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Short communication

Evaluation of silver nanoparticles as a surface-enhanced Raman spectroscopy substrate for tacrolimus detection in spiked human plasma

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Introduction

Tacrolimus is a macrolide immunosuppressive drug widely used for the prevention of organ transplant rejection and is routinely monitored through therapeutic drug monitoring (TDM) due to its narrow therapeutic window. Conventional methods, including immunoassays and liquid chromatography coupled with tandem mass spectrometry, provide high sensitivity but often require expensive instrumentation and extensive sample preparation (Zhan et al., 2024).

Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive vibrational spectroscopic technique that enables rapid and non-destructive molecular analysis. Signal enhancement is primarily attributed to localized surface plasmon resonance (LSPR), a phenomenon involving collective oscillation of conduction electrons on noble metal nanostructures, generating highly amplified electromagnetic fields near the nanoparticle surface. Silver nanoparticles are particularly attractive SERS substrates because of their strong plasmonic properties and excellent signal enhancement capability (Lai et al., 2024). However, direct determination of tacrolimus in plasma remains challenging due to its low concentration and the complexity of the biological matrix. The aim of this study was to evaluate the potential of silver nanoparticles deposited in the form of thin films as SERS-active substrates for the detection of tacrolimus in spiked human plasma samples and to investigate the future potential of zeolitic imidazolate framework-8 (ZIF-8) for analyte enrichment and matrix clean-up prior to SERS analysis.

Materials and methods

Sample collection and plasma preparation

Human blood was collected from a healthy volunteer in an ethylenediaminetetraacetic acid (EDTA) collection tube. Plasma was obtained by centrifugation using a C28 centrifuge (BOECO, Germany) and separated for further analysis.

Preparation of tacrolimus-spiked plasma

A tacrolimus standard solution was prepared by dissolving 8.04 mg of Tacrolimus Monohydrate CRS (EDQM) in 10 mL of 96% (*V/V%*) ethanol, resulting in a concentration of 0.804 mg mL^{-1} ($9.8 \times$

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10^{-4} mol L^{-1}). Spiked plasma samples were prepared by mixing 1 mL of plasma with 1 mL of the tacrolimus standard solution, followed by vortex mixing using a V-2H vortex mixer (BOECO, Germany).

Preparation and characterization of SERS substrates

Nanoplasmonic thin films built up by close packed silver nanoparticles were deposited on glass substrates utilizing a recently developed sonochemically-assisted colloidal bottom-up approach (Pejova et al., 2023). The structure, surface morphology and optical properties of the synthesized materials were characterized by X-Ray diffraction (XRD), atomic force microscopy (AFM) and optical spectroscopy in the UV-Vis spectral region.

Raman measurements

Deposition of the investigated tacrolimus water solution and the spiked human plasma on the nanoplasmonic surfaces, acting as SERS substrates, as well as on standard laboratory glass substrates (as reference substrates) was carried out by drop casting and immersion. Raman spectra of the deposited samples on glass and nanoplasmonic surfaces were measured using a LabRam300 spectrometer by Horiba Jobin–Yvon, using the red 632.817 nm HeNe laser line and a backscattering mode.

Results and discussion

Raman spectra of pure tacrolimus deposited on glass substrates show all the characteristic vibrational bands reflecting its macrolide lactone structure. These include the C-O(H) (carbon-hydroxyl oxygen) stretching and bending modes (~ 1440 cm^{-1}) , the unsaturated C=C (~ 1650 cm^{-1}) and carbonyl C=O stretching (~ 1720 cm^{-1}) modes, the in-plane bending modes due to aromatic and aliphatic segments (~ 1130 cm^{-1}), as well as the out-of-plane bending motions of the C-O-C, C-C-C and C-H fragments (~ 850 cm^{-1}).

Samples of human plasma spiked with tacrolimus exhibited a more complex Raman spectral profile due to contributions from proteins, lipids, carbohydrates and other endogenous compounds. Nevertheless, spectral windows allowing distinction of tacrolimus-related modes were identified. Deposition of tacrolimus and spiked plasma samples on the SERS substrates resulted in increase of the Raman intensities for 2-3 orders of magnitude, opening the possibilities for detection of the title species in complex matrices using predefines spectral windows.

Conclusion

Our preliminary findings indicate the potential applicability of silver nanoparticles as a SERS-active substrate for tacrolimus detection in spiked human plasma samples. Nevertheless, plasma represents a complex biological matrix containing proteins, lipids and various endogenous compounds that may interfere with analyte detection and affect analytical sensitivity. To further improve method performance, future work will focus on the synthesis of ZIF-8-based nanocomposites (Zhan et al., 2024), which, owing to their high surface area and adsorption capacity, may facilitate analyte enrichment and matrix clean-up prior to SERS analysis.

Furthermore, the incorporation of silver nanoparticles within the ZIF-8 structure may promote the accumulation of tacrolimus molecules in close proximity to plasmonic SERS “hot spots”, resulting in enhanced signal intensity and improved detection sensitivity. This approach is particularly important considering that therapeutic tacrolimus concentrations are typically in the low nanogram-per-milliliter range, requiring enhanced sensitivity for future clinical applications.

References

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