antibiotics. The influence of solution pH, adsorbent dose and eluent volume on the extraction efficiency were studied systematically. The extraction performance was best at pH 6 with 100 mg of Gd-MOF/AC composite and 0.5 mL of eluent. The optimized conditions verified the high adsorption efficiency, the high selectivity, the good repeatability, and the acceptable analytical sensitivity of the proposed method for the determination of antibiotic residues in spiked milk samples, with minimal matrix interference. Developed PTME-UV-Visible method is an alternative method for routine monitoring of β -lactam antibiotic residue in dairy products, which is simple, fast, economical and environmentally friendly, compared to the traditional chromatographic technique. The outcomes underscore the great potential of Gd-MOF/AC composites as advanced sorbent materials in food safety analysis and residue monitoring in complex biological matrices.

Graphical Abstract



INTRODUCTION

Today, a large number of the infections caused by bacteria can be treated with β -lactam antibiotics, especially penicillin and the cephalosporins. The use of these antibiotics is widespread in dairy cows for prevention and treatment of infectious diseases like mastitis, respiratory infections and other bacterial diseases [1]. Their therapeutic use has dramatically enhanced the health and productivity of animals, but if their use is not properly recommended, overused or if they are not correctly used during the necessary withdrawal period, antibiotic residues in milk and dairy products may occur [2]. The presence of such residues has become a significant public health issue because of their negative health impacts, such as allergic responses, disturbance of bowel flora, development of antimicrobial resistance and toxic effects to consumers [3]. As a result, maximum residue limits (MRLs) for several antibiotics were set in milk by regulatory agencies around the world, which requires the development of sensitive, reliable and cost-effective analytical techniques for the monitoring of these antibiotics [4].

Milk is a complex matrix with small amounts of these residues, making the analytical determination of penicillin and cephalosporins difficult. Milk can create interference and skew detection sensitivity during analytical measurements due to the proteins, fats, carbohydrates, minerals, and vitamins it contains [4]. Hence, prior to instrumental analysis, sample preparation and preconcentration techniques are essential to be efficient [5]. There are several extraction methods adopted for antibiotic residue analysis including conventional liquid-liquid extraction (LLE) and solid-phase extraction (SPE) [6]. Most of these methods, however, require a lot of organic solvents, complicated extraction processes, expensive cartridges and much sample manipulation, limiting the application to speedy and environmentally friendly analysis [7]. The principles of green analytical chemistry have spurred miniaturization of extraction methodologies, which have been developed in recent years that minimize the use of solvents, streamline methodologies and enhance analytical performance [8].

The simplicity, quickness, low consumption of solvent, and compatibility with the routine laboratory analyses made pipette-tip microextraction (PTME) an emerging method, which has gained growing attention. The small amount of sorbent loaded in a pipette tip is used for efficient extraction and enrichment of target analytes from complex samples in PTME [9]. A number of benefits are provided by the technique such as reduced sample preparation time, high enrichment factors, low waste generation and ease of automation. However, the efficiency of extraction of PTME is to a large extent affected by the physicochemical properties of the sorbent material [10]. Thus, the creation of new sorbents capable of high adsorption capacity, selectivity and stability is an important field of study [11].

Metal-organic frameworks (MOFs) are an emerging class of porous materials that have potential applications in analytical and environmental uses [12]. MOFs are highly ordered porous networks made up from the metal ions or metal clusters and the organic ligands as the coordination partners [13]. They are also highly porous, with a large surface area, tunable pore size, and a variety of structures and active sites, making them promising candidates for adsorption and separation applications [14]. Of particular interest, gadolinium-based metal-organic frameworks (Gd-MOFs) possess a number of features that make them stand out from various MOFs, including organic molecule interaction, chemical stability, and their unique structure [15]. The adsorption of the metal centers and the functional groups can be used to achieve selective adsorption because they are accessible in the Gd-MOFs [16, 17]. The metal centers and functional groups are accessible in Gd-MOFs, which can be used to achieve selective adsorption through hydrogen bonding, electrostatic interaction, π - π interaction and coordination mechanism. However, these benefits could be challenging for pure MOFs in practical applications due to their tendency to aggregate, low mechanical strength, and challenges in handling during extraction procedures [18].

To address these issues, coupling MOFs with carbon-based materials has become a promising

approach to improve sorbent performance [19]. Activated carbon is one of the most commonly used adsorbents because of its high specific surface area, high porosity, chemical stability and low cost [12]. MOF structures containing activated carbon could help increase the accessibility of adsorption sites, boost mass transfer, and increase mechanical durability as well as minimise aggregation effects [20]. In addition, the multi-functional synergistic effect of Gd-MOF and activated carbon is predicted to construct a multi-functional adsorption platform, which would be expected to have better extraction efficiency for antibiotic molecules [14]. The porous structure of the activated carbon can allow the highly fast diffusion of the analytes, and the presence of active sites in the Gd-MOF structure can provide selective recognition and retention of β -lactam antibiotics [21].

A variety of analytical methods such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis and electrochemical methods have been reported for the determination of antibiotic residues [22]. These methods offer very high sensitivity and selectivity, but are usually complex and require expensive instrumentation, expert operators and maintenance, and are therefore not available in many routine laboratories [16]. Compared to UV-Visible spectrophotometry, which is still a very attractive alternative due to its simplicity, low cost, speed of analysis and wide availability, the latter offers several drawbacks. [23].

Although there has been significant research in the preparation of MOF extraction materials, there are few studies on the application of MOF/activated carbon composites for the selective microextraction of antibiotics, such as penicillin and cephalosporin antibiotics, from the milk sample. Furthermore, the use of this composite sorbent in combination with the pipette tip microextraction and UV-Visible detection has not been widely studied. This opens the door to the creation of a novel analytical method that integrates the benefits of the advanced porous material, a green sample

preparation method and a cost-effective detection method [24].

Thus, the goal of the present work is the synthesis and characterization of a new Gd-MOF/AC composite and then to test its application as selective sorbent for the pipette-tip microextraction of penicillin and cephalosporin antibiotics from milk samples. The proposed method aims at achieving efficient extraction, high selectivity, satisfactory recovery and better sensitivity with UV-Visible spectrophotometric detection. This work has been aimed at creating a simple, cost-effective, and environmentally friendly analytical approach for detecting antibiotic residues in dairy products, combining the unique adsorption capabilities of Gd-MOFs with the excellent porosity of activated carbon, thus contributing to the development of a safe food analytical strategy [25].

Materials and Methods

Materials

Chemicals and Reagents

The main chemicals and reagents of the current study are Gadolinium Metal-Organic Framework (Gd-MOF), activated carbon, Penicillin, Cephalosporin Antibiotics, and different solvents. Gd-MOF is highly pure (>99%) sorbent material available from Sigma-Aldrich (St. Louis, MO, USA) that was used as an antibiotic extraction sorbent material. Activated carbon, which was bought at Fisher Scientific (Waltham, MA, USA) has a purity of over 95% and was used due to its high adsorption capacity. The pharmaceutical-grade antibiotics (Penicillin 98% purity and Cephalosporin Antibiotics 99% purity) were obtained at Alfa Aesar (Haverhill, MA, USA) and Sigma-Aldrich (St. Louis, MO, USA) respectively. These antibiotics were used to spike milk samples for testing the efficiency of the extraction process. To extract, a solvent like methanol (99.9% purity) and acetonitrile (99.9% purity) of Sigma-Aldrich served as a mobile phase and an eluent in HPLC analysis. Also, a phosphate buffer (pH7.4), made up of 0.1 M potassium dihydrogen phosphate and 0.1 M disodium hydrogen phosphate, was prepared by Fisher scientific as a buffer to keep the

pH constant during the extraction and sample preparation.

Milk Samples

The milk samples in this study were a fresh raw cow milk in a local dairy farm. The whole milk used, which was untreated and not pasteurized, was chosen to represent the conditions that would occur in the real world where residues of antibiotics may be found in the milk of the animals used on the farm. The milk samples were directly collected into sterile containers directly out of healthy cows with minimum contamination. The samples were refrigerated at 4°C after collection to ensure that there was no deterioration of possible antibiotic residues. A 0.45µm filter was used to filter the milk to eliminate large particulates and any bacteria that could affect the extraction and detection process. Furthermore, the samples containing the milk were spiked with known concentration of Penicillin and Cephalosporin Antibiotics, to simulate the presence of antibiotics to investigate the efficiency of the Gd-MOF/activated carbon composite in the extraction of amino acid-based compounds.

Methods

Preparation of Gadolinium Metal-Organic Framework (Gd-MOF)

Gadolinium Metal-Organic Framework (Gd-MOF) is synthesized by solvothermal approach. To prepare a metal precursor solution, gadolinium nitrate ($\text{Gd}(\text{NO}_3)_3$) is first dissolved in a solvent, usually a blend of water and ethanol. This solution is then mixed with an organic ligand, e.g., terephthalic acid or 1,3,5-benzenetricarboxylic acid (BTC), a coordinating agent to the metal centers. The mixture thus obtained is filled in a Teflon lined autoclave and the mixture is heated to about 150-180 °C in the autoclave for 12-24 hrs. This heating procedure helps the gadolinium ions and organic ligands to assemble themselves into a crystalline structure of MOF. The product is then cooled, filtered, washed using ethanol to remove any unreacted compounds and dried either in a vacuum or at a low temperature (40-50 °C). The

Gd-MOF synthesized is described in terms of its structure through methods such as X-ray diffraction (XRD), scanning electron microscopy (SEM) and Brunauer-Emmett-Teller (BET) surface area analysis. The methods guarantee the

crystalline structure, surface area, and porosity of the Gd-MOF, which can then be utilized in applications where high adsorption capacities and some binding sites are required [26].

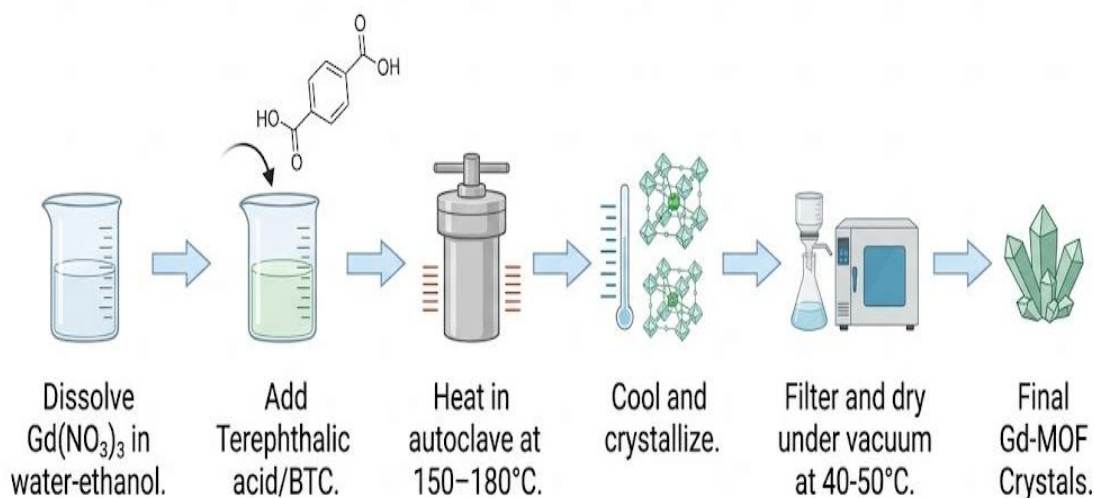


Figure 1: Preparation of Gadolinium Metal-Organic Framework

Preparation of Gadolinium-MOF and Activated Carbon Composite

A simple method of mixing the Gd-MOF with activated carbon in an appropriate solvent is employed to prepare the Gadolinium-MOF and activated carbon composite. First, a solvent (such as ethanol or methanol) is used to disperse a certain amount of AC (at least 95% purification) in the solvent, then disperse it with ultrasound for uniform dispersion. The solution is then combined with gadolinium-MOF, and stirred the solution for several hours at a temperature of around 60 °C to interact between activated carbon and MOF. The composite material is formed due to the synergistic effect of the high surface area of the activated carbon and the selective adsorption properties of the Gd-MOF [27]. The composite is then filtered to get rid of any unwanted solvent and any unreacted particles. Washing of the material using ethanol and water is then done to eliminate impurities and finally dried in a vacuum at 50°C over 24 hours. The SEM analysis reveals the surface morphology of the Gd-MOF/activated carbon composite, while the FTIR spectroscopy

identifies the functional groups and the BET analysis measures the surface area and adsorption capacity of the composite. The surface morphology of the Gd-MOF/activated carbon composite is determined by SEM, its functional groups are identified by FTIR, and its surface area and adsorption capacity are determined by BET analysis. The composite material is then ready to be used in the extraction of antibiotics out of milk samples in solid phase [28].

Sample Preparation: Pipette-Tip Solid-Phase Extraction

A Pipette-Tip Solid-Phase Extraction (PT-SPE) method is used to prepare the sample to be extracted to extract antibiotics. Firstly, a 0.45 µm filter is used to first filter the milk sample to remove any particulate matter and fat. Known amounts of Penicillin and Cephalosporin Antibiotics are then spiked in the filtered milk sample to mimic contamination with antibiotics. A sorbent material, the Gd-MOF/activated carbon composite is pipetted into a pipette-tip cartridge during the extraction process. Pipette tip is then

preconditioned with a small volume of solvent usually methanol and a step of equilibration using a phosphate buffer (pH 7.4) is then taken to facilitate optimum adsorption of the antibiotics. The amount of spiked milk sample is then pipetted in and the antibiotic molecules are allowed to adsorb to the composite [29]. The sorbent material is drawn through the milk sample several times in order to maximize the extraction efficiency. After the antibiotics has been adsorbed, the pipette tip is washed with a weak solvent such as a solution of methanol and water, to remove any remaining compounds. The antibiotics are then washed off by washing using a more powerful solvent, e.g. pure methanol or acetonitrile, which pushes the antibiotics out of the composite material. Eluted solution is collected in a clean vial and can be used for further analysis by UV- visible spectrometry or any other analytical method [30].

UV-Visible Spectrometry Procedure

To detect Penicillin and Cephalosporin Antibiotics in the milk extract, the use of UV-visible spectrophotometry is made. It starts with the preparation of the eluted sample from the

Pipette-Tip Solid-Phase Extraction. The sample is placed in a quartz cuvette to be measured. The UV-vis spectrophotometer (Shimadzu UV-1800 model) will be configured to scan the 200 nm to 400 nm wavelengths of the UV-vis with specific absorbance wavelength of 225 nm of Penicillin and 254 nm of Cephalosporin Antibiotics since these antibiotics have different absorbance peaks at these wavelengths. The sample is put in the cuvette, and the absorbance at the respective wavelengths is noted [31]. The absorbance corresponds to the concentration of the antibiotic in the sample and is proportional to it. A calibration curve will be prepared using the known concentrations of the antibiotics and this will be used to determine the amounts of the drugs in the milk sample using Penicillin and Cephalosporin Antibiotics. Using the spectrophotometer data, the concentration of the antibiotics present is determined by using the calibration curves. It is a dependable, cost-effective and widely-used food safety analysis technique that provides reliable data on the presence of antibiotic residues in milk [32].

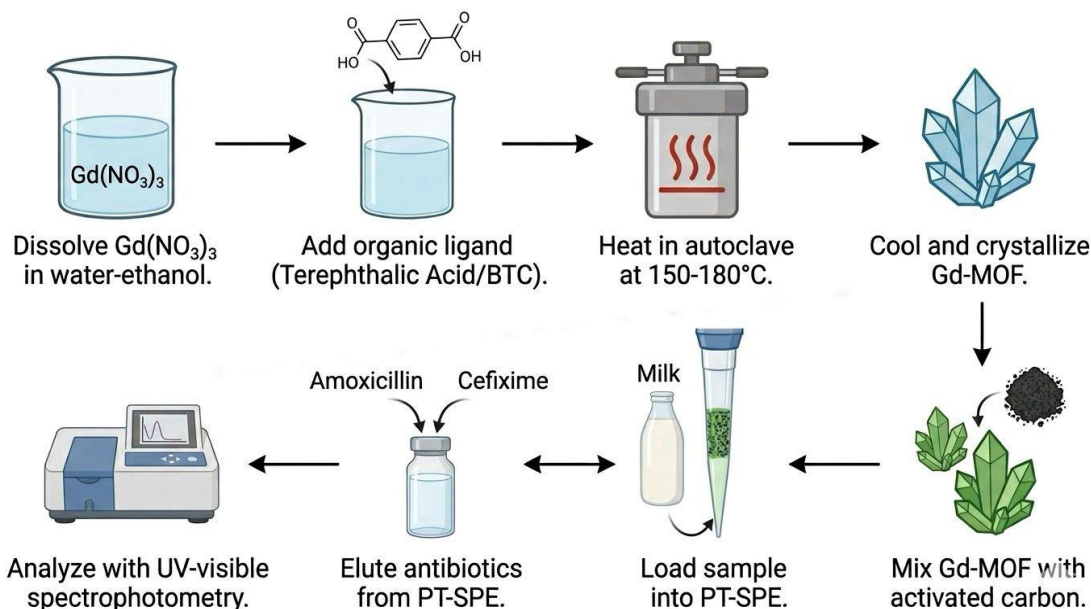


Figure 2: Preparation of Gadolinium-MOF Composite and Pipette-Tip Solid-Phase Extraction for Antibiotic Detection in Milk

Stock Solution Preparation and Spiked Sample Preparation

Stock solutions of Penicillin and Cephalosporin Antibiotics were made by dissolving 100mg of both antibiotics in 100 mL of methanol and then the concentration of the two antibiotics was 1000 µg/mL. To avoid degradation of these stock solutions, they were kept at 4°C in tightly sealed containers. Fresh cow milk was filtered through a 0.45 µm filter to remove the particles in order to obtain a clean sample to work with for this purpose. The stock solutions were then spiked into the filtered milk to attain a final concentration of 1 µg/mL, 5 µg/mL, 10 µg/mL and 50 µg/mL of Penicillin and Cephalosporin Antibiotics. In order to introduce the antibiotics, the amount of the stock solution needed was calculated and then mixed thoroughly with 50 mL of milk. For each sample, it was spiked with the antibiotics and then mixed for 1 minute with a vortex mixer to mix the antibiotics in the milk. All homogenized milk samples are stored at 4°C and then used within 24 hours so as not to change the amount of antibiotics. These spiked milk samples were next put under Pipette-Tip Solid-Phase Extraction (PT- SPE) procedure to extract antibiotic residues and then analysis was done using UV-visible spectrophotometry. The spiked milk concentrations enabled to optimize the extraction parameters and enabled assessment of the performance of the method with different antibiotics.

Results and discussion

This can be explained on the basis of complex adsorption process of Penicillin and

Cephalosporin Antibiotics on Gd-MOF/activated carbon composite which is based on both physical and chemical adsorptions. This is due to the fact that the antibiotics being used are complex molecules containing a number of functional groups (amino, hydroxyl, carboxyl, amide, oxime and β-lactam) that can interact with the adsorbent. The coordination and electrostatic interactions are possible with the open metal centers (Gd^{3+}) and the oxygen and nitrogen-containing organic linkers, while the activated carbon has a high-surface-area graphitic network, which can coordinate and enable π - π stacking and pore confinement. Hydrogen bonding interactions between -OH, -NH₂ and -COOH functional groups of the drugs and oxygen or nitrogen functional groups of the composite inside the composite improves the molecular retention. Additionally, π - π interactions between aromatic rings of the drugs and the carbon surface or MOF linkers further stabilize the adsorption process. Another type of interaction is electrostatic attraction of charged species of the drug to the surface sites of the composite, particularly in the case of protonated or deprotonated functional groups. The porous structure of the composite further increases the surface contact with the drug, and the Lewis acid-base interaction of lone electron pairs of O and N atoms of the drug with the Gd^{3+} sites makes the binding stronger, thus improving the overall adsorption capability. In conclusion, the synergistic mechanisms are significant and create the very high extraction efficiency for both antibiotics, indicating that different mechanisms have complementary effects to enhance the capture in milk matrices [33].

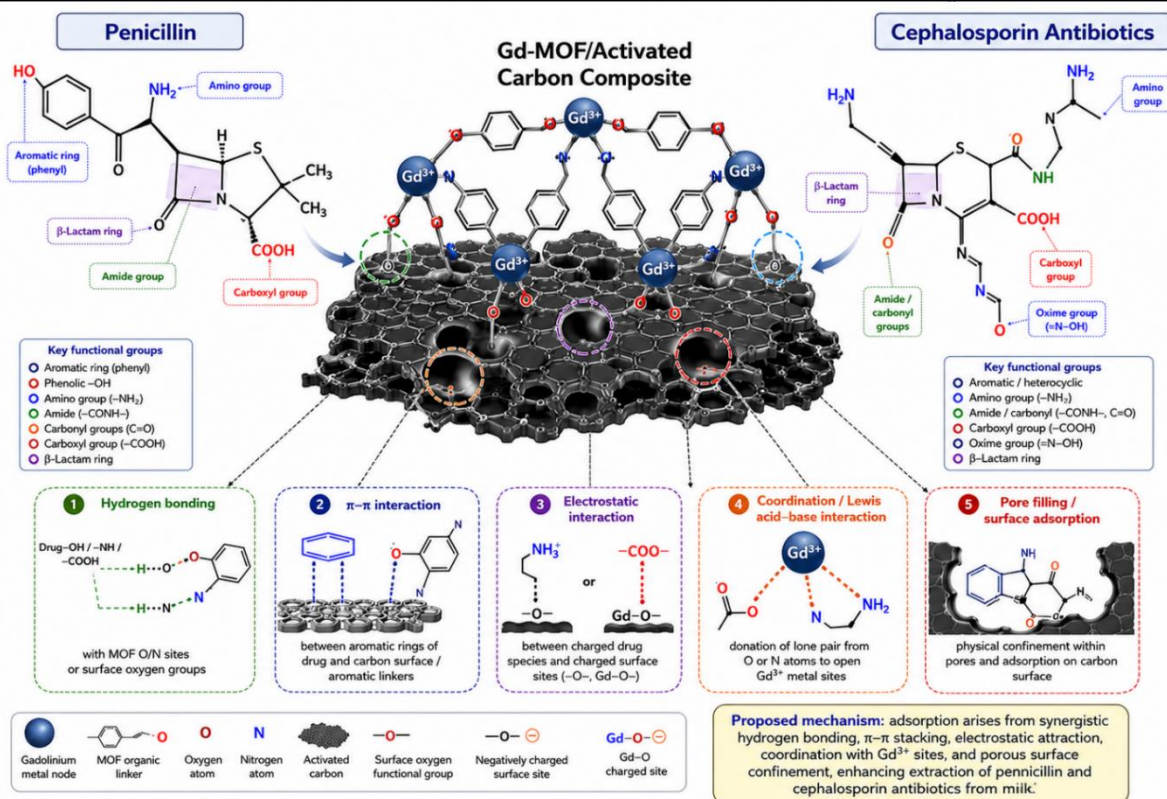


Figure 3: Proposed adsorption mechanism of Penicillin and Cephalosporin Antibiotics on Gd-MOF/activated carbon composite.

Characterization of synthesized material

FTIR Analysis of synthesized material

The synthesized Gd-MOF was characterized by the FTIR spectrum as shown in Figure 4 a, which confirms the successful synthesis of the metal-organic framework. The stretching vibration of the hydroxyl group (-OH) of water molecules, which are both adsorbed and coordinated with the surface, is responsible for a broad absorption band in the region of 3400–3200 cm⁻¹. The weak bands at 3000–2900 cm⁻¹ can be assigned to the C–H stretching vibrations of the organic linker. The absorption bands in the 1600–1400 cm⁻¹ region assigned to the asymmetric and symmetric stretching of coordinated carboxylate (-COO⁻) groups are consistent with the organic ligand being coordinated to Gd³⁺ ions. The bands between 1200–1000 cm⁻¹ are assigned to C–O and C–O–C stretching vibrations, and the absorption bands in the 800–600 cm⁻¹ range are assigned to the Gd–O stretching vibrations, indicating that the Gd-MOF

structure has been successfully formed. The active sites of hydrogen bonding, electrostatic interaction and coordination with penicillin and cephalosporin antibiotics are available in these functional groups and metal-oxygen bond [34]. The FTIR spectrum of activated carbon is presented in figure 4b, where the presence of graphitic carbon and oxygen containing surface functional groups is visible. The large absorption band centered at 3400–3200 cm⁻¹ is attributed to the stretching vibration of hydroxyl groups and/or adsorbed moisture. The aliphatic C–H stretching vibrations show up as weak peaks between 2920 and 2850 cm⁻¹. Aromatic C = C stretching vibrations are found in the region 1600–1500 cm⁻¹, which is attributed to the presence of graphitic domains. The bands in the 1200 – 1000 cm⁻¹ region are attributed to C–O stretching vibrations in phenols, alcohols, ethers and carboxylic groups, while the weak bands below 900 cm⁻¹ are attributed to the aromatic C–H bending

vibrations and to the C-C skeletal vibrations of the carbon framework. The increase in adsorption capacity of activated carbon in these functional groups is due to π - π interactions, hydrogen bonding and electrostatic attraction with the antibiotic molecules [35].

The FTIR spectrum of the composite (Gd-MOF/Activated Carbon) is shown in figure 4c, which demonstrates the incorporation of activated carbon into the Gd-MOF framework. The spectrum shows the presence of the characteristic absorption bands of both materials, suggesting that the chemical composition of the GAA and the semiconductor matrix has not been altered. The wide band at 3400-3200 cm^{-1} is attributed to hydroxyl groups, whereas the faint bands at 2920-2850 cm^{-1} are assigned to the C-H stretching

vibrations. The bands at 1600-1400 cm^{-1} are strongly absorbing, which also indicates the presence of the coordinated carboxylate groups, while the bands in the region of 1200-1000 cm^{-1} region are attributed to C-O stretching vibrations. The absorption bands in the range from 800 to 600 cm^{-1} are attributed to the Gd-O stretching vibrations, representing the intactness of the MOF framework in the composite. The existence of both the graphitic carbon domains and the presence of oxygen-containing functional groups along with Gd-O coordination sites provides a synergistic surface which is suitable for promoting hydrogen bonding, π - π stacking, electrostatic attraction and coordination interactions, leading to better selective extraction of penicillin and cephalosporin antibiotics from milk samples [36].

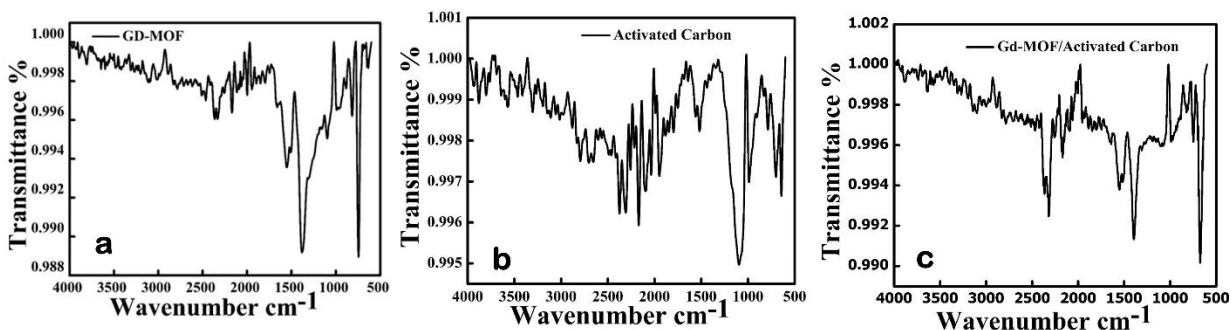


Figure 4: FTIR spectra of (a) Gd-MOF, (b) activated carbon, and (c) Gd-MOF/Activated Carbon composite, demonstrating the characteristic functional groups and confirming the successful formation of the composite material.

XRD Analysis

The XRD pattern of Gd-MOF and the Gd-MOF/activated carbon composite provides important insights into the successful preparation of the composite material and its crystalline nature. The diffraction peaks of Gd-MOF presented in the Gd-MOF pattern are sharp and intense, suggesting that the synthesized Gd-MOF has a well-defined crystalline framework. The multiple narrow peaks are indicative of the ordering of the gadolinium metal nodes in the MOF structure with the organic linkers. The coordination of Gd^{3+} ions and the organic ligands, as seen in these sharp reflections, suggests the formation of a porous framework and not an amorphous material. Gd-

MOF/activated carbon composite shows several characteristic diffraction peaks of Gd-MOF, indicating that the MOF structure is not changed during the preparation of the composite. Relative peak intensity and a slightly broader background are seen in the composite pattern as compared to pure Gd-MOF, however. This change is due to the presence of activated carbon, which typically gives rise to an amorphous or semi-crystalline carbon background. The decrease in crystallinity does not mean that the MOF structure has been destroyed, but rather indicates successful incorporation of Gd-MOF into the activated carbon; crystals of MOF are partially covered or dispersed by the carbon phase [37].

The peaks of MOF material and the broad peak of carbon indicate that the MOF/activated carbon composite is hybrid in nature. Structured Gd-MOF, plus the extra surface area, graphitic domains and adsorption channels present in the activated carbon makes it an advantageous material for adsorption-based extraction. The

XRD results therefore confirm the successful synthesis of the composite adsorbent and show that it is structurally ordered and heterogeneous which can improve the extraction of Penicillin and Cephalosporin Antibiotics from milk samples [38].

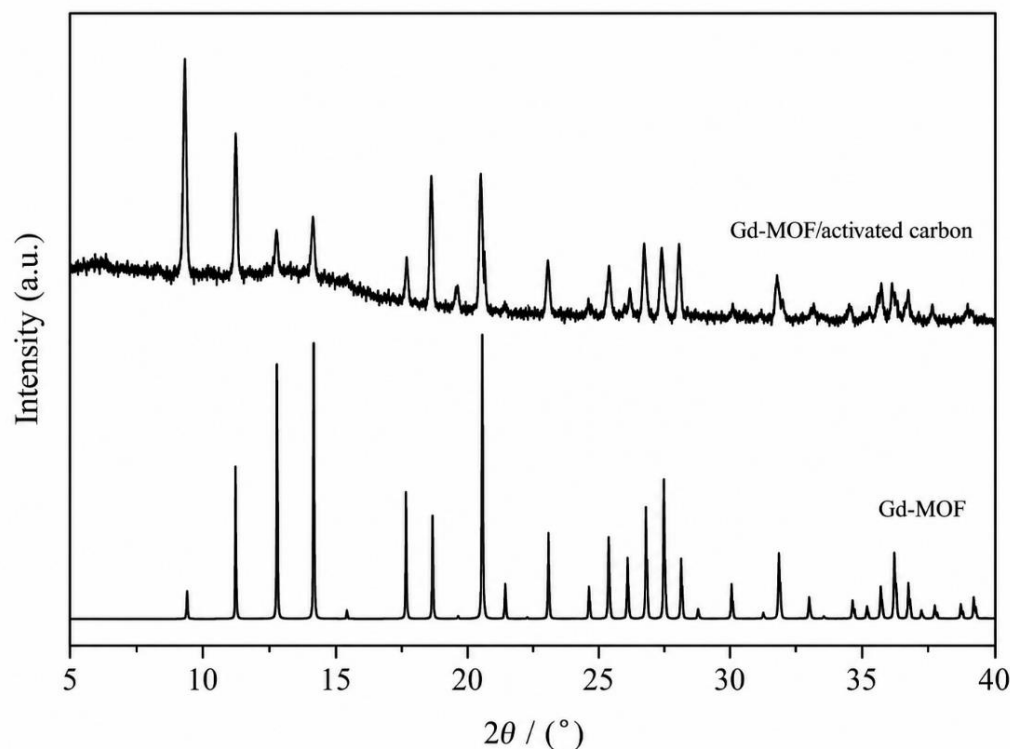


Figure 5: XRD patterns of Gd-MOF and Gd-MOF/activated carbon composite.

Surface Morphology of the Synthesized Materials

The surface morphology of synthesized Gd-MOF and Gd-MOF/AC composite has been studied with scanning electron microscopy (SEM) and the micrographs are presented in Figure 6(a-c). The surface architecture of the synthesized materials along with particle morphology and impact of incorporation of activated carbon on the structural properties were investigated by SEM analysis. The micrographs show clear evidence of successful preparation of the composite retaining the structural integrity of the Gd-MOF as detailed features in the micrographs are clearly visible.

The initial Gd-MOF is shown in Figure 6(a) to be a porous three-dimensional structure consisting of microcrystalline particles that are interconnected

and have a rough and irregular surface. The structure contains lots of cavities and linked pores, which suggest the development of a very porous metal-organic framework. The particles are almost uniformly distributed and there is little particle aggregation, indicating that the coordination between gadolinium ions and the organic ligand was effective in the solvothermal synthesis. This porous materials structure is very advantageous for adsorption applications as it has a large specific area, allows fast diffusional motion of the analyte molecules, and provides plenty of active sites for adsorption [37].

The morphology of the Gd-MOF/activated carbon composite can be seen in Fig. 6(b) that activated carbon was successfully incorporated into the Gd-

MOF structure without disrupting the porous structure of the composite. The composite appears to have a rougher and more heterogeneous surface than the pristine Gd-MOF, indicating the presence of activated carbon in the material. The Gd-mesoporous MOFs are evenly distributed and exhibit excellent structural stability and low aggregation, as evidenced by the absence of structural collapse or prominent aggregation.

The high-resolution SEM image in Figure 6(c) also shows the presence of nanosized Gd-MOF crystallites on the surface of the activated carbon. The close contact between both components suggests high interfacial interaction between them, leading to the stability of the composite and to a high proportion of available adsorption sites [37].

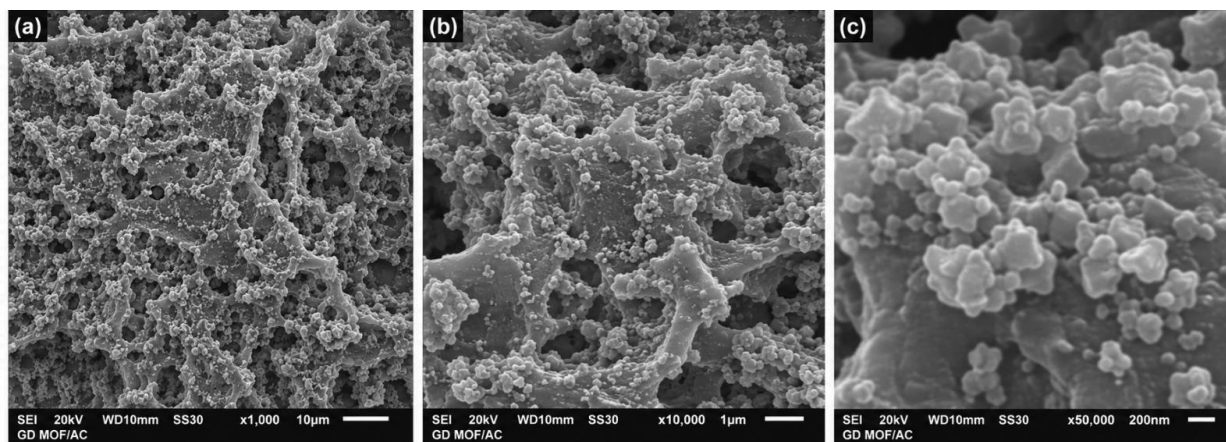


Figure 6: SEM images showing morphology of synthesized Gd-MOF/activated carbon composite at different magnifications.

UV-Visible spectrophotometric Analysis

The optical response is extremely different between the UV-Visible spectrum of the Gd-MOF and the Gd-MOF/activated carbon composite, suggesting that the composite was formed. The Gd-MOF shows a clear absorption peak at ~ 259 nm that is believed to be predominantly from the $\pi \rightarrow \pi^*$ transitions of aromatic organic linkers, and potentially the $n \rightarrow \pi^*$ transitions of oxygen-containing functional groups. The above is a sign of absorption which indicates the presence of conjugated organic linker units in the framework of Gd-MOF.

The high peak to background ratio shows that the electronic transition is not being dominated by the carbon absorption, but by the MOF absorption. The absorption maximum of Gd-MOF is blue-shifted and intensified upon adding of activated carbon to Gd-MOF. As can be seen, there is a red shift in the range of 259-282nm which is due to the shift in the electronic environment of Gd-MOF after the interaction with the activated carbon. The change could result from π - π interactions between the aromatic linkers of the MOF and graphitic domains of the activated carbon, and/or electronic coupling between the MOF and the activated carbon.

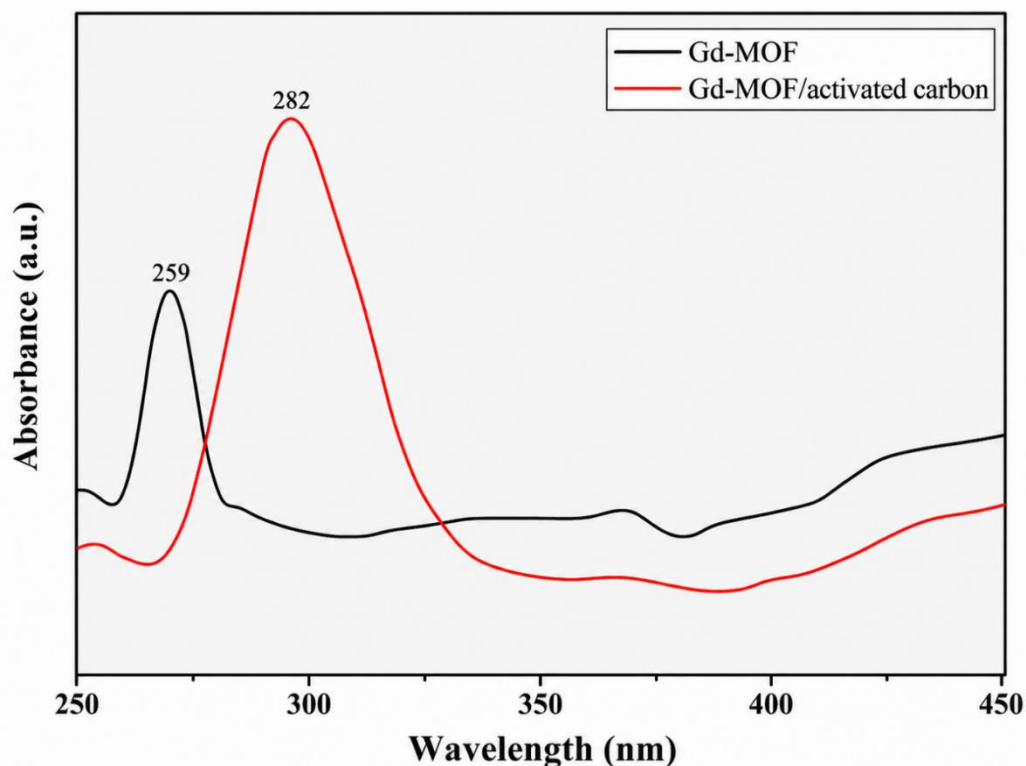


Figure 7: UV-Visible absorption spectra of Gd-MOF and Gd-MOF/activated carbon composite, showing absorption maxima at 259 nm and 282 nm, respectively.

Optimization of pH

The absorbance of the Gd-MOF/AC composite at various pH levels (pH 4, 5, 6, 8, blank) is observed in the UV-Visible result from the image. Although the amount of text omitted is small, it is evident that absorbance is higher as the pH is increased, and is highest at around pH 6, showing that the optimum pH for adsorption of the analyte is pH 6. The lower absorbance values at pH 4 and pH 5 indicate that there is less interaction between the antibiotic and the MOF, which may be attributed to the protonation of functional groups on both the MOF and the carbon surface. The absorbance at higher pH (pH 8) decreases slightly, which is probably because the antibiotics are deprotonated which causes repulsion between the negatively charged sites on the adsorbent and the anionic form of the antibiotics.

The pH of the solution greatly influences the adsorption of Penicillin and Cephalosporin Antibiotics on Gd-MOF/activated carbon composite. The maximum adsorption of the antibiotic molecule was noted at pH 6, which is assumed to be due to the balance of protonation and deprotonation of the surface functional groups on the composite and ionization state of the antibiotic molecules. As the pH decreases, carboxyl and hydroxyl groups will be protonated and hydrogen bonding and electrostatic interactions will be lessened, leading to a decrease in adsorption. At higher pH, deprotonation of these groups and the antibiotics could lead to some electrostatic repulsion which slightly reduces the adsorption efficacy. Thus, it is evident that during pipette-tip adsorption process it is important to control the pH of the solution, as the optimum pH for the effective extraction is pH 6.

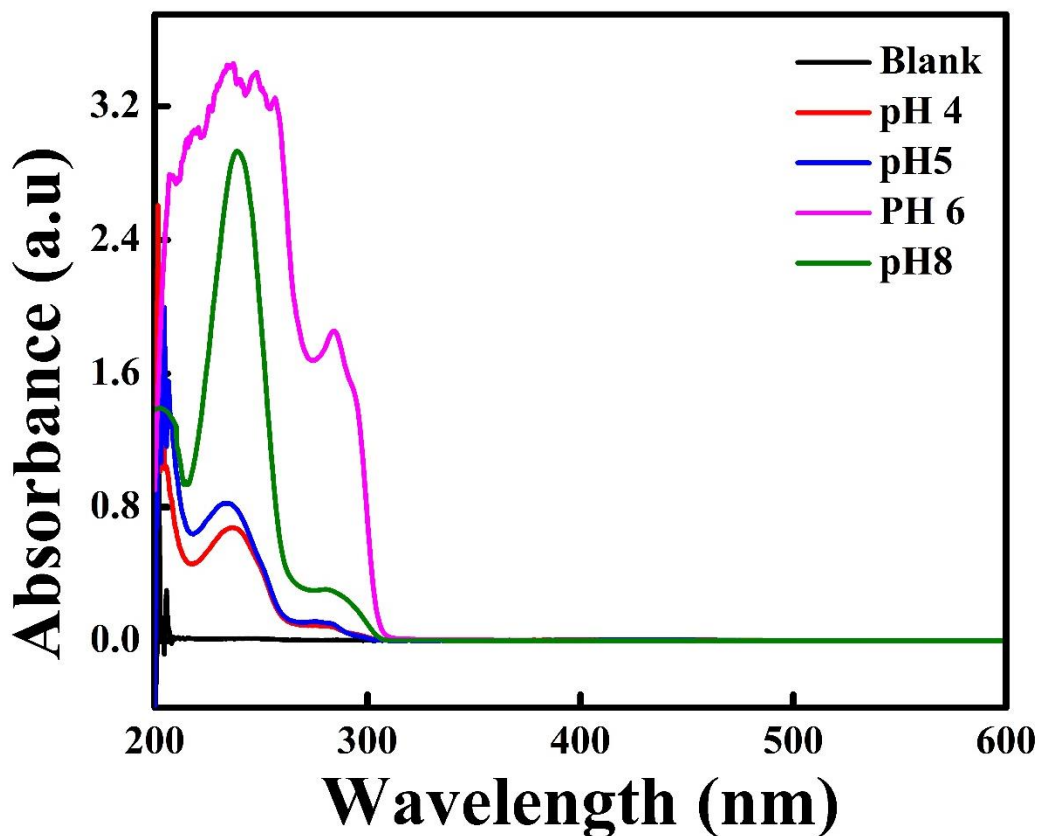


Figure 8: Effect of pH on the adsorption of Penicillin and Cephalosporin Antibiotics onto the Gd-MOF/activated carbon composite. Maximum adsorption is observed at pH 6, indicating optimal interaction between the adsorbent surface functional groups and the antibiotic molecules.

Optimization of concentration of drugs

The UV-Visible data of different adsorbent doses (20 mg, 40 mg, 60 mg and 100 mg) shows the effect of the concentration (amount of adsorbent) on the extraction efficiency of the Cephalosporin Antibiotics (Figure a) and Penicillin (Figure b). The absorbance of both antibiotics increases with the increasing dosage of Gd-MOF/AC composite, and higher the dosage more will be the interaction sites. Cephalosporin Antibiotics: an increment is noticed from 20 mg to 60 mg and beyond 100 mg the increment stops as there are adsorption sites that are saturated at high doses. Likewise, with Penicillin, absorbance increases with the concentration of the adsorbent and approaches a maximum of 100 mg; this suggests that there is a need for adequate adsorbent to obtain the best extraction.

This is because the extraction efficiency of pipette-tip method depends on the level of adsorbent so that if the amount of adsorbent is too low in the pipette-tip then the extraction will be incomplete, but if too much is added to the tip then the saturation of the adsorption sites will not significantly increase the extraction. The flat absorbance at the highest dose level suggests that an equilibrium exists between the analyte in solution and the surface of the adsorbent. Therefore, 100 mg of Gd-MOF/AC composite was chosen as the optimal dosage for the removal and preconcentration of Penicillin and Cephalosporin Antibiotics from milk samples, which provides maximum capacity of the adsorption and minimum number of materials.

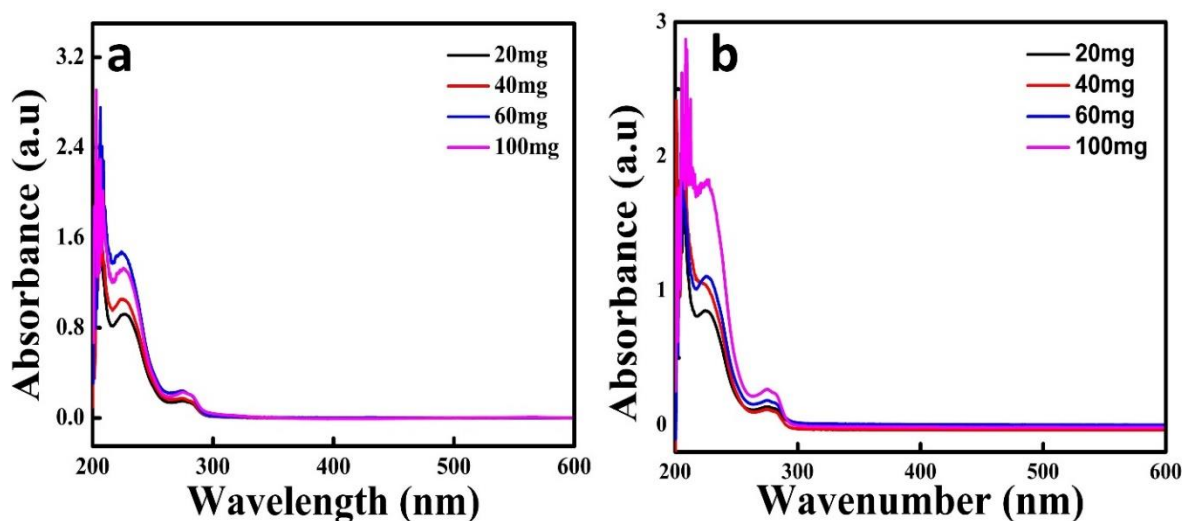


Figure 9: Effect of adsorbent concentration on the UV-Visible absorbance response of (a) Penicillin and (b) Cephalosporin Antibiotics using Gd-MOF/activated carbon composite. The increase in absorbance with adsorbent dosage from 20 to 100 mg indicates enhanced adsorption due to the greater availability of active binding sites for antibiotic extraction.

Effect of adsorbent dose

The effect of the dose of the adsorbent on the extraction of the antibiotics is demonstrated by the UV-Visible data. This indicates that the more the amount of Gd-MOF/ activated carbon composite is increased from 10 mg to 60 mg, the more the absorption is increased, revealing that the number of binding sites is increased. When the amount of adsorbent is less, there are less sites available which

make it less efficient of an extraction. If absorbance is increased beyond 60mg, it means that there are no significant increases and it can be assumed that the adsorption sites are saturating the addition of more adsorbent. Depending on the concentration of the antibiotic molecules, this indicates that 60 mg of adsorbent is sufficient to provide the interaction with the molecules and also be efficient without wasting material.

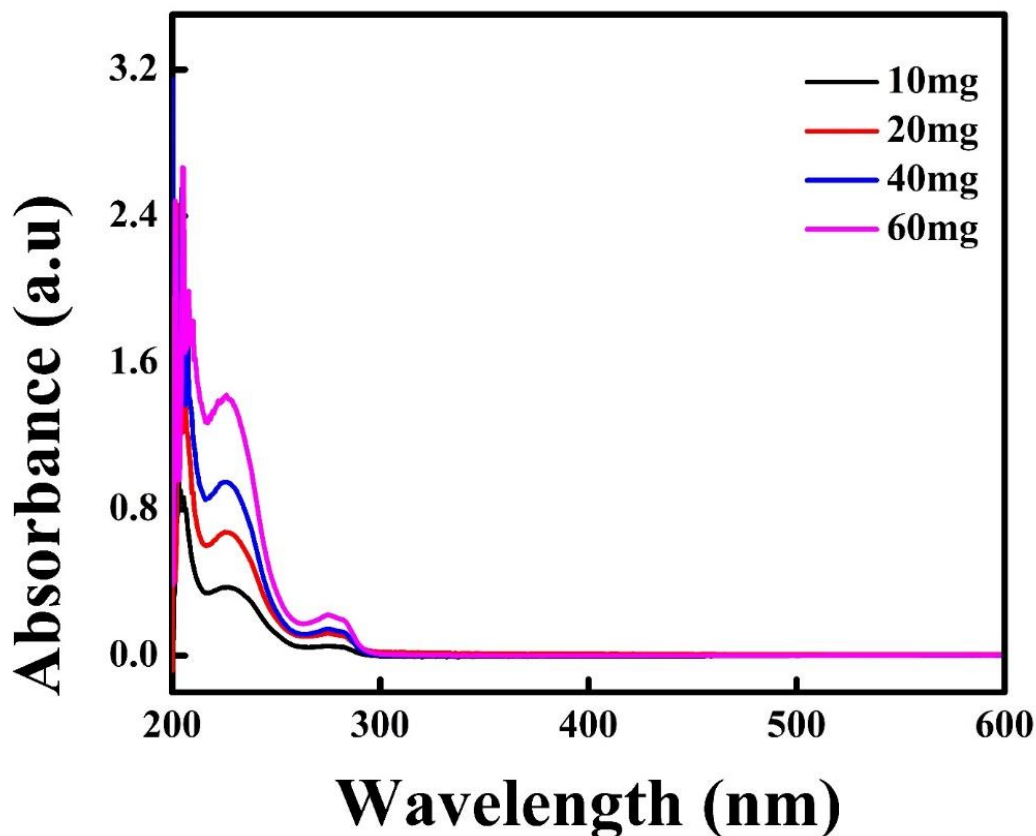


Figure 10: Effect of adsorbent dose on the UV-Visible absorbance of antibiotics using Gd-MOF/activated carbon composite.

Eluent volume

Significant amount of eluent used in the extraction process has been investigated which greatly influences the desorption and responses of the extracted antibiotics with UV-Visible. It can be seen that the highest absorbance was achieved for the volume of eluent equal to 0.5mL, which was enough to elute the retained analytes from the Gd-MOF/activated carbon composite but at the same time the concentration of analytes in the final solution was high. The absorbance decreased as the volume of eluent was increased from 1.0 to 3.0 mL, which could be due to the main reason of dilution of the desorbed antibiotics with the larger volume of eluent. Larger volumes of eluent will give more complete desorption, but the final

concentration of the analyte in the eluate will decrease, and so will be the absorbance measured. From trend this means that less eluent is better to achieve optimal analytical sensitivity and higher preconcentration factor. The amount of eluent used in the adsorption-based extraction method should be sufficient to completely remove the analytes from the surface of the adsorbent, but not to excessively dilute the sample. The results obtained at 0.5 mL is good enough to make the drug molecule to desorb, indicating good interaction between the drug molecule and the eluent. Hence, the volume of eluent selected as 0.5 mL was found to be the optimal volume since it yielded the most response with minimum dilution of Cephalosporin Antibiotics and Penicillin during extraction.

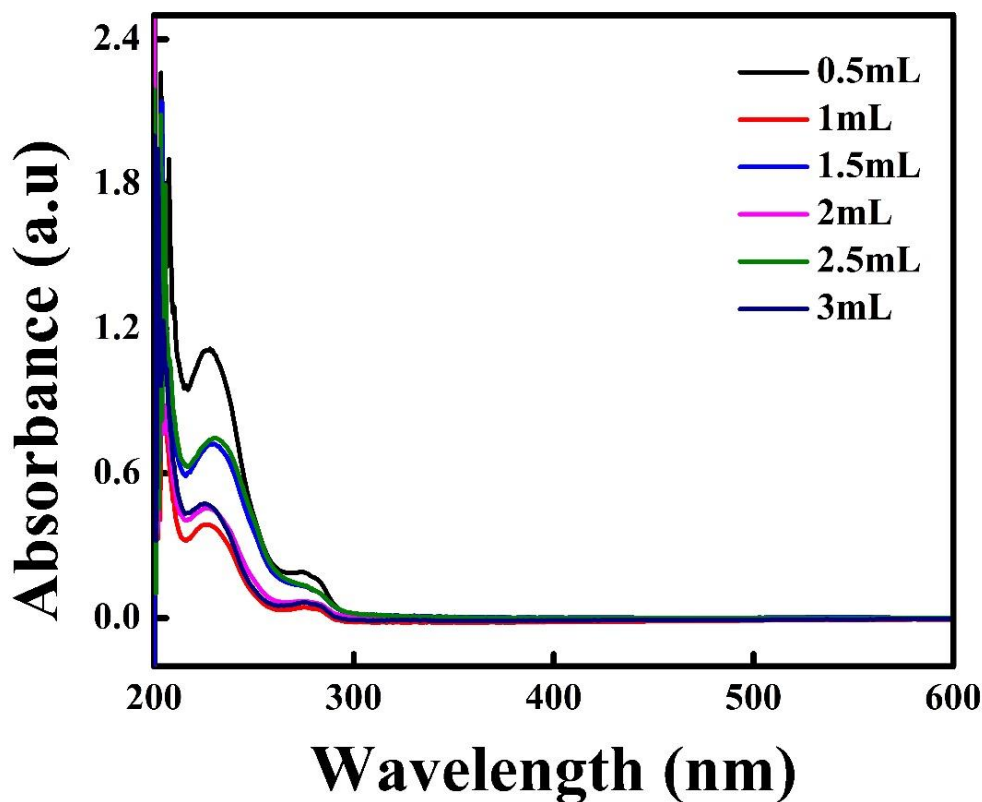


Figure 11: Effect of eluent volume on the UV-Visible absorbance response of antibiotics extracted using Gd-MOF/activated carbon composite.

Real sample analysis

The results obtained by analyzing the real sample with UV-visible showed that the method developed using the Gd-MOF/AC pipette-tip is applicable to the analysis of UV-vis of the drug residues extracted from the milk sample. It can be observed that the blank sample showed near zero significant absorbance at all the wavelengths measured, which is indicative of the fact that there is very little interference in the measured range (400-1000nm) from the unspiked matrix post-extraction. The absorbance response and intensity of the absorbance response were found to be good for the spiked milk samples with 40 ppm, 60 ppm, 100 ppm and 200 ppm spiked and the intensity of the absorbance response was related to the concentration of the spiked analyte. The absorbance increased gradually indicating that the developed extraction and detection method is sensitive enough to detect different

concentrations in the complex food matrix, milk. The spectra also showed that there was no significant matrix effect as a result of milk that would suppress the analytical response as the characteristic absorbance pattern was still observed on all fortified samples.

The absorbance of the concentration with 200 ppm of the sample was the highest, the absorbance of the other concentrations (100, 60 and 40 ppm) were in ascending order, showing that the absorbance was directly proportional to the tested concentration, thereby supporting the possibility of the proposed method to quantify the tested concentrations. The good difference between blank and spiked samples shows good selectivity and efficacy of the extraction system for the target antibiotics from real milk samples. Hence, these results demonstrated that the Gd-MOF/activated carbon composite is applicable to the real sample

analysis and can be used as an effective adsorbent to detect the antibiotic residues in dairy samples.

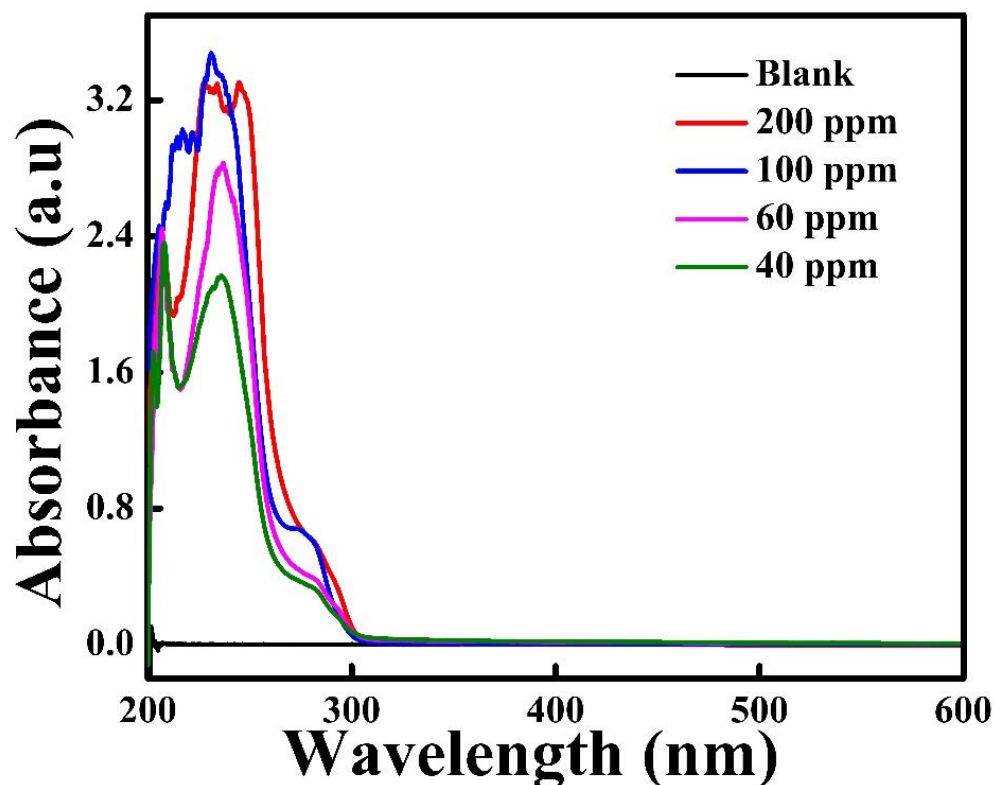


Figure 12: UV-Visible spectra for real milk sample analysis after pipette-tip extraction using Gd-MOF/activated carbon composite.

Reproducibility

Repeatability of the method developed by the Gd-MOF/AC-based pipette tip extraction was studied by taking repeated UV-Visible spectra at the optimized experimental parameters. The spectra have very similar absorbance profiles, and only a slight difference in peak intensity, indicating a consistency of the extraction and detection process in terms of analytical response. The repeated spectra is similar, which suggests the conditions for the packing of the adsorbent, the adsorption and desorption process, and the UV measurement were stable during analysis. The results therefore support the idea that Gd-MOF/activated carbon composite is able to interact with the targeted antibiotics and can be reused several times to extract analytes from the sample matrix with good precision.

It is possible that the differences noted between the repeated absorbance curves are due to small experimental differences, including pipetting error, slight differences in distribution of the adsorbent in the pipette tip and slight variations in elution and UV measurement. The differences, however, are not large enough to significantly affect analytical performance of the method. The reproducible answer illustrates the ability of the proposed method to be used routinely for the detection of antibiotic residues; consistency and precision for analysis of milk samples is of critical importance. The results showed a good operational reproducibility for the developed pipette-tip extraction method for the determination of Penicillin and Cephalosporin Antibiotics, in general.

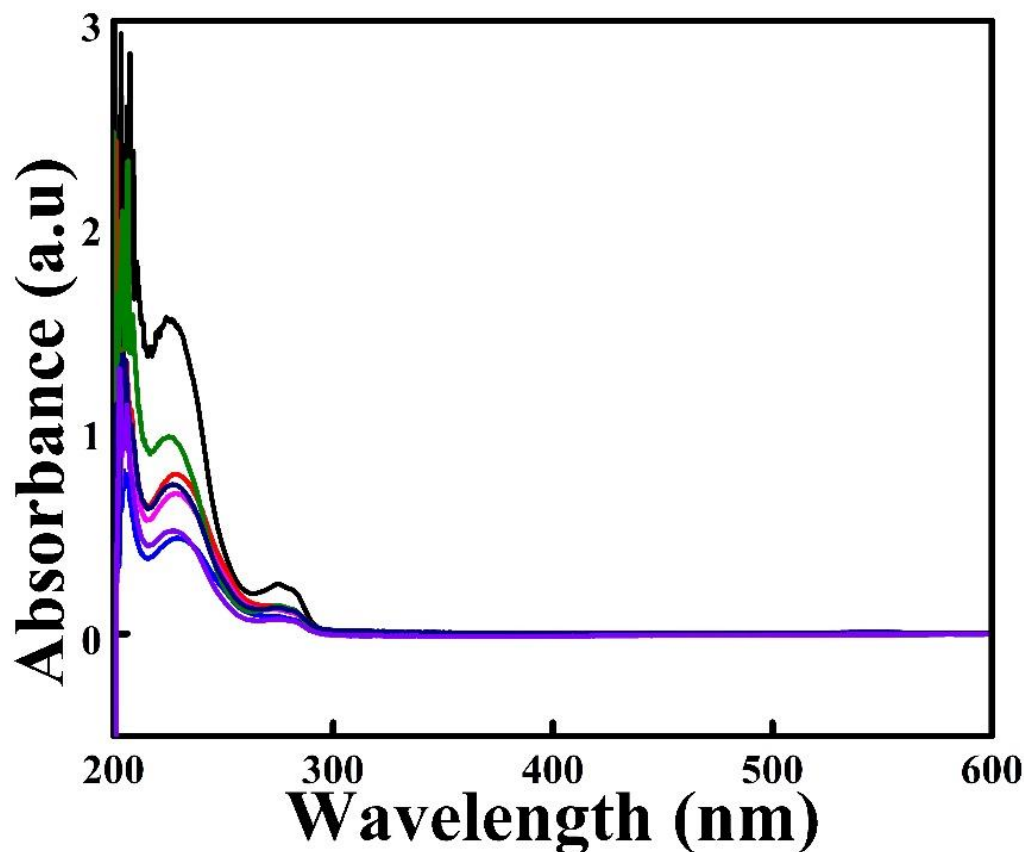


Figure 13: Reproducibility study of the Gd-MOF/activated carbon-based pipette-tip extraction method using repeated UV-Visible measurements.

Conclusion

Gd-MOF/activated carbon conjugate as an efficient sorbent material for solid phase extraction of Penicillin and Cephalosporin Antibiotics from milk samples by pipette tips was successfully used. The high porosity, large surface area and active metal sites, functional groups of the composite material gave rise to a high synergistic adsorption effect of Gd-MOF. The properties of these enhanced the hydrogen bonding, electrostatic attraction, π - π interaction, coordination bonding and pore confinement between adsorbent and antibiotic molecule. Therefore, the developed composite exhibited good potential for extracting and preconcentrating antibiotic residues from a complex milk matrix. Different experimental parameters such as pH, amount of adsorbent, and volume of eluent were optimized for the extraction process. The highest

adsorption efficiency was at pH 6, the interaction between the antibiotic molecules and the composite surface was most favorable.

The optimized amount of adsorption obtained was 100 mg which enough amount of binding sites to get effective extraction of both the Penicillin Antibiotics and Cephalosporin Antibiotics. The absorbance spectrophotometric results of the target antibiotics confirmed the successful identification of the target antibiotics after the extraction from the bacterial cells. The absorbance of the blank milk sample was very low and the absorbance of the spiked milk sample increased with the increase in concentration of the antibiotic which demonstrated good sensitivity and low matrix effect. Overall, the suggested method of Gd-MOF/AC for detection of the Penicillin and Cephalosporin Antibiotics residues in milk is a simple, fast, economic and

environmental-friendly approach. This can be a less complex option than traditional methods, which would need more expensive equipment and complex sample preparation, making it an easier method for routine food safety monitoring practices. Good repeatability, selectivity and applicability in real milk samples validate the developed method in determining antibiotic residues in dairy products. Therefore, the present study contributes to the development of analytical methods that are useful for the milk safety and are also useful for protecting consumers in case of contamination by antibiotics.

Future perspective

The application of Gd-MOF/AC composites in the extraction of antibiotics from milk successfully demonstrates high efficiency, selectivity and environmental friendliness. Future studies are anticipated to be directed towards the designing of MOF-carbon composites with a higher selectivity for other broader spectrum of antibiotic residues which can involve the molecular imprinting or functionalization of the ligands to target the specific antibiotic structures. These developments could enable these materials to be used for other complex food matrices as well as milk to be used in food safety monitoring. The second important point that requires further research is the development of the said composites materials for applying in the automated pipette-tip based extraction system for high throughput screening (HTS) which will not only help to enhance sample preparation but also reduce solvent consumption and help to process a huge number of samples in dairy and food-processing industries.

The growing emphasis on sustainable and eco-friendly analytical methods will also play an important role in optimizing cycles for regeneration and mechanically stability systems, both for longevity and cost effectiveness. Furthermore, MOF-carbon composites with various multifunctional detection methods (e.g., electrochemical sensors, fluorescence, LC-MS/MS) could lead to simultaneous preconcentration and rapid, sensitive quantitative and multivariate analysis of a number of antibiotic

residues. In upcoming years, these difficulties with matrix interferences, reproducibility in complex food samples, and scalability can be further addressed by MOF-carbon composites, enhancing their versatility and usability for regulatory compliance, consumer safety, and environmental monitoring applications.

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