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# A fluorescence biosensor for therapeutic drug monitoring of vancomycin using *in vivo* microdialysis

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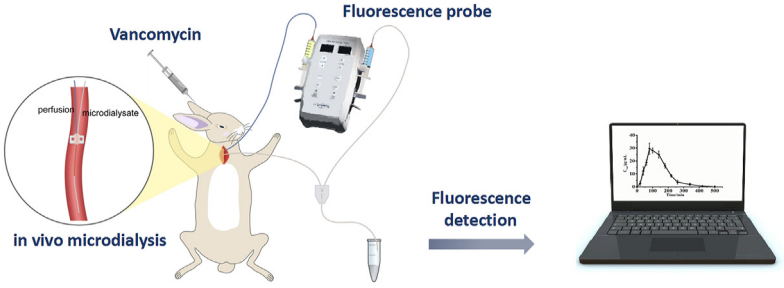
## HIGHLIGHTS

- Microdialysis sampling enables vancomycin (Van) monitoring by a biosensor.
- Pharmacokinetics of Van was recorded using the biosensor in normal and CRF rabbits.
- Van multidose in normal and CRF rabbits achieved steady state at therapeutic range.
- Fluorescent Van biosensor with microdialysis sampling may replace blood sampling.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Clinical vancomycin (Van) treatment is administered over a prolonged period to achieve a sustained therapeutic effect. Accurate, rapid, and continuous Van detection is an unmet medical monitoring need that can provide data-based guidance for doctors to adjust the dosage and treatment plan in real-world settings. In this study, we created a Van-specific, fluorescent biosensor that was combined with a microdialysis sampling technique to develop a rapid, simple, accurate, and sensitive detection method, which was validated for Van *in vivo*. A Van-specific probe was created by separately conjugating each of the two peptide chains of a dimeric derivative of the Van binding peptide L-Lys-D-Ala-D-Ala to a fluorescent dansyl chloride group. Subject-specific pharmacokinetics of Van was recorded in normal rabbits and rabbits with adenine-induced chronic renal failure (CRF), which indicated the feasibility of therapeutic drug monitoring *in vivo*. The area under the concentration–time curve ($AUC_{0-last}$) was 10,715 min μg $mL^{-1}$ (95% CI = 8,892 to 12,538) in the normal rabbits, and 14,822 and 19,025 min μg $mL^{-1}$ in two selected CRF rabbits. Furthermore, using pharmacokinetic dosing of Van in a rabbit study, we designed the dosage and drug administration interval to achieve a sustained therapeutic effect. The normal rabbits received three Van doses, 20, 5, and 5 mg $kg^{-1}$, administered at 0, 500, and 900 min, respectively. The CRF rabbits received two Van doses, 20 and 5 mg $kg^{-1}$, administered at 0 and 600 min, respectively. Thus, we established an effective method for the continuous monitoring of Van. This

*Abbreviations:* $AUC_{0-last}$, area under the concentration–time curve from 0 h to the last measured value; BUN, blood urea nitrogen; $C_{max}$, peak drug concentration; CRF, chronic renal failure; CV, coefficient of variation; HE, hematoxylin and eosin staining; LLOQ, lowest limit of quantification; MD, microdialysis (MD); PAS, Periodic acid–Schiff stain (PAS); SCr, serum creatinine; TDM, therapeutic drug monitoring; Van, vancomycin.

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method facilitates the detection of Van in clinical treatment and provides the scientific basis for an effective approach to monitor blood drug levels during the clinical treatment of various diseases.



## 1. Introduction

Vancomycin (Van) is a broad-spectrum, glycopeptide antibiotic, mainly used in the prophylaxis and treatment of serious or severe infections caused by Van-susceptible strains of methicillin-resistant *Staphylococcus aureus* [1]. It is often reserved as the "drug of last resort" when other antibiotics are not effective. However, high Van blood concentrations can lead to serious adverse reactions, such as ear and renal toxicity, as well as thrombotic phlebitis. To prevent over- or under-dosing, therapeutic drug monitoring (TDM) of Van is recommended [2,3]. TDM requires patients to visit medical facilities for a painful, invasive procedure involving conventional blood sampling methods from their fingers using needles. Therefore, developing a painless TDM method is critical for implementing precision medicine, which reduces the risk of adverse drug events in patients [4].

Once a Van-treatable disease is diagnosed, it is critical to implement a precise, individualized treatment regimen. However, following current clinical treatment guidelines, patients are at risk of developing Van resistance due to high dosages and prolonged treatment periods. Furthermore, individual differences in the pathophysiology may lead to different drug metabolism in patients, resulting in insufficient dosage or adverse drug reactions. Thus, to achieve individualized and precise Van treatment, continuous and instantaneous TDM is required [5], which can reduce adverse drug reactions during treatment and provide the scientific basis for precise adjustments of clinical protocols.

Methods for clinical monitoring of Van levels are immunoassay and HPLC based, which often require multiple samples and sample pre-treatment before analysis, resulting in long processing periods and potential delays. To expand the options for Van monitoring, the proposed analytical methods include detection via luminol–$H_2O_2$–CuO nanosheet chemiluminescence [6], direct fluorescence polarization [7], peptide-functionalized cantilever array sensor [8], and spectrophotometry [9–11], along with electrochemical detection [12]. However, these Van monitoring approaches have some limitations, such as expensive instruments, time-consuming procedures, not suitable for automated analysis, and the need for toxic solvents. Thus, these approaches cannot provide efficient and comprehensive guidance for the timely adjustment of clinical treatment regimens, especially in the event of adverse drug reactions.

Microdialysis (MD) is a minimally invasive membrane sampling technique that has been widely applied to study drug metabolism in humans and animals [13]. Because the MD technique does not involve body fluid loss, the patient can be continuously monitored for drug or metabolite concentrations over several hours, days, or even weeks [14]. Based on this, the concentration–time curve can be constructed. Bue et al. [15] reported the feasibility of determining Van drug concentrations using MD probes in pigs, and a plasma pharmacokinetics analysis of Van was performed over 12 h.

In this study, we focused on developing an *in vivo* Van test with high specificity and sensitivity. The tripeptide L-Lys-D-Ala-D-Ala is known for its high affinity to Van, and it has been demonstrated that linking the tripeptide to a dansyl chloride group creates a Van-specific fluorescent probe ($N^{\alpha}$-dansyl-$N^{\varepsilon}$-Ac-L-Lys-D-Ala-D-Ala) with a high association constant. Furthermore, it has been reported that the dimeric derivative of the Van binding peptide has an even higher affinity to Van than the monomeric tripeptide [16,17]. Based on these previous findings, we designed a probe in which each peptide chain of the dimeric Van binding peptide derivative was separately linked to a dansyl chloride group. Thus, we were able to develop a Van probe with improved fluorescent properties (*d*-Dansyl-KAA) (Scheme 1). To achieve *in vivo* detection of Van, we combined an MD-based *in vivo* sampling technology with the fluorescent probe detection method. We evaluated the selectivity, precision, and linear range of the Van test. Moreover, we used the test in a pharmacokinetic study of Van in rabbits to develop the theoretical foundation for future practical applications.

## 2. Materials and methods

### 2.1. Chemicals

Van hydrochloride, chromatographic grade acetonitrile, and isopropanol were purchased from Sigma-Aldrich (St. Louis, MO, USA); *s*-Dansyl-KAA and *d*-Dansyl-KAA were synthesized by ChinaPeptides Co., Ltd. (Shanghai, China); Urea Assay Kit and Creatinine Assay Kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The Van ELISA Kit was purchased from Jinning Shiye Co. Ltd. (Shanghai, China). Other reagents were of analytical grade. All aqueous solutions were prepared with MilliQ water (18 MΩ cm) (Millipore, Eschborn, Germany).

### 2.2. Equipment and instrument settings

UV–vis and fluorescence spectroscopy of *s*-Dansyl-KAA and *d*-Dansyl-KAA were recorded by a microplate reader (Infinite M200 Pro, TECAN, Mannedorf, Switzerland). The excitation wavelength was 340 nm, and the emission was collected from 400 to 700 nm. Samples were analyzed by HPLC-UV (LC-30A, Shimadzu, Kyoto, Japan) using a Shim-pack XR-ODS III column (column dimensions, 2.0 mm *i.d.* × 75 mm; particle size, 1.6 μm; Shimadzu). The Van detection wavelength was 220 nm.

### 2.3. Preparation of standard and working solutions

#### 2.3.1. Stock solutions

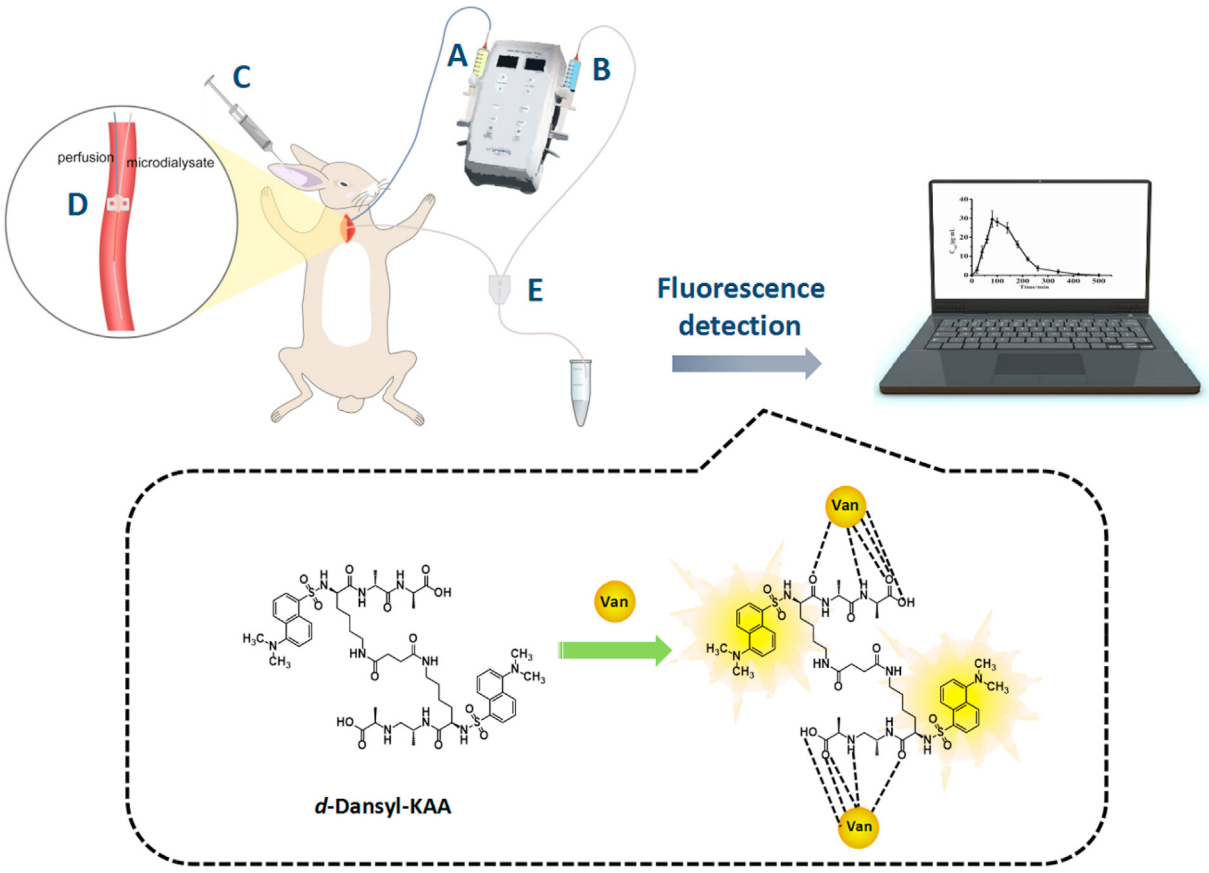
A 1 g $L^{-1}$ stock solution of Van was prepared by dissolving 10 mg Van in 10 mL double distilled water. Stock solutions of 1 mM s-Dansyl-KAA and 1 mM d-Dansyl-KAA were prepared with deionized water. Stock solutions were diluted to obtain appropriate working solutions. All solutions were stored at 4 °C.

#### 2.3.2. Calibration samples

Microdialysate calibration samples were prepared by mixing appropriate volumes of Van working solution with the microdialysate from anticoagulated whole blood to prepare Van concentrations of 0.02, 0.05, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 μg $mL^{-1}$.

#### 2.3.3. QC samples

Microdialysate QC samples were prepared by serial dilution of Van working solutions with the microdialysate from anticoagulated




**Scheme 1.** Schematic diagram of the vancomycin (Van) detection system. (A): perfusion fluid; (B): fluorescent probe (*d*-Dansyl-KAA); (C): vancomycin; (D): microdialysis probe; (E): three-way connector.

whole blood to obtain spiked matrix samples containing 0.1, 0.25, 1, 10, and 50 μg $mL^{-1}$ Van.

### 2.4. Fluorescence measurement

The *d*-Dansyl-KAA (40 μL, 10 μM) was added to the collected microdialysate samples (40 μL; 1:1, *v/v*) and mixed for 2 min at room temperature (25 °C). The fluorescence intensity of the solution was then measured at an excitation wavelength of 340 nm and emission wavelength of 570 nm.

### 2.5. Analytical validation

Microdialysate calibration samples of Van were mixed with *d*-Dansyl-KAA (10 μM) (1:1, *v/v*). The fluorescence intensity was plotted versus the Van concentration (in μg $mL^{-1}$) to construct the calibration curve and derive the regression equation. QC samples were used to assess the precision and accuracy of an assay. Replicates (n = 5) of each concentration were analyzed on the same day for intra-assay and on three separate days for inter-assay precision and accuracy determination. The precision should have a relative standard deviation within 15%, and its accuracy should be within 15% of the nominal concentration [18].

Van working solution was added to the microdialysate to obtain 0.1 μg $mL^{-1}$. The lowest limit of quantification (LLOQ) was defined as the lowest concentration within an acceptable range of 20% for accuracy and precision.

Van specificity was assessed to determine the suitability of the method for measuring Van in clinical samples. The optical response of *d*-Dansyl-KAA to various antibiotics, including teicoplanin, imipenem, meropenem, piperacillin, cefoperazone, ceftazidime, penicillin, tetracycline, gentamicin, and quinolone, was measured under the same conditions used for Van, i.e., excitation at 340 nm and emission at 570 nm.

### 2.6. Method comparison

Van concentrations in the microdialysate samples were analyzed with the proposed method (*d*-Dansyl-KAA) and an HPLC method using UV detection or the Van ELISA Kit in accordance with the manufacturer's instructions. Passing-Bablok regression analysis and Bland-Altman plot were performed to evaluate the agreement between the two different analytical methods.

### 2.7. Microdialysis (MD)

CMA/20 probes (membrane length, 10 mm; molecular weight cut-off, 20–100 kDa) and a CMA 402 Syringe Pump from CMA Microdialysis AB (Stockholm, Sweden) were used in this study. To determine the relative recovery for Van, the probe was perfused at 2 μL $min^{-1}$ of normal saline while immersed in a vial filled with a Van solution of known concentration. Samples (n = 9, at three concentrations) were collected at 30 min intervals. The relative recovery R(%) was calculated according to $R(\%) = C_{out}/C_{in} \times 100$ (Equation 1), where $C_{out}$ is the solution concentration in the dialysate calculated from the calibration curve, and $C_{in}$ is the known concentration of the Van solution. In our study, the calculated R(%) was 9.75% (CV 4.89%) on average (Table S1).

### 2.8. Animal experiments

Fourteen adult male New Zealand rabbits (2–2.5 kg, JSJ-Lab Co., Ltd., China) were randomly divided into the normal group (n = 7) and the chronic renal failure (CRF) group (n = 7). CRF was induced by feeding the rabbits with 0.75% adenine rabbit feed (Trophic Animal Feed High-tech Co., Ltd., China). All animals were maintained in a controlled environment (22 ± 1 °C, 12 h light-dark cycle) with *ad libitum* access to food and water. All procedures involving animals were approved by the Animal Ethics Committee at the East China Normal University, Shanghai, China (No. Rb 20190701).

Rabbits were anesthetized with thioethamyl (initial dose of 30 mg $kg^{-1}$ and continuous infusion with 20–40 mg $h^{-1}$). A flexible intravenous MD probe was implanted into the jugular vein under perfusion with heparin sodium (10 mg $mL^{-1}$) for approximately 20 min to prevent blood clotting around the dialysis fiber. After anticoagulation treatment, normal saline was perfused at 2 μL $min^{-1}$ for 30 min to equilibrate. Then, based on actual body weight, 5-20 mg $kg^{-1}$ Van was administered intravenously. Using non-stop perfusion of normal saline at 2 μL $min^{-1}$, the first dialysate was collected after initiation of Van infusion. Dialysates were collected at 20 min intervals during the initial 3 h period and at 40 min intervals during the following 20 h. The syringe installed in the CMA 402 Syringe Pump contained the d-Dandyl-KAA solution that was mixed with the microdialysate through a three-way connector. Fluorescence-labelled microdialysates were collected and dispensed onto microplates for quick analysis.

### 2.9. HPLC analysis

The analytical HPLC conditions were as follows: a sample injection volume of 5 μL was used, the mobile phase was composed of 57% acetonitrile/43% water with 0.025% hydrochloric acid, and a flow rate of 0.3 mL $min^{-1}$ was used. The column oven temperature was 40 °C. The UV detection wavelength was 220 nm. The analyte peaks in the chromatograms were integrated, and the peak areas were calculated using the LabSolutions software developed by Shimadzu.

### 2.10. Biochemical measurements

Blood samples were collected from each rabbit using the marginal ear vein, and the separated plasma samples were used to assess the renal function by measuring creatinine and urea levels. The blood urea nitrogen (BUN) and serum creatinine (SCr) were measured spectrophotometrically using commercial assay kits.

### 2.11. Histopathology

Kidney tissue samples were fixed in 10% neutral buffered formalin solution and embedded in paraffin. The samples were sectioned (5 μm) and stained with hematoxylin and eosin, periodic acid-Schiff (PAS) stain, and Masson's trichrome stain to verify histological details in the renal tissues.

### 2.12. Statistical analysis

Statistical analyses were performed with MedCalc (Ostend, Belgium). The correlation coefficient, intercept, and slope were estimated using Passing-Bablok regression analysis with 95% confidence intervals (95% CI). The agreement between methods was tested using a Bland-Altman plot, and the limits of the agreement were defined as the mean difference ±1.96 SD. A P-value less than 0.05 indicated statistical significance. Results are presented as mean (CV%) unless stated otherwise.

## 3. Results and discussion

### 3.1. Analytical performance parameters of Van test

Fluorescence spectra of *d*-Dansyl-KAA were recorded either without Van or at different Van concentrations (Fig. 1A). The maximum fluorescence intensity was measured at 570 nm when the excitation wavelength was 340 nm. The lowest fluorescence intensity was observed in the absence of Van. The fluorescence intensity of the *d*-Dansyl-KAA emission peak increased gradually in the presence of increasing Van concentrations. Thus, the enhanced fluorescence signal intensity was directly correlated with the Van concentration within a certain range, which made it possible to develop a sensitive Van detection method. The Van-dependent effect on the *d*-Dansyl-KAA fluorescence emission, which was observed using Van in the concentration range of 0.02–100 μg $mL^{-1}$, can be expressed with a nonlinear curve-fitting equation: $Y = 1.0618 + 0.1444\ (1\text{-exp}\ (-x/0.7195)) + 0.7434\ (1\text{-exp}\ (-x/29.5491))$ (regression coefficient, $R^2 = 0.9983$). A nonlinear relationship was also observed in the lower concentration range (0.02–5 μg $mL^{-1}$), which met the detection range of microdialysate samples. The calibration curve was described by the equation:

$$Y = 1.0360 + 0.5056\ (1\text{-exp}\ (-x/0.0458)) + 0.2502\ (1\text{-exp}\ (-x/1.9563))$$

with a regression coefficient, $R^2 = 0.9992$, where $Y$ represents the fluorescence ratio ($F/F_0$), and $x$ represents the Van concentration. Using *d*-Dansyl-KAA for Van detection was associated with an improved Van concentration range compared with that using *s*-Dansyl-KAA (Van concentration: 1–50 μg $mL^{-1}$) (Fig. S1). The chemical structures of *s*-Dansyl-KAA and *d*-Dansyl-KAA are shown in Scheme S1. The complex of D-Ala-D-Ala with Van is stabilized by an array of hydrophobic van der Waals contacts and five hydrogen bonds (H-bonds) lining the Van binding pocket (Scheme 1). The strong fluorescence signals emitted from the biosensor were observed because the probe could rapidly bind to Van via hydrogen bond, leading to a change in the electron cloud charge density. The divalent complex had an improved dissociation constant and enhanced binding (~$10^3$) compared with those of the corresponding interaction between Van and diacetyl-L-Lys-D-Ala-D-Ala [16,19].

### 3.2. Optimization of detection conditions

The concentration of *d*-Dansyl-KAA, reaction time, and temperature were optimised for Van detection performance. The fluorescence intensity ratios ($F/F_0$) were observed to initially increase, and subsequently decrease with increasing *d*-Dansyl-KAA concentration from 2 to 30 μM (Fig. S2A). Thus, 10 μM d-Dansyl-KAA was selected for subsequent experiments. Moreover, temperature was found to have negligible effects on Van detection. Therefore, these experiments were performed at ambient temperature (25 °C; Fig. S2B). The *d*-Dansyl-KAA fluorescence response to Van with reaction time is presented in Fig. S2C. Furthermore, at a reaction time of 2 min, the fluorescence intensity ratio of the system plateaued; thus, 2 min was selected as the reaction time for subsequent experiments.

### 3.3. Validation of the methods

The results of the intra- and inter-assay precision and accuracy evaluation met validation requirements (Table 1). Specifically, the Van test had an intra-assay precision of 2.4%–7.9% and inter-assay precision of 3.1%–7.5%; accuracy was in the range of 95.2%–114.0%

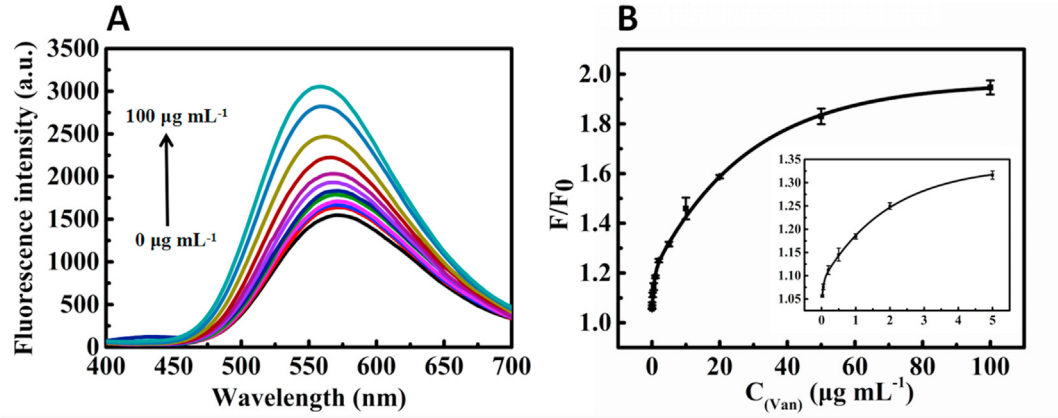



**Fig. 1.** Effect of Van on fluorescence emission of *d*-Dansyl-KAA. **A**: Fluorescence spectra of d-*d*-Dansyl-KAA measured in the presence of 0, 0.02, 0.05, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 μg mL$^{-1}$ Van. **B**: Plot of F/F$_0$ as a function of the Van concentration, where F$_0$ and F are the fluorescence intensities (at 570 nm) emitted from *d*-Dansyl-KAA in the absence and the presence of Van, respectively. Abbreviation: a.u., arbitrary units.

**Table 1**
Method validation parameters: precision and accuracy.

| Nominal concentration (μg mL$^{-1}$) | Intra-assay evaluation | | | Inter-assay evaluation | | |
|---|---|---|---|---|---|---|
| | Observed concentration (μg mL$^{-1}$) mean ± SD | Precision (CV %) | Accuracy (R %) | Observed concentration (μg mL$^{-1}$) mean ± SD | Precision (CV %) | Accuracy (R %) |
| 0.25 | 0.24 ± 0.01 | 5.9 | 95.2 | 0.24 ± 0.02 | 7.5 | 96.8 |
| 1.0 | 1.14 ± 0.09 | 7.9 | 114.0 | 1.16 ± 0.07 | 6.0 | 115.9 |
| 10.0 | 10.8 ± 0.26 | 2.4 | 107.6 | 10.7 ± 0.38 | 3.6 | 106.5 |
| 50.0 | 50.7 ± 1.44 | 2.9 | 101.3 | 50.3 ± 1.54 | 3.1 | 100.6 |

for intra-assay evaluation and 96.8%–115.9% for inter-assay evaluation. Using the QC samples at low concentrations, the LLOQ of the Van test was 0.1 μg mL$^{-1}$ with a CV of 13.95% (R 117.4%) for microdialysates. Thus, an LLOQ suitable for Van testing was achieved in microdialysate samples.

Selectivity is a critical parameter for potentially using the newly developed test for Van detection in real samples. We tested the optical response of *d*-Dansyl-KAA to various antibiotics, including teicoplanin, imipenem, meropenem, piperacillin, cefoperazone, ceftazidime, penicillin, tetracycline, gentamicin, and quinolone, using the same conditions with 340 nm as excitation wavelength and 570 nm as emission wavelength. Compared with the clear fluorescence emission increase by Van, no substantial change in fluorescence intensity was detected for other antibiotics, except for teicoplanin (Fig. 2). Furthermore, we confirmed that the fluorescence emission intensity of *d*-Dansyl-KAA was increased by adding teicoplanin and Van (see Fig. S3). Teicoplanin and Van are both glycopeptide antibiotics. Since they specifically bind peptides with D-Ala-D-Ala at the carboxyl terminus, they have a similar effect on the fluorescence emission of *d*-Dansyl-KAA [20].

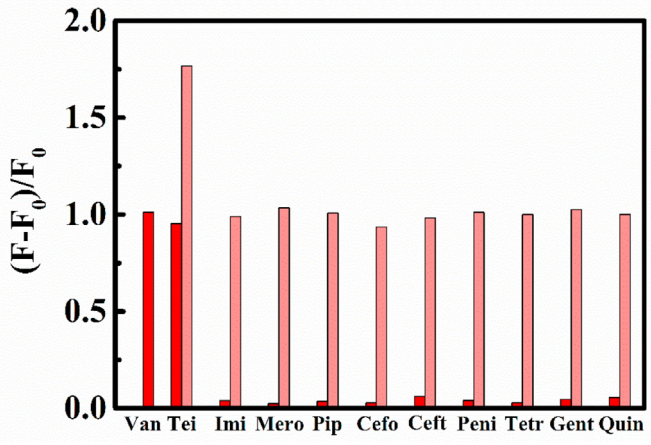



**Fig. 2.** Fluorescence response of *d*-Dansyl-KAA to various antibiotics. Red columns represent *d*-Dansyl-KAA fluorescence measured in the presence of different antibiotics (50 μg mL$^{-1}$), including Van. Pink columns represent *d*-Dansyl-KAA fluorescence after adding 50 μg mL$^{-1}$ Van combined with 100 μg mL$^{-1}$ of other antibiotics. Abbreviations: Tei, teicoplanin; Imi, imipenem; Mero, meropenem; Pip, piperacillin; Cefo, cefoperazone; ceftazidime; Peni, penicillin; Tetr, tetracycline; Gent, gentamicin; Quin, quinolone. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 3.4. Method comparison

Using the Bland-Altman method, the difference expressed as a percentage of the mean concentration values were plotted against the mean values derived from the results between the newly developed *d*-Dansyl-KAA assay and the HPLC assay using microdialysate samples (Fig. 3A). Fig. S4 shows the representative HPLC chromatograms of microdialysate samples analyzed before and after being spiked with Van. The mean difference between the two methods was −5.9%, with one sample outside the ±1.96 standard deviation range (−20.6% to +32.4%). Our test method had an acceptable mean difference from the HPLC method (<10%).

We also performed the Passing-Bablok regression analysis to investigate the correlation between the test results of the two methods (Fig. 3B). The linear regression model equation was near to ideal ($Van_{d\text{-Dansyl-KAA}} = 0.223 + 1.019\ Van_{HPLC}$). The correlation coefficient between the two methods was 0.981 without linearity deviations. The Passing-Bablok regression slope of the comparison between Van concentrations measured using the *d*-Dansyl-KAA and HPLC method did not detect a proportional bias (95%

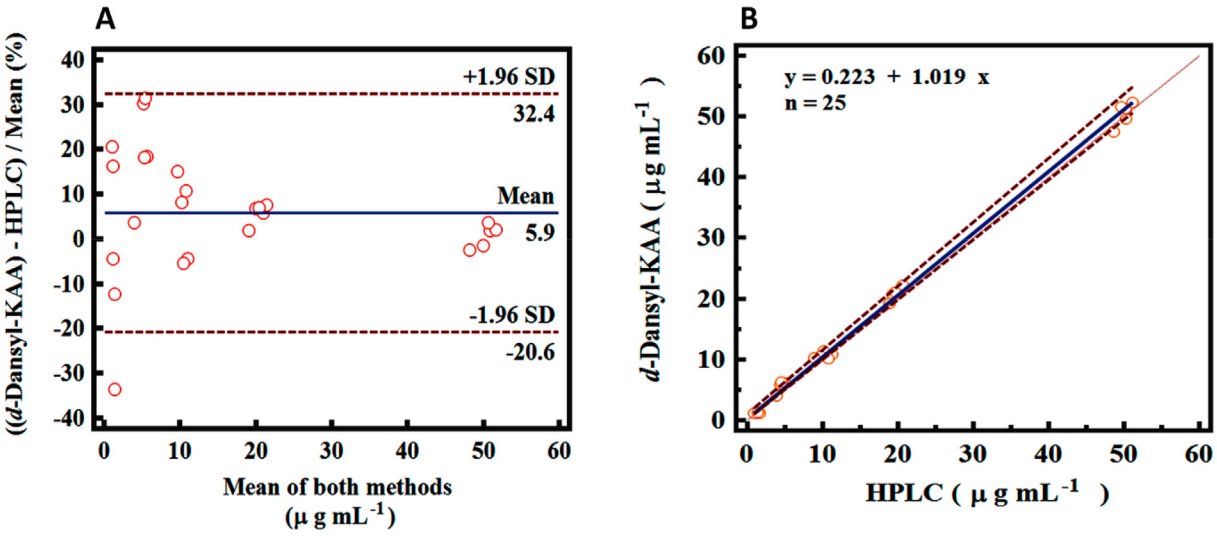



**Fig. 3.** Comparison of Van test results obtained with *d*-Dansyl-KAA and HPLC assay using Bland-Altman analysis (**A**) and Passing-Bablok regression analysis (**B**).

CI = 0.9884 to 1.0529). However, the intercept of the regression indicated a low systematic bias (95% CI = 0.1334 to 1.0258). Hence, we statistically confirmed a positive correlation between our new method and the HPLC method for measuring the Van concentration in microdialysates.

### 3.5. Pharmacokinetics of Van in rabbits

In our rabbit study, the SCr and BUN levels in normal rabbits were 38.81 ± 7.57 μmol $L^{-1}$ and 5.22 ± 0.92 mmol $L^{-1}$ (n = 5), respectively. The SCr level of 125.26 ± 25.19 μmol $L^{-1}$ in adenine-induced CRF rabbits (n = 5) was substantially higher than the normal level. Similarly, the BUN level of 12.22 ± 2.26 mmol $L^{-1}$ in adenine-induced CRF rabbits (n = 5) was substantially increased compared to that in normal rabbits. Thus, we showed that the SCr and BUN levels were significantly higher in rabbits with adenine-induced CRF than in normal rabbits ($P < 0.05$), indicating severe renal injury after feeding adenine-enriched for 30 d [21,22]. Fig. 4 shows the histological findings with the use of hematoxylin and eosin, PAS, and Masson's trichrome staining of kidney sections from normal control rabbits (A, B, C) and adenine-induced CRF rabbits (D, E, F) after the 30-d study period. Morphologically, the kidneys from the CRF rabbits displayed renal tube base membrane thickening, renal interstitial fibrosis, glomerular fiber hyperplasia, and glomerular damage manifested as glomerulosclerosis and hypertrophy [23,24]. Our observations indicated that this rabbit model displayed pathological features typically associated with CRF. Some of these characteristics are presented in Fig. 4.

Using the method as depicted in Scheme 1, we obtained a good correlation between the Van concentrations in the MD probes. Plasma concentration–time profiles of select rabbits (three normal rabbits; CRF rabbit 1 and 2) are shown in Fig. 5, and the corresponding PK parameters are provided in Table 2. A dose of 20 mg $kg^{-1}$ Van was intravenously administered based on actual

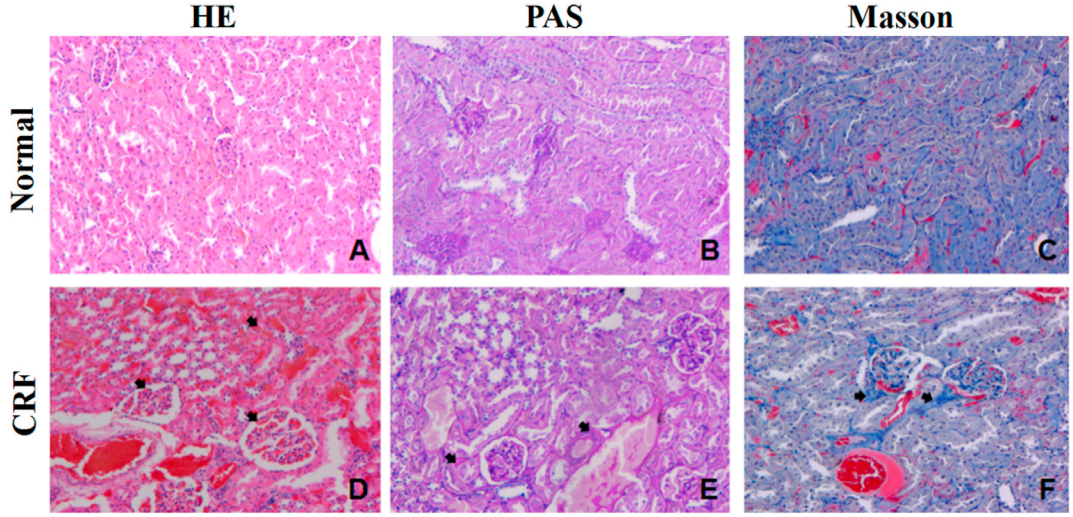



**Fig. 4.** Pathological differences between the kidneys of normal and CRF rabbits (×100). Kidney histopathology is compared between rabbits fed with a standard diet (A, B, C) and 0.75% adenine-enriched diet (D, E, F). A and D show the general morphology enhanced by hematoxylin and eosin staining (HE). In D, glomerular mesangial cells exhibit mild hyperplasia and hypertrophy; swelling of renal tubular epithelial cells can be observed with vacuole degeneration. B and E were processed using the Periodic acid–Schiff stain (PAS). In E, CRF is indicated by the thickening of the glomerular basement membrane (GBM) and tubular basal membrane with mesangial matrix expansion. C and F were processed by Masson's trichrome stain for collagen and fibres. In F, the stain revealed mild renal interstitial fibrosis.

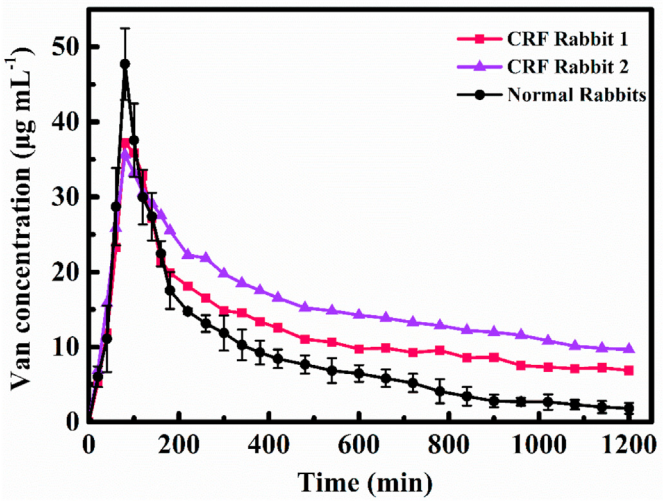



**Fig. 5.** Mean concentration–time profiles of Van in normal and CRF rabbits.

**Table 2**
Pharmacokinetic parameters of Van in normal rabbits (n = 3), CRF rabbit 1, and CRF rabbit 2.

| PK parameter | Normal rabbits[a] | CRF rabbit 1 | CRF rabbit 2 |
|---|---|---|---|
| $AUC_{0-last}$ (min·μg $mL^{-1}$) | 10,715 (8,892, 12,538) | 14,822 | 19,025 |
| $C_{max}$ (μg $mL^{-1}$) | 38.2 (33.0, 43.4) | 33.3 | 30.6 |
| $T_{max}$ (min) | 152 (127, 177) | 148 | 128 |
| $T_{1/2}$ (min) | 209 (167, 251) | 222 | 536 |

$AUC_{0-last}$, area under the concentration–time curve from 0 h to the last measured value; $C_{max}$, peak drug concentration; $T_{max}$, time to $C_{max}$; $T_{1/2}$, half-life at β phase.
[a] Values derived from three normal rabbits are given as the mean (95% CI) unless otherwise stated.

body weight. In CRF rabbit 1 (14,822 min μg $mL^{-1}$) and CRF rabbit 2 (19,025 min μg $mL^{-1}$), the area under the curve from 0 h to last time point ($AUC_{0-last}$) was higher than in the normal rabbits (10,715 ± 1,823 min μg $mL^{-1}$, mean ± SD, n = 3) ($P < 0.05$). There was also a difference between the means of the peak drug concentration ($C_{max}$); in CRF rabbit 1 (33.3 μg $mL^{-1}$) and CRF rabbit 2 (30.6 μg $mL^{-1}$), the $C_{max}$ was lower than in the normal rabbits (38.2 ± 5.2 μg $mL^{-1}$, mean ± SD, n = 3) ($P < 0.05$). Thus, $AUC_{0-last}$ and $C_{max}$ values varied significantly between the normal rabbits and both CRF rabbits. The Van half-life is approximately 3–9 h in normal rabbits. Excretion via the urine by glomerular filtration eliminates approximately 75%–80% of the Van dose, but renal impairment slows this excretion route [25]. In CRF rabbits, the kidney damage extended the average Van half-life of elimination by drug metabolism, which can be up to 9 h as in our experiment. The half-life time is prolonged in rabbits with varying degrees of kidney damage. Therefore, monitoring the Van plasma level is necessary owing to the potential for nephrotoxicity and ototoxicity, especially in populations with impaired renal function.

Meanwhile, Bland-Altman, as well as Passing & Bablok regression, analyses were employed to compare our developed assay to ELISA assays to evaluate the consistency of microdialysis system performance in Van pharmacokinetics in rabbits (Fig. S5). The Bland-Altman plot showed a mean difference between the methods of 6.8%, with 3/60 (5%) samples located outside the ±1.96 standard deviation range (−16.7 to +30.2%). Furthermore, Passing-Bablok regression analysis was performed to investigate the correlation. The linear regression model equation was $Van_{d\text{-Dansyl-KAA}} = 0.144 + 1.052\ Van_{ELISA}$ with a correlation coefficient of 0.991. Therefore, it can be concluded that the results of our method and the ELISA method are in good agreement.

### 3.6. Individualized administration of Van

Van treatment is challenging due to its narrow therapeutic window. The Van plasma AUC/minimum inhibitory concentration ratio of ≥400 is a good predictor of therapeutic success [26]. Therefore, Van plasma concentrations between 10 and 20 μg $mL^{-1}$ are considered within the therapeutic range [3,27]. This study established drug concentration–time profiles in blood, using an animal model. Based on the trendline, a polynomial equation of regression was derived. We calculated the dosage interval and duration of treatment by superposing the drug concentration–time profiles of different dosage concentrations, based on the steady-state conditions for examining the feasibility of TDM in our study. In normal rabbits (n = 3) and CRF rabbits (n = 3), we used different loading doses of 20 mg $kg^{-1}$, 10 mg $kg^{-1}$, and 5 mg $kg^{-1}$ to characterize the association, including both horizontal and vertical variations, between concentration and time (Fig. S6). The following equations were used for normal rabbits: 20 mg $kg^{-1}$ with a fitting equation $Y_1 = P_1x^5 + P_2x^4 + P_3x^3 + P_4x^2 + P_5x + P_6$ ($P_1 = -7.664\exp(-13)$, $P_2 = 3.279\exp(-10)$, $P_3 = 1.451\exp(-6)$, $P_4 = -0.001481$, $P_5 = 0.4118$, $P_6 = -4.242$); 5 mg $kg^{-1}$ with a fitting equation $Y_2 = P_1x^5 + P_2x^4 + P_3x^3 + P_4x^2 + P_5x + P_6$ ($P_1 = 4.103\exp(-12)$, $P_2 = -8.749\exp(-9)$, $P_3 = 6.551\exp(-6)$, $P_4 = -0.002136$, $P_5 = 0.2713$, $P_6 = -0.957$). The following equations were used for CRF rabbits: 20 mg $kg^{-1}$ with a fitting equation $Y_1 = P_1x^8 + P_2x^7 + P_3x^6 + P_4x^5 + P_5x^4 + P_6x^3 + P_7x^2 + P_8x + P_9$ ($P_1 = -9.184\exp(-21)$, $P_2 = 5.153\exp(-17)$, $P_3 = -1.207\exp(-13)$, $P_4 = 1.526\exp(-10)$, $P_5 = -1.121\exp(-7)$, $P_6 = 4.783\exp(-5)$, $P_7 = -0.01106$, $P_8 = 1.116$, $P_9 = -6.514$); 5 mg $kg^{-1}$ with a fitting equation $Y_2 = P_1x^5 + P_2x^4 + P_3x^3 + P_4x^2 + P_5x + P_6$ ($P_1 = -7.973\exp(-11)$, $P_2 = 5.950\exp(-8)$, $P_3 = -1.429\exp(-5)$, $P_4 = 9.106\exp(-4)$, $P_5 = 0.05625$, $P_6 = 0.04869$). Then, we superposed the coordinates of different 'time points ($x$)' to calculate the sum of the corresponding concentrations to reach and maintain the range of 10–20 μg $mL^{-1}$ (when $x_a < x_b$ and $Y_1(x_a) > Y_1(x_b)$, $10 < Y_1 + Y_2 < 20$). Based on the calculation, we administered a multiple-dose regimen (Fig. 6). The normal rabbit (n = 1) received a loading dose of 20 mg $kg^{-1}$ of Van (0 min), followed by a supplementary dose of 5 mg $kg^{-1}$ after 500 min and the last dose of 5 mg $kg^{-1}$ after 900 min. The CRF rabbit (n = 1) received a loading dose of

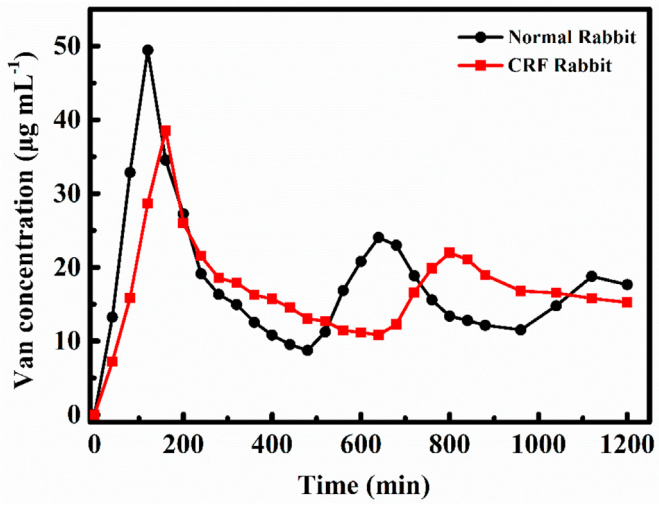



**Fig. 6.** Van concentration monitoring in rabbits. MD samples of one normal and one CRF rabbit were analyzed to determine the steady-state concentration of Van.

20 mg $kg^{-1}$ of Van (0 min), followed by a supplementary dose of 5 mg $kg^{-1}$ after 600 min. We obtained a good correlation between the calculations and the *in vivo* concentrations of Van, and we achieved adequate concentrations to maintain efficacy.

## 4. Conclusion

In this study, we developed a Van monitoring method that does not require blood sampling. The sensitivity, selectivity, and speed of the analysis, along with simple operation and continuous on-site monitoring, are the critical advantages of using direct spectrofluorimetry in this method. The pharmacokinetic profiles of Van in normal and CRF rabbits were compared, and Van concentrations were maintained at the steady-state level in normal and CRF rabbits after multi-dose administration. Our investigation provides guidance for using this Van monitoring method in human pharmacokinetic studies, and potentially, for Van TDM. It can reduce adverse drug events associated with Van regimens and create a scientific basis for the precise adjustment of clinical protocols. Next, we will develop high-precision clinical diagnosis and treatment equipment for collecting patient data that will provide accurate guidance on clinical individualization of the antibiotic therapy based on the sensor chip diagnosis and treatment system.

## CRediT authorship contribution statement

**Fangya Mu:** Conceptualization, Methodology, Investigation, Writing - original draft. **Xinguang Zhou:** Formal analysis, Validation. **Fang Fan:** Investigation, Writing - review & editing, Supervision. **Zhiyu Chen:** Methodology, Investigation, Validation. **Guoyue Shi:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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## Appendix A. Supplementary data

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