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SERS INVESTIGATIONS OF 2-METHOXY-2-METHYLPROPYLISONITRILE (MIBI) AND ITS TECHNETIUM COMPLEX

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ABSTRACT. Technetium radiopharmaceuticals remain the most widely available and cost-effective compounds used in nuclear medicine imaging. However, exact information regarding the chemical structure of these compounds is scarce. In this study we present a spectroscopic investigation of Hexakis (2-methoxy-2-methylpropylisonitrile) (MIBI) Technetium (^{99m}Tc) (^{99m}Tc-sestamibi). Surface Enhanced Raman Scattering spectra of the ligand and the Technetium sestamibi complex were recorded. For the later, the measurements were recorded in solution as administered to patients and at different moments from the time of preparation in order to monitor its stability. The information provided by the vibrational characterization of the MIBI ligand allowed us to understand spectroscopic features pertaining to the SERS spectra of the Technetium complex.

Keywords: *Technetium; radiopharmaceuticals; SPECT; MIBI; SERS.*

1. INTRODUCTION

^{99m}Tc-Sestamibi is a Technetium based radiopharmaceutical commonly used for Single Photon Emission Computed Tomography (SPECT) imaging. Since its introduction, as a cardiac imaging agent [1], it rapidly became a standard in nuclear medicine clinical practice for myocardial perfusion scans and parathyroid adenoma identification [2, 3]. The radiopharmaceutical, consisting of a ^{99m}Tc core bound to six 2-methoxy-2-methylpropylisonitrile (MIBI) ligands, presents an uptake mechanism

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dependent on mitochondrial and plasma membrane potentials, extending its use in oncology applications e.g. breast cancer imaging and Multidrug Resistance Mutation (MDR1) expression studies [4, 5].

Since molecular and electronic structure information is missing for both the Technetium complex and the MIBI ligand, we previously presented a joint experimental and computational study of the ligand. Using conformational and vibrational analysis based on DFT calculations in both harmonic and anharmonic approximations we obtained an accurate characterization of the Raman spectrum of MIBI [6].

The aim of the present study is to extend the previously reported investigation on the ^{99m}Tc-sestamibi complex. Due to the fact that in nuclear medicine studies radiopharmaceuticals are injected in very small quantities, we considered the Surface Enhanced Raman Scattering (SERS) technique as the best suited approach for this purpose. Thus, we present here an analysis of the SERS spectrum for the MIBI ligand, as well as the SERS spectrum of ^{99m}Tc-Sestamibi in the solution administered to patients. We will focus also on the observed differences that appear in the SERS spectrum at different time intervals after the radiolabelling of the Technetium complex.

2. EXPERIMENTAL DETAILS

2.1. Chemicals

As received aqueous solution of MIBI (Manchester Organics Ltd., 98.3% purity) was used without further purification.

Sodium pertechnetate solution was obtained following the daily elution of an Ultratechnekow^{TM} FM 12.9 GBq ^{99m}Tc generator purchased from Curium.

^{99m}Tc-Sestamibi solution was obtained using the Technescan^{TM} Sestamibi kit for radiopharmaceutical preparation purchased from Mallinckrodt Medical. The radiolabeling process was performed during regular clinical practice, following the manufacturer's instructions. Briefly, a 3ml saline solution of sodium pertechnetate was aseptically added to a vial from the preparation kit containing 1 mg [Tetrakis (2-methoxy-2-methylpropylisonitrile)copper(I)] tetrafluroborate. The vial was then placed in a water bath at 100^{o}C for 10 minutes and left to cool at room temperature for 15 minutes.

SERS measurements for both MIBI and ^{99m}Tc-Sestamibi were performed using an Ag-colloid as substrate, prepared by the Leopold-Lendl method [7]. We found that a 1:9 sample to substrate ratio yielded the best results for both MIBI and ^{99m}Tc-sestamibi measurements.

All radioactive solutions were handled according to radioprotection guides and local regulations.

2.2. Instrumentation

The SERS spectra were recorded on an Invia Reflex confocal Raman spectrometer (Renishaw, UK), equipped with a RenCam CCD detector and using the 532 nm excitation line from a Cobolt Diode Pumped Solid State (DPSS) laser and a diffraction grating with 1800 lines mm^{-1}.

3. RESULTS AND DISCUSSIONS

The interpretation of the SERS spectra is made in accordance with our previous work in which we presented a full assignment of the Raman spectra of the MIBI ligand based on DFT calculations, taking into account conformer analysis and both, the harmonic and anharmonic approximations [6].

Figure 1 shows the SERS spectrum of the MIBI ligand of 10^{-4}M concentration. It is worth mentioning that we previously found the limit of detection of 10^{-3}M by using the Raman technique [6]. Comparing the Raman and SERS spectra of MIBI it is evident that the main band observed in the Raman spectrum are also observed in the SERS spectrum, some of them being enhanced and/or shifted.

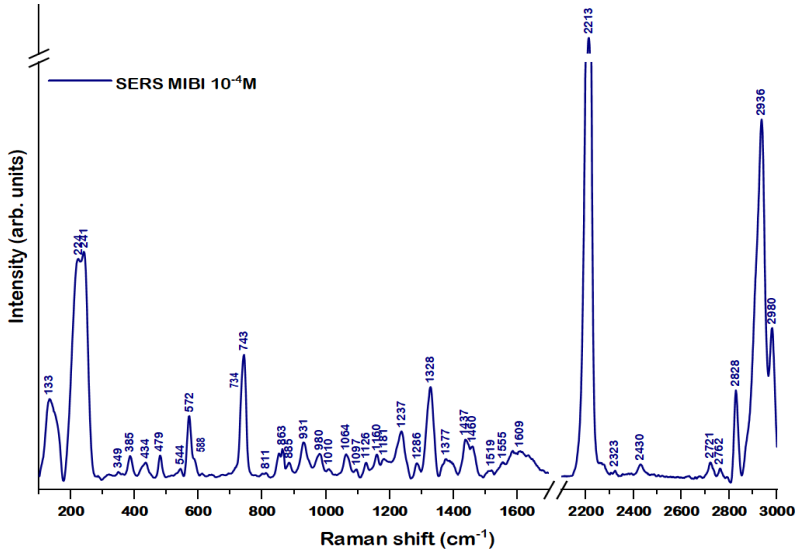

Figure 1. SERS spectrum of MIBI at 10^{-4}M

The most striking difference both in position and intensity is observed for the band at 2213 cm^{-1} in the SERS spectrum (2148 cm^{-1} Raman) assigned to a C≡N stretch mode [6]. The band suffers a significant blue-shift of 65 cm^{-1} and presents the largest enhancement. Other bands with significantly increased wavenumbers can be observed in the fingerprint region at 572, 479, 434 and 385 cm^{-1}. On the other hand the bands at 2936, 1377, 1328, 1286, and 1064 cm^{-1} exhibit red-shifts between 7 and 16 cm^{-1}.

Slight increases in relative intensity can be observed for most of the bands in the fingerprint region, while significant enhancements are seen for the combined C-CH_2 stretch and CH_2 wagging mode at 1328 cm^{-1}, the 1237 cm^{-1} band assigned to C-CH_3 stretch and COC bend, and the doublet at 588/572 assigned to a COC and CNC bend.

The doublets observed in the SERS spectrum at 743/734 and 588/572 were proved to be due to contributions from different conformers [6]. The shoulder at 734 observed is less pronounced than in the Raman spectrum. For the second doubled we see that the band at 572 cm^{-1} suffers a blue-shift, and an increase in relative intensity. These changes can be attributed to the signal enhancement of certain conformers.

Important to note is the band located at 1377 which suffers a significant increase in intensity when compared to the Raman measurements. This band is accurately reproduced by the harmonic and especially the anharmonic calculations but was very weak on the experimental Raman spectra at either at 1M or 10^{-1}M concentration.

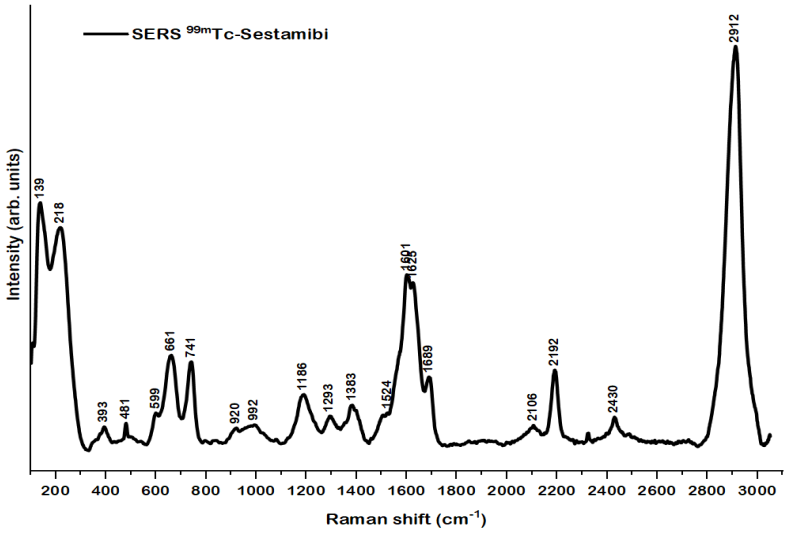

Figure 2. SERS spectrum of ^{99m}Tc-Sestamibi solution

In Figure 2 we present the SERS spectra of ^{99m}Tc-Sestamibi. The complex is considered to be stable for patient use within 8 hours after preparation. We can observe several bands characteristic to the MIBI ligand: 2912 cm^{-1} (CH_2 stretch), 2192 cm^{-1} (C≡N stretch), and 741 cm^{-1} (C-CH_3 and CO stretch). We note the presence of the band at 661 cm^{-1} that was not observed neither in the Raman or the SERS spectrum of the ligand. It is very probable that the band arises due to the excipients present in the kit that facilitate the formation of the Technetium complex.

In addition we performed measurements at different times from the moment of radiolabelling. Figure 3 shows the comparison between the SERS spectra of Tc-sestamibi at 4, 24, 54 and 106 hours from preparation. Comparing the first two we observe no significant change in band position and intensity in the first 24 hours. However we can observe three new, although weak, bands at 2952, 2835 and 1355 cm^{-1}. In contrast, the spectrum recorded at 56 hours from preparation shows significant changes. We observe new bands characteristic to the ligand, such as the doublets at 1455/1435 and 590/573 as well as the bands at 2829, 1288, 852, 808 and 349 cm^{-1}. Also, one can easily note the splitting of the band assigned to the C≡N stretch mode at 2193 cm^{-1}.

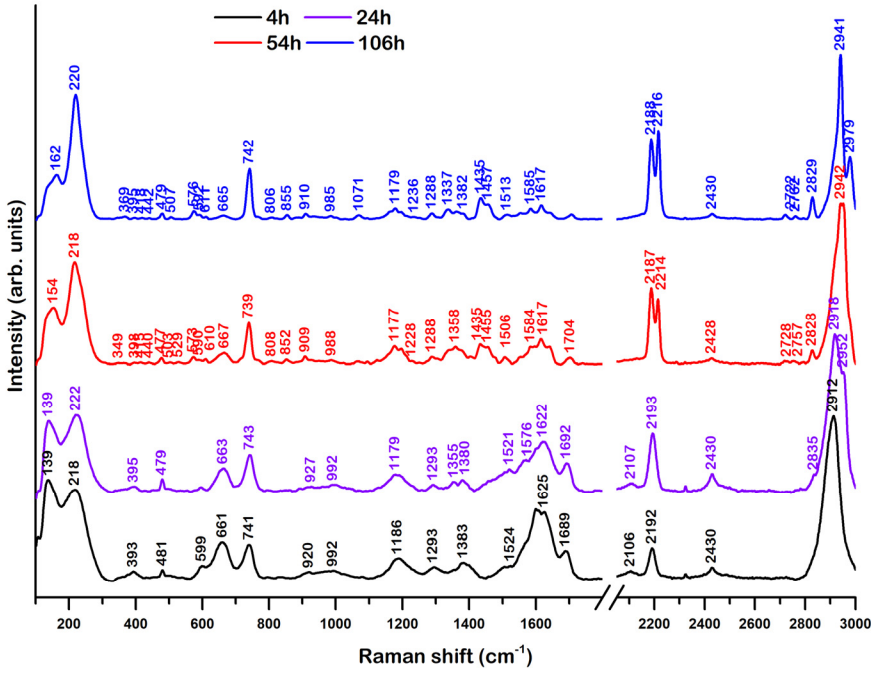

Figure 3. SERS spectra of ^{99m}Tc-Sestamibi at 2, 24, 54 and 106 hour from radiolabelling

The next spectrum, which was recorded at 106 hours, shows no significant differences in band positions with respect to that recorded at 54 hours after preparation. We can assume that the Technetium complex destabilizes between the second and third measurement.

The higher wavenumber component of the doublet recorded at 54 and 106 hours after the preparation (2216 cm^{-1}) is very close to that corresponding to the SERS spectrum of the unbound MIBI ligand (2213 cm^{-1}). Thus, most probably, the splitting of the C≡N band can be explained either by the appearance of free MIBI molecules adsorbed on the colloidal surface, besides the Tc-sestamibi complex which became slightly red-shifted with time.

4. CONCLUSIONS

SERS measurements on the MIBI ligand show good amplification of Raman bands even at concentration lower than the Raman detection limit, in particular the C≡N stretch at 2213 cm^{-1}. In addition we find good agreement between the experimental data and previously obtained results. This allows for a more confident characterization of the ^{99m}Tc-sestamibi. The SERS spectrum of the complex reveals several MIBI characteristic bands. We also observe that ^{99m}Tc-Sestamibi has a structural stability of at least 24 hours from the time of the radiolabelling process. However by this time most ^{99m}Tc atoms will have decayed to ^{99}Tc atoms which would make the complex inefficient for imaging.

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REFERENCES

[1] D. Berman, H. Kiat, J. Maddahi, The new ^{99m}Tc myocardial perfusion imaging agents: ^{99m}Tc-sestamibi and ^{99m}Tc-teboroxime, *Circulation*, 84(3 Suppl) (1991) I7-I21.

[2] D. Agostini, V. Roule, C. Nganoa, N. Roth, R. Baavour, J.J. Parienti, F. Beygui, A. Manrique, First validation of myocardial flow reserve assessed by dynamic 99mTc-sestamibi CZT-SPECT camera: head to head comparison with 15O-water PET and fractional flow reserve in patients with suspected coronary artery disease. *Eur. J. Nucl. Med. Mol. Imaging,* 45 (2018) 1079–1090.

[3] J. Whitman, I. Allen, E. Bergsland, I. Suh, T. Hope, Assessment and comparison of fluorocholine PET and sestamibi scans in identifying parathyroid adenomas: a meta-analysis, *J. Nucl. Med.*, 62(9) (2021) 1285-1291.

[4] S. Del Vecchio, M. Salvatore, Bonanno, O. Schillaci, ^{99m}Tc-MIBI in the evaluation of breast cancer biology. *Eur. J. Nucl. Med. Mol. Imaging*, 31 (2004) S88-S96.

[5] N.H. Hendrikse, E.J.F Franssen, W.T.A van der Graaf, C. Meijer, D.A. Piers, E.G.E. Vries, ^{99m}Tc-sestamibi is a substrate for P-glycoprotein and the multidrug resistance-associated protein, *Brit. J. Cancer,* 77 (1998) 353–358.

[6] G. Crisan, A. Macea, G. Andrieș, V. Chiș, Experimental and computational Raman spectroscopies applied to 2-methoxy-2-methylpropylisonitrile (MIBI) ligand of 99mTc-sestamibi radiopharmaceutical, *J. Mol. Struct.*, 1246 (2021) 131159.

[7] N. Leopold, B. Lendl, A New Method for Fast Preparation of Highly Surface-Enhanced Raman Scattering (SERS) Active Silver Colloids at Room Temperature by Reduction of Silver Nitrate with Hydroxylamine Hydrochloride, *J. Phys. Chem. B,* 107 (2003) 5723-5727.