RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2025, **15**, 25404

A colorimetric probe based on peroxidase-like activity of CuAl-LDH/agarose nanocomposite hydrogel for determination of vancomycin in exhaled breath condensate

Zahra Karimzadeh,[a] Aylar Keyvani,[b] Vahid Jouyban-Gharamaleki,[cd] Maryam Khoubnasabjafari[ef] and Elaheh Rahimpour *[b]

Background: Vancomycin is a glycopeptide antibiotic used to treat certain bacterial infections. It works by interfering with RNA synthesis, altering cell membrane permeability, and inhibiting peptidoglycan synthesis in the cell wall. The recommended therapeutic concentration of vancomycin in plasma and serum is 5.0–40 μg mL^{-1}, reflecting a narrow therapeutic window that can lead to toxic side effects and bacterial resistance if the concentration is too high or too low. Monitoring vancomycin in a low-cost and convenient way is significant in administering accurate dosages of vancomycin to prevent the development of bacterial resistance. *Results*: Herein, a colorimetric nanoprobe was established to detect vancomycin in exhaled breath condensate samples. This platform incorporated agarose hydrogels with nanozymes to initiate specific reactions and produce colorimetric signals. CuAl-layered double hydroxide was used as nanozyme, which exhibits peroxidase-like activity accelerating the oxidation of the colorless compound 3,3′,5,5′-tetramethylbenzidine (TMB) to the blue product oxTMB in the presence of hydrogen peroxide. The detection mechanism of vancomycin was its interaction with Cu in CuAl-LDH/agarose, inhibiting the oxidation process of TMB. The inhibiting effect of vancomycin led to a significant decrease in the analytical response across a concentration range of 0.04–0.3 μg mL^{-1}. The inter- and intra-day relative standard deviations of the nanoprobe were recorded at 1.2% and 4.4%, respectively. This proposed system was effectively employed to quantify vancomycin levels in patients receiving the medication, with samples collected from the expiratory circuit of a mechanical ventilator. *Significant*: As a result, LDH/agarose nanocomposite catalyzed H_2O_2 : TMB system's benefits of simplicity, versatility, and cost-effectiveness make this platform a promising tool for colorimetric detection of vancomycin in clinical settings. Notably, this sensing platform can be customized for optical assays targeting different molecules by altering the encapsulated substances within the hydrogel.

Received 19th May 2025
Accepted 8th July 2025

DOI: 10.1039/d5ra03521j

rsc.li/rsc-advances

1. Introduction

Vancomycin is a glycopeptide antibiotic frequently employed in the treatment or prevention of infections caused by a range of Gram-positive bacteria, particularly methicillin-resistant *Staphylococci*, as well as in individuals who are allergic to beta-lactam antibiotics.[1] The antibacterial action of vancomycin is achieved through several mechanisms of interfering with RNA synthesis within the cytoplasm, changing the cell membrane permeability, and inhibition peptidoglycan synthesis by avoiding the addition of *N*-acetylmuramic acid and *N*-acetylglucosamine to the peptidoglycan in the cell wall. According to the literature, vancomycin's therapeutic concentration range is 5.0–40 μg mL^{-1} in plasma and serum samples. This therapeutic efficacy indicates a narrow therapeutic window which is associated with toxic side effects caused by high concentration and bacterial resistance caused by insufficient drug concentration.[2] Therefore, to maintain effective therapeutic ranges and personalized dosage, monitoring the therapeutic drug is crucial in biological samples.[3] Numerous analytical methods have been employed to determine vancomycin in biological and pharmaceutical samples, including

[a]*Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
[b]*Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. E-mail: rahimpour_e@yahoo.com*
[c]*Kimia Idea Pardaz Azarbayjan (KIPA) Science Based Company, Tabriz University of Medical Sciences, Tabriz, Iran*
[d]*Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[e]*Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Science, Tabriz, Iran*
[f]*Department of Anesthesiology and Intensive Care, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

capillary electrophoresis,[4] chromatographic techniques such as liquid chromatography (LC)-mass spectrometry (MS),[5,6] HPLC-UV,[7,8] UV spectrophotometry,[9] spectrofluorimetry, and chemiluminescence.[10] Although all of these techniques exhibit advantages and drawbacks, developing a sensitive, accurate, and simple technique for the quantification of an analyte in biological matrices is a great challenge for researchers. The colorimetric assay possesses distinct superiorities in visualization, low cost, and simple operation which is an appropriate candidate for vancomycin detection.

An emerging category of nanomaterials with enzyme-like features known as nanozymes, has recently garnered significant interest. Compared to natural enzymes, nanozymes have been considered highly suitable for the development of colorimetric sensors due to their cost-effectiveness, exceptional stability, and notable catalytic efficiency.[11] There are several reports in literature for using nanozyme catalyzed systems for sensing goals such as determination of some pesticides using Pt nanozyme catalyzed colorimetric system,[12] determination of acetylcholinesterase activity and adrenaline using single-atom Rh nanozyme catalyzed colorimetric system,[13] determination of aspirin using sodium dodecyl sulfate modified silver nanoparticles catalyzed colorimetric system,[14] determination of chlordiazepoxide using UiO-66/Au NPs-polyvinyl alcohol nanocomposite hydrogel catalyzed colorimetric system[15] *etc.* which confirming their popularity1in analysis field.

Among different nanomaterials including, carbon-based nanomaterials, noble metals, layered double hydroxides (LDHs), and transition metal oxides, LDH represents a novel category of two-dimensional (2D) layered materials with the formula of $([M_{1-x}^{2+}M_x^{3+}(OH)_2]_x^+(A^{n-})_{x/n}\cdot mH_2O)$ that incorporate divalent (M^{2+}) and trivalent (M^{3+})metal ions located on the main laminate.[16] Here, x, m, and A^{n-} are the molar ratios of $M^{3+}/(M^{2+}+M^{3+})$, several interlayer water molecules, and an interlayer anion, respectively.[17] This class of materials has achieved considerable attention owing to its remarkable peroxidase-like activity and straightforward synthesis. Compared to monometallic catalysts, LDHs benefit from the synergistic effects of various components, resulting in promising nanozymes with improved catalytic performance.[18,19] Nevertheless, the minimal mechanical resistance during the reprocessing, dispersion, and low stability has led to the introduction of suitable supports to enhance their efficiency. An effective way to increase LDH stability and dispersion includes loading them in hydrogel matrices.[20] Hydrogels are three-dimensional (3D) polymers featuring various functional groups and biodegradability. The capability of holding a high amount of water besides the structural stability, has moved natural polymers with hydration properties to the forefront. Mixing LDH with natural polymers can fabricate nanocomposite hydrogel with enhanced and unique properties.[21]

In this research, we aim to design an easy, environmentally friendly, highly dispersed, and biocompatible nanocomposite hydrogel with an enzyme mimetic property utilizing copper aluminum (CuAl)-LDH as an efficient catalyst and agarose as a natural polymer. The embedded CuAl-LDH hydrogel enabled the reaction between hydrogen peroxide (H_2O_2) and 3,3,5,5-tetramethylbenzidine (TMB), serving as an optical nanoprobe for the detection of vancomycin in exhaled breath condensate (EBC) samples. EBC serves as an alternative biological sample that presents fewer interfering substances compared to urine, blood, and plasma matrices in drug detection.[22]

2. Experimental

2.1. Reagents and solutions

Analytical-grade materials were used in this work. For that, H_2O_2, and TMB, were received from Merck (Germany). For synthesizing LDH/agarose hydrogel, copper(II) nitrate trihydrate ($Cu(NO_3)_2\cdot 3H_2O$), aluminum nitrate nonahydrate ($Al(NO_3)_3\cdot 9H_2O$), urea, and agarose were sourced from Sigma-Aldrich. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were achieved from Scharlau (Barcelona, Spain) for adjusting different pHs. Double-distilled deionized water and vancomycin were reached from Ghazi and Dana Pharmaceutical Company (Tabriz, Iran), respectively.

2.2. Instruments and apparatuses

Shimadzu UV-1800 spectrophotometer fitted with a micro quartz cell was used for measuring the UV-vis absorption spectra (Shimadzu, Japan **https:\\www.shimadzu.com**). To examine the morphology and distribution of the synthesized nanocomposite hydrogel, a CM30 transmission electron microscope (TEM) and dynamic light scattering (DLS) apparatus (Philips, Leiden, The Netherlands) were employed. X-ray powder diffraction (XRD) data were obtained in the 2θ range of 4–60° utilizing a Siemens diffractometer (Tongda Co., China) equipped with filtered Cu-Kα radiation at 35 kV. Additionally, Attenuated total reflectance-Fourier transforms infrared (ATR-FT-IR) spectroscopy was accomplished on Bruker Tensor 270 to verify the chemical bindings.

2.3. Synthesis of CuAl-LDH based hydrogel

CuAl-LDHs were synthesized using a straightforward hydrothermal method.[23] In this process, $Cu(NO_3)_2\cdot 3H_2O$ (380 mg), urea (60 mg), and $Al(NO_3)_3\cdot 9H_2O$ (150 mg) were thoroughly dissolved in 20 mL of deionized water while being vigorously stirred at room temperature. The resultant mixture was subsequently placed into a Teflon-lined stainless-steel autoclave and held at a temperature of 130 °C for 8 hours. Following cooling to room temperature, the product was washed with deionized water and ethanol after centrifugation. Then, the obtained product was dried in a vacuum oven overnight before use. The incorporation of synthesized CuAl-LDH into a polymeric network was achieved through a post-synthetic approach within agarose matrices. For that, 33.4 mg of agarose was dissolved in 50 mL of water and heated to 90 °C for 30 minutes. Subsequently, 10 mg mL^{-1} of LDH solution was introduced into the mixture. Following the vigorous stirring, the resulting product was then allowed to cool to room temperature to form a nanocomposite hydrogel. The resultant product was stored at 4 °C and diluted with deionized water for confirming experiments.

2.4. Samples preparation

EBC does not necessitate any sample pretreatment or preparation for the analysis procedure of the collected samples as they are highly diluted and low-protein aqueous matrix. The samples of EBC were gathered using a laboratory-designed apparatus with no preparation step.[22] Optimization and calibration processes were conducted utilizing samples from healthy volunteers. To assess the effectiveness of the procedure, the used EBC samples were sourced from four patients undergoing treatment with vancomycin. Before collecting samples, all donors provided informed consent by signing a form that was approved by the ethical committee of Tabriz University of Medical Sciences, referenced as IR. TBZMED.REC.1402.859.

2.5. General procedure

A batch analysis method was employed in a 2 mL microtube. Initially, 10 μL of a 0.1 mol L^{-1} phosphate buffer (pH 5.0) was introduced into the microtube comprising either EBC of patients or EBC of healthy people spiked with standard vancomycin. Subsequently, 80 μL of the CuAl-LDH/agarose nanocomposite hydrogel, 110 μL of H_2O_2 (8.75% v/v) and 55 μL of TMB (0.33 mmol L^{-1}) were added. The resulting solution was then diluted to a final volume of 500 μL and the analytical response was immediately recorded by measuring the absorbance at 650 nm.

3. Results and discussions

3.1. Choice of probe for vancomycin determination

The demand for efficient, quantitative, and rapid drug monitoring has driven the need for advanced materials. To identify the optimal probe for target analyte sensing, we evaluated LDH alone, and its composite with hydrogel. As can be seen in Fig. 1A, with similar nanozyme amount, a 1.5-fold difference in catalyzed system intensity was observed when using the LDH/agarose nanocomposite hydrogel. Notably, the LDH/agarose nanocomposite exhibited superior system intensity stability in compared to alone LDH as can be seen in Fig. 1B. Consequently, LDH/agarose nanocomposite hydrogel was selected as the probe for vancomycin determination in the next experiments.

3.2. Characterization of the CuAl-LDH/agarose hydrogel

To assess the morphology and particle size of the nanozymes, TEM was employed. According to Fig. 2A(I), CuAl-LDH have been relatively uniformly distributed with a flower-like shape with an average particle size of less than 80 nm. With the size distribution histogram of the nanomaterial achieved from TEM images, the nanoparticles size was found to be around 43.5 ± 3.1 nm (Fig. 2A(II)). Additionally, zeta potential measurements revealed a negative zeta potential of −27.5 mV for CuAl-LDH/agarose hydrogel. This negative zeta potential can be attributed to the interplay of the functional groups from the LDH and the agarose contributing an overall negative zeta potential observed for the nanocomposite hydrogel.

To show the crystalline structure of the nanocomposite and confirm the successful integration of CuAl-LDH into the agarose hydrogel matrix, XRD analysis was conducted on the prepared samples and shown in Fig. 2B. The XRD pattern of the CuAl-LDH/agarose hydrogel displayed clear peaks at the 2θ angle of 10.12° and 20.46°, which correspond to the pattern of the CuAl-LDH reported in the ref. 23. Furthermore, the pattern also exhibited a peak at a 2θ angle of 19.0°, indicating the semi-crystalline nature of agarose hydrogel.[24] Fig. 2B presented the XRD patterns for pure agarose, CuAl-LDH, and the CuAl-LDH/agarose hydrogel, confirming the successful synthesis of the nanocomposite.

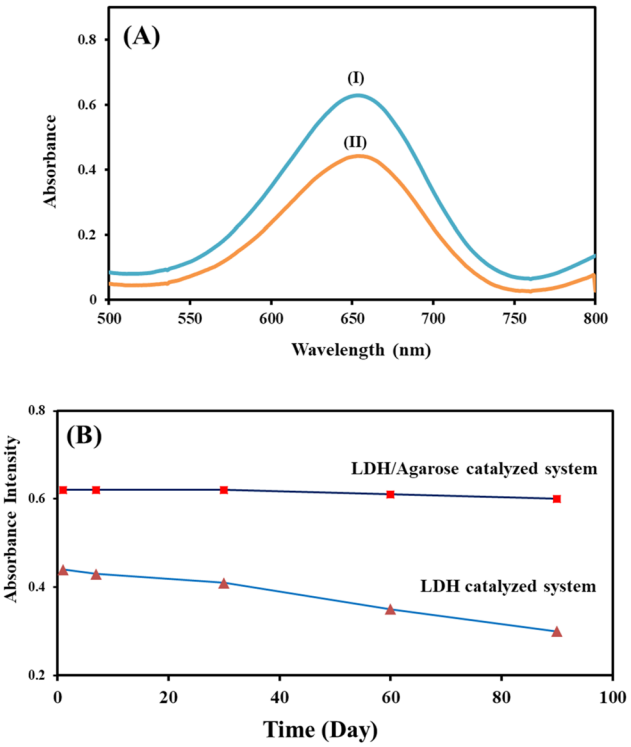

Fig. 1 (A) The absorbance spectra of (I) LDH catalyzed TMB + H_2O_2 system and (II) LDH/agarose nanocomposite hydrogel catalyzed TMB + H_2O_2 system and (B) stability study of the TMB + H_2O_2 system intensity in the presence of LDH with/without agarose.

3.3. Probe response toward vancomycin

The performance of the synthesized CuAl-LDH/agarose with peroxidase-like activity in the system of TMB:H_2O_2 has been studied, after confirming the successful synthesis. Furthermore, the response of the system was examined with the different concentrations of vancomycin. The chromogenic reaction of TMB : H_2O_2 showed a weak absorbance in the absence of a catalyst suggesting that the oxidation process could not take place (Fig. 3I). In the presence of CuAl-LDH/agarose as an efficient nanozyme, TMB oxidation was efficiently induced by H_2O_2 and the absorbance peak was significantly enhanced at 650 nm, signifying the outstanding catalytic ability of CuAl-LDH/agarose nanocomposite (Fig. 3(II)). In the initial investigations, it was found that vancomycin can decrease the absorbance of H_2O_2 : TMB: LDH/agarose system (Fig. 3(III)). The possible interaction could be due to the hydrogen bonds of

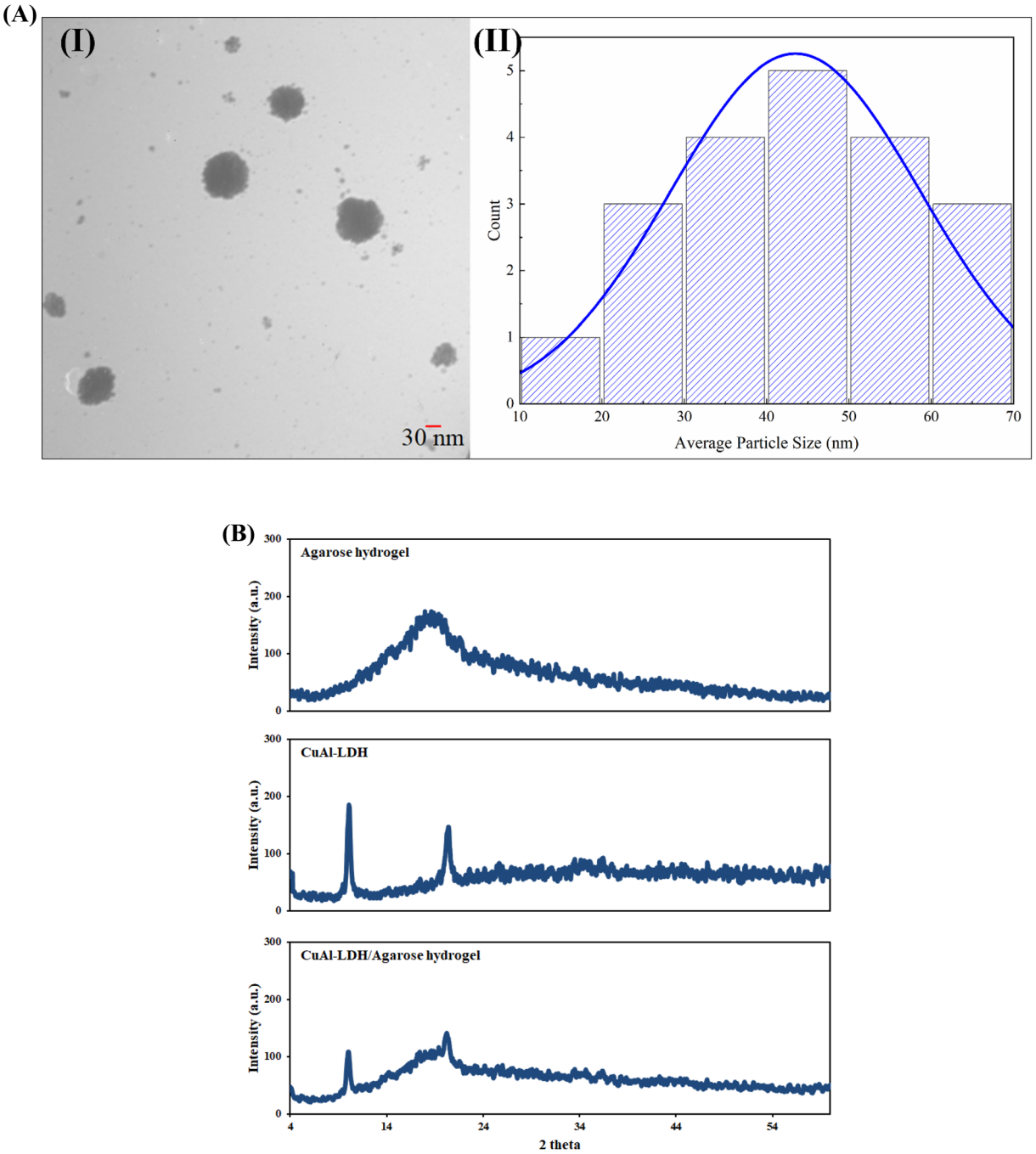

Fig. 2 Characterization analyses (A) TEM image (I) and size distribution (II) of CuAl-LDH/agarose hydrogel, and (B) XRD pattern of pure agarose, CuAl-LDH, and the CuAl-LDH/agarose hydrogel.

hydroxyl and amino groups in vancomycin with the surface hydroxyl groups or metal sites of CuAl-LDH. Moreover, according to previous reports,[25] vancomycin could coordinate with Cu ions in CuAl-LDH/agarose nanocomposite, inhibiting the oxidation process of TMB. This interaction was also proved by ATR-FT-IR. As can be seen in Fig. 4, the agarose peak at 1637 cm^{-1} related to HOH bending band showed a slight shift after interaction with vancomycin with CO stretching in this region. Moreover, in the presence of vancomycin, two new peaks were appeared at 2063 cm^{-1} (CC stretching) and 745 cm^{-1} (aromatic CH bending), corresponding to the structure of vancomycin, in the mixed sample. This confirmed the influence of vancomycin on the functional groups of the hydrogel. Therefore, this system can be utilized for the development of sensitive probe for vancomycin sensing in biological samples.

3.4. Optimizing reaction conditions

To obtain a sensitive system, the influence of diverse parameters such as pH, amount of H_2O_2 and TMB, the volume of nanozyme, and incubation time were investigated. The

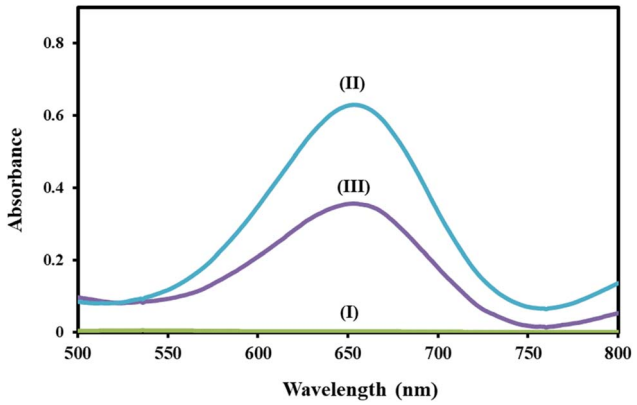

Fig. 3 The absorbance spectra of (I) TMB + H_2O_2 (II) LDH/agarose catalyzed TMB + H_2O_2 system and (III) LDH/agarose nanocomposite hydrogel catalyzed TMB + H_2O_2 system in the presence of vancomycin.

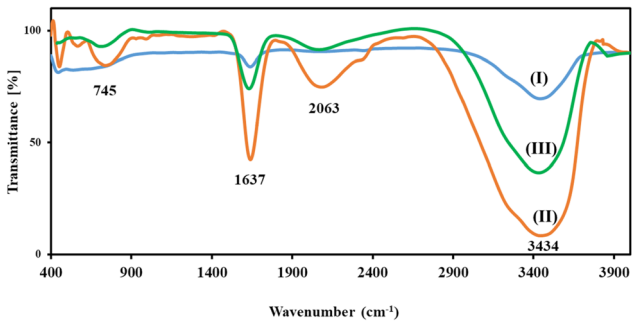

Fig. 4 The ATR-FT-IR spectra of (I) LDH/agarose hydrogel (II) vancomycin and (III) LDH/agarose hydrogel + vancomycin.

analytical response was determined by the variation in absorbance intensity observed with (I) and without (I_0) the addition of vancomycin ($\Delta I = I - I_0$). As shown in Fig. 5A, the optimal pH for the vancomycin sensing was determined to be 5.0. At this pH, positively charged vancomycin ($pK_a = 7.75$) can electrostatically interact with negatively charged CuAl-LDH inside the agarose matrix. Another critical parameter examined was the concentration of H_2O_2 and TMB, which served as redox reagents. These parameters were analyzed based on the concentration of TMB to H_2O_2. As can be seen in Fig. 5B and C, the highest response was obtained with 1.92% v/v of H_2O_2 and 0.33 mmol L^{-1} of TMB. The investigation on the influence of CuAl-LDH/agarose nanocomposite volume revealed that the response of the system enhanced as its amount increased up to 80 μL. However, at higher amounts, the signal stabilized and exhibited minimal variation. Consequently, 80 μL of CuAl-LDH/agarose was employed in subsequent experiments (Fig. 5D). Furthermore, to achieve optimal sensing performance, the impact of contact time between vancomycin and the nanozyme catalyzed system was examined. As illustrated in Fig. 5E, the response decreased over time, in which immediate measurement was performed in the next experiments.

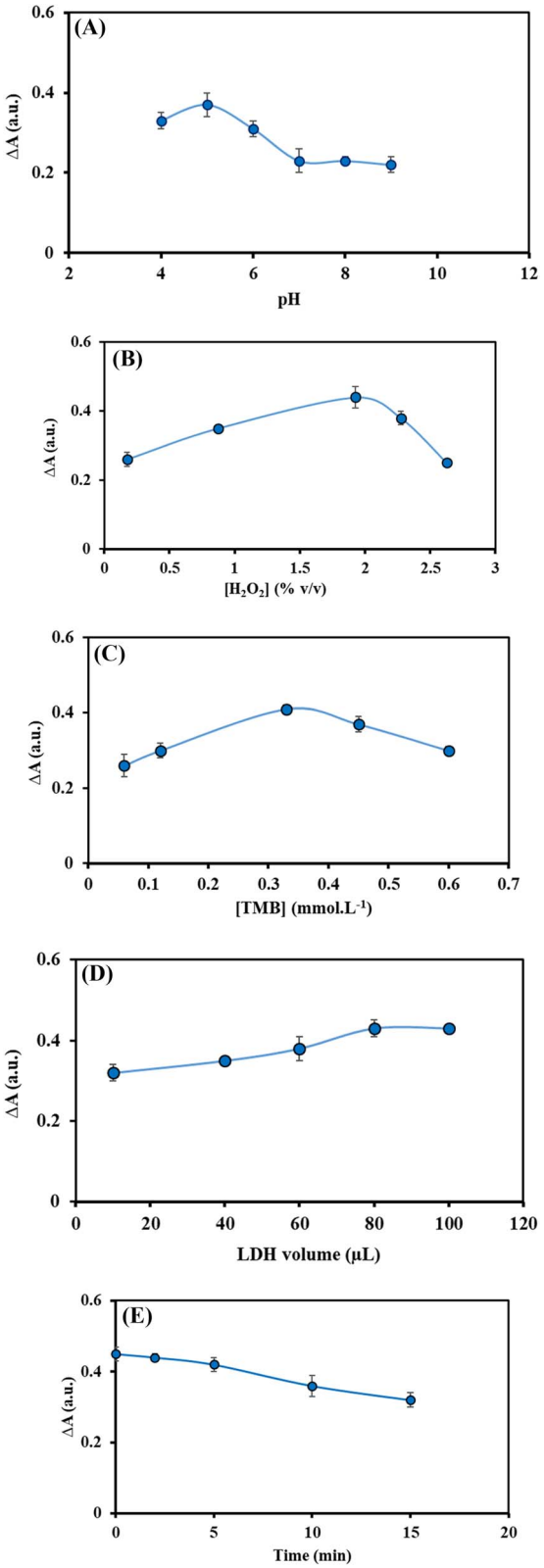

Fig. 5 Effect of pH (A), the concentration of H_2O_2 (B), TMB (C), CuAl-LDH/agarose nanocomposite (D), and time (E) on the response of the proposed probe.

3.5. Analytical figures of merit

As described above, the reaction of LDH/agarose nanocomposite catalyzed H_2O_2 : TMB system was inhibited in the presence of vancomycin. To study the linearity, various standard concentrations of vancomycin in EBC samples were prepared and analyzed under optimal conditions. The findings indicate a robust correlation, with a correlation coefficient of 0.9959, between the analytical response and the concentration of vancomycin within the range of 0.04–0.3 μg mL^{-1} (Fig. 6). The regression equation established was $\Delta A = 1.6755\ C + 0.0519$, where ΔA signifies the analytical response derived from the difference in absorbance intensity in the presence and absence of vancomycin, and C signifies the vancomycin's concentration in μg mL^{-1} unit. The limit of detection (LOD) was determined to be 0.009 μg mL^{-1}, calculated as $3S_b/m$. The LOD is established on the principle that the detection limit corresponds to a signal that is distinguishable from the blank by a certain factor, typically three times the standard deviation of the blank (S_b). The value of S_b is obtained by assessing the absorbance signal of five blank samples (which do not contain vancomycin) under optimum experimental conditions. The standard deviation of these blank readings is then calculated, demonstrating the intrinsic noise or variability of the method in the absence of the analyte. Conversely, the slope of the calibration curve is derived by plotting the system response (ΔI) against varying concentrations of vancomycin. This slope (m) signifies the method's sensitivity; a steeper slope denotes a more sensitive method, where minor concentration variations lead to significant changes in the instrument's response. The factor of three in the numerator is a standard criterion for establishing a dependable detection limit, grounded in statistical principles that suggest that at three times the standard deviation of the blank, the signal is unlikely to arise from random noise, thereby confirming the detection's significance.

To study the system's precision, intra- and inter-day of 0.2 μg mL^{-1} vancomycin was performed by the relative standard deviations (RSD) from replicated measurements ($n = 5$). The results exhibited 1.2% and 4.4%, respectively. Furthermore, RSD% for measurement from different experimenters was obtained 8.2%.

3.6. Investigation of interferences

To evaluate the specificity of the suggested technique for the target analyte, the study was conducted in optimum conditions in the existence of other laboratory-available over-the-counter or co-administered medications, including nicotinamide, clonazepam, celecoxib, diltiazem, ibuprofen, pantoprazole, diazepam, paracetamol, gentamicin, naproxen, tobramycin, amikacin, and caffeine. The system's response was examined with concentrations of each pharmaceutical five times greater than that of the target analyte. The results in Fig. 7, clearly indicated that the tested compounds (except for gentamicin, tobramycin, and amikacin) do not have a significant impact on the probe's response. These outcomes demonstrated a high level of selectivity for the precise determination of vancomycin. About the interfered pharmaceuticals of gentamicin, tobramycin, and amikacin, it should be noted that the validated method may not be enough precise in their presence. Given the structural similarity of vancomycin to such drugs, this interference may arise from competition for binding sites on the CuAl LDH hydrogel due to the inherent chemical properties of these aminoglycoside antibiotics. The functional groups such as

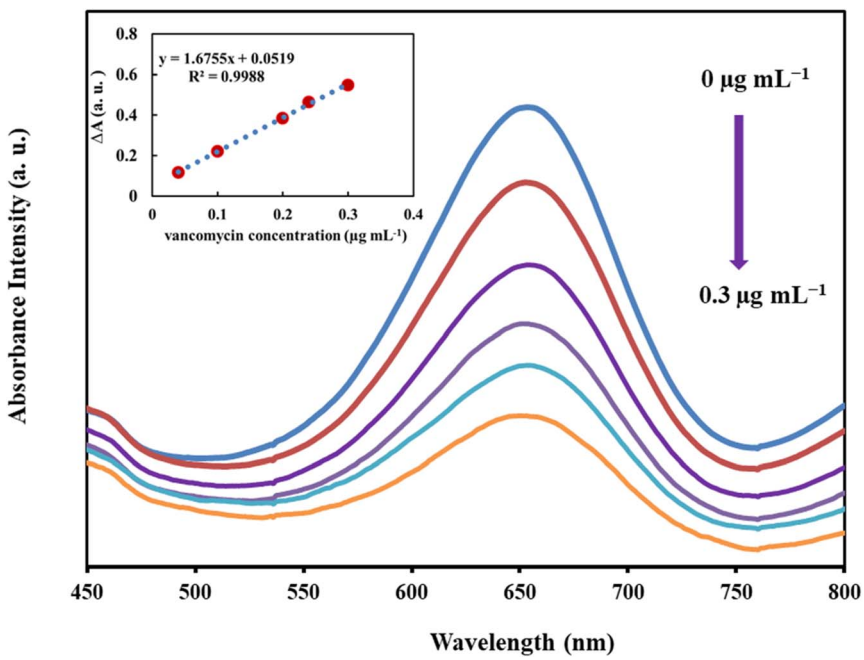

Fig. 6 Absorbance spectra of LDH/agarose nanocomposite catalyzed H_2O_2 : TMB system in the absence and presence of vancomycin within the range of 0.04–0.3 μg mL^{-1} (inset: calibration curve).

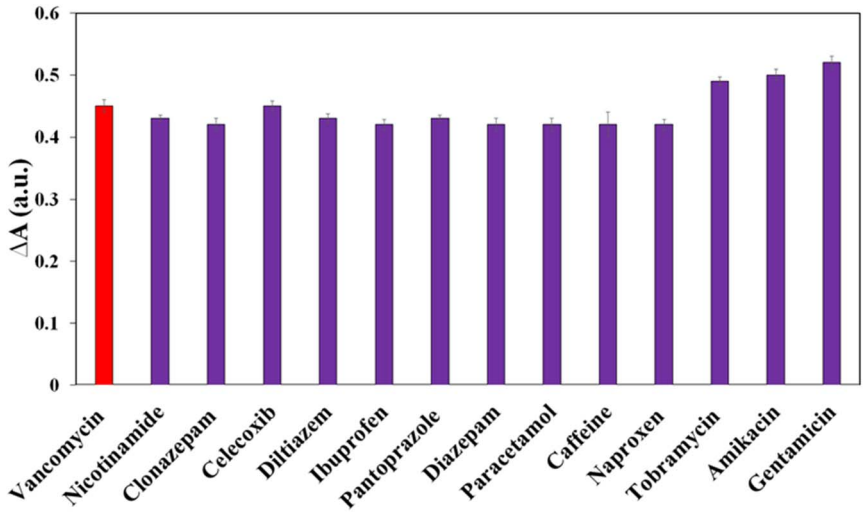

Fig. 7 The selectivity study of the method in the presence of interferences (some possible over-the-counter or co-administrated drugs). Vancomycin concentration was 0.2 μg mL^{-1} and the interfering compounds were 1.0 μg mL^{-1}.

Table 1 The comparison of the current approach with alternative methods

Method	Sample	LOD (μg mL^{-1})	Linear range (μg mL^{-1})	Refs
LC-MS	Human serum	0.001	0.05–10	26
UHPLC-UV	Human serum	—	2.5–120	8
UHPLC-MS/MS	Human plasma	—	1–100	27
Immunoassay method	Cerebospinal fluid	0.4	0—15	28
Spectrofluorimetry based on NSPCl-doped CDs	EBC	0.005	0.01–2	29
Synchronous spectrofluorimetry based on copper nanocluster	EBC	0.06	0.1–8	25
UV-spectrophotometric	Pharmaceutical preparations	—	20—100	30
Spectrophotometry	Human serum	0.99	12.5–200	31
Spectrophotometry	Lyophilized powder	0.72	50—150	32
LDH/agarose nanocomposite catalyzed H_2O_2 : TMB spectrophotometry system	EBC	0.009	0.04–0.3	This work

hydroxyl and amino groups present in the structure of these antibiotics can interact with the CuAl LDH hydrogel and lead to competition with vancomycin for available binding sites on the hydrogel, thus affecting catalytic proficiency and diminishing signal intensity. Consequently, these factors together decline the accuracy of vancomycin quantification. To address these interferences, the implementation of separation techniques such as microfluidic devices or immunoaffinity-based separation may enhance accuracy in the presence of these interfering drugs. This approach could be explored in future work to enhance the specificity of the probe. Moreover, incorporating a multicomponent or multiplexed detection system could allow for the simultaneous detection of multiple antibiotics, thereby differentiating vancomycin from other antibiotics based on their unique interaction profiles.

3.7. Comparison of the present method with other reported methods

The evaluation of the current technique with alternative methods was presented in Table 1. This Table summarized the analytical characteristics of each system, offering insights into their effectiveness in the determination of vancomycin. It is clear that the current method demonstrated a satisfactory and comparable performance toward vancomycin comparing other methods.

3.8. Real samples analysis

For evaluating the method's applicability, the validated approach was employed to measure vancomycin levels in MVEBC of four real samples collected from premature or newborn babies receiving vancomycin under mechanical ventilation. A summary of the results was provided in Table 2. To determine the method's accuracy, a recovery was conducted by adding some known quantity of vancomycin (0.05, 0.1 and 0.2 μg mL^{-1}) to the samples under analysis. The recovery varied between 96% and 104%, thereby affirming the method's accuracy for vancomycin quantification in patient EBC samples. Furthermore, samples 1 and 2 were analyzed by HPLC-UV as a reference method. The measured vancomycin concentrations were 0.112 ± 0.003 μg mL^{-1} (sample 1) and 0.194 ± 0.002 μg mL^{-1} (sample 2). A paired two-tailed *t*-test

Table 2 Vancomycin determination using the developed probe in MVEBC samples

No.	Gender	Co-administered drugs	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)
1	Female	Ampicillin, vitamin K, caffeine	—	0.11	—
			0.05	0.159	98
			0.1	0.206	96
			0.2	0.308	99
2	Male	—	—	0.19	—
			0.05	0.241	102
			0.1	0.286	96
			0.2	0.38	95
3	Female	Ampicillin	—	0.16	—
			0.05	0.212	104
			0.1	0.257	97
			0.2	0.354	97
4	Male	Ampicillin, calcium, caffeine, heparin	—	0.22	—
			0.05	0.269	98
			0.1	0.318	98
			0.2	0.422	101
5	Male	Ampicillin, calcium, caffeine	—	0.14	—
			0.05	0.194	108
			0.1	0.242	102
			0.2	0.343	101.5
6	Male	Calcium, caffeine, vitamin A	—	0.09	—
			0.05	0.142	104
			0.1	0.198	108
			0.2	0.281	95.5

comparison with the validated proposed method (0.110 ± 0.010 and 0.190 ± 0.009 μg mL^{-1}, respectively) showed no statistically significant difference ($p > 0.05$, 95% confidence level). These results demonstrate that the proposed method is reliable for quantifying vancomycin in the studied biological matrices.

4. Conclusion

In this study, a colorimetric probe integrating hybrid hydrogels was developed for the straightforward determination of vancomycin in EBC samples. This platform utilizes a hydrogel to immobilize nanozyme. Notably, CuAl-LDH was utilized as a nanozyme to enhance and indicate colorimetric signals. The remarkable peroxidase-like activity of CuAl-LDH/agarose for H_2O_2 : TMB system was utilized for vancomycin analysis. The findings indicated that the absorbance of the CuAl-LDH/agarose catalyzed H_2O_2: TMB system diminished with increasing the vancomycin concentration. A notable linear correlation was established between absorbance and vancomycin concentration, demonstrating a strong relationship within a range of 0.04–0.3 μg mL^{-1}. The system displayed low inter- and intra-day variations and remarkable precision suggesting its suitability for vancomycin monitoring. Importantly, this sensing platform has the potential to be adapted for optical assays of other targets by modifying the molecules encapsulated within the hydrogel. Therefore, with its advantages of simplicity, versatility, and cost-effectiveness, this platform paves the way for colorimetric detection of vancomycin in clinical applications.

Sample ethical statement

All experiments were performed in accordance with the guidelines of Helsinki declaration, and experiments were approved by the ethics committee at Tabriz University of Medical Sciences. Informed consents were obtained from human participants of this study.

Data availability

Data supporting this study are included within the article.

Author contributions

Zahra Karimzadeh; investigation, writing, Aylar Keyvani; investigation, Vahid Jouyban-Gharamaleki; validation, Maryam Khoubnasabjafari; validation, Elaheh Rahimpour: supervision, methodology, writing – review & editing. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgements

Research reported in this publication was supported by Elite Researcher Grant Committee under grant number 4021271 from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran. We would like to appreciate of the

cooperation of Clinical Research Development Unit, Imam Reza General Hospital, Tabriz, Iran in conducting this research.

References

1 F. Bruniera, F. Ferreira, L. Saviolli, M. Bacci, D. Feder, M. Pedreira, M. S. Peterlini, L. Azzalis, V. C. Junqueira and F. Fonseca, *Eur. Rev. Med. Pharmacol. Sci.*, 2015, 19.
2 C. A. Hammett-Stabler and T. Johns, *Clin. Chem.*, 1998, **44**, 1129–1140.
3 V. P. Gaspar, S. Ibrahim, R. P. Zahedi and C. H. Borchers, *J. Mass Spectrom.*, 2021, **56**, e4788.
4 R. I. Abed and H. Hadi, *Arabian J. Sci. Eng.*, 2020, **45**, 4751–4763.
5 H. Zhou, R. Liu, Q. Chen, X. Zheng, J. Qiu, T. Ding and L. He, *Food Chem.*, 2022, **369**, 130886.
6 N. B. Andriguetti, L. L. Lisboa, S. R. Hahn, L. R. Pagnussat, M. V. Antunes and R. Linden, *J. Pharmaceut. Biomed. Anal.*, 2019, **165**, 315–324.
7 P. Milla, F. Ferrari, E. Muntoni, M. Sartori, C. Ronco and S. Arpicco, *J. Chromatogr. B*, 2020, **1148**, 122151.
8 F. Fang, N. Li, C. Xu, R. Tan, J. Yang and Z. Zheng, *Curr. Pharm. Anal.*, 2021, **17**, 847–852.
9 H. S. A.-w. Al-ward, M. Q. Al-Abachi and M. R. Ahmed, *Baghdad Sci. J.*, 2023, **20**, 0245.
10 A. Cetinkaya, E. Yıldız, S. I. Kaya, M. E. Çorman, L. Uzun and S. A. Ozkan, *Green Anal. Chem.*, 2022, **2**, 100017.
11 T. Bu, S. Zhao, F. Bai, X. Sun, K. He, Q. Wang, P. Jia, Y. Tian, M. Zhang and L. Wang, *Anal. Chem.*, 2021, **93**, 6731–6738.
12 F. Li, J. Jiang, H. Peng, C. Li, B. Li and J. He, *Sens. Actuators, B*, 2022, **369**, 132334.
13 J. Guan, M. Wang, R. Ma, Q. Liu, X. Sun, Y. Xiong and X. Chen, *Sens. Actuators, B*, 2023, **375**, 132972.
14 S. M. Abachi, H. Rezaei, M. Khoubnasabjafari, V. Jouyban-Gharamaleki, E. Rahimpour and A. Jouyban, *Pharm. Sci.*, 2022, **29**, 368–375.
15 Y. Sefid-Sefidehkhan, Z. Karimzadeh, A. Jouyban, M. Khoubnasabjafari, V. Jouyban-Gharamaleki and E. Rahimpour, *RSC Adv.*, 2024, **14**, 29143–29150.
16 Y. T. Liu, L. Tang, J. Dai, J. Yu and B. Ding, *Angew. Chem., Int. Ed.*, 2020, **59**, 13623–13627.
17 G. Fan, F. Li, D. G. Evans and X. Duan, *Chem. Soc. Rev.*, 2014, **43**, 7040–7066.
18 Z. Wang, Y. Chen, X. Li, G. He, J. Ma and H. He, *Environ. Sci. Technol.*, 2021, **56**, 1386–1394.
19 H. Wang, L. Ji, Y. Zhang, R. Wang, Z. Cao, D. Wang, J. Cheng and C. Liu, *Anal. Lett.*, 2024, 1–17.
20 X.-J. Yang, P. Zhang, P. Li, Z. Li, W. Xia, H. Zhang, Z. Di, M. Wang, H. Zhang and Q. J. Niu, *J. Mol. Liq.*, 2019, **280**, 128–134.
21 S. J. Peighambardoust, S. I. Zardkhaneh, M. Foroughi, R. Foroutan, H. Azimi and B. Ramavandi, *Environ. Res.*, 2024, **258**, 119428.
22 M. K. Jouyban, K. Ansarin and V. Jouyban-Gharamaleki, Breath sampling setup, *Iranian Pat.*, 81363, 2013.
23 D. Luo, X. Liu, S. Dai, J. Yi, N. Tang, Y. Cai, X. Bao, M. Hu and Z. Liu, *Inorg. Chem.*, 2024, **63**, 10691–10704.
24 P. Date, A. Tanwar, P. Ladage, K. M. Kodam and D. Ottoor, *Chem. Pap.*, 2020, **74**, 1965–1978.
25 E. Rahimpour, M. Khoubnasabjafari, M. B. Hosseini and A. Jouyban, *J. Pharmaceut. Biomed. Anal.*, 2021, **196**, 113906.
26 T. Zhang, D. Watson, C. Azike, J. Tettey, A. Stearns, A. Binning and C. Payne, *J. Chromatogr. B*, 2007, **857**, 352–356.
27 J. Huo, Y. Guo, B. Zhang, Z. Zhao, G. Shi and S. Mei, *J. Mass Spectrom.*, 2023, **58**, e4925.
28 L. Marks, S. Duchein, I. I. González, H. A. Suárez and S. Rivolta, *Rev. Fac. Cien. Med.*, 2019, **76**, 101–106.
29 K. Shirazi, Z. Karimzadeh, M. B. Hosseini, V. Jouyban-Gharamaleki, M. Khoubnasabjafari, J. Soleymani, E. Rahimpour and A. Jouyban, *Heliyon*, 2024, **10**, e37253.
30 S. Pande and J. R. Parikh, *Journal of Drug Delivery and Therapeutics*, 2019, **9**, 116–118.
31 M. Tariq, H. Naureen, N. Abbas and M. Akhlaq, *Journal of Analytical & Bioanalytical Techniques*, 2015, **6**, 239.
32 P. Nascimento, A. Kogawa and H. R. N. Salgado, *Drug Analytical Research*, 2021, **5**, 39–45.