

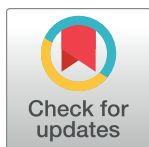
RESEARCH ARTICLE

Biogenic synthesis of ZnO and Al₂O₃ nanoparticles using *Camellia sinensis* and *Origanum vulgare* L. leaves extract for spectroscopic estimation of ofloxacin and ciprofloxacin in commercial formulations

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Abstract

The current study describes the biogenic synthesis of two metal oxides zinc oxide (ZnO), aluminum oxide (Al₂O₃) nanoparticles using *Camellia sinensis*, and *Origanum vulgare* L. leaves extract, respectively. The synthesized metal oxide nanoparticles were investigated using spectroscopic and microscopic techniques to confirm the formation of their nanostructures. Accurate and precise spectrofluorometric probes were proposed for the quantification of Ofloxacin (OFX) and Ciprofloxacin (CPFX) in their bulk and commercial formulations. The extraordinary properties of Zinc oxide and aluminum oxide nanoparticles (ZnONPs and Al₂O₃NPs) enhance the fluorescence intensity in the presence of 0.5 mL and 1.0 mL of sodium dodecyl sulfate (SDS, 1.0% w/v) as organizing agent for the detection of OFX and CPFX, respectively. The optical detection of both drugs at $\lambda_{ex/em}$ range 250–700 nm displayed linearity with a main correlation coefficient >0.999 at 1–300 (OFX-SDS-ZnONPs) and 0.5–100 (OFX-SDS-Al₂O₃NPs) ng mL⁻¹, 10–400 (CPFX-SDS-ZnONPs) and 0.1–50 (CPFX-SDS-Al₂O₃NPs) ng mL⁻¹. The detection and quantification limits were found to be 0.04, 0.03, and 0.02, 0.04 ng mL⁻¹, 0.13, 0.10, and 7.24, 0.09 ng mL⁻¹ for the above-mentioned fluorescence systems, respectively. The suggested spectrofluorometric probes were validated and potentially applied for the estimation of OFX and CPFX in their bulk and commercial formulations.

Introduction

Microbial infections are important human diseases caused by a variety of microorganisms such as viruses, parasites, fungi, and bacteria [1]. These diseases can be transmitted to humans or animals through direct or indirect contact with the source of infection [2]. Unsuitable or unregulated pharmaceutical doses are one of the main causes of death worldwide, particularly among children and young people [3].

project administration, funding acquisition, and conceptualization.

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Antibacterial drugs have recently been utilized to reduce or kill the growth of a variety of Gram-positive and Gram-negative infections [4]. The broad-spectrum antibacterial fluoroquinolones pharmaceuticals are among these antibiotics, and they are commonly used for a variety of hospital and community-acquired illnesses [5].

The current investigation focused on two fluoroquinolone antibiotics (ofloxacin, OFX, and ciprofloxacin, CPFX). They are used to treat infections of the respiratory system, community-acquired pneumonia, urinary tract, bone, and soft tissue [6, 7]. Chromatographic approaches [8, 9], spectrophotometric methods [10, 11], chemiluminescence methods [12, 13], and electrochemical methods [14, 15] have all been presented for determining OFX and CPFX in various analytical matrices.

Despite the fact that these previously stated separation techniques have excellent selectivity and sensitivity, they require expensive apparatus, highly skilled analysts, complex preconditions, and lengthy procedures. As a result, they still have some restrictions. Furthermore, electrochemical procedures are rapid to perform; the recorded data is obtained as electrical impulses; yet, these techniques have a history of analytical errors, necessitating environmental protection to reduce toxicity. A fluorescence approach for detecting a specific wavelength has the advantages of simplicity, high sensitivity, non-destruction, and susceptibility to background interferences compared to other analytical techniques [16–18].

With advancements in all domains of technology and industry, new nanoscale materials with distinct chemical and physical properties have been discovered [19, 20]. To improve the sensitivity of the analytical detection of various sensing probes, nanomaterials such as noble metals, quantum dots, nanocomposites, and nanometal oxides have been used as alternatives to standard fluorophores [21–24]. To increase the fluorescence intensity, nanostructures have also been used as sensor substrates. Recent studies have shown that it is possible to boost the fluorescence of affordable and non-toxic nanometal oxides, which holds tremendous promise for biomedical applications [25].

Metal oxides, which have visual properties, good chemical reactivity, outstanding electrical conductivity, and potential catalytic and chemical reactivity, are among the nanomaterials that can be made in an environmentally acceptable manner [26]. Because of their exceptional physicochemical properties, zinc oxide (ZnONPs) and aluminum oxide (Al_2O_3 NPs) nanostructures have recently attracted a lot of attention. Thin films, quantum dots, nanoflowers, and nanorods are all examples of ZnO nanostructures that are widely used as major semiconducting materials for various sensing and biosensing applications [27, 28]. At room temperature, these ZnO nanostructures are optically stable, with a large band gap (3.36 eV) and significant exciton energy (60 meV). Furthermore, ZnO nanostructures absorb UV rays and hence serve as effective signal enhancers for FL-based sensors [29], sensors and biosensors [30–33], food additives [34], electronic systems [35], industrial productions [36], catalysis [37], and medication delivery systems [38] are only a few of the new applications for these nanoparticles. The most fascinating metal oxides with unique features are aluminum oxide or alumina (Al_2O_3) nanoparticles. Over a large temperature range, they are thermodynamically stable particles. Surface charge, shape, and particle size all influence the behavior of alumina nanoparticles. They're also employed in fluorescence-based biosensors [39], electrochemical sensors [40–43], clinical applications [44], and biomedical applications [45].

The literature survey addressed many reports concerned with different developed methods for ZnO and Al_2O_3 nanoparticles synthesis including co-precipitation [46, 47], sol-gel [48, 49], microwave-assisted methods [50, 51], and thermal decomposition [52, 53]. The size and shape of the nanostructured metal oxides can be changed by varying the synthesis conditions used. However, certain drawbacks such as bioaccumulation, loss of mechanism understanding, high toxicity, the natural damage accompanied by the elevation of toxic materials, wastes generated

during various analytical processes, and instability can be observed [54, 55]. Therefore, these drawbacks opened great chances in the research area to prospect the use of green chemistry to improve environmental protection and to enhance the economics of chemical industries. The green chemistry notion shows an attractive technology to industrialists, scientists, researchers, and chemists for innovative chemistry, biology, and sensing applications. The perspective of plant extracts in the biogenic synthesis of nanomaterials such as metals and metal oxides involve the existence of bi-product metabolites, which act as reducing, stabilizing agents, or both. Furthermore, the same plant extract can be involved in the preparation of different types of nanomaterials; e.g., lemon extract has been utilized for the nanoengineering of noble metals and metal oxides [56, 57]. Many reports have described the green synthesis of nanomaterials [58–60] and their tremendous applications including, biosensors [61], catalysis [62], and biomedical studies [63].

Natural extracts such as *Camellia sinensis* (green tea) leaves and *Origanum vulgare* L. (wild oregano) leaf extracts were recommended in this study for the environmentally friendly preparation of ZnONPs and Al₂O₃NPs, respectively. The metal oxide nanoparticles were synthesized utilizing a simple green process with reactant (zinc sulfate and aluminum nitrate) precursors. This method offers various advantages, including simple preparation, precise temperature control of the reactant, ease of handling, and a one-step reaction. Different spectroscopic and microscopic approaches were used to comprehensively analyze the metal oxide nanomaterials that were obtained. The produced ZnONPs and Al₂O₃NPs nanoparticles were also utilized to determine the spectrofluorometric concentrations of two antibiotics, OFX and CPFEX, in bulk powders and commercial formulations.

Experimental

Materials and reagents

Pure grade chem Natural extracts such as *Camellia sinensis* (green tea) leaves and *Origanum vulgare* L. (wild oregano) leaf extracts were recommended in this study for the environmentally friendly preparation of ZnONPs and Al₂O₃NPs, respectively. Different spectroscopic and microscopic approaches were used to comprehensively analyze the metal oxide nanomaterials that were obtained. The produced ZnONPs and Al₂O₃NPs nanoparticles were also employed to determine the spectrofluorometric concentrations of two antibiotics, OFX and CPFEX, in bulk powders and commercial formulations were used throughout this study. Sigma Aldrich (Hamburg, Germany) provided zinc sulfate monohydrate (99.5%), aluminum nitrate monohydrate (99.9%), acetonitrile (99.9%), ethanol (99.9%) and methanol (99.9%). Sodium dodecyl sulfate (SDS, 98.5%), sodium hydroxide (98.0%) and triton X-100 were acquired from WIN-LAB (East Midland, UK). Britton-Robinson buffer (pH 2–12) was prepared by mixing 0.04 mol L⁻¹ of boric acid (99.5%, BDH Ltd., Poole, UK), 0.04 mol L⁻¹ acetic acid (BDH Ltd., Poole, UK), and 0.04 mol L⁻¹ phosphoric acid BDH Ltd. (Poole, UK) that has been titrated to the desired pH with 0.2 mol L⁻¹, cetylpyridinium chloride (CPC, BDH, Poole, UK). The pure grade of each OFX and CPFEX was obtained from Tabuk pharmaceutical Co., Tabuk, Saudi Arabia. The commercial formulations including Oflox[®] 0.3 w/v eye drop (Allergan pharmaceuticals LTD, Washington, USA) and Ciproxin[®] 500 mg CPFEX/tablet (Jamjoom Pharma, Jeddah, Saudi Arabia), were purchased from local drug stores (Riyadh, Saudi Arabia).

Apparatus

All spectrofluorometric detections were performed using a Shimadzu RF-5301pc luminescence spectrometer (Shimadzu, Kyoto, Japan) equipped with a 150W Xenon arc lamp, excitation grating, and emission monochromators. The excitation and emission monochromators

were set at 5 nm slit width. The outcomes were automatically PC controlled and data acquisition was assessed by fluorescence intensity software, version 4.00.03. The spectrophotometric (UV-Vis) and Fourier transform infrared (FTIR) characterizations of the synthesized ZnONPs and Al₂O₃NPs were investigated using Ultrospec 2100-Biochrom spectrophotometer, (Biochrom Ltd., Cambium, Cambridge, UK) and a PerkinElmer FTIR spectrophotometer (PerkinElmer Ltd., Yokohama, Japan), respectively. The surface morphologies of ZnONPs and Al₂O₃NPs were measured by JEM-2100F scanning electron microscope (JEOL Ltd., Tokyo, Japan) and JEM-1400 transmission electron microscope (JEOL Ltd., Tokyo, Japan). The elemental composition of ZnONPs and Al₂O₃NPs was studied at 20 kV using an Energy-dispersive X-ray (EDX, JSM-7610F; JEOL, Tokyo, Japan). The XRD patterns of the synthesized ZnONPs and Al₂O₃NPs were recorded by Siemens D-5000 diffractometer (Siemens, Erfurt, Germany). To adjust the pH conditions of the analytical solutions, Metrohm pH-meter model-744 (Metrohm Co., Herisau, Switzerland) was used. Deionized water obtained from Lonenaustauscher-SG, (Lonenaustauscher, Burgwedel, Germany) was used throughout the experimental studies.

Preparation of analytical solutions

Preparation of standard solutions of OFX and CPFX. Separate standard solutions (1000 µg mL⁻¹) of each pure OFX and CPFX were prepared by dissolving 0.1 g in 100 mL of deionized water. The testing solutions were prepared in the concentration ranges of 1.0–300 and 10.0–400 ng mL⁻¹ for the analysis of OFX and CPFX in the presence of ZnONPs. Others were prepared in the concentration ranges of 5–100 and 0.1–50 ng mL⁻¹ for the determination of the same drugs in the presence of Al₂O₃NPs, respectively.

Preparation of eye drop and tablet samples. The standard solution of Oflox[®] 0.3 w/v % eye-drop equivalent to 3 mg/mL of OFX was prepared by transferring 0.3 mL eye drop to a 100-mL volumetric flask and mixed with 50 mL of deionized water, followed by sonication for 20 min. The volume of the solution was adjusted up to the mark with deionized water to obtain a final concentration of 10 µg mL⁻¹ of OFX. Ten tablets of Ciproxin[®] 500 mg CPFX/tablet were weighed and finely powdered. An accurate amount (0.268 g) of the powder equivalent to 200 mg of CPFX was transferred into a small conical flask, in which 0.2 mol L⁻¹ HCl was added to dissolve the powder. Then the solution was filtered and the residue was washed several times. The obtained solution was diluted to the desired concentration with deionized water.

Preparation of *Camellia sinensis* and *Origanum vulgare* L. leaves extract. *Camellia sinensis* leaves were collected and washed with deionized water, air-dried, and ground into fine powder. Approximately, 100 mL of deionized water was added to 5 g of *Camellia sinensis* powder and magnetically stirred for 2 h at 80°C. The resulting extract was cooled to room temperature and filtered through Whatman filter paper No.1 [64]. The obtained extract was then stored at 4°C until employed for the preparation of ZnONPs for *Origanum vulgare* L. extract, approximately 500 g leaves were chopped into small pieces and macerated in 2.5 L of deionized water. The obtained mixture was refluxed for 4 h and the aqueous mixture was filtered, and then dried at 60°C under vacuum using a rotary evaporator. A dark brownish color extract of 30 g was collected and stored at 4°C [65].

Green synthesis of ZnONPs and Al₂O₃NPs. The green synthesis of ZnONPs was performed obeying the previously reported method [66] with slight modification. Briefly, For ZnONPs preparation, 30 mL of *Camellia sinensis* extract was mixed with 70 mL of zinc sulfate monohydrate (0.2 mol L⁻¹) and stirred. Pale-white ZnONPs was formed and dries for 12 h at 60°C. The product was calcined for 1 hour at 100°C and stored for further experiments. Whereas slight modification of the procedure [67] was performed to synthesize Al₂O₃NPs. It

was conducted by adding 10 mL of *Origanum vulgare* L. leaves extract to 40 mL of aluminum nitrate (2.0 mol L^{-1}) and magnetically stirring for 25–30 min. A yellowish-brown Al_2O_3 precipitate was formed. Then resulting Al_2O_3 NPs were centrifuged and filtered using Whatman filter paper No 1, dried, and stored in a container for further experiments. Stock solutions of ZnONPs and Al_2O_3 NPs were prepared by suspending 0.1 g of each nanopowder in 100 mL deionized water, then sonicated for 10 min and kept at 4°C in the refrigerator. S1 Fig in [S1 File](#) represents the potential mechanism for green synthesis of ZnO and Al_2O_3 NPs using plant extract.

General analytical procedure. At room temperature, the fluorescence intensity for OFX and CPFEX determination was examined in the presence of ZnONPs, Al_2O_3 NPs, and SDS (1.0% w/v). The working solutions of the selected medications were examined using 10 mL volumetric flasks to estimate the pharmaceuticals in their pure samples and tablets. For OFX in the presence of ZnONPs and Al_2O_3 NPs and SDS (1.0% w/v), and for CPFEX in the presence of the same nanoparticles and SDS (1.0% w/v), the fluorescence detection was carried out at room temperature using λ_{em} 480 nm and 470 nm after λ_{ex} 294 nm, respectively. A regression equation was utilized to determine each of the tested drugs using the calibration graphs. The difference in the emission wavelengths can be attributed to the variation in the surface plasmon resonance of each compound in the presence of nanoparticles, and also the difference in ZnONPs and Al_2O_3 NPs features of their crystalline phases. To the best of our knowledge that the strongest band gap the is shorter wavelength. The band gap of Al_2O_3 NPs is higher than that of ZnONPs and hence the wavelength will shift towards less values than that in the presence of ZnONPs [68, 69].

Results and discussion

Characterization of ZnONPs and Al_2O_3 NPs

The optical behavior of the prepared nanomaterials was investigated by applying UV-vis spectroscopy. Two broad absorption peaks were recorded at 220 and 300 nm for ZnSO_4 and another peak at 273 nm for *Camellia sinensis* leaves extract. A distinct peak due to the surface plasmon resonance (SPR) of ZnONPs was recorded at 347 nm (Fig 1A). The obtained spectra were in agreement with the literature [70]. Furthermore, the absorption spectra in Fig 1B, revealed the appearance of two significant absorption peaks, which are corresponding to *Origanum vulgare* L. extract at 285 and 325 nm, and one peak at 238 nm corresponding to Al_2O_3 NPs. The obtained spectra matched those in the literature [71]. The formula $E_g = h\nu$ was used to calculate the bandgap energy of the synthesized ZnONPs and Al_2O_3 NPs, where E_g is the bandgap energy, h is a blank constant, and ν is the frequency. The calculated bandgaps were found as 3.57 and 5.21 eV for ZnONPs and Al_2O_3 NPs, respectively. The red shift of absorption spectra of ZnONPs and the blue shift of Al_2O_3 NPs were found to be at 347 and 238 nm, respectively, and the decrease of bandgap values indicated the presence of nanostructure materials. These different peaks, which have particle diameters between 50 and 100 nm, respectively, are thought to be the creation of ZnONPs and Al_2O_3 NPs, according to the literature [66, 67]. The quantization effect is responsible for increasing the diameter of nanoparticles as the bandgap value decreases, although it never reaches zero [72].

The active functional groups or bonds of inorganic and organic compounds in the as-prepared ZnONPs were screened using FTIR spectroscopy. The observed peak in the region 417 cm^{-1} represents the formation of ZnO nanoparticles (Fig 1C). Various stretching vibration bands were recorded at 3431, 1581, and 1419 cm^{-1} related to O-H, C-O bonds, and N-H bonds for intercalated water and phytochemicals functional groups, respectively. These results matched the previously reported results for phytochemicals in plant extracts, which serve as

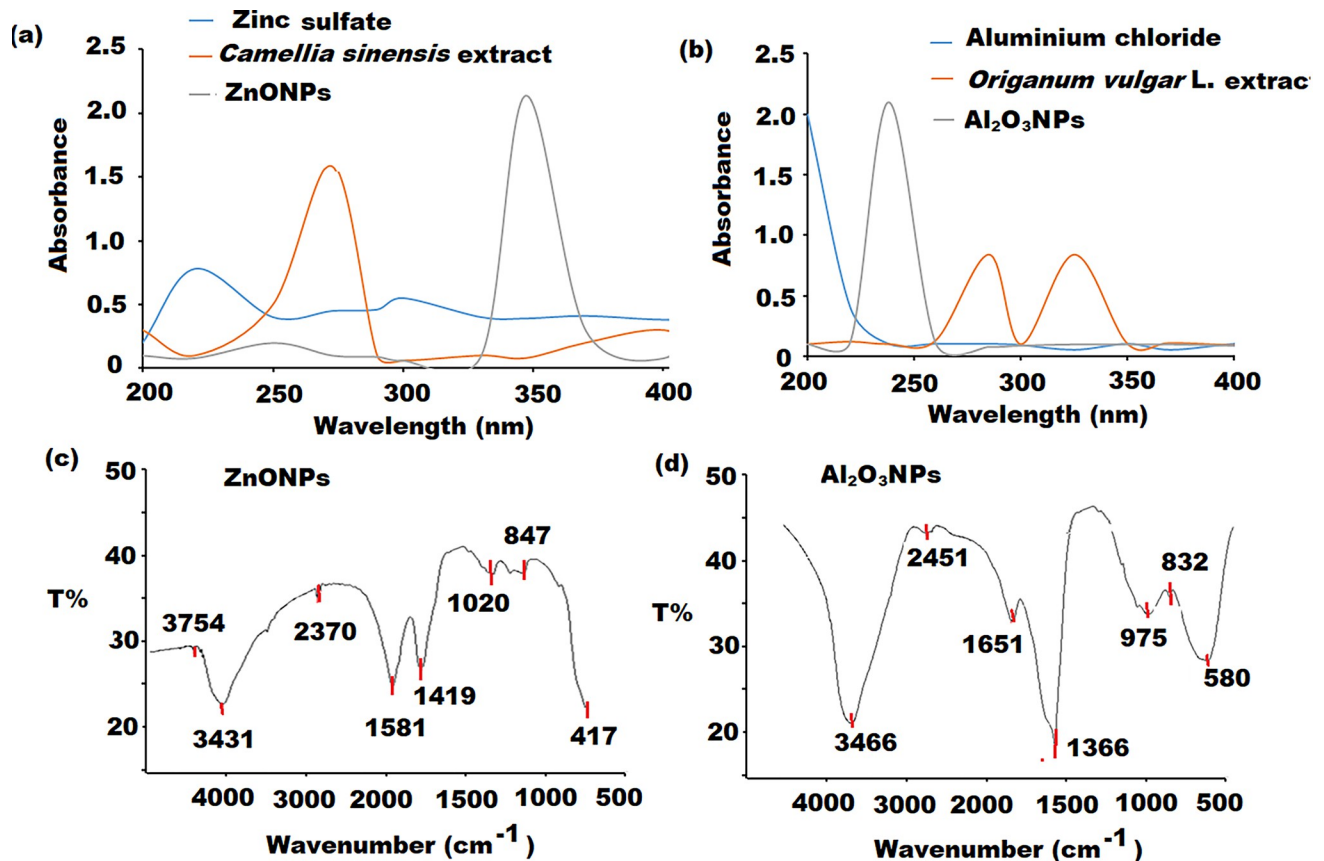


Fig 1. UV-Vis absorption spectra of green synthesized (a) ZnONPs (347 nm) and (b) $\text{Al}_2\text{O}_3\text{NPs}$ (238 nm) and FTIR spectra in the range of 4000–500 cm^{-1} of green synthesized (c) ZnONPs and (d) $\text{Al}_2\text{O}_3\text{NPs}$ using *Camellia sinensis* and *Origanum vulgare* L. leaves extract, respectively.

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capping and stabilizing materials on the surface of biosynthesized nanoparticles [70]. The FTIR spectrum (Fig 1D) of $\text{Al}_2\text{O}_3\text{NPs}$ in the range of 500–4000 cm^{-1} showed two absorption peaks at 3466 and 1651 cm^{-1} representing the presence of stretching and bending O-H vibrations of the intermolecular layer of water, respectively. Broadband of Al–O vibration has appeared at 832 cm^{-1} . The noticed peak at 580 cm^{-1} represents the vibration of Al–O–Al [73]. The particle size distribution of ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$ was determined using the Zetasizer Ultra particle size analyzer (Malvern Panalytical Ltd, Malvern, UK).

As demonstrated in Fig 2A and 2B, the particle size distributions were 94.25 nm and 114.8 nm for ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$, respectively. The obtained value confirmed that the green synthesized ZnO and Al_2O_3 using *Camellia sinensis* and *Origanum vulgare* L. extracts are in nano-scale form.

The zeta potential of green-synthesized ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$ with negative values of about -15.9 and -23.5 mV indicated a strong negative charge (Fig 2C and 2D). The negative surface zeta potential of ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$ suggests that the reduction process could be conducted through the surface-capped plant phytochemicals such as polyphenols compounds present in *Camellia sinensis* and *Origanum vulgare* L. extracts that were adsorbed on the surface of metal oxide nanoparticles.

To study the crystal shape of the green synthesized ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$, X-ray diffraction patterns were detected over the 20–80° 2 θ range. Fig 3A demonstrated sharp and distinct peaks, which revealed that the obtained metal oxide nanoparticles possess excellent

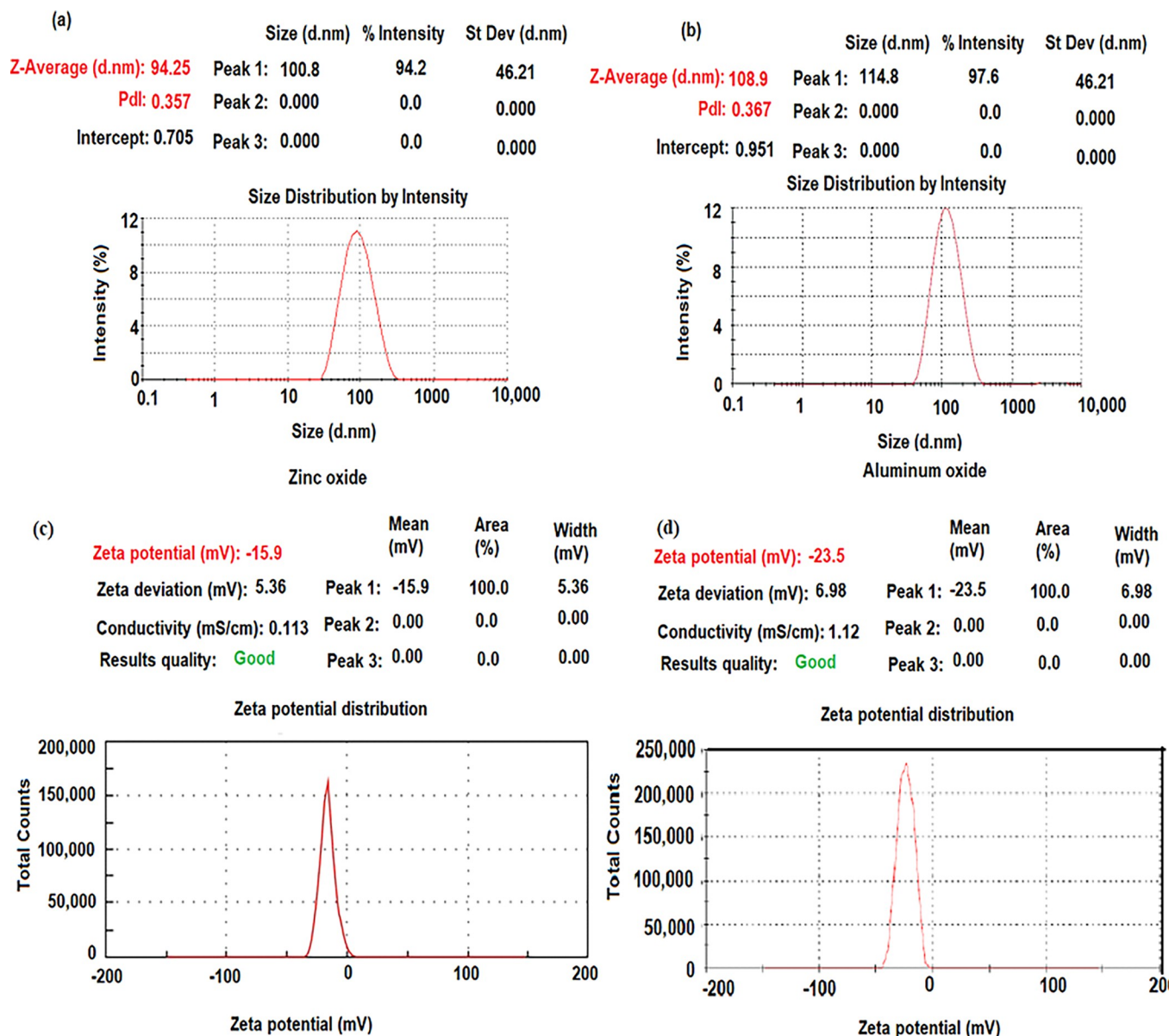


Fig 2. Particle size distribution of (a) ZnONPs and (b) Al₂O₃NPs and Zeta potential spectra of the green prepared (c) ZnONPs and (d) Al₂O₃NPs synthesized using *Camellia sinensis* and *Origanum vulgare* leaves extract, respectively.

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crystallinity with approximately larger crystallites and consider the influence of synthesis conditions on the formation of the crystal.

The XRD pattern of ZnONPs showed significant peaks at 2θ values of 31.73° (1 0 0), 34.44° (0 0 2), 36.15° (1 0 1), 47.35° (1 0 2), 56.50° (1 1 0), 62.90° (1 0 3), 68.11° (1 1 2) and 69.2° (2 0 1). The obtained values are closely in agreement with the literature values of the standard Joint Committee on Powder Diffraction Standards (JCPDS card no.36-1451) of ZnO [74]. Furthermore, no other significant peaks were recorded revealing the high purity of the prepared sample. The Debye-Scherrer formula, $D = 0.89 \lambda / \beta \cos \theta$ was used to calculate the average crystalline size of ZnONPs. Where λ (1.54 Å) is the wavelength of X-ray, θ is Bragg's diffraction angle and β is the full width at half maximum. The estimated crystalline size value was 29 nm

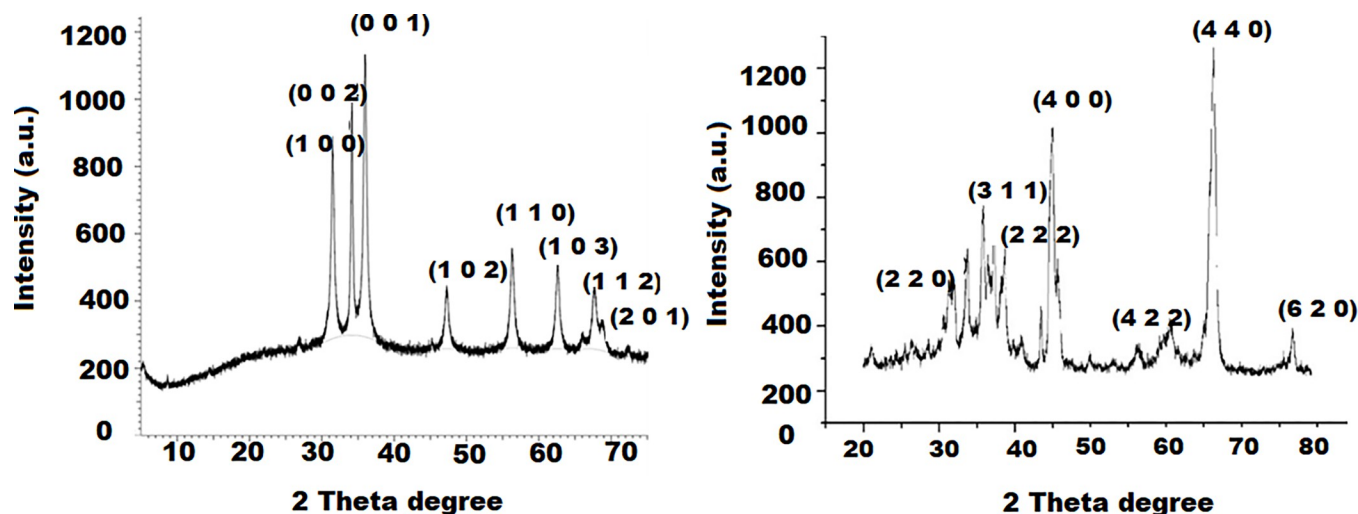


Fig 3. XRD patterns of (a) ZnONPs and (b) Al_2O_3 NPs synthesized using *Camellia sinensis* and *Origanum vulgare* leaves extract, respectively.

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[75]. The XRD pattern of Al_2O_3 NPs showed cubic and symmetric crystals with a face-centered lattice (Fig 3B). The obtained 2θ values were found at 32.5° (2 2 0), 35.1° (3 1 1), 38.7° (2 2 2), 46.5° (4 0 0), 62.4° (4 2 2), 67.2° (4 4 0), 78.4° (6 2 0). These values were in agreement with the standard Joint Committee on Powder Diffraction Standards (JCPDS-79-1558) [76]. The average crystal size was calculated using the above-mentioned formula and was found to be 31 nm.

EDX analysis was carried out to evaluate the chemical contents of the green synthesized ZnONPs and Al_2O_3 NPs using *Camellia sinensis* and *Origanum vulgare* L. leaves extract, respectively. The outcomes demonstrated that the weight and atomic percentages of Zn and O in ZnONPs were 70.27%, 29.73%, and 37.71%, 62.29%, respectively (Fig 4A and 4B). However, in Al_2O_3 NPs, the weight and atomic percentages of Al and O were 69.88%, 30.12%, and 39.93% 60.07%, respectively (Fig 4C and 4D). No additional peaks were recorded relating to other elements, proving the purity of the synthesized ZnONPs and Al_2O_3 NPs.

TEM and SEM were used to screen the surface morphology of the synthesized ZnONPs and Al_2O_3 NPs at 200,000-x magnification. The TEM images of ZnONPs and Al_2O_3 NPs (Fig 5A and 5B) demonstrated that the prepared metal oxide nanoparticles are uniformly distributed, hexagonal (ZnONPs), and short or long rods (Al_2O_3 NPs) in shape, and their sizes are between 50–100 nm, respectively. The SEM images in (Fig 5C and 5D) showed hexagonally, agglomerate, and nanorod in nature of nanocrystallites with particle size around 100 nm for ZnONPs and Al_2O_3 NPs, respectively [74, 77].

Optimization of experimental parameters

The experimental conditions of the suggested spectrofluorometric probes including, the effect of various parameters (type of solvent, volume of nanoparticles, type, and volume of surfactant, pH, detection time, and temperature) on the analytical estimation of the tested drugs were optimized. Table 1 summarized the optimized conditions of the suggested spectrofluorometric probes.

The suggested spectrofluorometric systems detected OFX and CPFX samples prepared in various solvents such as distilled water, methanol, ethanol, and acetonitrile. According to the nature of each solvent, the absorption maxima were recorded (Fig 6A). Dissolving the selected drugs (OFX and CPFX) in distilled water gave maximum fluorescence intensities in the

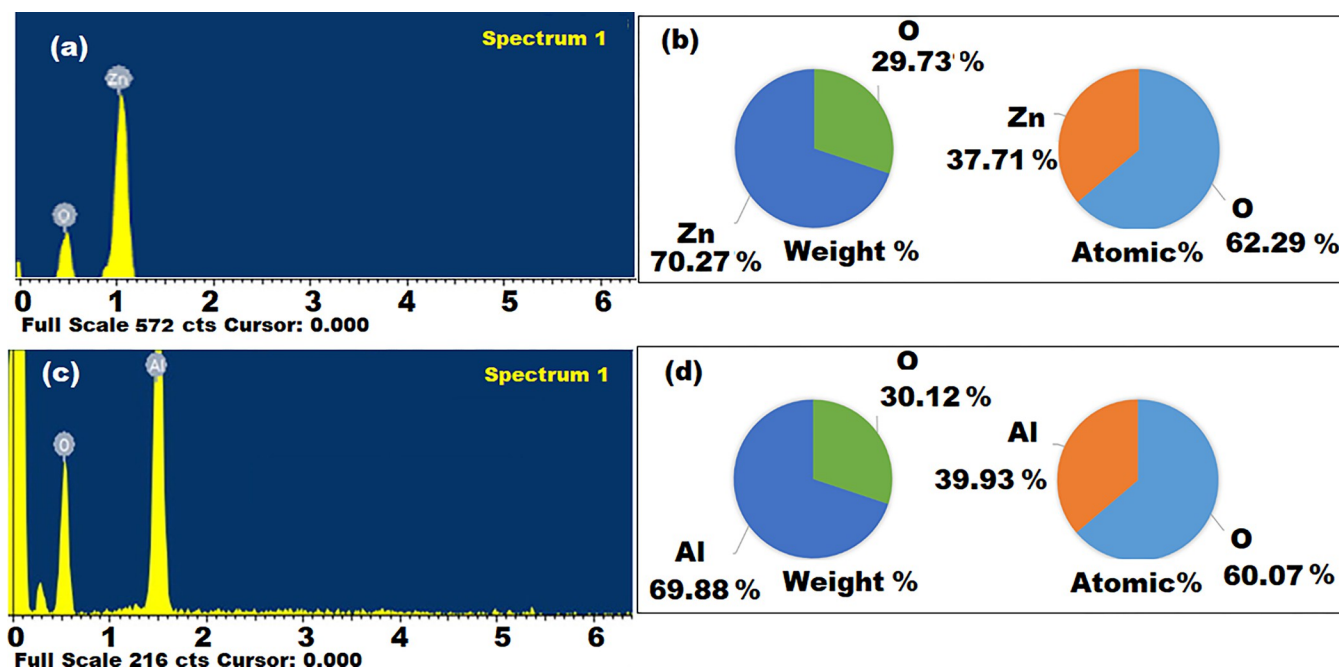


Fig 4. EDX spectra of: (a,c) the green synthesized ZnONPs and Al₂O₃NPs using *Camellia sinensis* and *Origanum vulgare* L. leaves extract, respectively, (b,d) the weight % and atomic % of the same metal oxides, respectively.

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absence of nanoparticles at wavelengths λ_{ex} 294 and λ_{em} 275, λ_{ex} 480 and λ_{em} 425 nm for OFX and CPFX, respectively. The fluorescence intensity of OFX and CPFX in aqueous and various micellar media were investigated using different types of surfactants such as anionic surfactant (SDS), cationic surfactant (CPC), and nonionic surfactant (Triton X-100). The addition of SDS to OFX and CPFX samples in the presence of ZnONPs and Al₂O₃NPs showed a substantial enhancement in the fluorescence intensity. Whereas, the other added surfactants caused a decrease in fluorescence or even have no significant effect on the FI of OFX and CPFX (Fig 6B).

To detect the effect of SDS volume on the fluorescence intensity of OFX and CPFX, different volumes (0.1–5 mL) of SDS (1.0% w/v) were tested. The maximum fluorescence intensity was achieved after adding 0.5 mL of SDS to both OFX and CPFX in the presence of ZnONPs. In the presence of Al₂O₃NPs, 0.5 mL and 1.0 mL of SDS were selected for OFX and CPFX, respectively (Fig 6C).

Because the volume of the as-prepared ZnONPs and Al₂O₃NPs can alter the fluorescence intensity of the measured medicines, 50 ng mL⁻¹ OFX and CPX solutions were used to test 0.1–5 mL of each manufactured nanoparticle. After adding 1.0 mL of ZnONPs to OFX and CPX, respectively, or 0.5 mL of Al₂O₃NPs to the same medications, the greatest fluorescence intensities were recorded. As a result, these volumes were chosen for additional investigations (Fig 6D).

The 0.1 mol L⁻¹ Britton- Robinson buffer was used to investigate the effect of pH on fluorescence intensity over the pH range of 3–11. The maximal fluorescence intensity for OFX-SDS-ZnONPs was obtained by adjusting the test samples to pH 5. However, no effect of pH on the samples containing OFX-SDS-Al₂O₃NPs, CPFX-SDS-ZnONPs, and CPFX-SDS-Al₂O₃NPs.

The response time was also studied by performing the experimental analysis at various time intervals of 2–10 min to evaluate the reaction time between each drug sample and the added

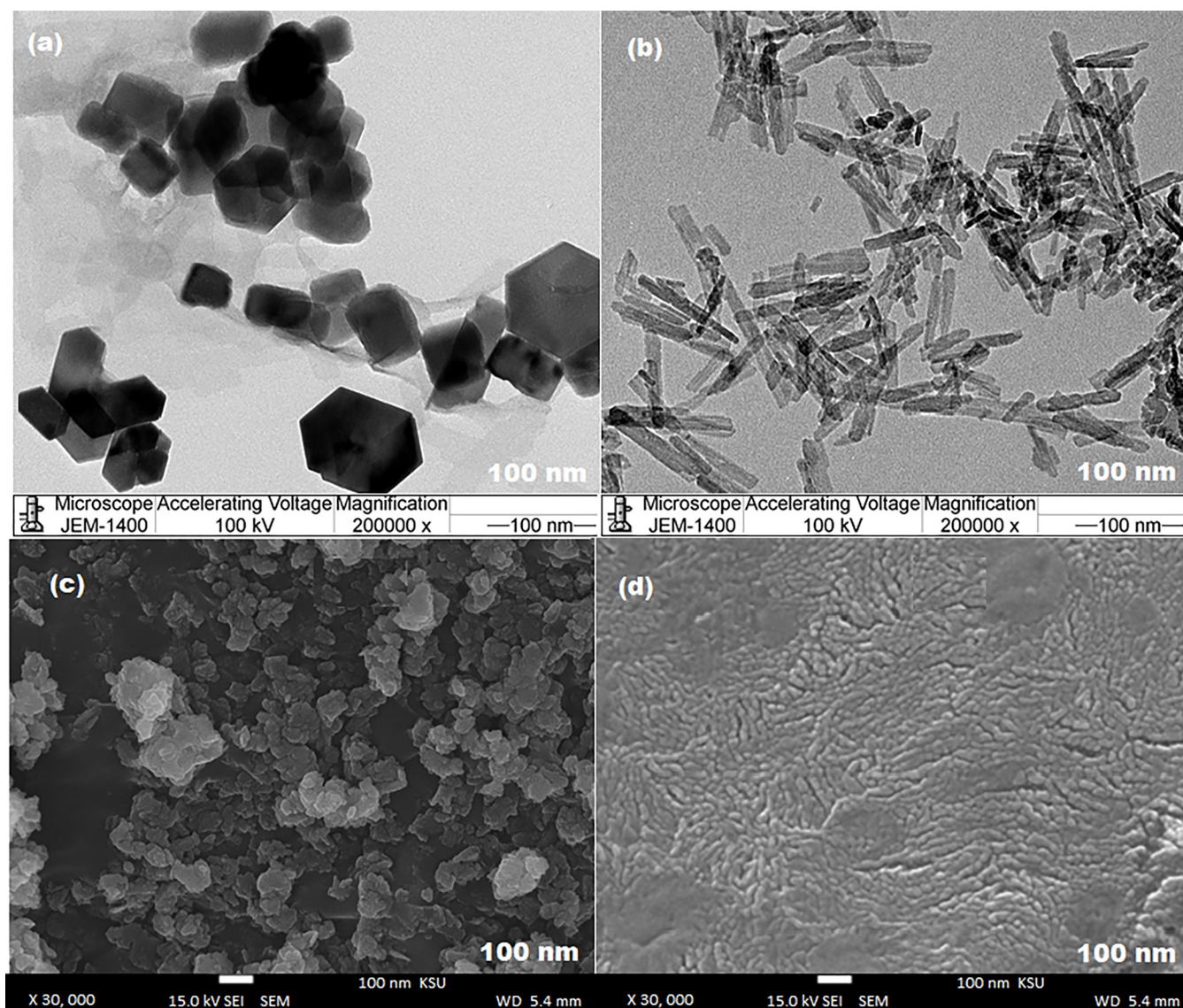


Fig 5. The TEM images (a) and (b) represent ZnONPs and Al₂O₃NPs, synthesized using *Camellia sinensis* and *Origanum vulgare* L. leaves extract, respectively, SEM (c) and (d) images represent the same nanoparticles, respectively.

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nanoparticles. Fast responses were recorded within 3 and 4 min for OFX and CPFX samples in the presence of SDS-ZnONPs, respectively. However, in the presence of SDS-Al₂O₃NPs, 4 min was suitable for detection. The fluorescence intensity reached a constant value within 2–4 min and remained constant (Fig 7A).

The influence of heat on the spectrofluorometric determination of OFX and CPFX was investigated by heating the samples and blank at 25–50°C in a water bath. It was observed that raising the temperature by more than 25°C reduced the fluorescence intensity of the two medicines. Therefore, the suggested spectrofluorometric systems for the determination of the OFX and CPFX were performed at ambient temperature (Fig 7B). The tested OFX and CPFX were found to exhibit weak emission bands at 470 and 425 nm after excitation wavelength at 294 and 275 nm, respectively. The addition of ZnONPs or Al₂O₃NPs to each fluorescence system,

Table 1. Optimized conditions for the determination of OFX and CPFX using the suggested spectrofluorometric systems in the presence of ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$, respectively.

Parameter	Studied range	OFX		CPFX	
		ZnONPs	$\text{Al}_2\text{O}_3\text{NPs}$	ZnONPs	$\text{Al}_2\text{O}_3\text{NPs}$
$\lambda_{\text{ex/em}}$ (nm)	250–700	294/480	294/470	275/427	275/445
NPs, volume (mL)	0.1–5	1	0.5	1	0.5
Surfactant type	SDS, CPC and CMC	SDS	SDS	SDS	SDS
Surfactant concentration	1.0%	1.0%	1.0%	1.0%	1.0%
Buffer pH	2–11	5	decrease	decrease	decrease
Brffer volume (mL)	0.1–5	1	-	-	-
Time (min)	1–10	3	2	4	2
Temperature ($^{\circ}\text{C}$)	20–50	25 $^{\circ}\text{C}$	25 $^{\circ}\text{C}$	25 $^{\circ}\text{C}$	25 $^{\circ}\text{C}$

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OFX-SDS-ZnONPs, OFX-SDS- $\text{Al}_2\text{O}_3\text{NPs}$, CPFX-SDS-ZnONPs, and CPFX-SDS- $\text{Al}_2\text{O}_3\text{NPs}$, enhances the emission bands for each system (Fig 8A–8D).

Method validation

The suggested spectrofluorometric methods using ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$ were validated using the previously recommended guidelines [78] to prove their accuracy and suitability for the determination of OFX and CPFX in their authentic samples. The calibration graphs of

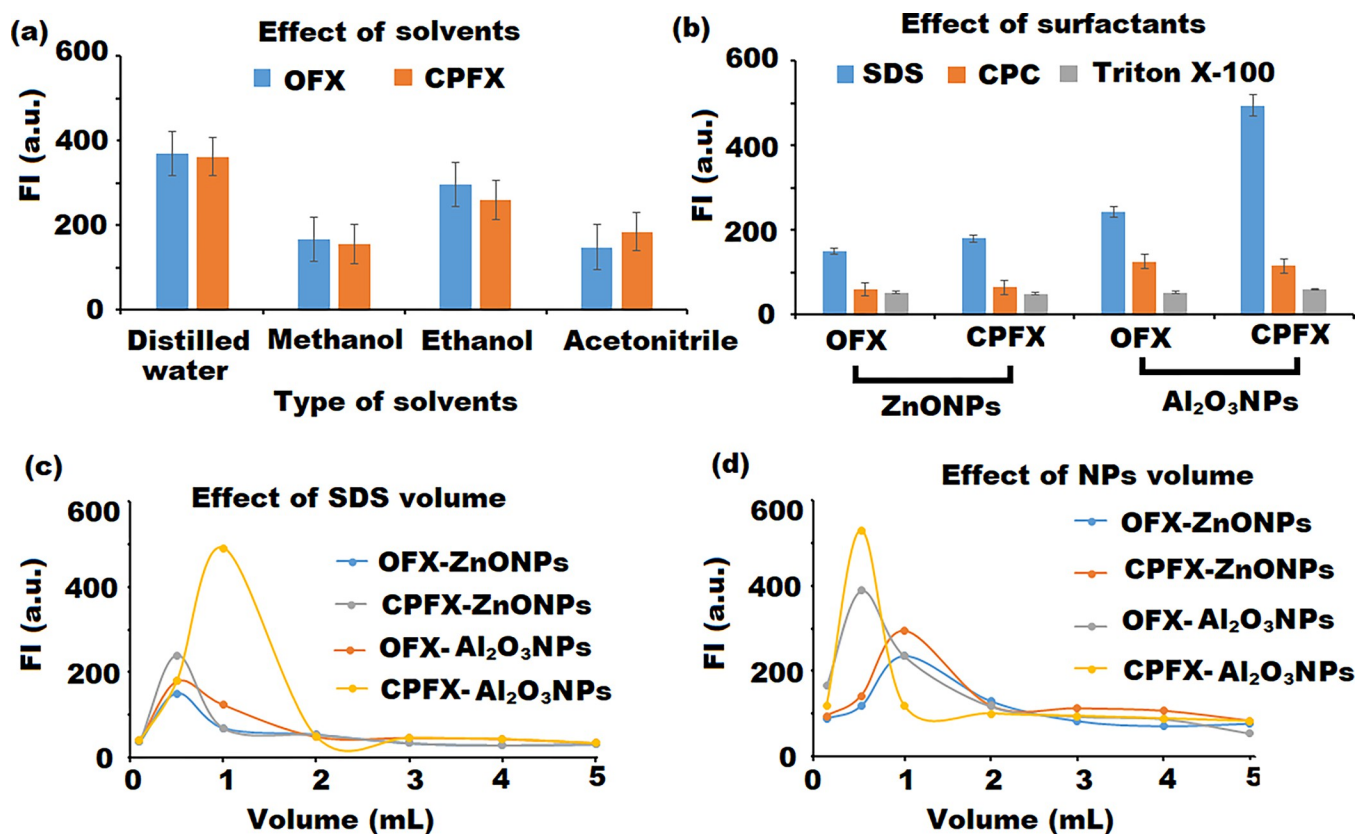


Fig 6. Optimization conditions: (a) Effect of solvents on the FI of OFX and CPFX dissolved in different solvents and in the absence of nanoparticles, (b) effect of type of surfactants (CPC, SDS and Triton x-100), (c) effect of volume of SDS and (d) effect of ZnONPs and $\text{Al}_2\text{O}_3\text{NPs}$ volume.

<https://doi.org/10.1371/journal.pone.0286341.g006>

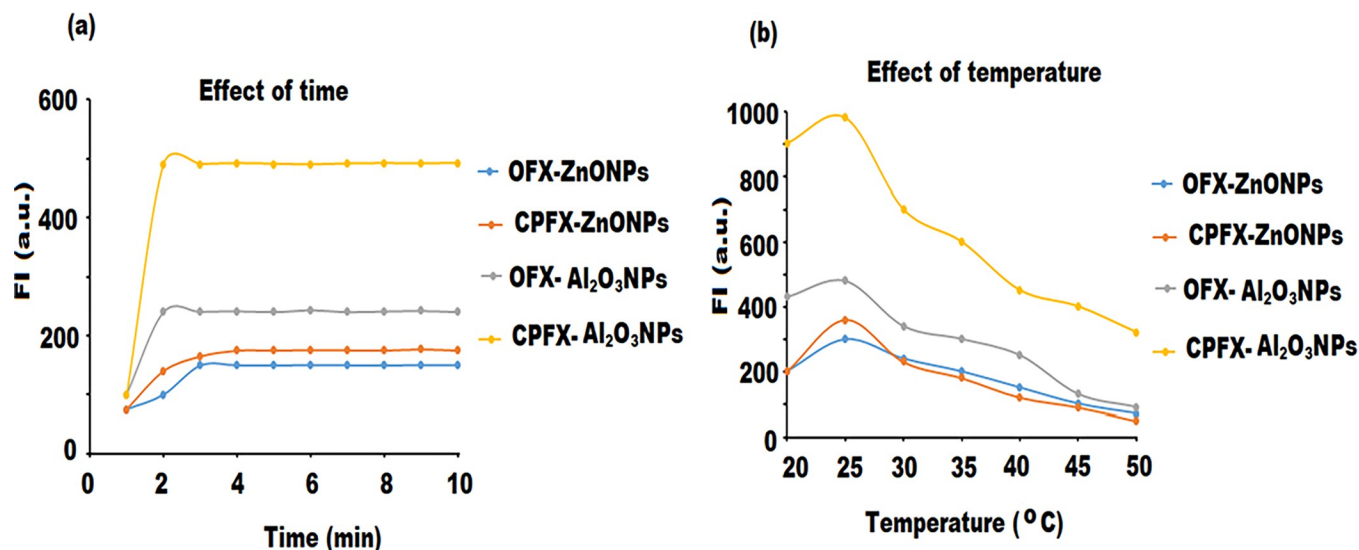


Fig 7. (a) Effect of time and (b) Effect of temperature on the fluorescence of OFX and CPFX determined in the presence of ZnONPs and Al₂O₃NPs.

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both investigated drugs were constructed by plotting the fluorescence intensity vs. the increase in drug concentrations. Rectilinear relationships were obtained over the concentration ranges of 1.0–300 and 10.0–400 ng mL⁻¹ for OFX and CPFX in the presence of SDS-ZnONPs, respectively, and 0.5–100 and 0.1–50 ng mL⁻¹ for the same drugs in the presence of SDS-Al₂O₃NPs (Table 1). The created linear graph was revealed by statistical analysis of the data, which produced greater correlation coefficients (*r*) and lower intercept and slope standard deviations (*S_a* and *S_b*), as shown in Table 2.

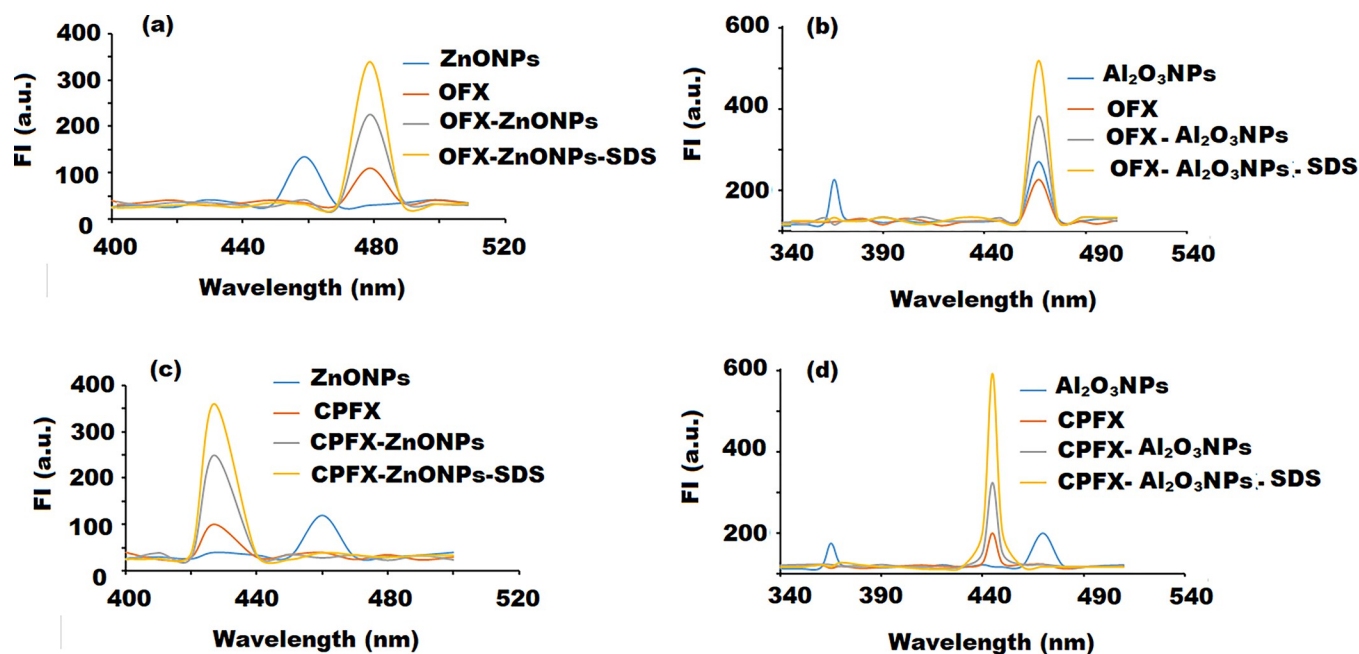


Fig 8. The fluorescence spectra of 50 ng mL⁻¹ of (a, b) OFX and CPFX solutions in the presence of ZnONPs and (c, d) for the same drugs in the presence of Al₂O₃NPs, respectively.

<https://doi.org/10.1371/journal.pone.0286341.g008>

Table 2. Outcomes resulted from the determination of OFX, and CPFX using the suggested spectrofluorometric systems in the presence of ZnONPs and Al₂O₃NPs, respectively.

Samples	λ_{em} (nm)	Conc. range ng mL ⁻¹	Regression equation	Correlation coefficient (r)	LOD, (ng mL ⁻¹) ^a	LOQ, (ng/mL ⁻¹) ^b	(S _b) ^c	(S _a) ^d
OFX-ZnONPs	480	1–300	FI = 1.9519C + 192.27	0.9993	0.04	0.13	4.25	0.026
OFX-Al ₂ O ₃ NPs	470	0.5–100	FI = 6.0164 C + 185.64	0.9996	0.03	0.10	3.23	0.065
CPFX-ZnONPs	427	10–400	FI = 1.3885C + 286.83	0.9997	0.02	7.24	2.93	0.01
CPFX-Al ₂ O ₃ NPs	445	0.1–50	FI = 16.039 C + 187.71	0.9995	0.04	0.09	4.83	0.195

^aLOD = 3.3 S_a/slope, ^bLOQ = 10 S_a/slope, ^c(S_b) and ^d(S_a) = slope and intercept (SD)

<https://doi.org/10.1371/journal.pone.0286341.t002>

The lower limits of detection (LOD) and quantification (LOQ) of the suggested spectrofluorometric methods were calculated. The LODs of the investigated OFX-SDS-ZnONPs and CPFX-SDS-Al₂O₃ systems were (0.04 and 0.02 ng mL⁻¹) and (0.03 and 0.04 ng mL⁻¹) for the two probes, respectively. Using the ratio 3.3(S_a)/b, the LOD was calculated by determining the lowest level at which the analyte can be consistently identified. However, LOQ was quantified using the relation 10(S_a)/b as the least quantity of analyte in a sample that can be acceptably detected with precision and accuracy values using the same optimized analytical conditions and was found to be (0.13 and 7.24 ng mL⁻¹) and (0.1 and 0.09 ng mL⁻¹) for the above-described systems (Table 2).

To prove the accuracy of the suggested fluorescence systems, OFX and CPFX were determined in their authentic samples. The accuracy of the newly developed fluorescence probs was stated as estimated % recoveries 98.93 ± 0.84, 98.91 ± 0.91 in the presence of ZnONPs and 98.92 ± 0.88, 99.01 ± 0.60 for OFX and CPFX in the presence of Al₂O₃NPs, respectively (Table 3).

The precision of the suggested systems was evaluated using intra-day and inter-day assays. The relative standard deviation percentage (RSD%) was computed from the results of three duplicate tests using three analytical concentrations of OFX or CPFX (n = 3). The obtained mean RSD % was found to be 0.62%, 0.89% and 0.94%, 0.64% for intra-day assay of OFX, and CPFX in the presence of ZnONPs and Al₂O₃NPs, respectively. However, the inter-day assay showed mean RSD % of 0.58%, 0.8, % and 0.93%, 0.87% of the selected analyte in the presence of SDS-ZnONPs and SDS-Al₂O₃NPs, respectively. These results are <2%, indicating acceptable precision of the suggested systems.

The selectivity of the suggested spectrofluorometric systems for the quantification of OFX and CPFX in the presence of ZnONPs and Al₂O₃NPs was evaluated in the presence of various possible inactive ingredient species used as co-formulated materials in the production of tablets. Under the optimized conditions the tolerable level which was determined as the number of foreign species that produce an error of less than 5% was calculated using OFX and CPFX samples (1.0 ng mL⁻¹). The obtained values of the selectivity levels proved that the suggested systems possess good selectivity for the determination of OFX and CPFX in the existence of SDS-ZnONPs or SDS-Al₂O₃NPs (Table 4).

Table 3. Results of validation calculated from the determination of OFX and CPFX in bulk powder using the suggested spectrofluorometric systems in the presence of ZnONPs and Al₂O₃NPs, respectively.

Samples	Accuracy (n = 9)	Intra-Day (n = 3)	Inter-Day (n = 3)	Repeatability (RSD %, n = 6)	Robustness	Ruggedness
OFX-ZnONPs	98.93 ± 0.84	0.66%, 0.5%, 0.71%	0.57%, 0.66%, 0.51%	0.65	99.58 ± 0.82	98.71 ± 1.21
OFX-Al ₂ O ₃ NPs	98.92 ± 0.88	0.93%, 0.84%, 1.05%	1.31%, 0.5%, 0.98%	0.51	99.05 ± 0.61	99.21 ± 0.74
CPFX-ZnONPs	98.91 ± 0.91	1.0%, 1.04%, 0.63%	1.02%, 1.0%, 0.39%	0.80	98.25 ± 0.54	98.45 ± 0.31
CPFX-Al ₂ O ₃ NPs	99.01 ± 0.60	0.26%, 0.38%, 1.28%	0.6%, 1.4%, 0.61%	1.12	99.45 ± 1.30	99.74 ± 0.43

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Table 4. Effect of interfering substances on the determination of OFX, and CPFX using the suggested spectrofluorometric systems in the presence of ZnONPs and Al₂O₃NPs, respectively.

Interference	Tolerable Values			
	OFX Tolerable values		CPFX Toelrable values	
	ZnONPs	Al ₂ O ₃ NPs	ZnONPs	Al ₂ O ₃ NPs
Lactose	80	60	100	70
Povidone	240	350	180	200
Microcrystalline cellulose	200	180	150	180
Magnesium stearate	600	400	350	300
Anhydrous colloidal silica	450	520	420	610
Red ferric oxide	250	380	550	400
Titanium dioxide	100	250	220	150

<https://doi.org/10.1371/journal.pone.0286341.t004>

The robustness of the suggested approaches for the analysis of OFX and CPFX was evaluated by carrying out slight deviations in the analytical parameters. The robustness of the techniques was conducted by increasing or decreasing the pH value of (± 1), varying the volume of SDS (± 0.1 mL), the volume of ZnONPs (± 0.2 mL) and the volume of Al₂O₃NPs (± 0.1 mL). The fluorescence intensity was not exaggerated by these variations. The found % recoveries were 99.58 ± 0.82 and 99.05 ± 0.61 for OFX in the presence of SDS-ZnONPs and SDS-Al₂O₃NPs, respectively, and 98.25 ± 0.54 and 99.45 ± 1.30 for CPFX in the presence of SDS-ZnONPs and SDS-Al₂O₃NPs, respectively.

The ruggedness of the fluorescence determination probe for the detection and quantification of OFX and CPFX was investigated by estimating the same samples under different conditions, such as other analysts, laboratories, and devices. The fluorescence was not affected by these changes. The calculated % recoveries were (98.71 ± 1.21 , 99.21 ± 0.74) and (98.45 ± 0.31 , 99.74 ± 0.4) for OFX and CPFX in the presence of SDS-ZnONPs and SDS-Al₂O₃NPs, respectively.

Analytical applications

The proposed spectrofluorometric method was used to quantify OFX and CPFX in their pure powder (Table 5).

The obtained results were 99.50 ± 0.76 and 98.59 ± 0.66 for OFX and CPFX in the presence of SDS-ZnONPs. However, the mean percentage recoveries were 99.29 ± 0.97 and 99.42 ± 0.87 for the same drugs in the presence of SDS-Al₂O₃NPs, respectively. The obtained data indicated that the presence of metal oxide nanoparticles enhances the sensitivity of the suggested spectrofluorometric systems and this can be attributed to ZnONPs and Al₂O₃NPs possessing large surface areas and excellent optical properties. The suggested spectrofluorometric method was used to estimate OFX and CPFX in their commercial formulations (Oflox[®] 0.3 w/v OFX eye drop and Ciproxin[®] 500 mg CPFX/tablet) and the outcome data were presented in Table 6.

Table 5. Determination of OFX and CPFX in their pure powder using the suggested spectrofluorometric systems in the presence of ZnONPs and Al₂O₃NPs, respectively.

Samples	Taken ($\mu\text{g mL}^{-1}$)	Found Range ($\mu\text{g mL}^{-1}$)	% Recovery	Mean $\pm$ SD	n	Variance	% SE	% RSD
OFX-ZnONPs	1.0–100	1.0–99.25	98.0–100.01	99.50 ± 0.76	7	0.58	0.29	0.76
OFX-Al ₂ O ₃ NPs	0.5–100	0.50–99.0	98.0–100.25	99.29 ± 0.97	7	0.94	0.37	0.98
CPFX-ZnONPs	10–400	9.90–401.0	97.5–99.33	98.59 ± 0.66	7	0.44	0.25	0.67
CPFX-Al ₂ O ₃ NPs	1.0–50	0.99–50.0	98.0–100.5	99.42 ± 0.87	7	0.76	0.33	0.88

<https://doi.org/10.1371/journal.pone.0286341.t005>

Table 6. Assay of OFX, and CPFX in their commercial dosage forms applying the suggested spectrofluorometric systems in the presence of ZnONPs and Al₂O₃NPs, respectively.

Samples	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	% Recovery	Mean $\pm$ SD	n	Var.	% SE	% RSD	Ref. methods [39–41]	t-Test (2.228) *	F-Test (5.05) *
OFX-ZnONPs	1.0–100	0.985–99.8	98.5–99.9	99.21 $\pm$ 0.69	6	0.48	0.28	0.70	99.89 $\pm$ 0.36	2.140	3.673
OFX-Al ₂ O ₃ NPs	0.5–100	0.5–99.5	98.0–100.1	99.25 $\pm$ 0.76	6	0.58	0.31	0.77		1.864	4.456
CPFX-ZnONPs	10–400	10.0–399.0	99.0–101.33	100.26 $\pm$ 1.15	6	1.32	0.47	1.15	99.58 $\pm$ 1.255	0.978	1.190
CPFX-Al ₂ O ₃ NPs	1.0–50	0.98–50.1	98.0–100.2	99.05 $\pm$ 0.72	6	0.52	0.29	0.73		0.913	3.038

*Tabulated t- and F values at $p < 0.05$.

<https://doi.org/10.1371/journal.pone.0286341.t006>

The collected results were found to be 99.21 ± 0.69 and 100.26 ± 1.15 for OFX and CPFX in the presence of SDS-ZnONPs. The % recoveries were recorded as 99.25 ± 0.76 and 99.05 ± 0.72 for the same analytical samples in the existence of SDS-Al₂O₃NPs, respectively. Mathematical assessment for outcomes was performed using Student's t-test and variance ratio F-test [79] and the results were matched with those resulting from other published techniques [80, 81]. It was observed that the proposed spectrofluorometric systems exhibited excellent sensitivity for the determination of OFX and CPFX in the presence of SDS-ZnONPs and SDS-Al₂O₃NPs. These findings are explained by the enhancing effect of metal oxide on the collective oscillations of conduction electrons and localized surface plasmon resonances that strongly couple to light at particular wavelengths and give materials their exceptionally high optical characteristics. These metal oxide nanoparticles' high fluorescence activity was also a result of their large surface area, tunable features that developed during the synthesis of their nanostructures, and capacity to take up the oxygen groups of the drugs under study and improve their spectrofluorimetric determination. The outcomes also revealed that the addition of ZnONP for the detection of OFX and CPFX exhibited excellent sensitivity with wider concentration range than the addition of Al₂O₃NPs. This can be attributed to the high exciton binding energy of ZnO (~ 60 meV) would allow for excitonic transitions even at room temperature, which could mean high radiative recombination efficiency for spontaneous emission. However, in Al₂O₃NPs the exciton binding energy (~ 51.16 meV) [82, 83].

Furthermore, results from the suggested spectrofluorometric systems were compared to previously published analytical techniques such as spectrophotometric, fluorescence, electrochemical, and chromatographic approaches. The outcomes have been summarized in Table 7.

The suggested spectrofluorometric systems using metal oxide nanoparticles practically showed great sensitivity, cost advantages, simple manufacture, and environmental friendliness. They are also more accurate and precise and do not require high technical skills when compared to other chromatographic or electrochemical techniques.

Conclusion

This study described accurate and precise fluorescence probes based on metal oxide nanoparticles (ZnO and Al₂O₃) for the quantification of antibiotics (OFX and CPFX) in their commercial dosage forms. The experimental detection was based on the extraordinary optical properties of ZnONPs and Al₂O₃NPs which enhance the fluorescence intensities of the selected drugs. The recorded data from the suggested fluorescence probes showed excellent catalytic activity of ZnONPs and Al₂O₃NPs and revealed that the increase in fluorescence was directly proportional to the increase in drug concentrations. The outcomes displayed excellent linearity over concentration ranges of 1.0–300, 10.0–400 ng mL^{-1} for OFX and CPFX in the presence

Table 7. A comparison between the current findings from the assay of OFX, and CPR applying the suggested spectrofluorometric prob in the presence of ZnONPs and Al₂O₃NPs, respectively and the previously reported analytical techniques.

Analytical Techniques	Reagent	Linear conc. Range	LOD	Reference
Spectrophotometry	OFX, Two sulphonphthalein acid dyes	1.0–16 $\mu\text{g mL}^{-1}$	0.11, 0.09 $\mu\text{g mL}^{-1}$	[10]
	CPR, by utilizing a solvent system composed of methanol: water (50:50 v/v)	1–6 $\mu\text{g mL}^{-1}$	0.41 $\mu\text{g mL}^{-1}$	[84]
Fluorescence	OFX, gold nanoparticles	20–300 nM	1.66 nM	[85]
	CPR, riboflavin and carbon dots	0.5–200 μM	0.13 μM	[86]
Electrochemical	OFX, boron doped diamond electrode (BDDE)	1.0×10^{-7} – 3.5×10^{-6} M	1.76×10^{-8} M	[87]
	CPR, reduced graphene oxide/poly(phenol red) modified glassy carbon electrode	0.002–0.05 μM And 0.05–400 μM	2 nM	[88]
Chromatography	OFX RP-HPLC–UV method, methanol and 0.05% trifluoroacetic acid (TFA) (38:62 v/v)	0.014–20 $\mu\text{g mL}^{-1}$	8 ng mL ⁻¹	[89]
	CPR, HPLC–UV method, phosphate buffer (pH 2.7) and acetonitrile (77:23, v/v)	0.05–8 $\mu\text{g mL}^{-1}$	0.01 $\mu\text{g mL}^{-1}$	[90]
Proposed method	Spectrofluorometric measurement in the presence of ZnONPs and Al ₂ O ₃ NPs	1.0–100 ng mL ⁻¹	0.04 ng mL ⁻¹	OFX–ZnONPs
		0.5–100 ng mL ⁻¹	0.03 ng mL ⁻¹	OFX–Al ₂ O ₃ NPs
		10–400 ng mL ⁻¹	0.02 ng mL ⁻¹	CPR–ZnONPs
		1.0–50 ng mL ⁻¹	0.04 ng mL ⁻¹	CPR–Al ₂ O ₃ NPs

<https://doi.org/10.1371/journal.pone.0286341.t007>

of SDS–ZnONPs and 0.5–100, 0.1–50 ng mL⁻¹ in presence of SDS–Al₂O₃NPs, respectively. The estimated least square equations for the above-mentioned systems were FI = 1.951C + 192.27 (r = 0.9993), FI = 6.0164C + 185.64 (r = 0.9996), FI = 1.3885C + 286.83 (r = 0.9997) and FI = 16.039C + 187.71 (r = 0.9995) in the presence of SDS–ZnONPs and SDS–Al₂O₃NPs, respectively. The proposed fluorescence probes were potentially used for the determination of OFX and CPFX in their commercial formulations with mean percentage recoveries of 99.21 ± 0.69, 99.25 ± 0.36, 100.26 ± 1.15 and 99.05 ± 0.72 in the presence of ZnO and Al₂O₃ nanoparticles, respectively. Statistical analysis of the resulting data was performed and the results were in good agreement with those reported from previously published methods. The suggested fluorescence methods, using the unique features of the synthesized ZnONPs and Al₂O₃NPs, revealed high catalytic activity and sensitivity due to the enhancement of surface plasmon resonance and collective oscillations of conduction electrons that coupled with light causing their strong optical features.

Supporting information

S1 File.

(DOCX)

S1 Scheme.

(TIF)

Author Contributions

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Writing – original draft: Hadeel A. Alabdulmonem.

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