

RESEARCH ARTICLE

Matrix-Corrected Impedimetric Quantification of Vancomycin in Milk and Serum-like Media Using Printed Carbon–Gold Aptasensors

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Abstract

Vancomycin quantification in milk and serum-like media requires more than target recognition because proteins, salts, lipids, carbohydrates, and nonspecific surface transport can shift the same aptamer-gating response in different directions. This study asks whether a printed carbon–gold impedimetric aptasensor can assign matrix-corrected vancomycin concentrations by coupling percentage charge-transfer-resistance opening with recovery factors measured in each sample class. The analytical record contained electrode geometry, gold-nanostructure deposition conditions, aptamer-affinity measurements, blocked and target-exposed resistance states, calibration constants, recoveries in three milk-fat levels and artificial serum, and interferent-retention responses. The printed strip had a geometric working-electrode area of 0.196 cm² and an electroactive area of 0.226 cm². Gold nanostructures were electrodeposited from 10 mM tetrachloroauric acid in acidic histidine solution at –100 μ A for 600 s, followed by immobilization of a thiolated vancomycin aptamer and blocking with 6-mercapto-1-hexanol. Percentage opening of the receptor layer was converted to concentration using a linear relation from 0.05 μ M to 1.00 μ M, with a slope of 56.83% per micromolar, an intercept of –0.853%, $R^2 = 0.981$, a detection limit of 1.721 nM, and a quantification limit of 5.216 nM. At a 1.00 μ M spike level, recoveries were 99.10% in 0.1% fat milk, 98.10% in 1.5% fat milk, 104.40% in 3.0% fat milk, and 96.20% in artificial human serum. Matrix correction returned the concentration estimates to 0.996 μ M to 0.999 μ M. Lactose, glucose, and gentamicin produced only 9.78–13.03% independent response, while vancomycin mixtures retained 89.05–96.58% of the target signal. The results show that this aptasensor supports concentration assignment only when the impedance opening, calibration interval, recovery factor, and selectivity retention are evaluated together for the stated matrix.

Keywords: vancomycin; impedimetric aptasensor; printed carbon electrode; gold nanostructures; milk residue analysis; serum monitoring; matrix correction; electrochemical spectroscopy

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1. Introduction

Vancomycin remains a clinically important glycopeptide antibiotic because it is used against serious infections caused by resistant Gram-positive bacteria, especially methicillin-resistant *Staphylococcus aureus*. Its use also creates a demanding measurement problem. Underdosing can compromise bacterial control, whereas excessive exposure increases the risk of toxicity and requires careful therapeutic mon-

itoring. Current therapeutic-drug-monitoring practice increasingly emphasizes exposure-guided interpretation rather than reliance on a single convenient concentration value, which makes accurate measurement in biological matrices essential [1, 2]. Reliable measurement therefore affects antimicrobial exposure assessment, patient safety, and antimicrobial stewardship.

Dairy analysis creates a different but related analytical challenge. Antibiotic residues in milk are important because they can affect food

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safety, interfere with fermentation processes, and serve as indicators of inappropriate antimicrobial use in animal production. The analytical matrix is chemically complex: casein micelles, whey proteins, lipids, lactose, calcium phosphate, and salts can all influence sample pretreatment and sensor response. Residue screening in milk has long depended on laboratory-grade chromatographic confirmation or broad screening tests, but decentralized and low-cost technologies remain desirable when rapid screening choices are needed near the point of production [3–5].

Liquid chromatography and tandem mass spectrometry provide high selectivity for vancomycin and related compounds. Such methods are indispensable when regulatory confirmation, multi-residue analysis, or reference-laboratory accuracy is required [6–8]. Immunoassays are also practical in routine therapeutic drug monitoring because they are automated and compatible with established clinical laboratory operations [1]. These approaches, however, are less suitable when the intended use is inexpensive field screening, disposable measurement, or small-instrument testing. The analytical need is not only lower detection limits; it is the combination of selectivity, portability, matrix tolerance, and transparent concentration translation.

Electrochemical aptasensors are attractive in this context because they join synthetic molecular recognition with compact electrical readout. Aptamers can be chemically synthesized, terminally modified, truncated, spaced, and immobilized on electrode surfaces with reproducible design control. Electrochemical aptamer-based sensors have been explored for therapeutic drug monitoring, environmental analysis, and antibiotic detection because target binding can be converted into an electrical change without requiring bulky optical instrumentation [9–12]. For vancomycin, the aptamer approach is especially relevant because the target is structurally specific and clinically meaningful, and because emerging aptamer work has already shown that sequence length, immobilization density, and surface format strongly influence analytical performance [13, 14].

Printed carbon electrodes provide the instrumentation side of this strategy. Their compact geometry, low cost, and compatibility with drop-cell or small-cell measurements make them useful for disposable electroanalysis. Gold nanostructures add a second level of control by improving electron-transfer accessibility and supplying Au–S anchoring sites for thiolated aptamers. The resulting carbon–gold interface can be tuned into a receptor-bearing impedance gate, where a molecular binding event changes charge-transfer resistance in a ferro/ferricyanide probe solution. This mode of operation fits within electrochemical spectroscopy because the signal is governed by interfacial charge transport, receptor organization, redox-probe access, and matrix-dependent transport rather than by bulk concentration alone [15–17].

The unresolved problem is calibration transfer. A buffer-derived impedance calibration may be visually strong and numerically linear, but milk and serum-like media can alter wetting, protein adsorption, ion screening, diffusion, and redox-probe access. As a result, the apparent concentration can be biased even when the aptamer remains chemically selective. The central research question is therefore specific: can percentage resistance opening at a printed carbon–gold vancomycin aptasensor be converted into matrix-corrected concentration estimates for low-fat milk, semi-skimmed milk, whole milk, and artificial human serum? This question is different from asking whether the aptasensor responds to vancomycin; it asks whether the response can be assigned as a defensible concentration after matrix displacement is explicitly quantified.

The analytical route is introduced in Fig. 1. Sample identity, printed-electrode impedance measurement, calibration conversion, and matrix correction are arranged as consecutive calculation steps. This arrangement clarifies why each concentration value depends on two measured quantities: the vancomycin-specific impedance opening and the recovery factor assigned to the matrix.

The sequence in Fig. 1 defines the calculation used throughout the

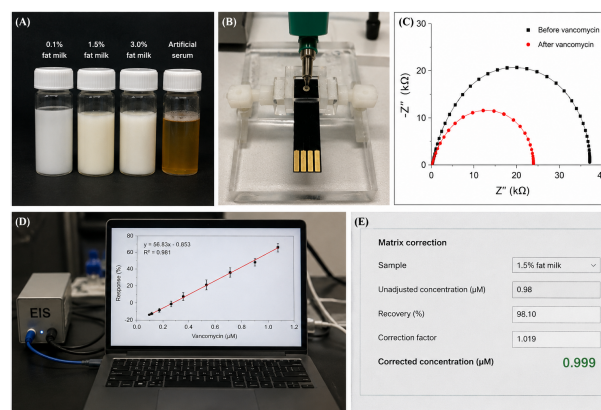


Figure 1: Matrix-corrected vancomycin readout.

study: identify the sample class, measure the before/after impedance change, translate the change through the calibration equation, and correct the result using the recovery behavior of the same matrix. The final concentration is therefore not a raw resistance value; it is a matrix-specified concentration assignment derived from linked electrochemical and recovery measurements.

2. Analytical record and study design

The analytical record contains six linked measurement groups: electrode geometry, gold deposition, receptor-layer construction, calibration response, matrix recovery, and selectivity retention. These values are analyzed together because concentration assignment in complex samples is controlled by both the target-induced impedance opening and the matrix-dependent recovery behavior. The design therefore evaluates how a vancomycin aptasensor should translate impedance information when sample composition changes.

The values in Table 1 show why a single performance number is insufficient. Electrode geometry controls the reproducibility of the active area, the gold layer controls immobilization and electron transfer, the aptamer layer controls molecular recognition, the calibration translates signal into concentration, the recovery layer corrects matrix displacement, and the selectivity layer defines when the signal remains chemically attributable to vancomycin. A concentration result is therefore accepted only when these measurements are mutually consistent.

The study design follows the same order. First, the electrode architecture is assessed as an impedance gate. Second, the calibration constants define the measurable concentration interval. Third, recovery behavior is converted into a matrix factor. Fourth, selectivity values are used to check whether the corrected signal remains dominated by vancomycin. In this arrangement, recovery is part of the concentration calculation rather than a detached validation percentage.

3. Materials and methods

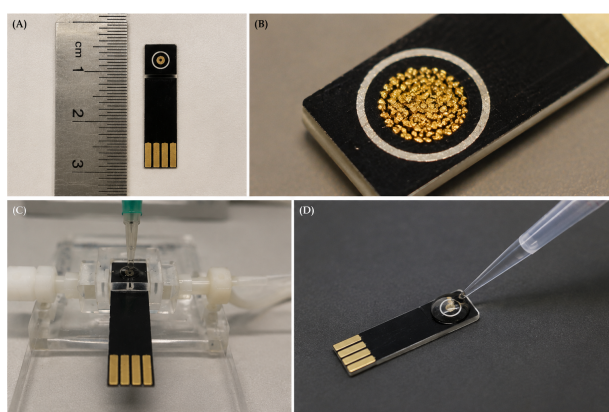
3.1. Printed carbon–gold aptasensor platform

The disposable sensing platform consisted of a printed carbon strip modified with electrodeposited gold nanostructures. The strip measured 1.5 cm by 1.0 cm, the geometric working-electrode area was 0.196 cm², and the electroactive area was 0.226 cm². Gold was deposited from 10 mM tetrachloroauric acid in 0.5 M sulfuric acid containing 150 mM histidine under a cathodic current of $-100 \mu\text{A}$ for 600 s. This preparation produced cauliflower-like gold nanostructures on carbon, increasing conductive surface accessibility and providing Au–S binding sites for aptamer immobilization.

Table 1: Analytical record for matrix-corrected impedance interpretation.

Measurement layer	Values used	Role in concentration assignment
Electrode geometry	Strip size of 1.5 cm by 1.0 cm; geometric area of 0.196 cm ² ; electroactive area of 0.226 cm ²	Defines the physical sensing domain and constrains interfacial current distribution
Surface preparation	Gold deposition from 10 mM tetrachloroauric acid with 150 mM histidine at −100 μ A for 600 s	Establishes conductive Au nanostructures for thiolated aptamer anchoring
Recognition layer	Thiolated vancomycin aptamer; 6-mercapto-1-hexanol blocking; affinity constants of 13.35 μ M by ITC and 18.31 μ M by SPR	Supplies molecular selectivity and target-induced interfacial rearrangement
Calibration response	Linear interval of 0.05 μ M to 1.00 μ M; slope 56.83% per micromolar; intercept −0.853%; $R^2 = 0.981$	Converts percentage impedance opening into an uncorrected concentration
Matrix recovery	Recoveries of 96.20–104.40% across milk and artificial serum at 1.00 μ M	Provides matrix-specific recovery factors for concentration correction
Selectivity retention	9.78–13.03% interferent-only response; 89.05–96.58% retained vancomycin response in mixtures	Tests whether the correction step is applied to a selective receptor response rather than to nonspecific opening

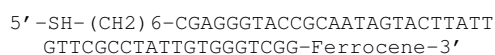
The physical platform is shown in Fig. 2. Its value for the method is that the printed strip, the gold-enriched working area, and the measurement cell are visible as a compact analytical unit. A portable assay cannot be evaluated only by molecular selectivity; it also requires a reproducible strip geometry, stable liquid contact, and a defined region where the recognition layer interacts with the redox-probe solution.

**Figure 2:** Printed carbon-gold strip and measurement cell.

The images in Fig. 2 support the practical interpretation of the assay. The working electrode is sufficiently small for low-volume measurement, but it is still large enough to allow visual inspection of liquid coverage and connection alignment. This matters because impedance measurements are highly sensitive to contact area, incomplete wetting, and electrode-to-cell positioning; the platform image therefore belongs in the method rather than in supplementary decoration.

3.2. Recognition-layer construction

The receptor strand was a thiolated vancomycin aptamer with a terminal hexyl-thiol spacer and a ferrocene terminus:



The terminal thiol allowed the aptamer to bind to the electrodeposited gold surface, and 6-mercapto-1-hexanol was used to occupy remaining gold sites and organize the interfacial film. Although the aptamer includes a ferrocene label, concentration assignment in this study uses charge-transfer resistance in a ferro/ferricyanide probe solution because that impedance variable provided the quantitative signal for matrix correction.

The interfacial sequence is shown in Fig. 3. It separates bare carbon, gold-enriched carbon, blocked receptor, and target-exposed states. The

panel set makes the resistance changes chemically interpretable: a low-resistance gold surface becomes a blocked receptor film, and target binding partially opens that film to the redox probe.

The sequence in Fig. 3 explains why the method uses each electrode as its own reference. Printed carbon alone is not the analytical endpoint, and gold alone is not selective. The blocked aptamer state establishes the high-resistance reference state, while vancomycin binding creates the measured opening. Expressing the response as a percentage change reduces dependence on absolute electrode-to-electrode resistance while retaining the chemistry of the gating process.

3.3. Impedance response definition

Electrochemical impedance spectroscopy was used to quantify charge-transfer resistance before and after vancomycin exposure. The blocked aptamer/mercaptohexanol layer restricted access of ferro/ferricyanide to the carbon-gold surface, while target binding reduced the resistance by rearranging the receptor layer. The analytical response was therefore defined as percentage opening of the blocked interface:

$$S_{\text{open}} = 100 \times \frac{R_{\text{ct,blocked}} - R_{\text{ct,target}}}{R_{\text{ct,blocked}}} \quad (1)$$

The definition in Eq. 1 gives a physically meaningful signal because it compares each electrode after target incubation with the same electrode before incubation. A high value indicates strong opening of the receptor film, whereas a low value indicates weak target-induced change or strong residual blocking. This relative definition is especially important for disposable electrodes because fabrication and immobilization differences can change absolute resistance.

The impedance sequence is summarized in Fig. 4. The visual sequence links the blocked state, target incubation, and reduced Nyquist semicircle after vancomycin binding. This figure is placed after the response definition so that the mathematical signal can be read directly against the bench-level measurement process.

The impedance sequence in Fig. 4 supports a signal-on interpretation in which target binding decreases resistance rather than increasing it. That direction is important for matrix correction: a matrix that makes the interface appear more open can cause positive bias, while a matrix that suppresses opening can cause underestimation. The correction step must therefore account for the sign and magnitude of recovery displacement rather than applying a uniform adjustment.

3.4. Matrix-corrected concentration calculation

The uncorrected concentration was obtained from the linear calibration relation between percentage opening and vancomycin concentration.

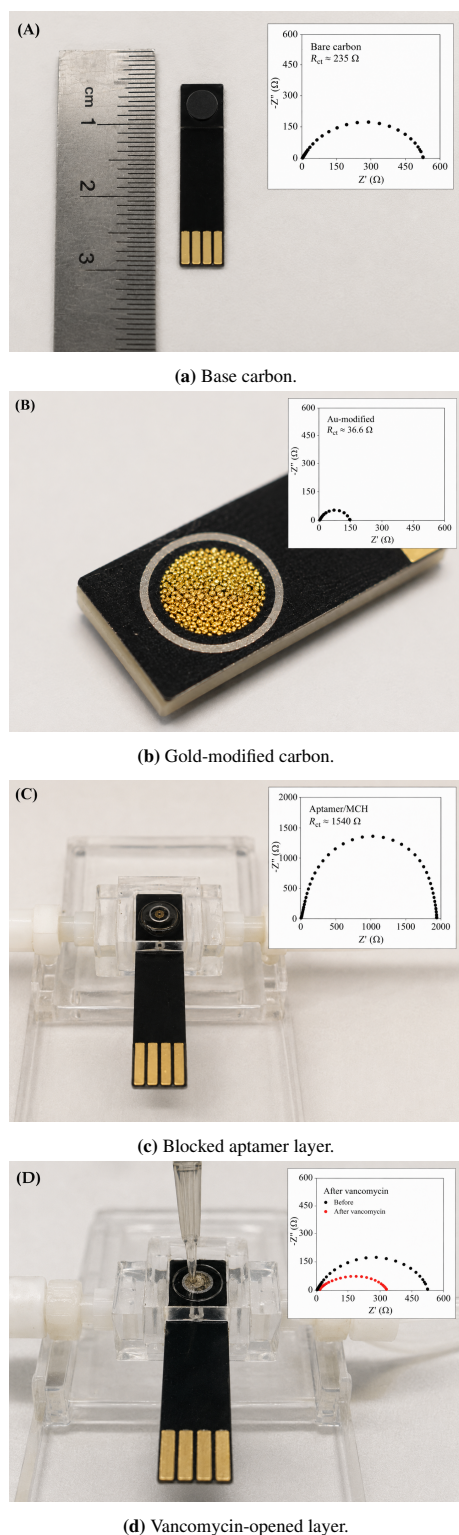


Figure 3: Surface states of the aptamer-gated electrode.

Because the calibration slope was 56.83% per micromolar and the intercept was -0.853%, the concentration estimate was calculated by subtracting the intercept from the observed response and dividing by the slope. The matrix-qualified concentration was then obtained by multiplying the uncorrected concentration by a recovery factor derived from the corresponding sample class:

$$C_{\text{matrix}} = \left(\frac{S_{\text{open}} + 0.853}{56.83} \right) \times \frac{100}{\text{Recovery}_{\text{matrix}}} \quad (2)$$

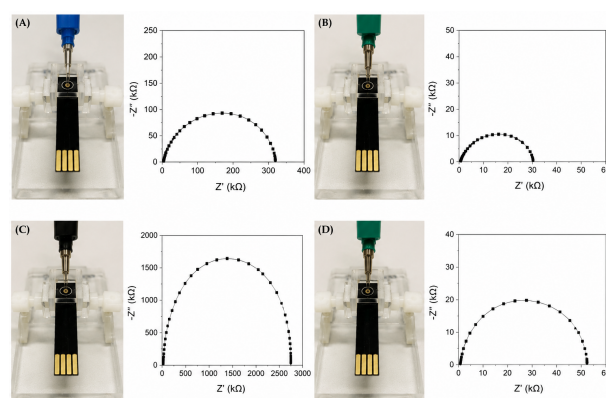


Figure 4: Impedance change after vancomycin binding.

The correction in Eq. 2 treats recovery as a matrix-transfer coefficient. When recovery is below 100%, the matrix has caused under-recovery and the concentration is adjusted upward. When recovery is above 100%, the matrix has caused positive displacement and the concentration is adjusted downward. Recovery therefore becomes an operational part of the concentration result rather than a retrospective statement that the assay passed validation.

The instrument-facing measurement sequence is displayed in Fig. 5. The panels move from impedance-cell connection to after-incubation readout, resistance comparison, calibration display, and matrix correction. The arrangement shows how the calculation can be embedded in a portable potentiostat interface or a simple tablet-based reporting system.

The sequence in Fig. 5 demonstrates that the method is designed for concentration reporting, not merely for observing resistance changes. The user would not receive a bare resistance value; the instrument would calculate the percentage opening, apply the calibration, select the matrix recovery factor, and return a corrected concentration with the matrix identity attached. This step is essential for avoiding false comparability between low-fat milk, whole milk, and serum-like samples.

3.5. Acceptance rules

A sample result was considered valid for concentration assignment only when three conditions were satisfied. The measured response had to show a decrease in charge-transfer resistance after vancomycin incubation, the calculated percentage opening had to fall within the validated linear interval of 0.05 μM to 1.00 μM , and the sample matrix had to correspond to a recovery class included in the analytical record. These rules prevent the matrix factor from being applied outside its evidence base. They also clarify that correction is not a substitute for selectivity, calibration validity, or matrix validation.

4. Results

4.1. Interfacial resistance states

The transition from printed carbon to gold-nanostructured carbon reduced the charge-transfer resistance from approximately 235 Ω to 36.6 Ω . This drop indicates that gold deposition improved redox-probe access and electronic communication at the working electrode. After aptamer immobilization and mercaptohexanol blocking, resistance increased to approximately 1540 Ω , showing that a compact receptor layer had formed on the conductive surface. Exposure to vancomycin then reduced resistance, which is consistent with target-induced rearrangement of the aptamer film.

The meaning of these resistance states is analytical rather than merely

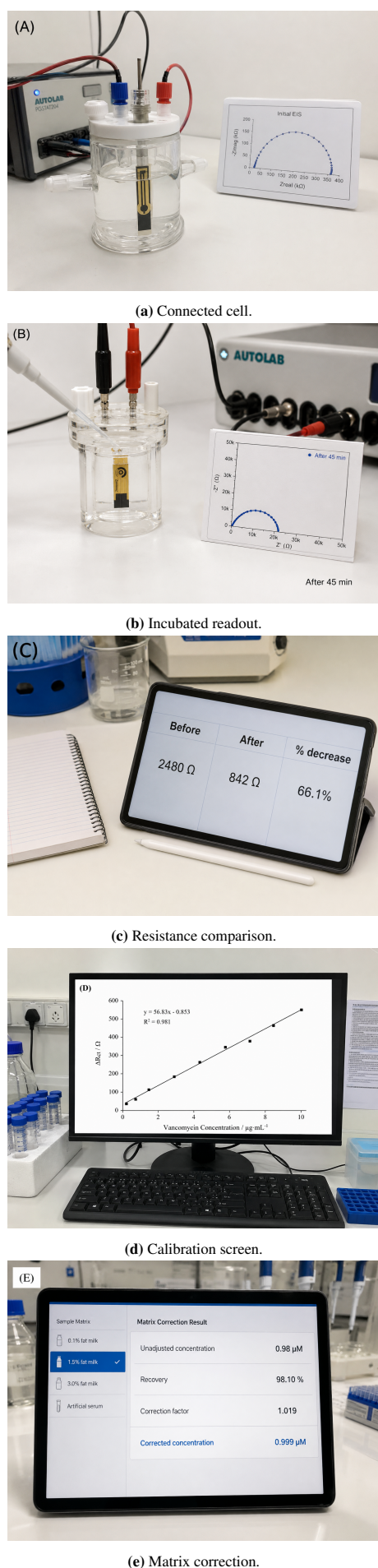


Figure 5: Instrument-to-concentration translation sequence.

descriptive. The low-resistance gold state supplies an electronically favorable base; the high-resistance blocked state supplies a large dynamic window; and the vancomycin-opened state supplies the measurable response. A sensor that did not pass through these three functional states would not support the matrix-corrected calculation because the calibration would have no stable interfacial origin.

4.2. Calibration and sensitivity

The calibration results define the concentration interval over which the matrix-corrected calculation can be applied. The response was linear from 0.05 μM to 1.00 μM . The slope was 56.83% response per micromolar, the intercept was -0.853%, and the coefficient of determination was 0.981. The detection limit was 1.721 nM, and the quantification limit was 5.216 nM. These values indicate that the impedance gate has enough signal contrast to operate well below the upper boundary of the calibration interval.

Table 2: Electroanalytical constants for the vancomycin matrix-corrected calculation.

Parameter	Value and analytical meaning
Printed strip dimensions	1.5 cm by 1.0 cm; compact disposable format
Geometric working-electrode area	0.196 cm ² ; defined physical area for liquid contact
Electroactive area	0.226 cm ² ; conductive area estimated from electrochemical response
Gold deposition condition	10 mM tetrachloroauric acid, 150 mM histidine, -100 μA , 600 s; nanostructured Au anchor layer
Aptamer affinity by ITC	13.35 μM with uncertainty of 2.74 μM ; solution binding evidence
Aptamer affinity by SPR	18.31 μM with uncertainty of 1.15 μM ; surface-sensitive binding evidence
Linear working interval	0.05 μM to 1.00 μM ; allowable concentration range for direct reporting
Calibration relation	Slope of 56.83% per micromolar and intercept of -0.853%; basis for concentration assignment
Coefficient of determination	0.981; evidence of near-linear calibration behavior
Limit of detection	1.721 nM; low-concentration signal detectability
Limit of quantification	5.216 nM; lower boundary for quantitative reporting

The constants in Table 2 show that the assay combines three types of performance evidence. Geometry and deposition conditions describe whether the electrode can be made reproducibly. Aptamer-affinity values show that the receptor has a measurable vancomycin-binding event. Calibration and detection-limit values show that the interfacial event can be converted into a quantitative impedance signal. All three are necessary for an instrument-facing method.

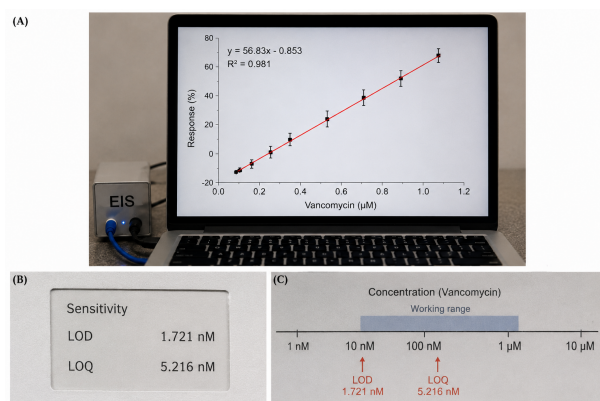
The calibration and conversion displays are presented in Fig. 6. The two panels place the linear response, the detection and quantification limits, and the concentration-conversion logic in a readable format. A short caption is used, but the surrounding text provides the interpretive detail needed for analytical assessment.

The displays in Fig. 6 are important because they distinguish sensitivity from valid concentration assignment. A low detection limit shows that small vancomycin-induced openings can be detected, whereas the linear interval defines where direct concentration conversion is justified. The matrix-corrected method therefore uses the calibration display as an acceptance boundary: responses below quantification or outside the linear window should not be corrected into routine concentration values without additional validation.

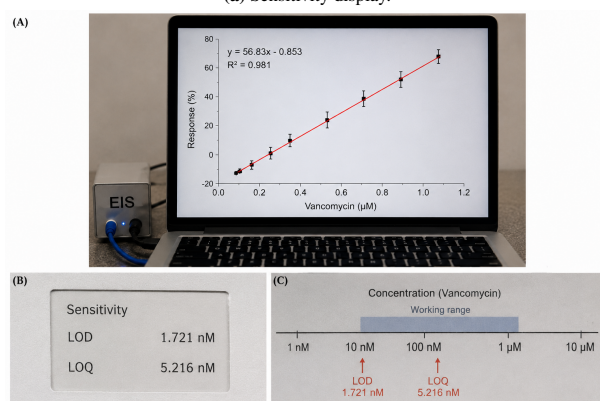
4.3. Matrix recovery and corrected concentration

Four sample classes were examined for recovery: 0.1% fat milk, 1.5% fat milk, 3.0% fat milk, and artificial human serum. Each was evaluated at a 1.00 μM vancomycin spike. The found concentrations ranged from 0.96 μM to 1.04 μM , while recovery ranged from 96.20% to 104.40%. Precision remained within a practical range, with relative standard deviation between 0.08% and 3.68%.

The recovery data in Table 3 reveal different matrix directions. Low-fat milk was almost neutral, with 99.10% recovery and very low RSD.



(a) Sensitivity display.



(b) Conversion display.

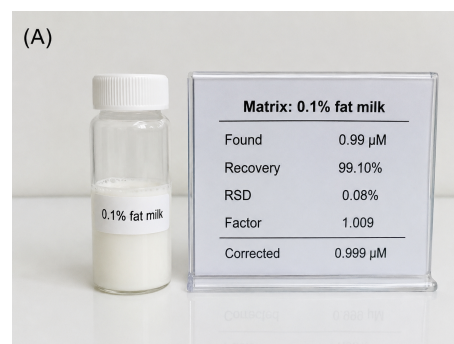
Figure 6: Calibration and concentration conversion.**Table 3:** Recovery correction of vancomycin in milk and serum-like media.

Matrix	Add.	Found	Rec., %	RSD, %	Factor	Corr.
Milk, 0.1% fat	1.00	0.99	99.10	0.08	1.009	0.999
Milk, 1.5% fat	1.00	0.98	98.10	2.32	1.019	0.999
Milk, 3.0% fat	1.00	1.04	104.40	2.56	0.958	0.996
Artificial human serum	1.00	0.96	96.20	3.68	1.040	0.997

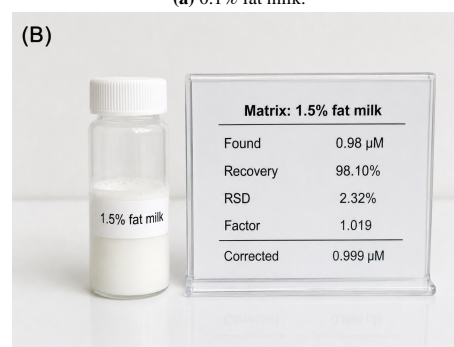
Semi-skimmed milk showed a mild negative bias. Whole milk produced a positive bias, suggesting that higher lipid content may change wetting or redox-probe access in a way that increases apparent opening. Artificial serum showed the largest under-recovery and the highest RSD, consistent with a more fouling-prone and ionically complex medium. These differences justify using matrix-specific recovery factors instead of one universal correction factor.

The corrected values after recovery correction converged to $0.996 \mu\text{M}$ to $0.999 \mu\text{M}$. This convergence is the central quantitative result. The recovery values are not used only to demonstrate acceptable assay performance; they are transformed into concentration-correction factors that bring matrix-specific estimates back toward the nominal spike. This result answers the research question at the tested concentration. The four recovery cases are visualized in Fig. 7. Each panel displays a different matrix rather than forcing all matrices into a single pooled plot. This presentation is useful because the direction of bias differs between whole milk and the other matrices.

The matrix panels in Fig. 7 show that a visually simple correction table hides an important analytical distinction. A concentration estimate in whole milk must be moved downward, whereas estimates in low-fat milk, semi-skimmed milk, and artificial serum must be moved upward. This directionality is exactly what a portable reporting interface should preserve; otherwise, matrix selection could become a hidden cause of systematic error.



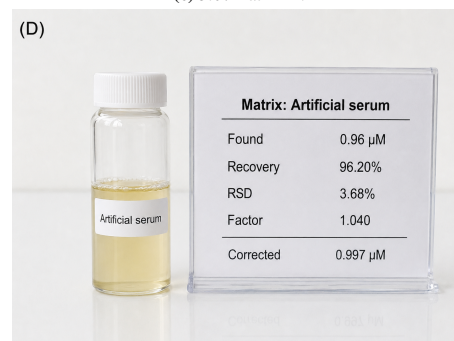
(a) 0.1% fat milk.



(b) 1.5% fat milk.



(c) 3.0% fat milk.



(d) Artificial serum.

Figure 7: Recovery correction in four matrices.

4.4. Selectivity and interferent retention

Selectivity testing used lactose, glucose, and gentamicin. Lactose and glucose represent common carbohydrate interferents in dairy or biological samples, while gentamicin represents a second antibiotic that could create nonspecific electrostatic interaction with the DNA layer. The individual interferent responses were weak relative to vancomycin, and the mixtures retained most of the vancomycin signal.

Table 4: Relative selectivity response of the aptamer-gated impedance assay.

Interferent	Alone, %	Vancomycin mixture, %	Interpretation
Lactose	9.78	94.37	Low independent opening; strong retention of target response
Glucose	10.65	89.05	Low independent opening; largest mixture attenuation
Gentamicin	13.03	96.58	Slightly higher independent opening; strongest retained target response

The selectivity results in Table 4 indicate that matrix correction is applied to a vancomycin-led signal rather than to broad nonspecific drift. None of the interferents alone exceeded 13.03% of the vancomycin signal. In the mixture tests, the retained response remained between 89.05% and 96.58%. Glucose caused the largest attenuation, but the remaining signal was still high enough to support screening when the sample matrix is validated.

The visual selectivity summary is provided in Fig. 8. The display places interferent responses and vancomycin with interferents together, which makes the retention pattern easier to evaluate than a numerical table alone.

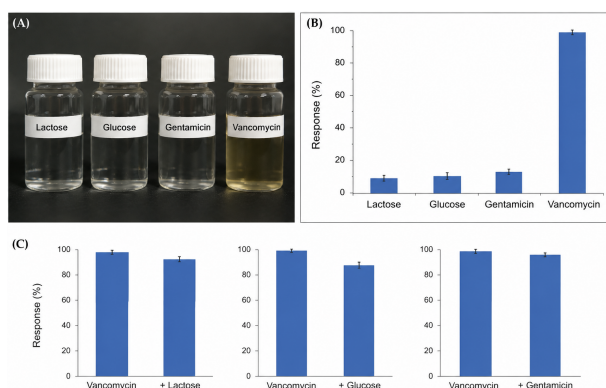


Figure 8: Selectivity against carbohydrate and antibiotic interferents.

The selectivity display in Fig. 8 supports two conclusions. First, lactose and glucose do not appear to trigger the aptamer gate in a target-like manner. Second, gentamicin produces a slightly larger independent response, plausibly because a cationic aminoglycoside can interact electrostatically with a negatively charged DNA layer, but this effect does not dominate when vancomycin is present. Selectivity is therefore adequate for the matrix-corrected concentration calculation, though broader antibiotic panels would be needed for routine multi-drug screening.

4.5. Matrix-class and quality-control displays

Two additional displays specify how the assay should be documented when more sample classes or electrode batches are tested. The first display, shown in Fig. 9, organizes matrix-class reporting around the same numerical fields used in Table 3: sample identity, recovery direction, correction factor, and corrected concentration.

The matrix-class display in Fig. 9 emphasizes that additional sample types cannot be merged into the method by name alone. Each milk or

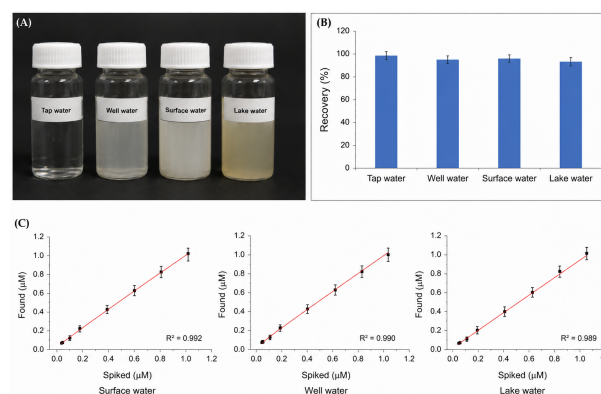


Figure 9: Matrix-class reporting format.

serum class requires its own recovery value and correction factor. A graphical panel is useful only when it is tied to the numerical recovery measurements that justify concentration assignment.

The second display, shown in Fig. 10, addresses reproducibility and storage control. Disposable printed electrodes require batch checks because gold morphology, aptamer density, storage humidity, and blocking efficiency can all influence the blocked-state resistance and the target-induced opening.

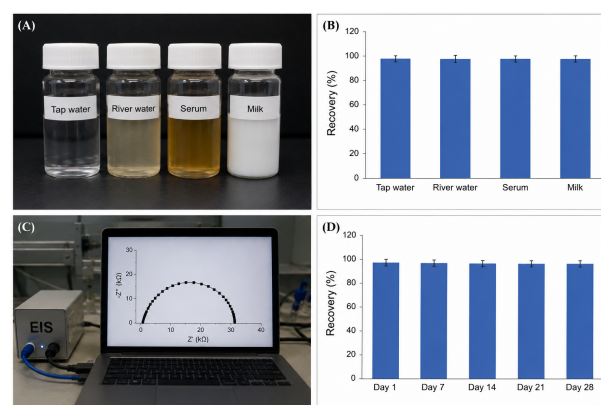


Figure 10: Quality-control view for disposable strips.

The quality-control display in Fig. 10 identifies the manufacturing checks required before routine use. A matrix correction factor is only meaningful if electrode batches remain stable enough for the same calibration and recovery factor to be used. Batch-to-batch reproducibility, storage-time response retention, and control-strip acceptance limits should therefore accompany the concentration calculation.

5. Discussion

5.1. Why matrix correction changes the analytical claim

The main analytical claim is not limited to aptamer binding, because vancomycin recognition by aptamer-modified electrochemical interfaces is supported by the broader literature on electrochemical drug sensing [9, 13, 14]. The more consequential claim is that a concentration can be assigned only after the response has passed through a matrix-aware translation step. In impedance sensing, measured resistance is shaped by the receptor layer and by every process that changes redox-probe access to the electrode surface. Milk fat, casein, salts, and serum proteins can therefore shift the apparent signal even without changing the molecular identity of the target.

Recovery correction addresses this problem by converting validation

evidence into a correction parameter. The approach is modest but useful: it does not claim that one correction factor can cover all possible milk or serum samples, and it does not replace full matrix-matched calibration when regulatory confirmation is required. Its value lies in making the calculation logic explicit. A concentration is not merely read from the calibration line; it is read from the calibration line under a stated matrix profile.

5.2. Interpretation of matrix-specific behavior

The recovery pattern suggests that diluted milk matrices do not behave as a simple linear function of fat content. Low-fat milk was almost unbiased, semi-skimmed milk showed slight under-recovery, and whole milk showed a positive bias. This pattern could arise from competing mechanisms: proteins may suppress redox-probe access by fouling the interface, while lipids and colloidal structures may alter wetting or partitioning in a way that increases apparent opening after rinsing. Artificial serum showed under-recovery and higher variability, consistent with protein adsorption and ionic screening near the DNA layer.

The recovery factor therefore should be interpreted empirically rather than mechanistically over-fitted. It provides a practical correction derived from the observed recovery at 1.00 μM , but it should not be generalized to untested concentrations without additional experiments. Multi-level recovery testing would show whether each matrix has a constant proportional effect, a concentration-dependent effect, or a nonlinear boundary near the low end of the calibration range.

5.3. Relationship to chromatography, immunoassay, and point-of-use sensing

Chromatographic methods remain essential for definitive vancomycin quantification, especially in clinical reference laboratories and food-residue confirmation. LC-MS/MS offers strong molecular specificity and can handle multi-analyte panels when appropriate sample preparation is available [2,4,6]. Immunoassays remain operationally attractive for hospital laboratories because they are automated and familiar [1]. A disposable impedance aptasensor should not be presented as a replacement for these methods in every setting.

The more realistic role of the aptasensor is rapid, low-cost, matrix-qualified screening. In milk, it could support preliminary assessment before confirmatory analysis. In serum-like media, it could support research-grade or decentralized monitoring studies after clinical validation. The matrix-corrected concentration calculation strengthens this role because it acknowledges the central weakness of electrochemical biosensing in complex samples: good receptor chemistry does not automatically guarantee unbiased concentration reporting.

5.4. Analytical limitations

Several limitations remain. The recovery factor was derived at a single 1.00 μM spike level, so it should not be assumed to apply across the full calibration range without multi-point validation. The serum matrix was artificial and therefore cannot represent all patient-serum protein loads, co-administered drugs, renal-failure metabolites, or ionic variation. The interferent panel included lactose, glucose, and gentamicin, but routine samples may contain additional antibiotics, preservatives, metabolites, or endogenous electroactive species. Finally, the quality-control displays do not replace batch-stability measurements; they identify the operational checks that should accompany routine deployment.

These limitations define the boundary of the claim. The results support matrix-corrected interpretation at the tested spike level and for the tested matrices. They do not establish universal quantification in unvalidated samples. This boundary is important for analytical sci-

ences because overstating sensor generality is a common reason why promising biosensors fail to translate into practical measurement tools.

6. Conclusion

This study asked whether percentage resistance opening at a printed carbon-gold vancomycin aptasensor can be converted into matrix-corrected concentration estimates for low-fat milk, semi-skimmed milk, whole milk, and artificial human serum. The results support that conversion within the tested concentration and matrix range. The electrode architecture produced the required interfacial sequence: printed carbon was converted into a conductive gold-nanostructured surface, aptamer/mercaptohexanol assembly produced a high-resistance recognition gate, and vancomycin binding decreased charge-transfer resistance in a concentration-dependent manner. The calibration interval of 0.05 μM to 1.00 μM , slope of 56.83% per micromolar, detection limit of 1.721 nM, and quantification limit of 5.216 nM show that the impedance response has sufficient analytical contrast for low-micromolar and diluted-sample interpretation.

The matrix-specific result answers the research question most directly. At a 1.00 μM spike, low-fat milk, semi-skimmed milk, whole milk, and artificial human serum produced recoveries from 96.20% to 104.40%. A buffer-only readout would therefore preserve small but directionally different matrix biases. Recovery correction reduced these differences and returned the concentration estimates to 0.996 μM to 0.999 μM . Selectivity values showed that the correction was applied to a vancomycin-dominant response: lactose, glucose, and gentamicin produced only 9.78–13.03% independent response, while mixtures retained 89.05–96.58% of the vancomycin signal.

The aptasensor is therefore best described as a matrix-qualified concentration tool rather than a matrix-independent resistance sensor. Its practical value lies in combining a disposable carbon-gold electrode, aptamer-gated impedance response, calibration conversion, recovery correction, and selectivity retention into a single concentration-assignment process. Before routine deployment, the method requires multi-level recovery testing, real patient serum, raw and processed milk variants, broader antibiotic interferent panels, and batch-stability acceptance limits. Within the tested evidence boundary, it provides a defensible route for translating vancomycin impedance responses into corrected concentrations in complex milk and serum-like matrices.

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