Research Article

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Anti-OTC antibody-conjugated fluorescent magnetic/silica and fluorescent hybrid silica nanoparticles for oxytetracycline detection

https://doi.org/10.1515/chem-2024-0066
received April 1, 2024; accepted June 24, 2024

Abstract: This study presents two alternative fluorescent nanoparticle-based oxytetracycline (OTC) detection methods in milk samples. Rhodamine 6G-coated fluorescent hybrid silica nanoparticles and fluorescent magnetic/silica nanoparticles functionalized with anti-OTC antibodies were used in this test. The sandwich test format was utilized to compare anti-OTC antibody-conjugated fluorescent magnetic/silica nanoparticles with OTC/OTC antibody-conjugated fluorescent hybrid silica nanoparticles in an Eppendorf tube with magnetic separators. The magnetic separator helps to quickly retain all of the OTC captured by fluorescent magnetic core–shell nanoparticles in the milk sample. As a result, the assay time was dramatically shortened. The obtained linear range was 1.34×10^{-6} to 2.10×10^{-8} (M) (R^2 = 0.9954), the detection limit was 4.76 ng/mL, and the total assay time was 90 min. This approach was used to determine the OTC concentration in milk samples, and the maximum percentage (%) of interference was less than 3.0%, with a recovery rate of greater than 97.0%. This approach offers a high potential for residue detection in milk samples. With a total analysis period of less than 90 min, this approach provided the best way to determine the capture and detector nanoparticles' response.

Keywords: OTC detection, magnetic separator, fluorescent hybrid silica nanoparticles, fluorescent magnetic silica nanoparticles, sandwich assay

1 Introduction

Food quality and safety have gained a lot of attention recently because chemical contaminants such as drug residues, pesticides, mycotoxins, and so on can be harmful to people health. As a result, monitoring drug residues in food is crucial. Antibiotics such as aminoglycosides, tetracyclines, macrolides, and sulphonamides are frequently used to prevent bacterial infections in the livestock business [1–4]. Excessive drug use in the livestock industry results in significant levels of antibiotic residues in milk, eggs, and edible tissues generated from the parent drug ingredient or metabolites/conjugates [5–9].

The European Medicines Agency constantly monitors the antibiotic content in food items, and it has already set many standards to monitor drug concentrations in veterinary goods and other food stuffs. Various preconcentration/extraction techniques such as liquid–liquid extraction, solid-phase extraction (SPE), liquid–liquid microextraction, solid-phase microextraction, and magnetic solid-phase extraction (MSPE) were initially used to evaluate antibiotic concentrations in foods and isolate antibiotic residues from various samples [10–12]. Following the introduction of chromatographic principles, the researchers developed liquid chromatography–tandem mass spectrometry (LC–MS/MS), ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS), and high-performance liquid chromatography with evaporative light-scattering

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detection (HPLC-ELSD) techniques. Later, surface-enhanced Raman spectroscopy, biosensors, capillary electrophoresis, and enzyme-linked immunosorbent assay (ELISA) [13–15] were developed. The use of nanoparticles for antibiotic residue detection provided several advantages, including a larger surface area that allows for higher biomolecule loading, biomolecule adsorption on nanoparticles which improves the stability, and easy separation from the reaction mixture using a centrifugal/magnetic field [16–18]. Tetracycline antibiotics are widely utilized in veterinary medicine because they are effective against both Gram positive and Gram negative microbial infections. Overuse of oxytetracycline (OTC) in farm animals leads to the accumulation of OTC residues in food items such as meat, milk, and chicken eggs, and researchers have made swift efforts to develop a sensing device for the enhanced detection of OTC in contaminated food products [19–23].

Internationally, the Codex Alimentarius, Food and Agricultural Organization, and World Health Organization periodically monitor the maximum residue levels for each medicine or pesticide in food items. Nowadays, technological advancements drive researchers to develop more sensitive and faster screening methods for residue detection in food products, with a wide detection range, high sensitivity, selectivity, and multiple detections in a single test in a short period of time. Microbiological techniques are quick, cheap, and easy to employ, but their sensitivity and specificity are low. Although instrumental analytical methods are exact and quantitative, they require extremely sophisticated, expensive equipment and elaborate sample pre-treatments. Methods for immunological analysis save time while giving high sensitivity and specificity [24–27]. Previously, a multicolour fluorescence sensing platform (CDs-Cit-Eu) [28], a nitrogen-doped and europium-based dual-response fluorescent probe [29], multicolour carbon dots as dual detection probes [30], and a ratiometric fluorescence sensor based on lanthanum-doped carbon dots [31] were described. The development of a drug residue detection system based on a dual method has received more attention (Figure 1).

The purpose of this research is to create a simple Eppendorf test to identify OTC residue in milk utilizing a dual detection approach. For this study, rhodamine 6G hybrid silica nanoparticles were synthesized and coupled with anti-OTC antibodies. The fluorescent magnetic core–shell nanoparticles were created using the sol–gel process and were linked with anti-OTC antibodies. Anti-OTC antibody-conjugated fluorescent magnetic/silica and anti-OTC antibody-conjugated fluorescent hybrid silica nanoparticles were used in magnetic separators to capture and detect OTC from milk. Sandwich reactions were carried out. The results demonstrate that this method can quickly detect OTC in milk with high sensitivity and specificity. This approach is especially effective for detecting OTC because of its unique properties. (1) The magnetic core with a fluorescent dye covered with a silica layer in a capture probe effectively prevents fluorophore leakage, and the surface functionalized with specific antibodies can bind to specific antigens, form immune complexes, and produce distinct signals based on analyte concentrations. (2) The anti-OTC antibody-linked capture probe binds directly to the OTC molecule, enabling rapid OTC detection. Various concentrations of OTC in a sample produce varying rates of electron shifting into the capture probe, resulting in fluorescence quenching of the tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate fluorescent dye. (3) The nanoparticles include rhodamine 6G dye molecules, which provide a strong signal and allow for straightforward quantification. (4) In this multiple probe detection method, when anti-OTC antibodies in a detection probe bind preferentially to OTC, they produce good intensity values at extremely low concentrations of the analyte in real samples. (5) The dye emission spectrum separations between fluorophores offer excellent reproducibility between analyses.

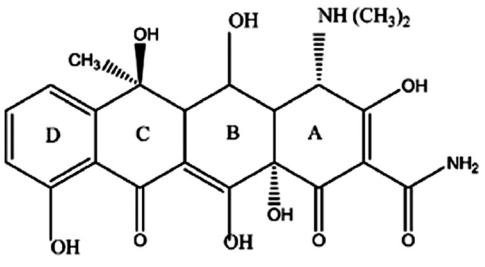

Figure 1: Structure of the OTC drug.

2 Materials and methods

2.1 Reagents

Reagents used in this study such as ferric chloride, ferrous chloride, oleic acid, tetraethyl orthosilicate (TEOS), Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate, 3-aminopropyltriethoxysilane (APTES), rhodamine 6G, and *N*-hydroxysuccinimide (NHS) were procured from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), bovine serum albumin (BSA), and *o*-phenylenediamine were obtained from TCI Chemicals. Anti-OTC antibodies were procured from Thermo Fisher Scientific.

2.2 Preparation of fluorescent magnetic/silica nanoparticles

Based on prior research [32,33], fluorescent magnetic/silica nanoparticles were created. In brief, a conical flask containing

10 ml of each of 1 M ferric chloride and 2 M ferrous chloride solutions were deoxygenated with nitrogen. To create oleic acid-decorated magnetic particles, 1 mL of oleic acid was added with 10 mL of ammonium hydroxide in a conical flask. It was heated with agitation at 80°C for 30 min. The flask was then allowed to cool to room temperature before being separated using a magnet and cleaned with distilled water and allowed to dry at 70°C. Finally, for the preparation of fluorescent magnetic/silica nanoparticles, 500 mg of oleic acid-coated iron oxide, 10 mL of Milli-Q water, and 10 mL of ethanol were added in a conical flask and mixed for 10 min. After that, 1.0 mL of TEOS, 5 mL of Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate fluorescent dye, and 1 mL of ammonium hydroxide solution were added, and the reaction mixture was mixed thoroughly for 3 h at room temperature. Then, 0.2 mL of APTES was added and stirred again for 1 h at room temperature to form the amine group on the surface, and the particles were separated using a magnet.

2.3 Synthesis procedure for fluorescent hybrid silica nanoparticles

To generate fluorescent hybrid silica nanoparticles, we followed a slightly modified procedure from our earlier study [34]. In a 50 mL conical flask, 20 mL of ethanol, 10 mL of 2% polyvinylpyrrolidone (PVP) solution, and 1 mL of TEOS were combined. They were mixed for 20 min, added with 5 mL of 6×10^{-3} mol/L rhodamine 6G fluorescent dye and 1 mL of ammonia hydroxide solution and stirred for 3 h, and then added with 1 mL of APTES and stirred for another 1 h to modify the surface.

The materials were examined by Fourier transform infrared (FTIR) analysis (PerkinElmer Spectrum One FTIR instrument), emission spectroscopy (multi-mode reader TECAN), scanning electron microscopy (SEM, Quanta 200 FEG), and dynamic light scattering (DLS) in the Sophisticated Analytical Instrumentation Facility at the Indian Institute of Technology, Chennai, using readily accessible methods.

2.4 Anti-OTC antibody conjugation

To conjugate anti-OTC antibodies with nanoparticles, 5 mL of NHS (1×10^{-3} mol/L), 5 mL of EDC (8×10^{-4} mol/L), 5 mL of anti-OTC antibodies (1.11 mg/mL), and 5 mL of amine group-modified nanoparticles (25 mg/mL) in a 25 mL vial and stirred for 2 h at room temperature. After that, the solution was centrifuged at 10 min at 8,000 rpm, the supernatant was collected from the vial, and the antibody-coated nanoparticles were dissolved in 10 mL of PBS solution (0.1 M) and stored at 4°C.

2.5 Quantification of OTC in samples

To 50 µL of 0.11 mg/mL anti-OTC antibody-conjugated fluorescent magnetic/silica nanoparticles, 50 µL serially diluted OTC samples at concentrations ranging from 5.5×10^{-3} to 8.25×10^{-11} (M) were added to an Eppendorf tube and agitated for 20 min (speed: 250 rpm/min) before being kept under magnetic separators to remove unbound OTC. The fluorescence quenching of capture probe fluorescent nanoparticles during interaction with OTC was studied (excitation (λ_{ex}) 455 nm; emission (λ_{em}) 600 nm). To create the sandwich complex, the anti-OTC-conjugated fluorescent magnetic silica/OTC complex was blocked with 10 mL of 2% BSA solution and magnetically separated for 30 min. Secondary fluorescent hybrid silica nanoparticles containing anti-OTC antibodies (50 µL at 0.12 mg/mL) were added and stirred for 20 min. Finally, the sandwich complex was magnetically separated and washed with PBS buffer. The fluorescence of anti-OTC-coupled rhodamine 6G and silica particles was measured (525 nm excitation; 550 nm emission), and the calibration curve was plotted as OTC concentration versus fluorescence intensity.

2.6 Comparative analysis: ELISA vs developed method

A reference ELISA was used to compare the generated assay. About 100 µL of anti-OTC antibodies (capture antibody diluted 1:5,000 in a carbonate buffer to a final concentration of 1 µg/mL) were coated in microplate wells. After covering the plates with sticky sealing tape, they were kept at 4°C for the whole night in a plastic bag containing wet paper towels. The following day, plates were gently agitated for 5 min while being washed three times with 300 µL of Wash buffer per well in an orbital shaker. By dumping into a sink and blotting with paper towels, the buffer was eliminated. After adding 300 µL of blocking buffer to each well, the plates were capped and left to stand at room temperature for 2 h while being stirred. In order to add them to the plate, the normal OTC samples were created. For the test, working dilutions of 8.20×10^{-11}–1.10×10^{-2} (M) OTC were utilized. After the blocking stage, 100 µL of each standard and sample were added to the plate in triplicate after the plates had been cleaned three times using the Wash buffer as previously mentioned. The only solution used as a blank was the Blocking buffer. The plates were shaken during the 2-h RT incubation period. Following that, the plates were poured into a sink and cleaned three times with Wash buffer. Following the emptying and washing of the above

solution, 100 µL of anti-OTC–HRP (HRP = horseradish peroxidase) was added to each well. (The mixture was diluted 1:100,000 in PBST-BSA to a final concentration of 5 ng/mL.) Plates were sealed, shielded from light, and incubated with agitation at room temperature for 1 h. Each well was added with 100 µL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate following the removal of the anti-OTC–HRP solution and washing. After 5 min, the reaction was halted by adding 100 µL of TMB Stop solution to each well and letting it equilibrate for an additional 5 min. After wiping the microplate's underside with 70% ethanol, the plate was read using a microplate reader at 450 nm, and the wavelength was corrected at 550 nm right away.

3 Results and discussion

Dual detection techniques were developed to identify OTC residue in milk samples. Fluorescent magnetic silica and fluorescent hybrid silica were synthesized for the test. Two fluorescent dyes, rhodamine 6G and $[Ru(bpy)_3]^{2+}$, were chosen for the dual detection test. PVP and TEOS 3D networks were developed for the hybrid fluorescent silica production based on the gelation of organic and inorganic components. PVP was easily dissolved in a water–ethanol solvent, promoting the ring opening of cyclic alkenyl monomers and polymerization between TEOS via a radical mechanism, and the binding processes were increased by heat. The water-soluble hydrogel was created, then the rhodamine 6G dye was added, and hybrid silica was formed by the sol–gel reaction, with ammonia acting as a catalyst. The developed approach yielded the most beneficial inorganic component of organic–inorganic hybrid materials with fluorescently readily changeable functional molecules, as well as a functional group for silane molecule binding. Figure 2 shows the characterizations of the particles evaluated using SEM (Figure 2a), particle size analysis (Figure 2b), FTIR spectroscopy (Figure 2c), and fluorescence emission spectroscopy (Figure 2d). DLS and SEM examinations confirmed that the hybrid fluorescent silica nanoparticles were 75–85 nm in size. The emission spectra revealed that the particles had been properly coated with rhodamine 6G dye. FTIR spectra of hybrid shell-coated rhodamine 6G nanoparticles showed characteristic bands at

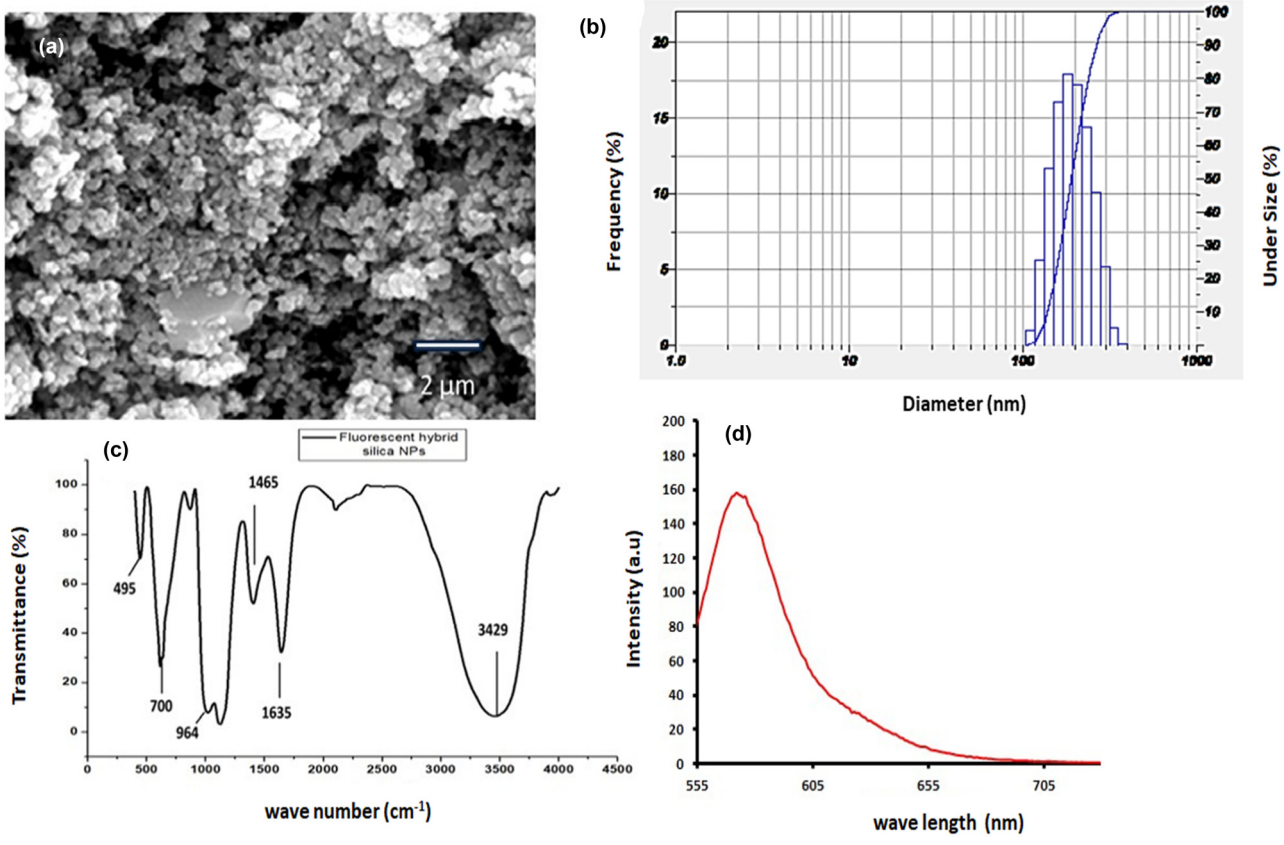

Figure 2: Shows the results of the physical characterization of the fluorescent hybrid silica nanoparticles obtained by (a) scanning electron microscopy (SEM), (b) particle size measurements using DLS, (c) FTIR surface characterization, and (d) emission spectra of the fluorescent hybrid silica nanoparticles (excitation wave length (λ) = 525 nm).

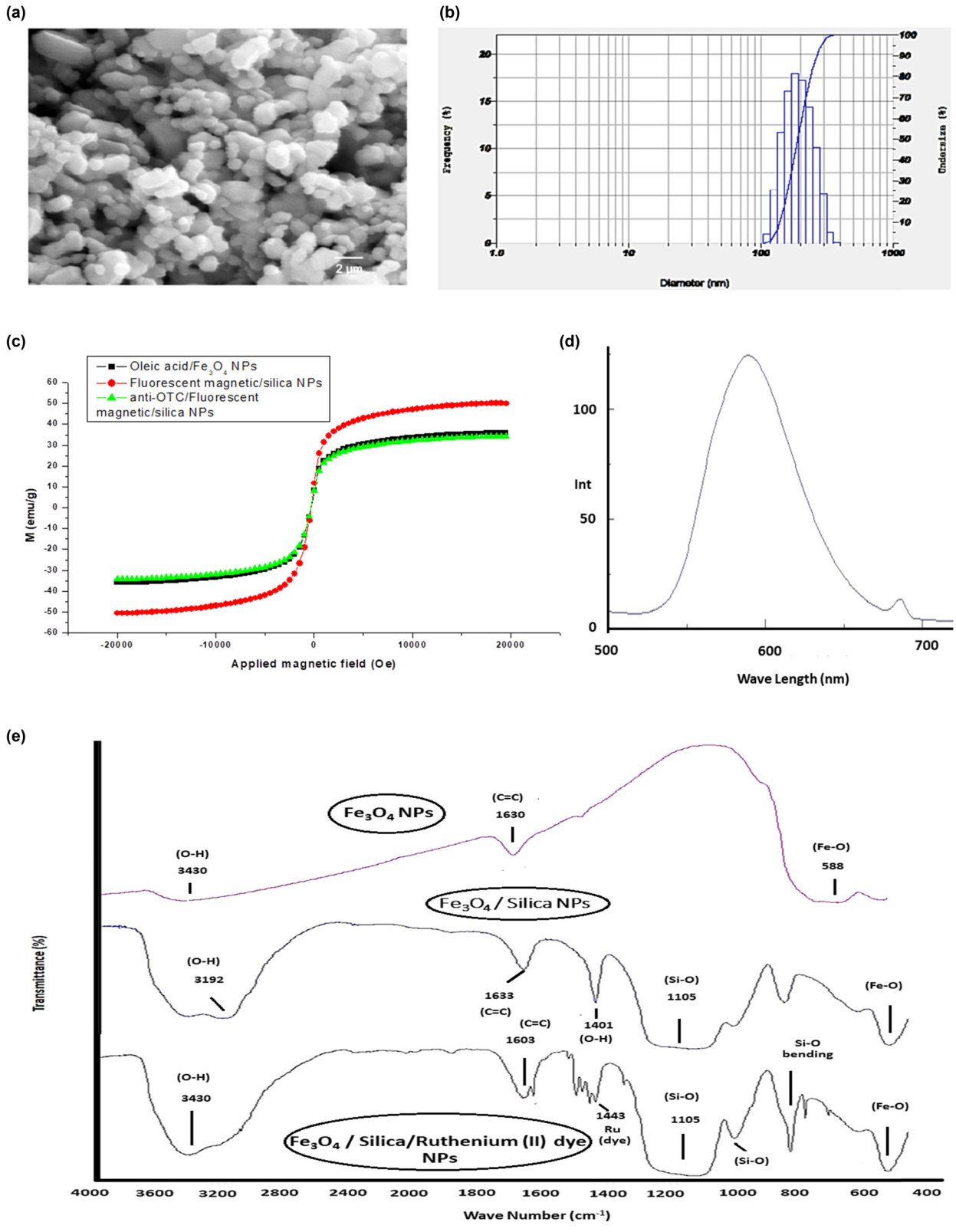

Figure 3: Characterization results of fluorescent magnetic and silica nanoparticles results of measuring particle size using DLS and scanning electron microscopy (SEM) are shown in (a) and (b). (d) Emission spectra of fluorescent magnetic/silica nanoparticles (excitation wave length (λ) = 455 nm), and (c) findings of an FTIR surface characterisation research. (e) Fluorescent magnetic/silica nanoparticle magnetization curves before and after coating with silica and over-the-counter antibodies.

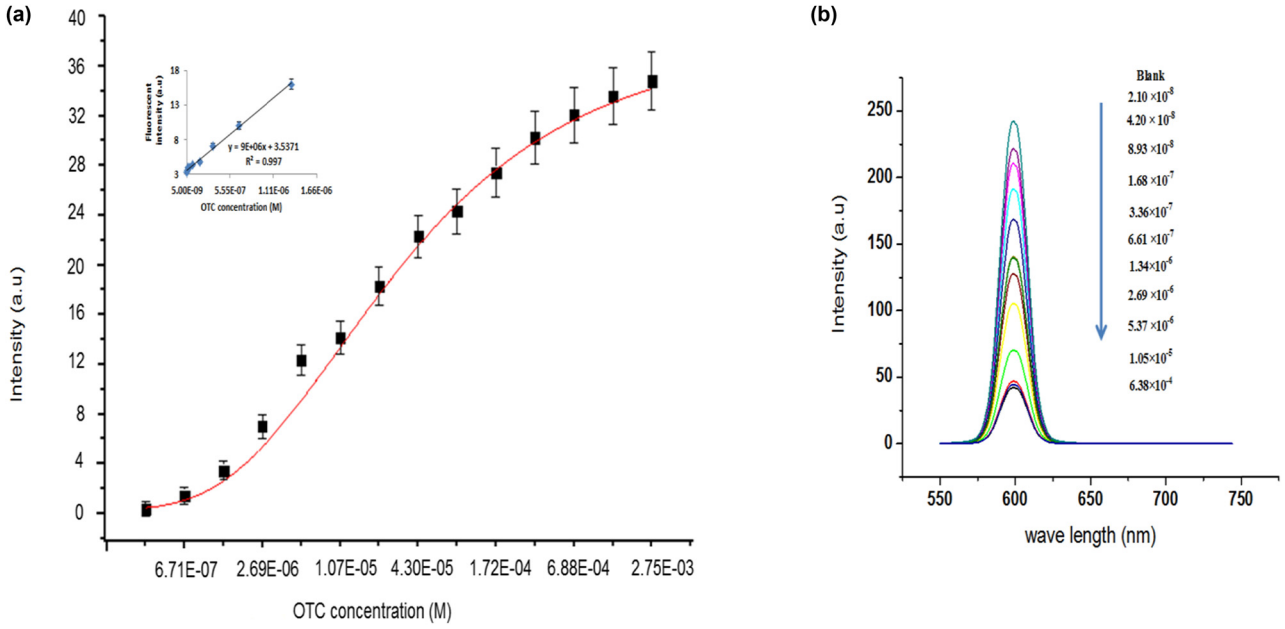

Figure 4: Results of the assay that was run (a) Sandwich testing results of OTC detected with fluorescent hybrid silica nano particles coated with anti-OTC antibodies – each data point represents the mean ± standard deviation of all three sub assays at each tested concentration (b). The anti-OTC conjugated fluorescent magnetic/silica nano particles' quenching effects on fluorescence.

3429.06 cm^{-1} (OH), 1653.69 cm^{-1} (PVP), 1465.23 cm^{-1} (Rhod), and 1082 cm^{-1} (Si–O–Si). For OTC capture, fluorescent magnetic/silica nanoparticles were produced. Using a co-precipitation method, oleic acid-coated magnetic nanoparticles were created. Their surfaces were then further changed by coating the fluorescent dye with a silica layer created using TEOS. The silica layer coating changed the polarity of the magnetic nanoparticles, and the layer made the particles hydrophilic and ideal for surface alterations, as illustrated in Figure 3. The size of the synthesized particles was examined using SEM and DLS, and the results verified that the size was around 110–120 nm (Figure 3a and b, respectively). Using the hysteresis loop, the magnetic sensitivity of the produced nanoparticles was investigated. Saturation magnetization and remanent magnetization measurements were typically used to calculate the magnetic sensitivity of the nanoparticles. The produced nanoparticles amply demonstrated that their magnetism value was roughly 20.69 emu/g, making them ideal for use in magnetic capture experiments (Figure 3c), and the emission spectra are shown in Figure 3d. FTIR spectral analysis was carried out to corroborate the particle characterization (Figure 3e). The fluorescent magnetic core–shell nanoparticles exhibited bands at 3432.54 cm^{-1} (OH), 1443.91 cm^{-1} (ruthenium(II) dye), 1088.88 cm^{-1} (Si–O–Si), and 586.75 cm^{-1} (Fe–O). The surface of the synthesized particles was further changed using APTES, which consists of three functional reactive ethoxy groups and one amine group.

Table 1: Stern–Volmer quenching kinetics

OTP concentration [*Q*] (M)	Average fluorescence	F_0/F	$F_0/F - 1$	$K_{sv} = F_0/F - 1/[Q]$
Blank	248.92			
2.10×10^{-8}	225.5	1.10	0.1	4.76×10^{6}
4.20×10^{-8}	215.2	1.16	0.16	3.73×10^{6}
8.93×10^{-8}	200.15	1.24	0.24	5.80×10^{6}
1.68×10^{-7}	170	1.46	0.46	2.76×10^{6}
3.36×10^{-7}	148.92	1.67	0.67	2.00×10^{6}
6.61×10^{-7}	125.86	1.98	0.98	1.48×10^{6}
1.34×10^{-6}	100.13	2.49	1.49	1.04×10^{6}
2.69×10^{-6}	88.68	2.81	1.81	6.72×10^{5}
5.37×10^{-6}	70.24	3.54	2.54	4.74×10^{5}
1.05×10^{-5}	49.63	5.02	4.02	3.82×10^{5}
6.38×10^{-4}	40.15	6.20	5.20	4.95×10^{5}

Through the processes of hydrolysis and condensation, the APTES molecules form amine groups and siloxane bonds (Si–O–Si) with the nanoparticles. The antibodies were bound to the surface of the nanoparticles using EDC–NHS chemistry. The primary amines in the nanoparticles react with NHS–EDC at physiological pH to generate Sulfo-NHS plus EDC (carbodiimide) crosslinking, which is notably more stable than the other intermediate. The antibodies were then conjugated. The antibody binding concentration on the surface of nanoparticles was evaluated using the 280 nm wavelength.

To mobilize the antibodies, 1.11 mg/mL anti-OTC antibodies were mixed with the nanoparticles and swirled overnight at 4°C. The unbound antibodies were then recovered by centrifugation and surface-blocked with 2% BSA solution. According to the calibration curve, the surface of the nanoparticles contains 6.25×10^{-6} mol/mg anti-OTC antibodies in fluorescent magnetic silica nanoparticles. The surface of the fluorescent hybrid silica nanoparticles contains 7.5×10^{-6} mol/mg OTC antibodies. Initially, assay parameters such as nanoparticle quantity and incubation duration were optimized for OTC detection for the assay validation. Each experiment required a maximum of 1.2 mg of anti-OTC antibody-conjugated fluorescent hybrid silica particles and 1 mg of anti-OTC antibody-conjugated fluorescent magnetic/silica nanoparticles. The anti-OTC antibody-conjugated fluorescent magnetic/silica nanoparticles required a maximum of 10 min for OTC enrichment from the samples, while the anti-OTC antibody-conjugated fluorescent hybrid silica nanoparticles required a maximum of 20 min for detection. The BSA blocking concentration was also optimized, the findings indicating that 2% BSA blocking solution is sufficient for nanoparticle surface blocking while minimizing background interferences. The OTC stock standard was created between 8.20×10^{-11} and 1.10×10^{-2} (M) to investigate the performance of the devised test. The anti-OTC antibody-conjugated fluorescent magnetic silica particles were exposed to varied concentrations of OTC for the experiment. Anti-OTC-conjugated fluorescent hybrid silica nanoparticles were used as the detection probe in the sandwich evaluation, and the results are displayed in Figure 4. The assay's linearity ranged from 1.34×10^{-6} to 2.10×10^{-8} (M) ($R^2 = 0.9954$). The minimum detectable concentration was 4.76 ng/mL, and the cumulative values (CVs) between 1.1 ng/mL and 476 µg/mL obtained for the inter-/intra-assay were 5.05, 6.05, 5.50, 6.34, 5.11, and 5.21%. The presented approach was extremely reproducible, as seen by the results, which indicated that the CVs were less than 10%. The anti-OTC antibody-conjugated fluorescent magnetic/silica nanoparticles were used as a capture probe. The antibody-conjugated nanoparticles formed the strongest non-covalent binding complex with OTC, and the complex simultaneously transferred the electrons and quenched the fluorescence. The quenching kinetics was investigated using the Stern–Volmer equation: $F_0/F = 1 + K_{SV}[Q]$, where F and F_0 stand for the fluorescence intensities measured in the presence and absence of a quencher, respectively, K_{SV} is the Stern–Volmer quenching constant, and $[Q]$ is the concentration of the quencher. Based on this, the calculated K_{SV} value is shown in Table 1. Amoxicillin was used to conduct a cross-reactivity study; the elevated concentration of amoxicillin was added to 78 µg/mL OTC and measured (Figure 4).

The results showed that the greatest changes were only 2.48%, and the assay results are displayed in Table 2. These findings revealed that the cross-reactivity in this proposed approach was negligible. For the real-sample analysis, the milk samples were spiked with different concentrations of OTC, and the assay was done; the findings showed that 100% of the spiked drug was recovered from the milk sample, as shown in Table 3. A comparative study was carried out to investigate their sensitivity and selectivity with commonly used ELISA kits. The sample analysis done in the devised approach is strongly associated with the ELISA results, as shown in Figure 5. The results revealed that the suggested approach is extremely suitable for detecting drug residues in milk samples, as well as offering miniaturization, integration, and multiplicity.

Table 2: Cross-reactivity study results (OTC concentration of 78 µg/mL was fixed as constant and different concentrations of amoxicillin were added)

Parameters	Concentration of OTC (78 µg/mL) spiked in milk samples and then mixed with different concentrations of amoxicillin (mg/mL) (n = 3)		
	305 mg/mL	156 mg/mL	78 mg/mL
Fluorescence intensity (a.u.)	20.581 ± 1.5	21.025 ± 1.5	21.890 ± 1.2
Percentage of interference	5.87	3.85	0

Table 3: Real-sample analysis results

Parameters	Concentration of OTC spiked in milk (n = 3)		
	2.5 mg/mL	312 µg/mL	78 µg/mL
Fluorescence intensity (a.u.)	34.402 ± 1.1	29.958 ± 0.86	21.806 ± 1.2
Percentage of recovery (%)	101	100.35	104.6
Percentage of interference	1.31	0	4.6

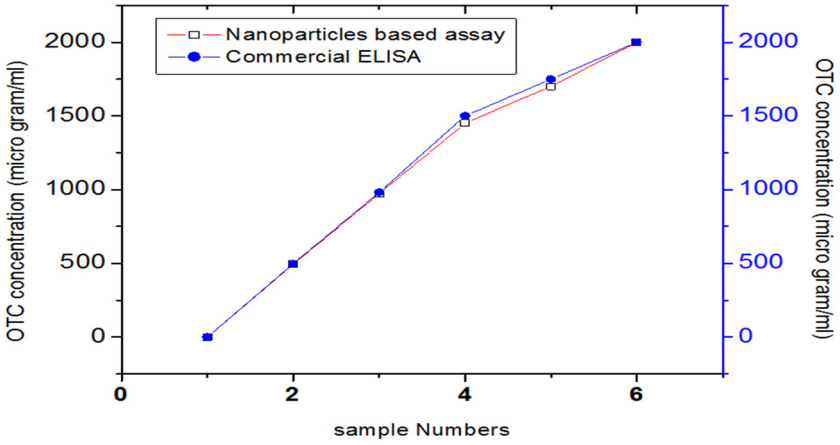

Figure 5: Correlation study findings of OTC-spiked milk samples using the suggested and commercial ELISA methods.

The proposed method is highly equivalent to existing OTC detection methods that have previously been reported such as colorimetric biosensing using the Fe_3O_4 nanoparticle assay (45 nM) [35], magnetic nanobead-based competitive enzyme-linked aptamer assay (14.7 ng/mL) [36], carbon dots and Fe_3O_4 MNP-based assay (9.5 nM) [37], magnetic nanotube-based assay (8.1 nM) [38], magnetic nanoparticle-based HPLC–UV detection system (50 µg/L) [39], magnetic nanoparticle-based immunofluorescence assay (50 ng/mL) [40], luminescent silicon-based nanoparticle-based assay (18 nM) [41], and lateral flow assay (5 ppb) [42], among which the low detection limit was exhibited by the colorimetric aptasensor (6.92 nM) [43] and HPLC-photodiode array detector (0.062 µg/g) [44]. Fluorescence assays utilizing single-emission probes are widely employed in diverse biochemical techniques. The primary determinant of assay efficiency in these approaches is the fluorescence intensity signal. However, the fluorescence intensity of the detection probe can be readily impacted by an array of external factors, such as the probe concentration, excitation light intensity, detection environment, and equipment efficiency. When compared to the single-mode detection method in this instance, the dual-mode detection method can eliminate the fluctuations in fluorescence intensity brought about by the previously mentioned factors because the capture probe used in the dual detection method is fluorescent, enabling self-calibration (in situations where knowledge of absolute concentrations is necessary) while retaining favourable selectivity, high sensitivity, and results that are visible to the naked eye. The capture probe conjugated fluorescent magnetic nanoparticles after bound with the specific analyte, and the fluorescence signal was quenched by the OTC, and it varied based on the concentration of the analyte and its rate was studied using the Stern–Volmer quenching kinetics equation. This technique has the advantage that the signal ratio is independent of the donor and acceptor absolute concentrations, in contrast to the approach that uses a quencher as the acceptor (capture probe). The capture probe fluorescence changes help to understand specific analyte binding, but in the sensors and other commonly used assays it needs specific approaches. This assay took less assay time than ELISA for OTC detection when compared to the standard ELISA approach. On the other hand, the newly developed test only needed a short incubation time (90 min) to achieve these limits, whereas the ELISA required extensive incubation times (~6 h). With no enzymatic step, a stable signal, an excellent signal-to-noise ratio, a flexible platform, and a short incubation time, this new immunoassay is incredibly sensitive. With no extra tools or expertise needed, the test format is simple. For high-throughput screening, it works better, much like ELISA.

4 Conclusions

A dual fluorescent nanoparticle system was created in this study to identify OTC residues in milk samples utilizing particular antibody-conjugated fluorescent nanoparticles. According to the results, the approach was very sensitive and selective for OTC in milk samples. The nanoparticles provided a high volume surface for antibody mobilization and were useful for OTC enrichment. The dual fluorescent probe allowed for simultaneous measurement of the capture and detection probes, and their fluorescence variations were very detectable after OTC binding. The external magnetic separation cut the assay time in half, and the secondary nanofluorescent probe was ideal for detecting OTC at low concentrations. As a result, the dual immunofluorescence approach detecting OTC residues in milk was effective, quick, and promising of parallel processes for additional antibiotic detection in milk samples.

Acknowledgements: The Researchers Supporting Project number (RSP2024R114) of King Saud University in Riyadh, Saudi Arabia, is acknowledged with gratitude from the authors.

Funding information: The Researchers Supporting Project number (RSP2024R114) of King Saud University in Riyadh, Saudi Arabia, is acknowledged with gratitude from the authors.

Author contributions: Viswanathan Kaliyaperumal – methodology, software, validation, original draft preparation, review and editing supervision, visualization and project in charge. Oleyan Al-Otibi – project administration, report preparation, over all supervision and funding acquisition. Ruth Sophila John and Raedah Ibrahim Alharbi. formal analysis, data curation and investigation, Dhinakar Raj Gopal – Study director and review and editing the reports, All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare that none of the work reported in this study could have been influenced by any known competing financial interest or personal relationships.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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