**ABSTRACT:** In this period when environmental pollution has become uncontrollable, the removal of drug active substances reaching the environment and the analysis of drug active substances in different matrix environments are important for both living life and a sustainable environment. Therefore, the production of multifunctional materials that can be used in these two different processes has gained importance in the literature. Based on this thought, in this study, a g-C₃N₄@TiO₂@Fe₃O₄ multifunctional nanohybrid material was synthesized and used for magnetic solid-phase extraction (MSPE) and photocatalytic degradation of trimethoprim and isoniazid, used together in tuberculosis treatment. All analyses were performed by high-performance liquid chromatography using a diode-array detection (HPLC-DAD) system. The synthesized material was characterized by X-ray diffraction spectroscopy (XRD), Raman spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Brunauer–Emmett–Teller (BET) method, ζ-potential analysis, field-emission scanning electron microscopy (FE-SEM), and energy-dispersive X-ray spectroscopy (EDX). Important analytical parameters for the MSPE method such as the pH value of the sample solution, the volume of the sample solution, the amount of the sorbent, the type and volume of the elution solvent, and extraction time were optimized. The optimized MSPE method was then applied to different environmental waters and pharmaceutical samples. The recovery percentages for these samples were found to be between 95 and 107%. For trimethoprim and isoniazid, the limit of detections (LODs) were 0.055 and 0.145 and the limit of quantifications (LOQs) were 0.167 and 0.439 ng·mL⁻¹, respectively. It was observed that ∼100% of trimethoprim and isoniazid active components were photocatalytically removed from the g-C₃N₄@TiO₂@Fe₃O₄ nanohybrid material in ∼120 min under UV light.

1. **INTRODUCTION**

The combined use of drug active ingredients for the treatment of diseases is important in the fight against diseases. Tuberculosis (TB), caused by *Mycobacterium tuberculosis* bacteria, one of the most serious diseases of the past years, is known to kill more than 2 million people annually according to the data from the World Health Organization.¹ The fact that the treatment of TB is very difficult and very costly has brought along the use of multiple drugs. In the treatment of TB, the combination of trimethoprim (TRM) and isoniazid (INH) drug active ingredients is used.² Trimethoprim is used to treat various bacterial infections of the respiratory, urinary, and gastrointestinal tract, while isoniazid is used as a prodrug in the treatment phase.³⁴ While the analysis of these drug active ingredients in the most frequently used drug forms is important for quality control, the control of their release to the environment after use is important for the sustainability of vitality. Therefore, it is essential in this direction to develop accurate and sensitive analytical methods for the analysis of drug active ingredients used in combination in this way.

Analysis techniques for drug active ingredients include high-performance liquid chromatography (HPLC),³ gas chromatography–mass spectroscopy (GC–MS),⁴ liquid chromatography–mass spectroscopy (LC–MS),⁵ and high-performance thin-layer chromatography (HPTLC).⁶ However, direct analysis is not always possible due to complications such as the lowest analyte concentration that these devices can measure, higher than the amounts found in the samples studied, and/or the matrix effects of foreign species present in the sample environment.⁷,⁸ Therefore, for the accurate and sensitive analysis of drug active ingredients in the instrumental detection system, a sample preparation method is required to separate...
drug active ingredients from the matrix environment and bring their concentration to a measurable level.9

MSPE known as a simple and inexpensive sample preparation method has been successfully applied for the separation and enrichment of drug components.10,11 The synthesis and use of new generation nanoadsorbents stand out as the most important developments that lead to different perspectives in solid-phase extraction techniques. Since the basic principle in the solid-phase extraction method is based on adsorption, the large surface areas of the produced nanomaterials maximize the adsorption capacity for analytes. At the same time, for the photocatalytic removal of the analyzed pollutants, the acquisition of photocatalytic properties during the synthesis of nanoadsorbents is an important issue in the literature.12−14

The fact that graphite carbon nitride (g-C3N4) has a large surface area and is a photocatalytic active material has led to its frequent use in the literature.12−14 Titanium dioxide (TiO2) is widely used in photocatalytic degradation studies due to its low bandwidth, very high surface area, and high photocatalytic efficiency.10,15−17 Despite the above-mentioned advantages of g-C3N4 and TiO2 NPs, they need to be isolated quickly and easily without agglomeration from the solution environment in solid-phase extraction and photocatalytic experiment steps. One of the effective solutions for this is the modification of nanomaterials with magnetic NPs such as Fe3O4 and α-Fe2O3. In this way, nanomaterials that gain magnetic properties can be easily isolated from the solution environment at any time by applying an external magnetic field.17

In the light of this information, a multifunctional nanohybrid material containing g-C3N4 NPs with high adsorption and photocatalytic properties, TiO2 NPs with high photocatalytic properties, and Fe3O4 NPs with high magnetism was produced. The g-C3N4@TiO2@Fe3O4 multifunctional nanomaterial was synthesized using a simple and green solvothermal synthesis procedure. The synthesized material was successfully used for magnetic solid-phase extraction and photocatalytic degradation of trimethoprim and isoniazid drug active ingredients.

2. EXPERIMENTAL SECTION

2.1. Materials, Reagents, and Equipment. Analytical grade reagents were used throughout the experimental study. TiO2 NPs had a particle size of 21 nm; FeCl3, CH3COONa, urea, and ethylene glycol were obtained from Sigma-Aldrich (St. Louis). All solutions used in HPLC-DAD analyses were of LC grade purity (Sigma-Aldrich, St. Louis). A Milli-Q system deionized water system (Millipore) was used to obtain deionized water (resistivity 18.2 MΩ•cm). 200 mg·L−1 stock solutions of isoniazid and trimethoprim were prepared in
For the production of g-C₃N₄ NPs, the BET-N₂ method was used. The C18 column was obtained from UEM research and development company (Kayseri, Turkey). A methanol:acetate buffer mobile phase at a flow rate of 1.2 mL·min⁻¹ was used. UV detection after chromatographic separation was performed at different absorption maxima depending on the used probe analyte. A photocatalytic reactor with a 400 W power of a UV lamp (Unitemrrm, Turkey) was employed for the photocatalytic degradation experiments. The structure and morphology of the synthesized g-C₃N₄ NPs and g-C₃N₄@TiO₂@Fe₃O₄ NPs were investigated using a field-emission scanning electron microscope (FE-SEM, Gemini 550). The crystallographic structures of g-C₃N₄ NPs and g-C₃N₄@TiO₂@Fe₃O₄ NPs were illuminated by employing a Bruker AXS D8 X-ray powder diffractometer with a simple cubic lattice and Cu Kα radiation (λ = 0.15406 nm), and the scan range (2θ) was from 5 to 90°. Raman spectra of TiO₂ NPs, g-C₃N₄ NPs, and g-C₃N₄@TiO₂@Fe₃O₄ NPs were recorded using a WITec alpha 300 M dispersive Raman spectrometer consisting of a He–Ne laser system (excitation wavelength is 532 nm). A Micromeritics Gemini VII BET analyzer was used to find the surface area, pore volume, and pore width for g-C₃N₄@TiO₂@Fe₃O₄ NPs. For this purpose, the BET-N₂ method was used. The ϵ-potentials of g-C₃N₄@TiO₂@Fe₃O₄ NPs were measured at different pH values using a Malvern ZEN2600 Zetasizer Nano ZS.

2.2. Fabrication of the g-C₃N₄@TiO₂@Fe₃O₄ Nano-Hybrid Material. For the production of g-C₃N₄ NPs, the calcination method as a simple and fast method proven in the literature was used. First, 1.0 g of urea powder was placed in a porcelain crucible and calcined in a muffle furnace at 550 °C at a heating rate of 2.3 °C·min⁻¹ in for 4 h, and then cooled at room temperature. At the end of this process, slightly yellowish g-C₃N₄ NPs were obtained. For the production of g-C₃N₄@TiO₂@Fe₃O₄ NPs, 0.5 g of g-C₃N₄ NPs was weighed and ultrasonicated for 20 min in 20 mL of an ethylene glycol solution. Then, 0.5 g of TiO₂ NPs was weighed in a second beaker and then ultrasonicated in 20 mL of ethylene glycol for 20 min. Next, 0.5 g of FeCl₃ and 2 g of CH₃COONa were weighed in a third beaker and then ultrasonicated in 15 mL of ethylene glycol until the homogenization process was completed. These three mixtures obtained were collected in a beaker. After mixing in a magnetic stirrer at 900 rpm for 5 min, it was transferred into a Teflon solvothermal synthesis vessel and subjected to a solvothermal synthesis process in a solvothermal unit at 180 °C for 16 h. After 16 h, g-C₃N₄@TiO₂@Fe₃O₄ NPs were collected by a neodymium magnet, and washing with ethanol and deionized water was performed. It was then left to dry at 80 °C in an oven.

2.3. Magnetic Solid-Phase Extraction Method. A total of 10 mL of a model solution containing trimethoprim and isoniazid at known concentrations was prepared in a phosphate buffer medium (25 mM, pH 6.0) and 50 mg of g-C₃N₄@TiO₂@Fe₃O₄ NPs was added to this solution. The mixture was mixed for 5 min with the help of a vortex to adsorb the drug molecules on the g-C₃N₄@TiO₂@Fe₃O₄ NPs. Finally, g-C₃N₄@TiO₂@Fe₃O₄ NPs were isolated from the sample solution by applying an external magnetic field with a neodymium magnet. The resulting waste phase was completely discarded. Next, g-C₃N₄@TiO₂@Fe₃O₄ NPs were dispersed in 0.25 mL of ethanol and vortexed for 5 min to desorb drug molecules from g-C₃N₄@TiO₂@Fe₃O₄ NPs. The same elution process was performed three times in total, and the eluents were collected in the same tube and the final eluent volume was 0.75 mL. In the last step, the eluent phase was taken using a micropipette, filtered through a 0.22 filter, and analyzed using an HPLC-DAD detection system. For the next MSPE treatment, g-C₃N₄@TiO₂@Fe₃O₄ NPs were washed with 25 mL of water twice. The same MSPE/HPLC-DAD procedure was applied to the standard solutions and blank samples. The MSPE/HPLC-DAD procedure is schematized in Figure 1A.

2.4. Real Sample Applications. Real sample experiments were carried out on two different drug samples commercially available in a tablet form, lake water, seawater, and drinking water samples. Overall, 10 tablets were taken from the drugs in a tablet form.
tablet and homogenized in a mortar. Further, 25 mg of each sample was weighed into a flask and dissolved in 250 mL of methanol. A certain volume of these samples was taken and pure water and buffer solution were added to it, and the developed MSPE/HPLC-DAD procedure was applied. Environmental water samples were filtered through a 0.22 μm filter before use and stored at 4°C.

2.5. Photocatalytic Degradation of Drug Molecules. The photocatalysis performance of g-C₃N₄@TiO₂@Fe₃O₄ NPs was evaluated in 100 mL of a model solution medium containing known concentrations of trimethoprim or isoniazid molecules. For this purpose, 100 mg of g-C₃N₄@TiO₂@Fe₃O₄ NPs was added to 100 mL of a model solution, and the mixture was stirred in a dark environment without light until the adsorption of the drug molecules was complete. Next, the mixture was transferred to a photocatalytic reactor and exposed to a 400 W UV halogen lamp (365 nm wavelength) located in the center of the reactor. To determine the rate of photocatalytic degradation versus time, aliquots of a 1.0 mL sample were taken for analysis at programmed time intervals (10 min/sample) and analyzed using HPLC-DAD. The degradation rate of each drug active species was calculated using the peak areas obtained from the spectra recorded by HPLC-DAD at increasing times. The photocatalytic degradation process of drug molecules is schematized in Figure 1B.

Figure 3. FTIR spectra of TiO₂ NPs, g-C₃N₄ NPs, and g-C₃N₄@TiO₂@Fe₃O₄ NPs.

Figure 4. XRD patterns of Fe₃O₄ NPs, g-C₃N₄ NPs, TiO₂ NPs, and g-C₃N₄@TiO₂@Fe₃O₄ NPs.
of g-C3N4 NPs is seen (Figure 2A). In the images in Figure 2B, C, absorption peaks observed between 830 and 930 cm
vibrations of the CN aromatic repeating units. The two and these nanospheres were surrounded by both g-C3N4 NPs
seen. Fe3O4 nanospheres with sizes varying in the range of 25

Fe3O4 nanospheres during synthesis. As can be understood from the
measurement procedure in the FE-SEM. The FE-SEM and
in the EDX spectrum is due to the gold plating originating from
Fe, Ti) doped in the structure can be seen. The unlabeled peak
is due to N
characteristic of the out-of-plane vibrations of triazine/s-triazine
rings in g-C3N4 NPs.20 The Raman shift at 1237 cm
is associated with the lattice vibration of the g-C3N4 crystal.

The Raman spectra of pure Fe3O4 NPs can be characterized
by three peaks at 286, 482, and 664 cm
appearing at 144 cm
The Raman shift at 1237 cm
are assigned to the Ti–O stretching vibrations for TiO2 NPs.

In Figure 4, XRD patterns of g-C3N4 NPs, Fe3O4 NPs, TiO2
NPs, and g-C3N4@TiO2@Fe3O4 NPs are shown. An intense
peak at 28 = 12.65 and 27.02° (JCPDS 087-1526) corresponds
to graphitic planes of C6N4 NPs. The XRD peak observed at
12.65°, corresponding to the (001) plane, is due to the in-plane
structural packing motif of the aromatic segments. The strongest
peak at 27.02°, corresponding to the (002) plane, is due to the
stacking of the conjugated aromatic system, which characterizes
the interlayer d-spacing of g-C3N4. Characteristic peaks for
anatase TiO2 NPs were confirmed by other studies in the
literature.16

For the XRD spectrum of Fe3O4 NPs, 28 = 31.0, 35.3, 43.8,
52.6, and 57.8° correspond to the planes of (220), (311), (400),
(422), and (511) of the magnetite Fe3O4 NP phase (JCPDS
Card No. 01-075-0449). The peaks obtained for Fe3O4 NPs are
in agreement with the cubic structure of magnetite Fe3O4-NPs
(space group: Fd-3m). When the spectrum of g-C3N4@TiO2@Fe3O4
NPs is examined, it is seen that the characteristic peaks of
Fe3O4 NPs, TiO2 NPs, and g-C3N4 NPs are obtained by
decreasing their peak intensities. This is due to the composite
structure of g-C3N4@TiO2@Fe3O4 NPs NPs.

In Figure 5, the Raman spectra of pure TiO2 NPs, Fe3O4 NPs,
and g-C3N4@TiO2@Fe3O4 NPs are shown. TiO2 NPs with an
anatase form have six Raman active modes (A1g + 2 B1g + 3 Eg)
appearing in the Raman spectrometer is used at very low energy, many false
peaks of hematite can be seen in the Raman spectrum of
Fe3O4 has a spinel structure, giving rise to
bands: three T2g, one E, and one A1g (Figure 5). The data
obtained for Fe3O4 NPs are

3. RESULTS AND DISCUSSION

3.1. Characterization of the g-C3N4@TiO2@Fe3O4
Multifunctional Nanomaterial. FE-SEM images of the
produced g-C3N4 NPs and g-C3N4@TiO2@Fe3O4 NPs at 25
kV at 50 000 times magnification and EDX images at 50 000–
10 000 times magnification are shown in Figure 2. As shown in
Figure 2, after calcination at 550
12.65 and 27.02
°
θ
of the modi-
cation of the species (C, N, O,
Value on MSPE E

Figure 5. Raman spectra of TiO2 NPs, Fe3O4 NPs, and g-C3N4@TiO2@Fe3O4 NPs.

ACS Omega 2022, 7, 23223–23233
https://doi.org/10.1021/acsomega.2c01311
ACS Omega http://pubs.acs.org/journal/acsodf
Article

23227
Trimethoprim and isoniazid are polar compounds containing acidic and carboxylic groups, so they can be found in a neutral or ionic form. If the pH of the solution is below the $pK_a$ value, these compounds are converted to a molecular form. The fact that most drug active species have more than one $pK_a$ value is another important factor to be aware of. It is assumed that the adsorption efficiency increases through effective intermolecular interactions between the analyte and the adsorbent. The effect of pH of the sample solution on the adsorption efficiency of the analytes was investigated in the range of 2.0–10.0 on model solutions containing 0.25 μg·mL$^{-1}$ of trimethoprim and isoniazid. (Figure 6A). The results obtained showed that the extraction efficiency of both active ingredients increases in parallel with the increasing pH value, reaching a maximum at pH 6.0, and the extraction efficiency decreases above pH values. Considering the results, the optimum pH value was selected as 6.0. $\zeta$-Potential analysis was performed to determine the surface charge of the synthesized g-C$_3$N$_4$@TiO$_2$@Fe$_3$O$_4$ NPs, and the results are given in Figure S1. It was observed that the surface of the g-C$_3$N$_4$@TiO$_2$@Fe$_3$O$_4$ NPs was positively charged when the pH of the aqueous solution was between 2 and 4, and it was negatively charged at higher pH values. It was observed that the surface of the g-C$_3$N$_4$@TiO$_2$@Fe$_3$O$_4$ NPs became more negatively charged as the pH increased. The $pK_a$ values of trimethoprim and isoniazid are 7.12 and 1.82, respectively. While the driving force for adsorption of trimethoprim was electrostatic interactions, the driving force for adsorption of isoniazid was predicted to be interactions such as van der Waals forces and hydrogen bonding.

3.2.2. Effect of the g-C$_3$N$_4$@TiO$_2$@Fe$_3$O$_4$ NP Amount on MSPE Efficiency. The effect of the g-C$_3$N$_4$@TiO$_2$@Fe$_3$O$_4$ NP amount on the adsorption efficiency of trimethoprim and isoniazid was investigated between 1 and 100 mg (Figure 6B). The obtained results showed that the increase in the amount of the adsorbent per unit analyte concentration caused an increase in the extraction efficiency. It was observed that the maximum extraction efficiency was reached with the addition of 50 mg of g-C$_3$N$_4$@TiO$_2$@Fe$_3$O$_4$ NPs, and there was no significant change in extraction efficiency with more additions. Therefore, 50 mg of g-C$_3$N$_4$@TiO$_2$@Fe$_3$O$_4$ NPs was used in subsequent experiments for both drug molecules.

3.2.3. Effect of the Eluent Type and Volume on MSPE Efficiency. In solid-phase extraction methods, the elution of analytes on the adsorbent before analysis is an important step. Since trimethoprim and isoniazid are analytes in an organic form, it is appropriate to use organic solvents in their elutions. For this purpose, methanol (MeOH), ethanol (EtOH), isopropyl alcohol (IPA), acetonitrile (ACN), and acetone were used in the desorption stage of trimethoprim and isoniazid. After the elution step, the analyte concentrations in the final phase were determined by HPLC. Analyses were carried out by preparing suitable standards for each eluent phase. The quantitative recovery values for trimethoprim and isoniazid were obtained using ethanol and methanol, but the highest recoveries were reached with ethanol (Figure 6C). Therefore,
ethanol was used as an eluent for subsequent experiments. It is important to perform the desorption process with the minimum possible volume of eluent to obtain a high enhancement factor, which is an important criterion for obtaining lower detection limits and consuming a low volume of an organic solvent. Desorptions of trimethoprim and isoniazid drug active ingredients adsorbed on g-C₃N₄@TiO₂@Fe₃O₄ NPs were performed with volumes of ethanol varying from 0.5 to 4 mL and sequential extraction (Figure 6D). The results obtained showed that both active pharmaceutical ingredients were recovered quantitatively with sequential extraction using 0.25 mL of ethanol three times. The eluents were collected in the same tube and the final eluent volume was 0.75 mL.

3.2.4. Effect of the Sample Solution Volume on MSPE Efficiency. The volume of the sample solution to which the method developed in sample preparation techniques can be applied is one of the most important parameters affecting the important analytical performance parameter such as the preconcentration factor, the limit of detection, and the limit of quantification. The developed MSPE method based on g-C₃N₄@TiO₂@Fe₃O₄ was applied to sample solutions changing from 15 to 100 mL under the optimum experimental conditions. The results obtained showed that the developed MSPE method can be applied up to 75 mL of the sample volume and that the extraction yields for both drug active ingredients at higher volumes were not quantitative (Figure S2). Therefore, the developed method was applied to real samples with a maximum volume of 75 mL.

3.2.5. Analytical Performance of the MSPE Method. For validation of the MSPE method, the following validation parameters were evaluated: linearity, sensitivity, precision, and preconcentration factor (Table 1). Calibration curves for trimethoprim and isoniazid were obtained after the application of the MSPE method to increasing analyte concentrations. The calculation of the LOD was based on dividing 3 times the standard deviation of the signal of the lowest concentration of the analyte concentration obtained by the slope of the calibration curve, while the LOQ was based on 10 times the standard deviation. The precision of the MSPE method was given as intraday RSD % and interday RSD % calculated by the standard deviation. The precision of the MSPE method was performed with volumes of ethanol varying from 0.5 to 4 mL and applying the MSPE method to 10 standard solutions containing 0.25 μg·mL⁻¹ trimethoprim and 0.5 μg·mL⁻¹ isoniazid. The precision of the MSPE method was evaluated according to the Langmuir–Hinshelwood kinetic model as given below.5

\[
\text{decomposition rate (\%) } = \frac{C_0 - C_t}{C_0} \times 100
\]

where \( C_0 \) and \( C_t \) (mg·L⁻¹) are the concentrations of probing molecules at the initial stage and the adsorption equilibrium concentration after irradiation at an irradiation time \( t \) (min), respectively. In chemical kinetics, when the reaction is first order, the reaction rate is measured based on the concentration of the reactants.

So, the rate of the first-order reaction is directly proportional to the concentration of the probe molecules. The kinetics of the photocatalytic degradation rate of the probe molecules was evaluated according to the Langmuir–Hinshelwood kinetic model as given below.5

![Table 1. Analytical Performance Parameters for the MSPE/HPLC-DAD Procedure](image)

| Parameters                  | Analytical performance results |
|-----------------------------|--------------------------------|
|                             | Trimethoprin                  | Isoniazid                   |
| Calibration curve equation  | \( y = 6470.9x^2 + 2844.4 \) | \( y = 4369.1x^2 - 985.42 \) |
| Correlation coefficients \( (R^2) \) | 0.9994                        | 0.9974                      |
| LOD, ng·mL⁻¹                | 0.055                         | 0.145                       |
| LOQ, ng·mL⁻¹                | 0.167                         | 0.439                       |
| Intraday RSD, %             | 1.25                          | 1.29                        |
| Interday RSD, %             | 3.02                          | 3.08                        |
| Average recovery, %         | 98 ± 2                        | 97 ± 4                      |
| PF                          | 100                           | 100                         |

"Peak area on the HPLC-DAD system. "Slope of the calibration curve.
The photocatalytic degradation efficiency of g-C₃N₄@TiO₂@Fe₃O₄ NPs for the trimethoprim and isoniazid drug active species was analyzed according to the Langmuir–Hinshelwood kinetic model. The results obtained showed that ∼100% of trimethoprim and isoniazid were photocatalytically degraded in ∼120 and 100 min, respectively (Figure 7A). The graph of the photocatalytic removal of drug molecules versus time and the kinetic study graph are shown in Figure 7B–D. The represented fitted straight lines were plotted with a slope equal to the apparent pseudo-first-order rate constant k (min⁻¹).

The UV–vis spectra were recorded for g-C₃N₄ NPs, TiO₂ NPs, and g-C₃N₄@TiO₂@Fe₃O₄ NPs (Figure 8). It was determined that TiO₂ NPs and g-C₃N₄ NPs have maximum absorptions at 520 and 324.7 nm wavelengths, respectively. The absorption edges of TiO₂ NPs and g-C₃N₄ NPs can be associated with the transfer of electrons from the valence band to the conduction band. The ternary nanocomposite g-C₃N₄@
TiO\textsubscript{2}@Fe\textsubscript{3}O\textsubscript{4} differed from other individual components in its absorption properties, giving a maximum absorption peak centered around 317.7 nm. This also can be attributed to the electronic transition from the valence band to the conduction band of the ternary composite and may also suggest significant interfacial contact between the g-C\textsubscript{3}N\textsubscript{4} NPs, TiO\textsubscript{2} NPs, and Fe\textsubscript{3}O\textsubscript{4} NP components in the final nanocomposite. Also, mixing the 4s orbital of Ti with the 4s orbital of Fe may result in the formation of the conduction band at low energy. Tauc's plot used to calculate direct band gaps showed that g-C\textsubscript{3}N\textsubscript{4} NPs, TiO\textsubscript{2} NPs, and g-C\textsubscript{3}N\textsubscript{4}@TiO\textsubscript{2}@Fe\textsubscript{3}O\textsubscript{4} NPs have direct band gaps of 2.39, 1.43, and 3.08 eV, respectively. These data further confirmed the formation of the intended heterojunction, enabling photocatalysis in the UV–visible light range.

The probable mechanism of the photocatalytic reaction of g-C\textsubscript{3}N\textsubscript{4}@TiO\textsubscript{2}@Fe\textsubscript{3}O\textsubscript{4} NPs is shown in Figure 9. TiO\textsubscript{2} NPs, from the wide-band-gap semiconductor class, have low UV light capture capacity to form light-induced electrons in the conduction band and accompanying holes in the valence band. g-C\textsubscript{3}N\textsubscript{4} NPs in g-C\textsubscript{3}N\textsubscript{4}@TiO\textsubscript{2}@Fe\textsubscript{3}O\textsubscript{4} NPs are the main component that successfully collect UV light and cause the excitation of electrons from the valence band to the conduction

Figure 8. UV–vis spectra of g-C\textsubscript{3}N\textsubscript{4} NPs, TiO\textsubscript{2} NPs, and g-C\textsubscript{3}N\textsubscript{4}@TiO\textsubscript{2}@Fe\textsubscript{3}O\textsubscript{4} NPs and the corresponding Tauc plot of g-C\textsubscript{3}N\textsubscript{4} NPs, TiO\textsubscript{2} NPs, and g-C\textsubscript{3}N\textsubscript{4}@TiO\textsubscript{2}@Fe\textsubscript{3}O\textsubscript{4} NPs.
band. Since the conduction band of TiO₂ has a higher reduction potential, electrons migrate from the conduction band of g-C₃N₄ to the conduction band of TiO₂ due to light. In addition, however, TiO₂ with the reduction potential of the conduction band higher than that of Fe₃O₄ NPs paves the way for further transfer of photogenerated electrons to the conduction band of Fe₃O₄ NPs. Electrons in the conduction band of TiO₂ tend to react easily with adsorbed oxygen (O₂) molecules to form superoxide anion radicals (°O₂⁻). Also, a simultaneous hole transfer occurred from the valence band of TiO₂ to the valence band of g-C₃N₄. As a result of the reaction of the holes in the valence band of g-C₃N₄ with water molecules (H₂O), hydroxyl radicals (°OH) are formed. These radicals then react with trimethoprim and isoniazid molecules, causing their degradation. Therefore, modifying a wide-band-gap semiconductor with a narrow-band-gap semiconductor is frequently used in photocatalyst applications. The resulting hybrid photocatalyst not only absorbs the photon in the UV−visible range but also has a suppressed recombination rate of the photogenerated charges. Starting from this point, in this study, while the g-C₃N₄ region of the g-C₃N₄@TiO₂@Fe₃O₄ nanohybrid material is the active site that collects light, the TiO₂ and Fe₃O₄ regions play the main role in the separation of charge carriers. Thus, it led to an increase in the photocatalytic efficiency of the designed photocatalyst.

4. CONCLUSIONS

The fact that trimethoprim and isoniazid are used together in the treatment of diseases such as tuberculosis makes it important to analyze both of them at the same time and to remove them from environmental waters after use. In this study, which was designed with this awareness, the usability of g-C₃N₄@TiO₂@Fe₃O₄ NPs as a magnetic adsorbent in magnetic solid-phase extraction for accurate and sensitive analysis of trimethoprim and isoniazid in different matrix media with the HPLC-DAD technique and as a magnetic photocatalyst for the degradation of trimethoprim and isoniazid in water medium was investigated. Due to its graphene-like structure, g-C₃N₄ NPs with high adsorption and photocatalytic properties were combined with TiO₂ NPs with high photocatalytic properties and Fe₃O₄ nanoparticles with strong magnetic properties to produce a new hybrid material offering three different properties for these two different process. The obtained results proved that g-C₃N₄@TiO₂@Fe₃O₄ NPs can be used successfully in the magnetic solid-phase extraction and the photocatalytic removal of trimethoprim and isoniazid.

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c01311.
ζ-Potential analysis figure of the synthesized g-C₃N₄@TiO₂@Fe₃O₄ NPs and figure of the effect of the sample solution volume on MSPE efficiency (PDF)

■ AUTHOR INFORMATION

Corresponding Author
Erkan Yilmaz — Department of Analytical Chemistry, Faculty of Pharmacy, Erciyes University, 38050 Kayseri, Turkey; ERNAM-Nanotechnology Research and Application Center and Technology Research & Application Center (TAUM), Erciyes University, 38039 Kayseri, Turkey; ChemicaMed Chemical Inc., Erciyes University Technology Development Zone, 38039 Kayseri, Turkey; orcid.org/0000-0001-8962-3199; Email: erkanyilmaz@erciyes.edu.tr

Author
Gokhan Sarp — Department of Analytical Chemistry, Faculty of Pharmacy, Erciyes University, 38050 Kayseri, Turkey; ERNAM-Nanotechnology Research and Application Center, Erciyes University, 38039 Kayseri, Turkey

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c01311

Notes
The authors declare no competing financial interest.
This manuscript is a part of Gokhan Sarp’s doctoral thesis.

■ ACKNOWLEDGMENTS

The authors are grateful for the financial support of the Unit of the Scientific Research Projects of Erciyes University (TDK-2022-11760).
REFERENCES

(1) The World Health Report 2002: Reducing Risks, Promoting Healthy Life; World Health Organization, 2002.
(2) Vilchés, C.; Jacobs, W. R. The Combination of Sulfamethoxazole, Trimethoprim, and Isoniazid or Rifampin Is Bactericidal and Prevents the Emergence of Drug Resistance in Mycobacterium Tuberculosis. Antimicrob. Agents Chemother. 2012, 56, 5142–5148.
(3) Yuvali, D.; Narin, L.; Soyalk, M.; Yilmaz, E. Green Synthesis of Magnetic Carbon Nanodot/Graphene Oxide Hybrid Material (Fe3O4@C-Nanodot@GO) for Magnetic Solid Phase Extraction of Ibuprofen in Human Blood Samples Prior to HPLC-DAD Determination. J. Pharm. Biomed. Anal. 2020, 179, No. 113001.
(4) Perez, E. R.; Knapp, J. A.; Horn, C. K.; Stillman, S. L.; Evans, J. E.; Arfsten, D. P. Comparison of LC−MS-MS and GC−MS Analysis of Benzodiazepine Compounds Included in the Drug Demand Reduction Urinalysis Program. J. Anal. Toxicol. 2016, 40, 201–207.
(5) Iwamoto, N.; Shimada, T. Structure-Indicated LC-MS/MS Bioanalysis of Therapeutic Antibodies. In Therapeutic Antibodies, Springer, 2022; pp 187–205.
(6) Gökbülbüt, A. High Performance Thin Layer Chromatography (HPTLC) for the Investigation of Medicinal Plants. Curr. Anal. Chem. 2021, 17, 1252–1259.
(7) Soyalk, M.; Yilmaz, E. Determination of Cadmium in Fruit and Vegetables by Ionic Liquid Magnetic Microextraction and Flame Atomic Absorption Spectrometry. Anal. Lett. 2015, 48, 464–476.
(8) Reclo, M.; Yilmaz, E.; Soyalk, M.; Andruch, V.; Bazel, Y. Ligandless Switchable Solvent Based Liquid Phase Microextraction of Nickel from Food and Cigarette Samples Prior to Its Micro-Sampling Flame Atomic Absorption Spectrometric Determination. J. Mol. Liq. 2017, 237, 236–241.
(9) Huang, L.; Shen, R.; Liu, R.; Xu, S.; Shuai, Q. Facile Fabrication of Magnetic Covalent Organic Frameworks for Magnetic Solid-Phase Extraction of Diclofenac Sodium in Milk. Food Chem. 2021, 347, No. 129002.
(10) Yilmaz, E.; Salem, S.; Sarp, G.; Aydin, S.; Sahin, K.; Korkmaz, I.; Yuvali, D. TiO2 Nanoparticles and C-Nanofibers Modified Magnetic Fe3O4 Nanospheres (TiO2@Fe3O4@C−NF): A Multifunctional Hybrid Material for Magnetic Solid-Phase Extraction of Ibuprofen and Photocatalytic Degradation of Drug Molecules and Azo Dye. Talanta 2020, 213, No. 120813.
(11) Yilmaz, E.; Sarp, G. Graphene-like MoS2-Modified Magnetic C-Dot Nanoflowers: An Efficient Magnetic Solid-Phase Extraction Adsorbent for Monitoring of Trace Amounts of Ibuprofen. Anal. Methods 2020, 12, 1570–1578.
(12) Fu, J.; Yu, J.; Jiang, C.; Cheng, B. G-C3N4-Based Heterostructured Photocatalysts. Adv. Energy Mater. 2018, 8, No. 1701503.
(13) Wen, J.; Xie, J.; Chen, X.; Li, X. A Review on G-C3N4-Based Photocatalysts. Appl. Surf. Sci. 2017, 391, 72–123.
(14) Zhu, X. D.; Wang, Y. J.; Sun, R. J.; Zhou, D. M. Photocatalytic Degradation of Tetracycline in Aqueous Solution by Nanosized TiO2. Chemosphere 2013, 92, 925–932.
(15) Liu, R.; Yang, W.; He, G.; Zheng, W.; Li, M.; Tao, W.; Tian, M. Ag-Modified g-C3N4 Prepared by a One-Step Calcination Method for Enhanced Catalytic Efficiency and Stability. ACS Omega 2020, 5, 19615–19624.
(16) Kibasomba, P. M.; Dhamini, S.; Maaza, M.; Liu, C.-P.; Rashad, M. M.; Rayan, D. A.; Mwakikunga, B. W. Strain and Grain Size of TiO2 Nanoparticles from TEM, Raman Spectroscopy and XRD: The Revisiting of the Williamson-Hall Plot Method. Results Phys. 2018, 9, 628–635.
(17) Soyalk, M.; Acar, D.; Yilmaz, E.; El-Khodary, S. A.; Morsy, M.; Ibrahim, M. Magnetic Graphene Oxide as an Efficient Adsorbent for the Separation and Preconcentration of Cu (II), Pb (II), and Cd (II) from Environmental Samples. J. AOAC Int. 2017, 100, 1544–1550.
(18) Castrejón-Sánchez, V. H.; Camps, E.; Camacho-López, M. Quantification of Phase Content in TiO2 Thin Films by Raman Spectroscopy. Superfície Vació 2014, 27, 88–92.
(19) Maślana, K.; Kalebiczuk, R. J.; Zielińska, B.; Mijowska, E. Synthesis and Characterization of Nitrogen-Doped Carbon Nanotubes Derived from g-C3N4. Materials 2020, 13, 1349.
(20) Wang, Y.; Yang, W.; Chen, X.; Wang, J.; Zhu, Y. Photocatalytic Activity Enhancement of Core-Shell Structure g-C3N4@TiO2 via Controlled Ultrathin g-C3N4 Layer. Appl. Catal., B 2018, 220, 337–347.