Research Article

Chemical Review and Letters
journal homepage: www.chemrevlett.com
ISSN (online): 2645-4947 (print) 2676-7279

Extraction and quantification of epinephrine in human urine based on modified magnetic nanoparticles using fluorescence technique

Mariam J Khinteel[1], Bassam F. Alfarhani[1, *],

[1]*Department of Chemistry, College of Science, University of Al-Qadisiyah, Iraq*

ARTICLE INFO

Article history:
Received 06 August 2024
Received in revised form 14 September 2024
Accepted 17 October 2024
Available online 24 October 2024

Keywords:
Doping,
Epinephrine,
Extraction,
Magnetic nanoparticles,
Human urine.

ABSTRACT

A simple, highly sensitive, and selective fluorescent method was developed to detect adrenaline, also known as epinephrine (EP). Adrenaline is a crucial hormone, neurotransmitter, and a substance used in drugs and doping. It utilized Fe3O4-APTS (3-aminopropyltriethoxysilane) nanoparticles to enhance the fluorescence of MNPs. Characterization of the synthesized MNPs was conducted through transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Fe3O4 -APTS demonstrated the highest extraction rates of adrenaline from aqueous solutions, synthetic urine, and human urine, with extraction ratios of 99%,96%, and 87% respectively. The detection limit (LOD) and the quantitative limit (LOQ) of adrenaline were determined using fluorescence spectroscopy at room temperature and were found to be 0.038871 µg/L and 0.11779 µg/L. A linear relationship between fluorescence intensity and adrenaline concentrations was established in the range of 4 to 12 µg mL−1. In addition, the experiment achieved approximately 90% disintegration of the bond between Fe3O4-APTS and adrenaline in an aqueous solution. It also achieved 84% disintegration of adrenaline in human urine. Furthermore, the successful application of this method to synthetic and human urine samples highlights its potential for diagnostic purposes.

1. Introduction

Doping entails the utilization of synthetic and frequently illicit chemicals, such as anabolic steroids, human growth hormones, stimulants, and diuretics, to obtain an unjust edge in athletic competitions. [1]

Developing effective anti-doping measures to detect the use of prohibited substances and methods by athletes requires detailed planning of all experimental, logistical, and administrative activities. This planning is essential for setting up a "laboratory system" capable of detecting unauthorized substances and methods, with response times as short as 24 hours from sample reception, especially for major international sports events.

The analysis of the chemicals above presents significant technological and financial difficulties and is only carried out by around 35 laboratories globally accredited by the World Anti-Doping Agency (WADA). Nevertheless, because of the constraints of laboratory equipment and the adeptness of doping athletes in evading detection, these substances may be the most frequently misused. Adrenaline, categorized as a stimulant by WADA, is one of these drugs. [27] Adrenaline, also referred to as Epinephrine [(R)-4-(1-hydroxy-2-(methylamino) ethyl) benzene-1,2-diol], is a hormone that is produced by the adrenal gland. It has been utilized as a therapeutic remedy for treating hypersensitivity caused by some drugs, particularly penicillin. Furthermore, it functions as a dilator of the pupil and aids in the reduction of intraocular pressure. [2,3]

Although it holds significant medicinal value, the improper utilization of this substance as a stimulant might result in severe health consequences. Consequently, the World Anti-Doping Agency (WADA)

∗ Corresponding author; e-mail: bassam.alfarhani@qu.edu.iq
https://doi.org/10.22034/crl.2024.471918.1394

has prohibited its usage in sports contests. Various analytical techniques are employed to determine adrenaline, such as chromatographic methods [4], voltammetry [5], capillary electrophoresis [6], flow injection [7], ion-selective electrode [8], spectrophotometric [9,10], and spectrofluorometric [11] approaches. Fluorescence measurement methods are widely recognized for their exceptional sensitivity.

A highly sensitive approach is required to detect molecules such as adrenaline in urine due to the extremely low concentration levels (sub-ppb) of these substances. Several analytical techniques have been devised to ascertain the presence of Adrenaline in urine samples. [16,17].

Currently, the ongoing trend in separation sciences involves the utilization of nanoparticles [12,28]. Magnetic nanoparticles (MNPs) are gaining significant interest as novel sorbents in solid-phase extraction techniques due to their superior advantages compared to conventional sorbents utilized in this process. [14,18] The great advantages of these sorbents over others are fast and effective extraction, high enrichment factor, high surface area, adjustable surface, facile isolation from matrix, biocompatibility, and often, reusability. [15,19]

Numerous studies have been dedicated to the advancement of chemical sensors [29,30], with nanomaterials garnering significant attention in recent years due to their exceptional physical and chemical properties stemming from their small size and large surface area. Magnetic nanoparticles serve a variety of purposes, including magnetic drug targets, materials for magnetic resonance imaging in clinical diagnosis, recording material, and catalysts, as well as environmental applications. Iron oxide nanoparticles, with diameters ranging from 1 to 100 nanometers, exist in two primary forms: magnetite (Fe3O4) and its oxidized form, maghemite (γ-Fe2O3). They have attracted widespread interest due to their superparamagnetic properties and potential uses across multiple fields. Iron oxide nanoparticles play a pivotal role in various domains of chemistry, physics, and materials science, owing to their unique attributes such as large surface area, high surface energy, low toxicity, good compatibility, superparamagnetic behavior, and high electron absorption and transfer capabilities. Recently, researchers have turned their attention to the development of nanostructured adsorbents for creating metal oxide nanostructures, which offer advantages such as large surface area and are well-suited for extracting or removing target molecules [31,33,34]. Additionally, there is increasing interest in applying magnetic nanoparticles in the food industry [28,32].

Currently, detecting Adrenaline in urine samples remains difficult due to the intricate nature of biological materials and the low levels of analytes. The classical pretreatment approaches used in the study of Adrenaline include liquid-liquid extraction (LLE) [20,29,30], separation using ion exchange resin [21], adsorption using activated alumina [26], and separation using a packing column [22] In recent times, magnetic adsorbents have gained significant popularity in several industries due to their quick and simple ability to separate and enhance procedures [23,24].

In this study, magnetic nanoparticles (MNPs) were coated with 3-aminopropyltriethoxysilane (APTS). The coated sorbent was used for the selective micro solid-phase extraction (MSPE) of epinephrine from urine samples. The selectivity of the analytes on the sorbent was compared to that of uncoated Fe_3O_4 MNPs. A fluorescence device was used to quantify the analytes in the extracted samples.

The study explores using APTS to modify the surface of Fe_3O_4 NPs directly. APTS offers the benefits of biocompatibility and a high concentration of surface functional group -NH_2, which allows for additional surface modification of these nanoparticles for biological applications. Figure 1 depicts the extraction process using modified nanoparticles. The study highlights the achievements in versatile surface modification techniques for these magnetic nanoparticles, creating new possibilities for detection and preconcentration applications.

The combination of solid support and fluorescence spectroscopy provides high sensitivity and selectivity. This allows for the simultaneous separation, preconcentration, and detection of the analyte. This method meets the requirements for precision, linearity, sensitivity, and specificity needed to quantify adrenaline in doped urine samples and urine from individuals undergoing medical treatment with adrenaline. Additionally, this cost-effective procedure eliminates the use of toxic organic solvents typically used in traditional extraction and elution methods, making it particularly valuable for monitoring adrenaline levels in small urine samples.

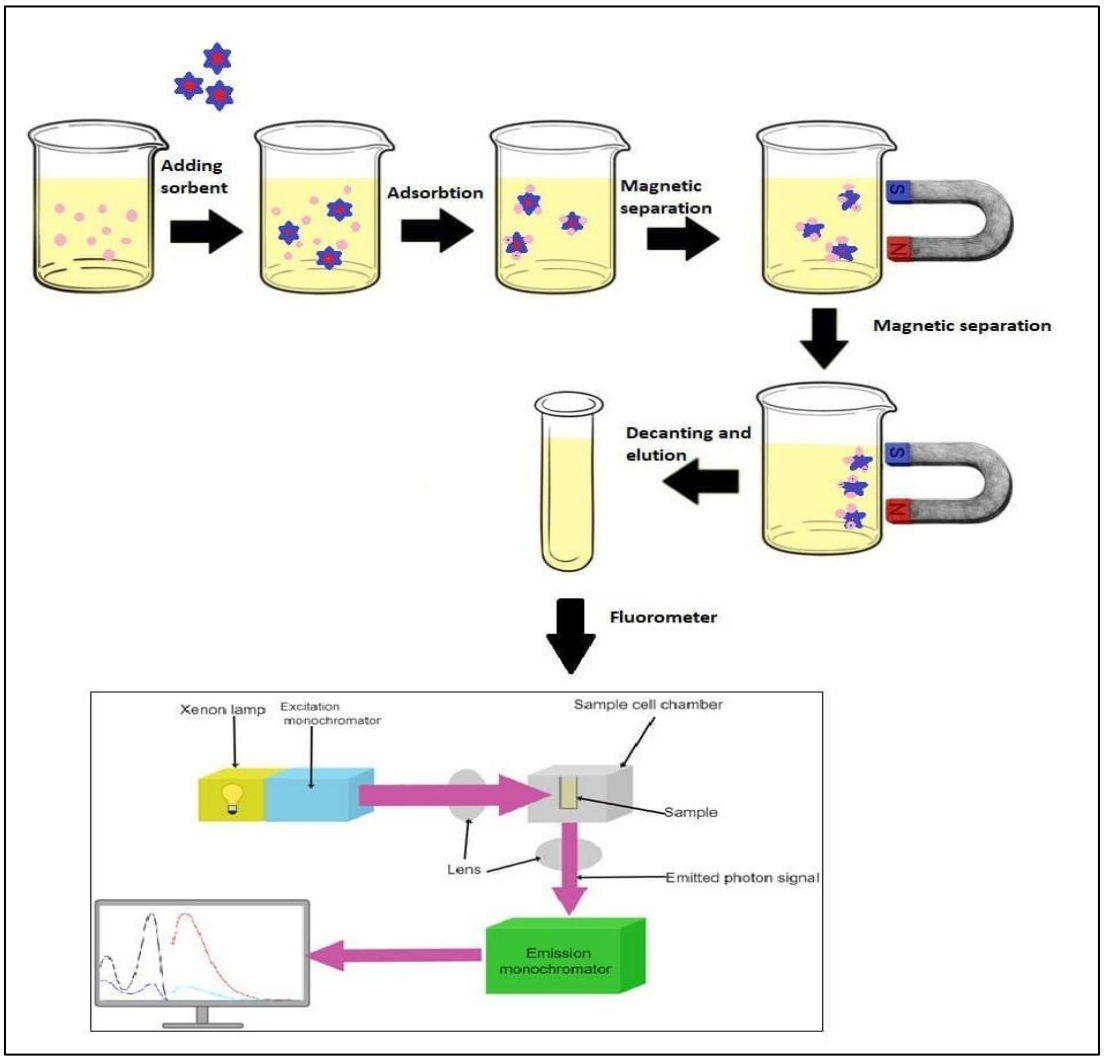

Fig. 1. Schematic diagram for extraction process.

2. Methodology

2.1 Chemical

Various chemicals were utilized in the experiment, including ethanol (Sigma-Aldrich, 99.9%), sodium hydroxide (Sigma-Aldrich, 98%), 3-aminopropyltriethoxysilane (Sigma-Aldrich, 99%), potassium chloride (Sigma-Aldrich, 99-100.5%), adrenaline (Alpha Chemka, 97%), hydrochloric acid (BDH, 35-38%), magnesium chloride (BDH, 99.0-102.0%), and dimethyl sulfoxide (DMSO) (BDH, 99.9%), ammonia (Sigma-Aldrich, 99.95), Iron(II) sulfate (Sigma-Aldrich, 99.99), Iron(III) chloride (Sigma-Aldrich, 99.99). All chemicals are of analytical purity and can be utilized without requiring additional purification. Water with a resistance of 18.2 MΩ was purified using the SUPER WATER-II water purification system.

2.2. Instrumentation

At room temperature, fluorescence measurements were conducted using a fluorescence spectroscopy RF-5301PC manufactured by Shimadzu, Japan. The aqueous solutions of adrenaline were placed in a quartz cuvette, and all windows were polished using a 3 nm slit-width for excitation and emission monochromators. Fourier transform infrared (FTIR) spectra were obtained using an FT-IR spectrophotometer (TENSOR 27, Bruker), and X-ray powder diffraction (XRD) patterns were captured using Aeris-Benchtop X-ray Diffractometers by Malvern Panalytical.

2.3 Synthesis of APTS-coated magnetite NPs

Magnetite nanoparticles were synthesized using a modified method developed by Molday [13]. To begin, a solution containing a mixture of $FeCl_3$ (0.01 M) and $FeSO_4$ (0.006 M) at pH 1.7 was mixed under N_2 flow.

Then, a 1.5 M ammonia aqueous solution was added to the mixture with vigorous stirring until the pH of the solution reached 9. The resulting magnetite was promptly washed five times with water and two times with ethanol using magnetic separation. The substance is subsequently dried into powder at room temperature under a vacuum.

The Fe_3O_4-APTS core was prepared using a 25 ml magnetite colloid ethanol solution and was diluted to 150 ml using ethanol and 1 ml H_2O. The solution was then treated with ultrasonic waves for 30 minutes. Following this, 35 µl of 3-aminopropyltriethoxysilane ($NH_2(CH_2)_3Si(OC_2H_5)_3$) was added to the solution and stirred rapidly for 7 hours. Afterward, the solution was washed with ethanol five times and dried into powder at room temperature under vacuum.

2.4 Preparation of a stock solution of adrenaline

10 mg of adrenaline was dissolved in 100 ml of ultra-pure water to create a stock solution. The stock solution was then stored in a dark place at 4°C. Before each measurement, the stock solution was examined for photolysis potential using fluorescence spectroscopy. All stock solutions were used within two weeks. Standard solutions were prepared daily as needed, using serial dilution by transferring specific volumes of the stock solution.

2.5 Extraction experiment

In the experiment, the extraction process commenced by preparing a 10 ml adrenaline solution. Subsequently, 50 mg of previously prepared Fe3O4-APTS was added, and the mixture was shaken in a shaking device (at 300 rpm) for 20 minutes at a temperature of 25 °C. Following this, the nanomaterial was separated from the solution using a neodymium magnet, effectively removing it from the solution. The solution obtained after the extraction process was collected, and its intensity was measured both before and after the extraction process using a fluorescence device. It is important to note that this entire process was carried out for adrenaline in both aqueous solution and artificial and human urine.

Post-extraction, experiments were conducted to test the potential for reusing the nanoparticles. This involved testing various solvents, and it was found that dimethyl sulfoxide (DMSO) is the most suitable solvent for this purpose. The elute process for the adrenaline involved adding 3 ml of DMSO to the nanomaterial previously used in the extraction process, followed by collection using a magnet**.**

3. Results and Discussion

3.1. Morphological analysis and characterization

Before application, the synthesized modified magnetic nanoparticles were subjected to characterization to verify their structural and optical features. X-ray diffraction technology is invaluable for determining materials' chemical composition and crystalline properties.

Using this technique, prepared nanoparticles were analyzed to gain insights into their crystal size, type of chemical bonding, lattice tension, lattice deviations, and arrangement of atoms. Scherrer's equation, which employs the constant K = 0.943, x-ray wavelength λ = 0.15405 nm, and the diffraction peaks half-height width β, was instrumental in estimating the average size of nanoparticles in the samples.

Figure 2 illustrates the XRD patterns of the Fe_3O_4-APTS nanocomposites, revealing a variety of peaks, with prominent peaks at 2Ɵ values of 30.5°,35.94°, 43.54°, 53,98°,57.56°,63.02°, and 74.64°. Notably, the 63.02° peak possibly corresponds to the characteristic peak of APTS, suggesting interactions between the Fe_3O_4 and APTS NPs. Our analysis using the Scherrer formula based on the full-width half maximum (FWHM) of the strongest reflection yielded an average crystallite size of 27.41 nm. [37]

Figure 3(d) The TEM image displays magnetite nanoparticles coated with APTS, revealing that the prepared particles have a dark spherical to semi-spherical nucleus with a brighter APTS matrix on the outside [38]. The majority of the particles are quasi-spherical and have an average diameter of 7.5 nm. The distribution of particle diameters is illustrated in Fig.3(e). Figure 3(b, c, f) shows the FE-SEM image of the Fe_3O_4-APTS and Fe_3O_4 NPs along with the corresponding EDX spectrum. The SEM image (Fig. 3a) depicts an average particle size of approximately 33 nm.

The microanalysis reveals the presence of Si, indicating the APTS functionalization. The image displays nanoparticles arranged in large agglomerates due to inter-particle interactions. The SEM-EDS (Fig. 3f) analysis showed a nearly complete APTS coating on the particle surface, further supported by a simple calculation. The EDX spectrum confirmed the presence of O, Fe, C, N, and Si as the principal elements. The calculated amounts of O, Fe, C, N, and Si elements are 45.08, 11.53, 32.56, 9.71, and 1.12%, respectively. These results further confirm the presence of the Fe_3O_4-APTS core-shell. [36]

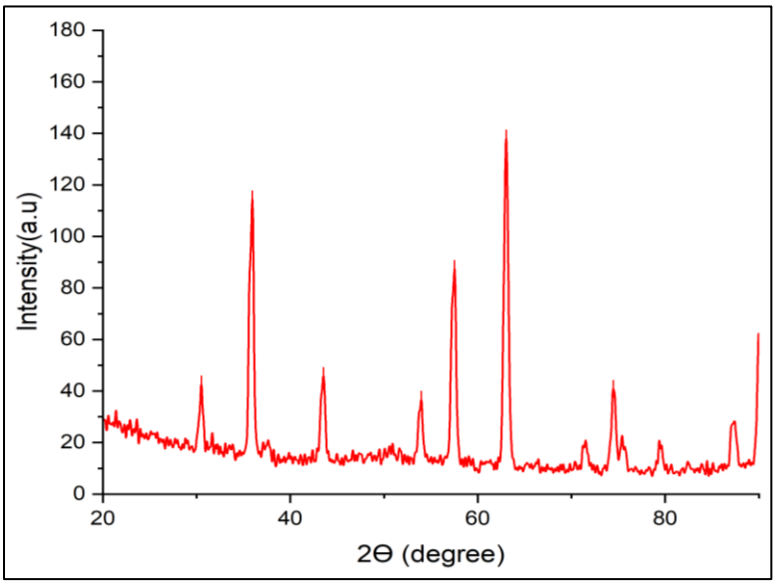

Fig. 2. X-ray diffraction spectrum of (Fe_3O_4-APTS) nanostructure.

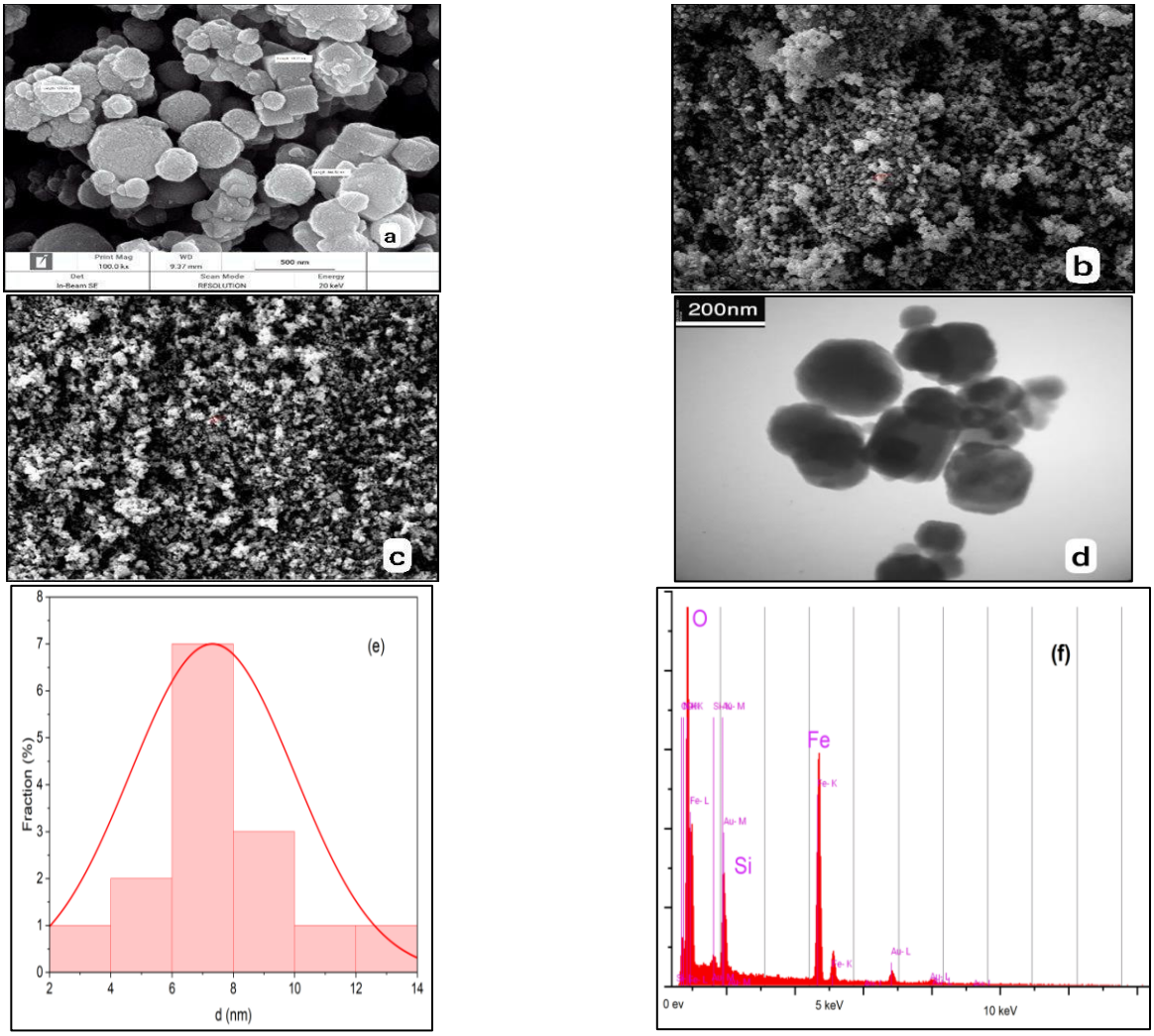

Fig. 3. (a, b) SEM of Fe_3O_4-APTS(c) SEM image of Fe_3O_4(d) TEM photography of the magnetite nanoparticles coated with APTS (e) Diameter distribution of magnetite nanoparticles coated with APTS(f) the distribution of the elements in the sample.

The ATR-FTIR spectra of Fe_3O_4-APTS are depicted in Figure 4. The fact was confirmed by comparing the FT-IR spectra of the coated and uncoated Fe_3O_4 nanoparticles, as shown in Fig. 4, it is evident that the coated Fe_3O_4 nanoparticles exhibit absorption bands at 2877.80 and 2918.74 cm^{-1}, indicating the stretching vibration of the C-H bond. Additionally, there are bands at 1195.32 cm^{-1} due to the stretching vibration of the C-N bond, 1122.93 cm^{-1} due to the stretching vibration of the Si-O bond, and 785.88 cm^{-1} due to the bending vibration of the $-NH_2$ group, all of which confirm the presence of APTS. Furthermore, absorption bands near 3414 and 1626 cm^{-1} in Fig. 4(a) and (b) are attributed to the vibration of residual H_2O in the samples, and the band near 3339.59 cm^{-1} in Fig. 4(b) also indicates the presence of $-NH_2$. It is noted that the characteristic absorption bands of the Fe-O bond of bulk Fe_3O_4 were previously reported to be at 591 and 410 cm^{-1}. However, in Fig. 4(a), these two bands shift to higher wavenumbers of about 600 and 450 cm^{-1} respectively, and the band near 600 cm^{-1} is split into two peaks at 688 and 577 cm^{-1}. [35,39]

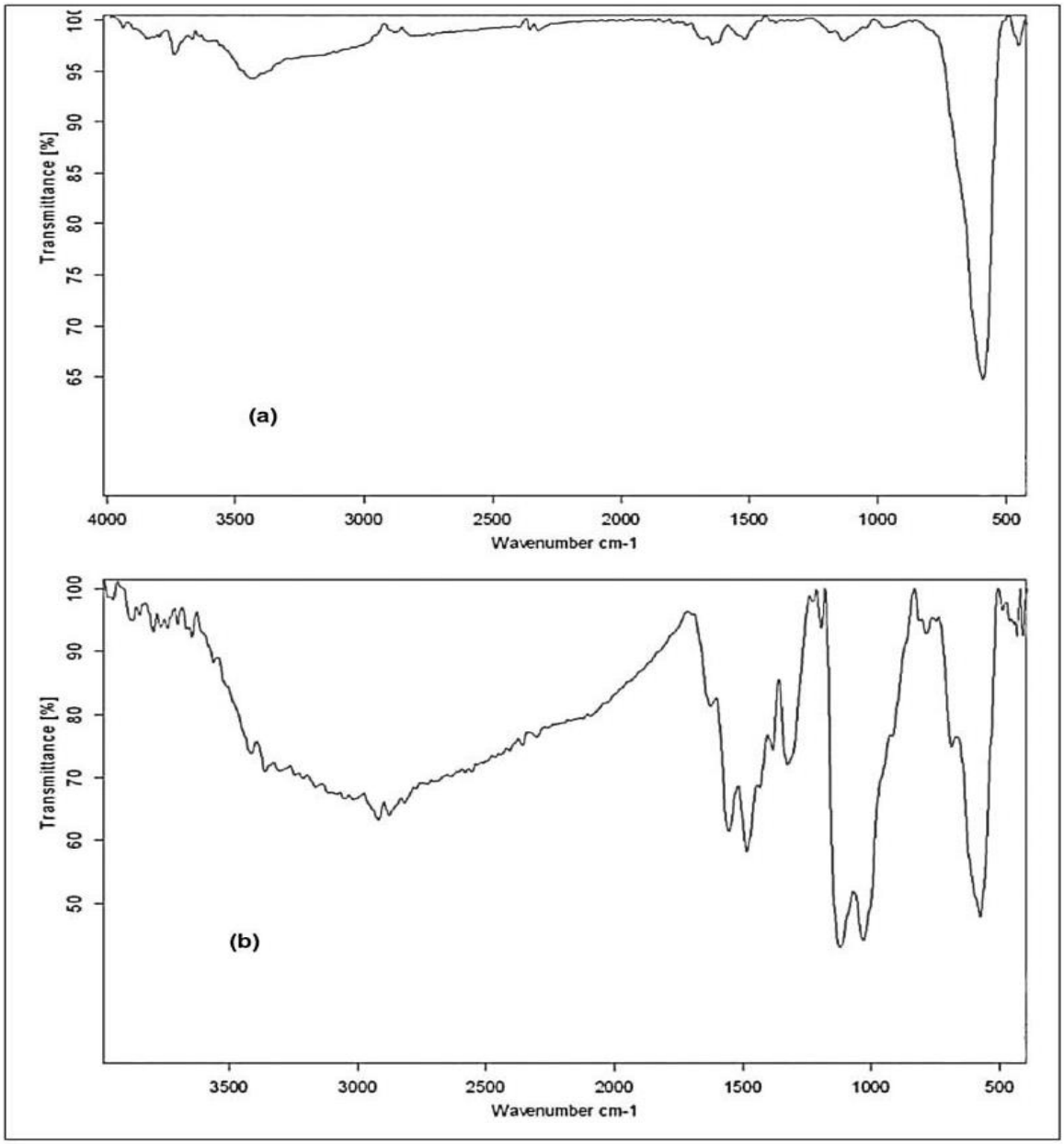

Fig. 4. FT-IR spectra of the uncoated (a) and coated (b) Fe_3O_4-APTS magnetite nanoparticles.

3.2- Fluorescence Spectroscopic Study

The excitation and emission spectra were measured for adrenaline compounds dissolved in a certain amount of distilled water. The excitation spectrum was used to determine the excitation and emission wavelengths. The measurements were taken at room temperature, and standard materials were used to monitor the device's performance and determine the radiation intensity at the highest spectrum. The results obtained were as follows:

The concentration of 10 µg. mL^{-1} was obtained by diluting a precise volume of the aqueous solution. The absorption and emission spectra of the compound were then measured, as illustrated in Figure 5 (a).

Synthetic urine has been proven to exhibit a significant matrix profile when analyzed using various analytical methods. Fluorescence spectrophotometry limits the matrix effect by utilizing the selectivity of fluorescence for certain molecules that impact the analysis. Monitoring the effect of the matrix by detecting adrenaline in artificial urine before analyzing real human urine was first tested. To achieve this goal, the selective sensing of adrenaline was carried out using excitation/emission wavelengths of 279/317 nm and a pH of 7. The excitation wavelength of 279 nm was chosen as it resulted in the strongest fluorescence emission. Thus, this specific wavelength was chosen to observe and measure the fluorescence intensity. The process of fluorescence is The Ex-Em spectrum of the compound adrenaline in synthetic urine was acquired, as shown in Figure 5(b). Subsequently,

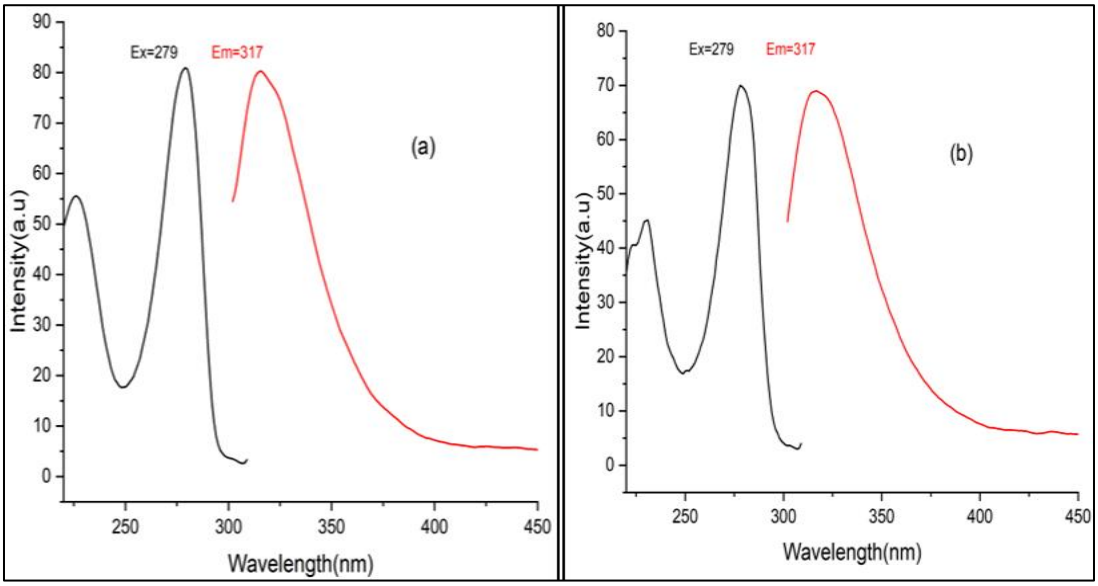

Fig. 5. Ex-Em spectrum of adrenaline(a) in aqueous solution (b) in synthetic urine.

To elucidate the potential detection of adrenaline, an endeavor was made to establish a correlation between the concentration of adrenaline and the luminescence intensity by employing the straight-line equation. Fig.6(a) depicts the fluorescence intensity plotted against the concentration of adrenaline. The linear relationship over the concentration range of adrenaline from 0.5 to 5 µg. mL^{-1} is notable. The regression value (R^2) was found to be 0.9987 which reflects a linear relation between the adrenaline concentration and fluorescence intensity. The formulas for determining the limit of detection (LOD, equation 1) and limit of quantification (LOQ, equation 2) are below. Also, the intensity of adrenaline fluorescence in synthetic urine was measured. A linear relationship was observed over the concentration range of adrenaline in synthetic urine from 4 to 12 µg. mL^{-1}. The regression value (R^2) was found to be 0.9947, indicating a strong linear relationship between the concentration of adrenaline in synthetic urine and fluorescence intensity A valid linear correlation was observed, as shown in Fig.6(b), demonstrating a strong linear association.

$$LOD = 3.3 * \sigma / S$$

$$LOQ = 10 * \sigma / S$$

where σ is the standard deviation of the intercept and S is the slope of the linear regression plot.

The LOD and LOQ were calculated to be 0.038871µg. L^{-1} and 0.1177µg.L^{-1}, in an aqueous solution respectively. The detection limit of adrenaline found in this study (table 1) was much lower than the reported studies (table 2) in the obtained LOD values in (Table1). The fluorescence spectrum in Figures 5a and 5b indicates that there is no effect of the synthetic urine matrix on the fluorescence intensity of the adrenaline.

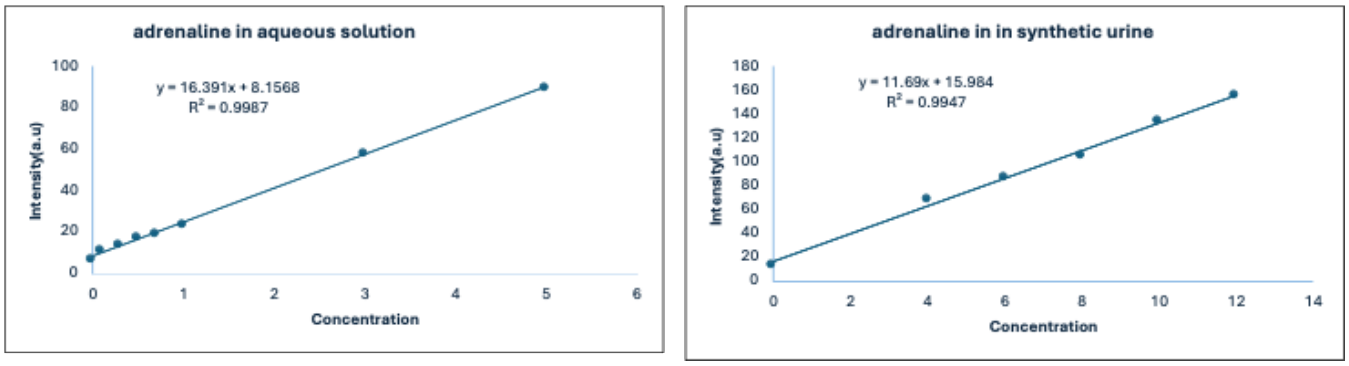

Fig. 6. (a) Calibration curve of adrenaline in aqueous solution(b) Calibration curve of adrenaline in synthetic urine.

Table 1. Determination and Quantification Limit Values for (adrenaline) using Fluorescence Spectrometry

Adrenaline in	LOD	LOQ	R^2
	µg/L	µg/L	
aqueous solution	0.038871±0.03475	0.11779±0.11257	0.9987
Synthetic urine	0.046939±0.02447	0.14224±0.64941	0.9947

Table 2. Comparison of detection limits for adrenaline estimation using various methods, including fluorescence.

methods	LOD µg/L	ref
fluorescent method	0.038871	This work
Spectrophotometric	0.5496	5
UHPLC-MS/MS	1.5	20
Electrochemical sensor	0.5239	22
HPLC-FLD	0.11	25

3.3. Using of Fe_3O_4 – APTS Core/Shell NPs for extraction of adrenaline

The extraction process was refined through a series of experiments to find the perfect combination of nanoparticle quantity and extraction time for optimal results. We begin by taking 0.05g of a specific weight from the nanocomposite (Fe_3O_4.APTS). Next, we add 10ml of 10 µg. mL^{-1} adrenaline solution and place the prepared solution in the shaker (at 300 rpm) for 20 minutes. After that, we use a Neodymium magnet to separate the solution. The nanoparticles will be attracted by the magnet to extract adrenaline. The solution separated from the nanoparticles will then be removed, and the measurement process will be performed.

This process was carried out on an aqueous solution, synthetic urine, and human urine. The intensity of absorption and emission is then measured using a fluorescent device both before and after the extraction. The Fe_3O_4-APTS core/shell was utilized to examine the interference with adrenaline in both aqueous solution, synthetic and human urine. The extraction process is illustrated in Figures 7(a, b, c), with extraction ratios of 99% in the aqueous solution, 96% in synthetic, and 87% in human urine.

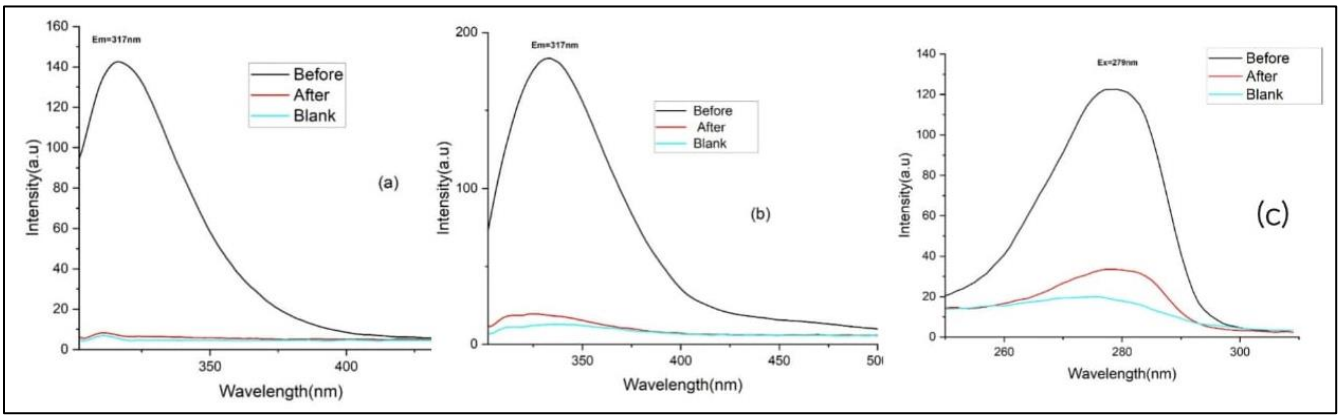

Fig. 7. (a) Em spectra of adrenaline in aqueous solution before and after extraction. (b) Em spectra of adrenaline in synthetic urine before and after extraction. (c) Ex spectra of adrenaline in human urine before and after extraction.

3.4 Determination of Optimum Conditions for Extraction

3.4.1 Shaking Time

It is crucial to control the shaking time during the extraction process because it determines how well the material to be extracted comes into contact with the adsorbent. Shaking enables the potential for electrostatic bonding. The extraction percentage of the adrenaline mentioned below was measured by keeping the extraction conditions constant while changing the time (5, 10, 15, and 20 minutes). It was found that the optimal time for extracting adrenaline is 20 minutes. This was confirmed by Figure 8, which illustrates the relationship between the shaking time and extraction percentage for adrenaline which shows the need for a longer shaking time (20 min) to ensure sufficient time for contact between the compound and nanoparticle.

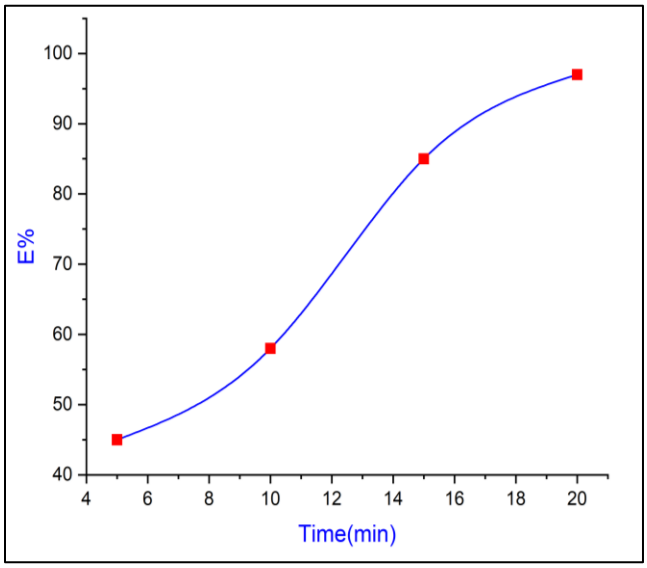

Fig. 8. Extraction rate at different times of shaking.

3.4.2 The maximum quantity of adrenaline that can be extracted.

The highest amount of adrenaline that could be extracted was determined by doing the extraction process with a fixed weight of nanoparticles (0.05 g). Various quantities of adrenaline (10, 15, 20, 25, and 30 µg. mL^{-1}) were employed, and the findings revealed that the optimal concentration for extraction was 10 µg. mL^{-1}. Figure 9 confirms the relationship between concentration and extraction rates for adrenaline.

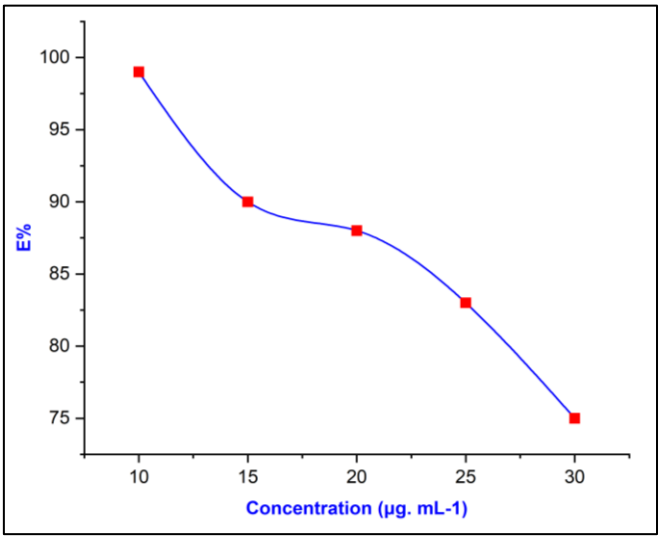

Fig. 9. The relationship between increased concentration and adrenaline extraction rates.

The results show that the extraction percentage decreases as the concentration increases. The relationship is represented by the equation: $n=N/N_A$

Where N stands for the number of molecules, n stands for the number of moles, and N_A is Avogadro's number, which is $6.022*10^{23}$ mol^{-1}. This happens because an increase in concentration results in a higher

number of adrenaline molecules that are not electrostatically linked to the nanoparticles in the solution. This change is observed in fluorescence spectroscopy as a decrease in the extraction percentage.

3.4.3 The Importance of APTS on the Surface of Synthesized Nanoparticles

The importance of having APTS on the surface of the previously prepared nanoparticle was studied. The extraction percentages of the nanoparticle with and without APTS on the surface to understand its necessity for achieving the highest extraction percentage. The extraction process using Nano Fe_3O_4 without any coating on the surface was performed, and it was found that the extraction percentage was 66%. Figure 10 illustrates the extraction process using Fe_3O_4. The obtained extraction percentage value reveals the importance of modifying the bare nanoparticle; APTS will provide the required sites that allow stronger electrostatic interaction with adrenaline.

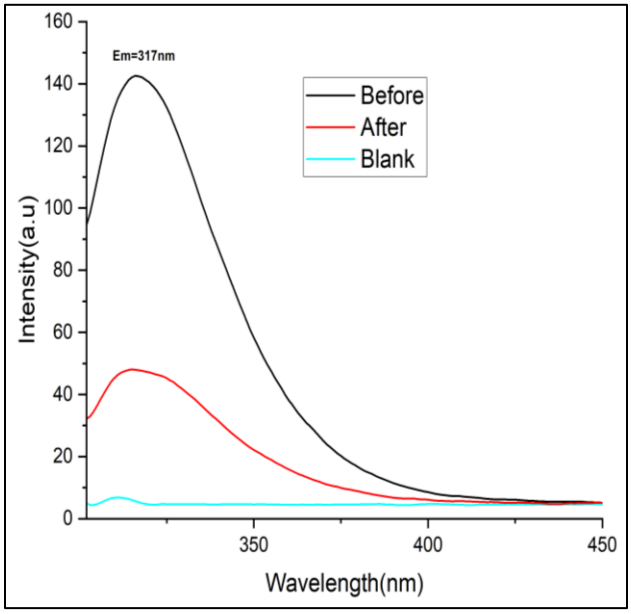

Fig. 10. Emission spectra of adrenaline before and after extraction with Fe_3O_4.

3.5 Adrenaline extraction and preconcentration from urine samples.

To investigate the impact of the matrix on the extraction process of adrenaline, a study was conducted by adding specific essential ions (Mg^{+2}, Na^{+1}, K^{+1}, and Cl^{-}) into the adrenaline-aqueous solution. The results indicated that the presence of the chosen ions did not affect the extraction efficiency of the adrenaline solution. Figure 11 suggests that most of the compounds examined do not cause interference.

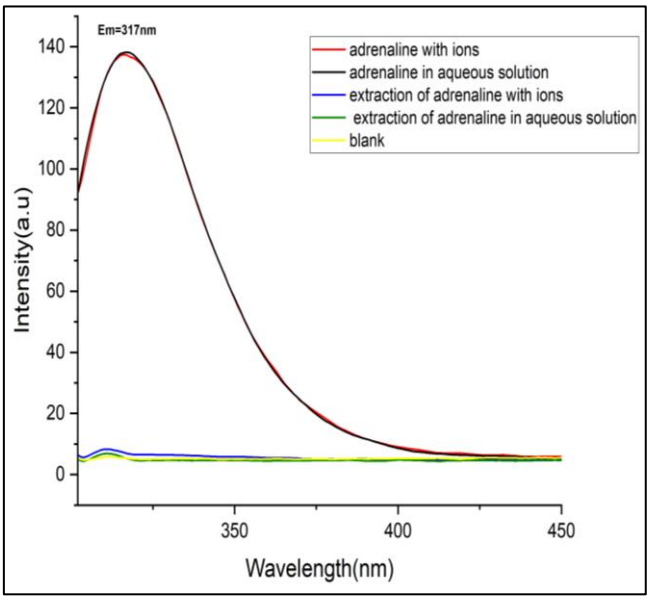

Fig. 11. The effect of ions on the extraction of adrenaline.

Subsequently, synthetic urine was employed as a more complex medium for conducting the extraction process. This involved spiking the synthetic urine solution with 10 µg. mL^{-1} and executing the extraction according to the modified approach. The analytical results demonstrate that the modified nanoparticles are capable of extraction, with a recovery value of around 95%. Figure 12(a) displays the measured concentration of adrenaline obtained by the use of synthetic urine. To test the practicality of using nanoparticles for selective extraction of a compound from human urine, a healthy volunteer's urine sample was spiked with 10 µg. mL^{-1}. The extraction was performed using the optimized procedure, resulting in a recovery value of approximately 95%. This high recovery value demonstrates the nanoparticles' suitability for extracting the compound even from real urine samples. Figure 12(b) displays the quantified concentration of adrenaline extracted from human urine. The relative standard deviations (RSDs %) for five replicate extractions were 0.77% and 0.69% for synthetic and actual urine, respectively. This indicates that the procedure has achieved sufficient precision and accuracy.

4. The possibility of reuse of the prepared nanoparticles after the removal process.

The process of reusing nanoparticles was completed by incorporating an appropriate organic solvent. Various solvents were tested, including acetone, n-hexane, methanol, and Dimethyl sulfoxide (DMSO). Among

these, DMSO emerged as the most effective solvent. It effectively severs the bond between the nanomaterial and

the previously extracted adrenaline, thereby allowing for the release of the nanomaterial for repeated use in extraction processes. This method involves adding 3 ml of DMSO to the compound, followed by placing the nanoparticles in a shaking device (at 300 rpm) for precisely 20 minutes, a duration determined through experimentation. Figure 13(c) illustrates the optimal time for the shaking process.

Subsequently, the mixture was subjected to centrifugation for another 20 minutes, and the emission intensity was measured using a fluorescence device post-separation. The analysis revealed that DMSO was capable of disintegrating approximately 90% of the connection between Fe_3O_4-APTS and adrenaline in an aqueous solution, and 84% adrenaline in human urine as illustrated in Figures 13(a)(b). This outcome demonstrates the potential for multiple applications of the nanocomposite in the extraction process.

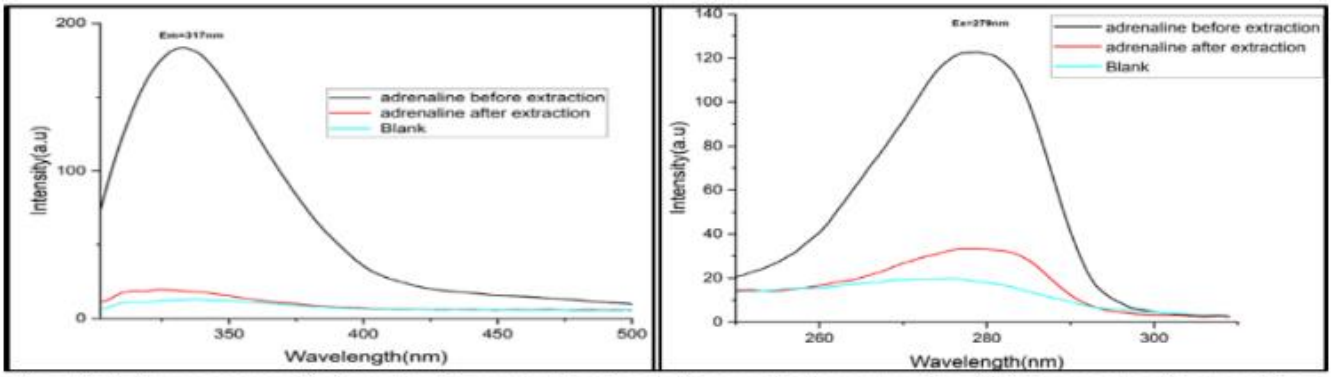

Fig. 12: (a)Em spectra of adrenaline in synthetic urine before and after extraction. (b) Ex spectra of adrenaline in human urine before and after extraction.

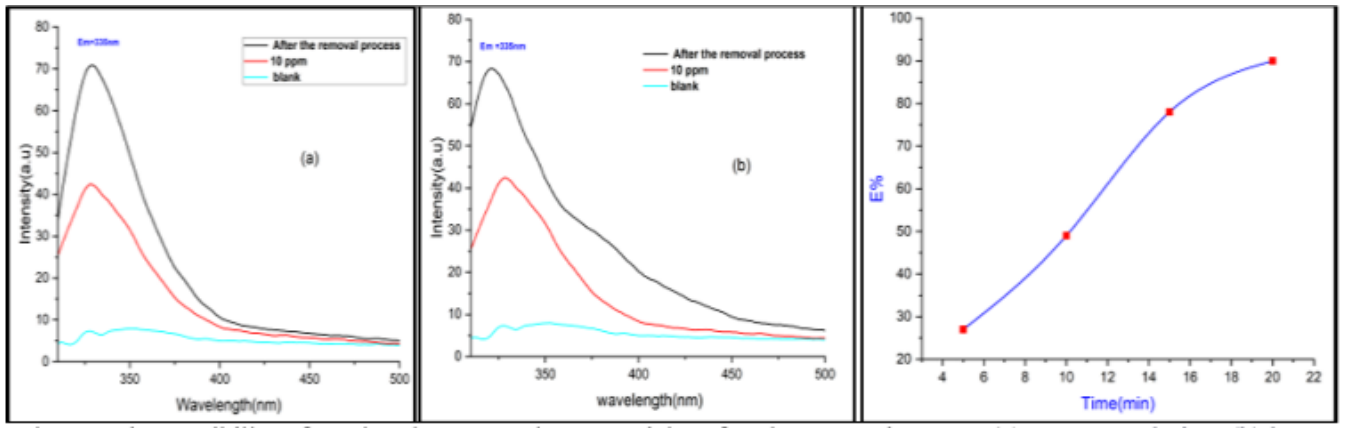

Fig. 13: The possibility of reusing the prepared nanoparticles after the removal process (a) aqueous solution. (b) human urine. (c) the optimal time for the shaking process

5. Conclusion

Fluorimetry is increasingly utilized in pharmaceutical analysis for two main reasons. This method is widely preferred for detecting small amounts of therapeutic and abusive drugs in biological materials, often as low as a few Nanograms per milliliter. Its added cost-effectiveness, simplicity, and flexibility have made it the recommended choice for assessing the purity of many drugs in pharmaceutical, forensic, and biomedical samples. A new, efficient method for sequentially determining adrenaline in urine using fluorimetry is proposed here. Solid support combined with fluorimetry

enhances sensitivity and selectivity, enabling the simultaneous separation, preconcentration, and detection of the analyte. Overall, this method meets the precision, linearity, sensitivity, and specificity requirements for quantifying adrenaline in doped urine samples and urine of individuals undergoing medical treatment with adrenaline. Additionally, this procedure is cost-effective and eliminates the need for toxic organic solvents

typically used in more traditional extraction and elution procedures. These factors make this method valuable for

monitoring adrenaline levels in small urine samples. This research used an advanced technique to extract adrenaline from water-based solutions, synthetic urine, and human urine. They prepared and studied APTS-coated magnetite nanoparticles with an average diameter of 7.5nm using TEM, XRD, FT-IR, and SEM-EDS techniques. The results showed that APTS and magnetic Fe3O4 played a crucial role in achieving high extraction efficiencies: 99% in water-based solutions, 96% in synthetic urine, and 87% in human urine. The study also demonstrated the potential for various applications of these nanomaterials, as they could be recovered using a suitable solvent such as DMSO for separation from the nanocomposite.

References

[1] World Anti-Doping Agency (WADA)

[2] S. S. Sharath and S. B. E. Kumara, Int. J. Electrochem. Sci., (2014).

[3] S. Masoomeh, J. L. Manzoori, and J. Abolghasem, Iranian Journal of Pharmaceutical Sciences, (2007).

[4] Bergh, M.S.-S., et al., Determination of adrenaline, noradrenaline, and corticosterone in rodent blood by ion-pair reversed-phase UHPLC–MS/MS. Journal of Chromatography B, (2018).

[5] FURLETOV, A. A., et al. Spectrophotometric determination of epinephrine using new analytical systems based on label-free silver triangular nanoplates. In: IOP Conference Series: Materials Science and Engineering. (2020).

[6] Weng, Y., et al, Separation and determination of dopamine and epinephrine in serum by capillary electrophoresis with inkjet introduction system. Chromatography, (2013).

[7] Michałowski, J and P. Hałaburda, Flow-injection chemiluminescence determination of epinephrine in pharmaceutical preparations using raw apple juice as enzyme source. Talanta, (2001).

[8] Zaidi, S.A., Utilization of an environmentally-friendly monomer for an efficient and sustainable adrenaline imprinted electrochemical sensor using graphene. Electrochimica Acta, (2018).

[9] Ekhtesasi, A. and M.R. Shishehbore, Sensitive determination of epinephrine using kinetic spectrophotometric method. Orient J Chem, (2016).

[10] Al-Ameri, S.A., Spectrophotometric determination of adrenaline in pharmaceutical preparations. Arabian Journal of Chemistry, (2016).

[11] Underberg, W. and G. Wiese, Spectrofluorometric determination of adrenaline and related compounds in pharmaceutical preparations. Pharmaceutisch Weekblad, (1980).

[12] Alfarhani, Bassam & Hammza, Rana. Fluorescence-based removal of higher molecular weight polycyclic aromatic hydrocarbons from aqueous solution using tannic acid modified Fe3O4 nanoparticle. Chemical Papers. (2022).

[13] R.S. Molday, US Patent 4452773, (1984).

[14] Alfarhani, Bassam & Al-Mousawi, Riyam & Aziz, Raghad & Shingar, Fadha Modified nanoparticles method for fluorescent dye removal from aqueous samples. IOP Conference Series: Earth and Environmental Science. (2021).

[15] . Alfarhani, Bassam & Al-Mousawi, Riyam & Alzaidy, Ali & Aziz, Raghad & Shingar, Fadha Magnetic nanoparticles for hydroxy-PAHs removal from synthetic urine. IOP Conference Series: Earth and Environmental Science. (2021).

[16] Weil-Malherbe H, Bigelow LB. The fluorometric estimation of epinephrine and norepinephrine: an improved modification of the trihydroxyindole method. Anal Biochem. (1968).

[17] Åse Marie Hansen, Jesper Kristiansen, Jeanet Løgsted Nielsen, Kirsten Byrialsen, Jytte Molin Christensen, Validation of a high-performance liquid chromatography analysis for the determination of noradrenaline and adrenaline in human urine with an on-line sample purification, Talanta. (1999)

[18] Wu, A., Zhao, X., Wang, J., Tang, Z., Zhao, T., Niu, L., … Wu, F. Application of solid-phase extraction based on magnetic nanoparticle adsorbents for the analysis of selected persistent organic pollutants in environmental water: A review of recent advances. Critical Reviews in Environmental Science and Technology. (2020).

[19] Öztürk Er, E., Dalgıç Bozyiğit, G., Büyükpınar, Ç., & Bakırdere, S. Magnetic Nanoparticles Based Solid Phase Extraction Methods for the Determination of Trace Elements. Critical Reviews in Analytical Chemistry. (2020).

[20] Zorina, M.; Dotsenko, V.V.; Nesterenko, P.N.; Temerdashev, A.; Dmitrieva, E.; Feng, Y.-Q.; Atapattu, S.N. Phthalylglycyl Chloride as a Derivatization Agent for UHPLC-MS/MS Determination of Adrenaline, Dopamine and Octopamine in Urine. Molecules (2023)

[21] B H Westerink, F J Bosker, J F O'Hanlon, Use of alumina, sephadex G10, and ion-exchange columns to purify samples for determination of epinephrine, norepinephrine, dopamine, homovanillic acid, and 5-hydroxyindoleacetic acid in urine., Clinical Chemistry. (1982).

[22] ELUGOKE, Saheed E., et al. Electrochemical sensor for the detection of adrenaline at poly (crystal violet) modified electrode: optimization and voltammetric studies. Heliyon, (2022)

[23] Tetiana Tatarchuk, Liubov Soltys, Wojciech Macyk, Magnetic adsorbents for removal of pharmaceuticals: A review of adsorption properties, Journal of Molecular Liquids. (2023).

[24] Atul Sharma, Divyanshi Mangla, Shehnaz, Saif Ali Chaudhry, Recent advances in magnetic composites as

adsorbents for wastewater remediation, Journal of Environmental Management. (2022).
[25] Xing, Yanyan & Li, Jianshu & Chen, Meiling & Wang, Xiangting & Hou, Xiao Hong. Tannic acid-directed synthesis of magnetic and boronic acid-functionalized metal-organic frameworks for selective extraction and quantification of catecholamines in human urine. Microchimica Acta. (2021).
[26] Silva R, Boldt S, Costa VM, Carmo H, Carvalho M, Carvalho F, Bastos Mde L, Lemos-Amado F, Remião F. Evaluation of GSH adducts of adrenaline in biological samples. Biomed Chromatogr. (2007).
[27] Geeraets, V. Ideology, Doping and the Spirit of Sport. Sport, Ethics, and Philosophy, (2017).
[28] Synthesis of Fe3O4/SiO2/Polypyrrole magnetic nanocomposite polymer powder: Investigation of structural properties and ability to purify edible sea salts. Advanced Powder Technology, 32(4), pp.1233-1246.
[29] Rapid determination of pyridine derivatives by dispersive liquid-liquid microextraction coupled with gas chromatography/gas sensor based on nanostructured conducting polypyrrole. Talanta, 87, pp.249-254.
[30] Nanoporous conducting polypyrrole gas sensor coupled to a gas chromatograph for determination of aromatic hydrocarbons using dispersive liquid-liquid microextraction method. IEEE Sensors Journal, 11(12), pp.3400-3405.
[31] Application of nano-sized poly N-phenyl pyrrole coated polyester fiber to headspace microextraction of some volatile organic compounds and analysis by gas chromatography. Current Analytical Chemistry, 12(5), pp.457-464.
[32] Extraction and determination of volatile organic acid concentration in pomegranate, sour cherry, and red grape juices by PPy-Ag nanocomposite fiber for authentication. Separation Science and Technology, 53(1), pp.117-125.
[33] Synthesis and characterization of magnetic nanocomposites based on Hydrogel-Fe 3O 4 and application to remove organic dye from wastewater. Main Group Chemistry, 16(2), pp.85-94.
[34] Application of nano-sized poly (N-methyl pyrrole-pyrrole) fiber to the headspace solid-phase microextraction of volatile organic compounds from yogurt. Journal of Chemistry Letters, 1(1), pp.39-46.
[35] Yan Wei, Bing Han, Xiaoyang Hu, Yuanhua Lin, Xinzhi Wang, Xuliang Deng, Synthesis of Fe3O4 Nanoparticles and their Magnetic Properties. (2012).
[36] YADAV, Neelam; SINGH, Amit; KAUSHIK, Mahima. Hydrothermal synthesis and characterization of magnetic Fe 3 O 4 and APTS coated Fe 3 O 4 nanoparticles: Physicochemical investigations of interaction with DNA. Journal of Materials Science: Materials in Medicine, (2020).
[37] Mohammad Jafarzadeh, Ebrahim Soleimani, Parastoo Norouzi, Rohana Adnan, Heshmatollah Sepahvand, Preparation of trifluoroacetic acid-immobilized Fe3O4@SiO2–APTES nanocatalyst for synthesis of quinolines, Journal of Fluorine Chemistry, (2015).
[38] Aekkaphon Mokkarat, Senee Kruanetr, Uthai Sakee, One-step continuous flow synthesis of aminopropyl silica-coated magnetite nanoparticles Journal of Saudi Chemical Society, (2022).
[39] Ming Ma, Yu Zhang, Wei Yu, Hao-ying Shen, Hai-Qian Zhang, Ning Gu, Preparation and characterization of magnetite nanoparticles coated by amino silane, Colloids and Surfaces A: Physicochemical and Engineering Aspects, (2003).