

Density Functional Theoretical

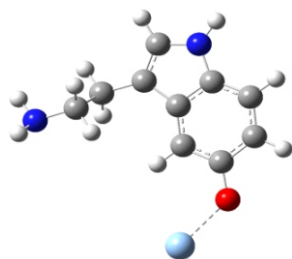
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Adsorption of Neurotransmitter, Serotonin on the Surface of Silver Nanoparticles: A Surface-enhanced Raman Scattering and DFT Study

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5HT (anion)-Ag. The colour codes used to identify the atoms are N (blue), O (red), C (grey), H (white) and Ag (sky blue)

ABSTRACT

Surface-enhanced Raman scattering (SERS) and density functional theoretical (DFT) studies of serotonin were investigated in order to have a better insight into the adsorption as well as the detection of the neurotransmitter at low concentrations specific to physiological levels. In addition to the sensitive detection of serotonin on the silver nanoparticles surface, the good agreement between the computed and experimental Raman spectrum indicated the presence of serotonin in the neutral and zwitterionic forms in solid and aqueous solution, respectively. The SERS and DFT results also revealed the presence of the analyte predominantly in its reduced neutral (anion) form on the silver nanoparticles surface with the probable adsorption through the oxygen/hydroxyl site.

KEYWORDS: Serotonin; Surface-enhanced Raman scattering (SERS); Density functional theory (DFT); Structural and vibrational analysis; Conformers

Introduction

Serotonin (5-hydroxytryptamine, 5HT) is an important neurotransmitter which controls various physiological and behavioural functions in the body, such as mood, anxiety, sleep and cognition functions [1]. It is well known that the serotonin (5HT) levels in the body can be correlated to several diseases such as depression, anxiousness, sleep and digestive problems, panic disorders, etc. [2]. Therefore, it is of prime importance to detect the neurotransmitter, 5HT at low concentrations, specific to physiological levels. Among the various methods used for detecting the 5HT biomarker, the surface-enhanced Raman scattering (SERS) technique has attracted great attention due to its high selectivity, sensitivity, cost-effectiveness and ease of operation [3]. In addition, to the amplified sensitivity in the Raman detection limit, the SERS technique has the potential to identify and fingerprint biomarkers, drugs, proteins, etc. [4-6]. SERS also provides valuable structural information regarding the interaction viz. the sites of binding and the orientation of the analytes on the nanomaterial substrates [7-9]. However, sensitive and precise detection of the 5HT levels using SERS require the fabrication of novel nanosubstrates.

In the present work, novel silver nanosubstrates, viz. colloidal silver nanoparticles (AgNPs) as well as nanostructured silver-coated films (NSCFs) were prepared for the detection of traces of the neurotransmitter, 5HT using the SERS technique. In addition to fingerprinting and detection of 5HT, the adsorption characteristics of the analyte on AgNPs

and NSCFs were investigated. The binding characteristics were probed by monitoring the observed changes in the Raman spectral features measured on the surface of AgNPs and NSCFs relative to that in aqueous solution. 5HT probably exists in the cationic, neutral, zwitterionic and anionic conformations at neutral to alkaline pH. The prevalent conformers of 5HT in solid and solution as well as the adsorbed species on AgNPs and NSCFs were identified by comparing the experimentally observed normal Raman and SERS spectrum with the DFT computed Raman spectrum of various conformers of 5HT and their Ag complexes. This study reveals that trace levels of the biomarker, 5HT can be easily detected using the SERS technique and the variation in its levels under physiological conditions can be correlated to mood swings, anxiety, and sleep as well as digestive disorders.

Materials and Methods

AgNPs were prepared using modified Creighton method [10]. NSCFs were fabricated using formamide as the reducing agent [11,12]. 5HT functionalized AgNPs (5HT-Ag) and 5HT capped NSCFs (5HT-NSCFs) were prepared by the addition of 5HT solution to AgNPs for the former and by dipping the NSCFs on the 5HT solution for 15 min for the latter, respectively. The AgNPs, NSCFs, 5HT-Ag and 5HT-NSCFs were then characterized using UV-vis absorption and Raman measurements. An insight into the experimental results was obtained by performing geometry optimization for all the conformers of 5HT using DFT (Gaussian 03 program) [13] with B3LYP functional and aug-cc-pVDZ basis set [14]. The molecular vibrations were computed for the optimized conformers and compared with the experimentally observed

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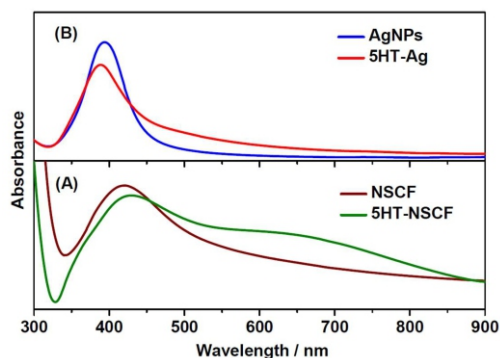


Fig.1: Absorption spectrum of (A) NSCF and 5HT (10 nM) functionalized NSCF (5HT-NSCF); (B) AgNPs and 5HT (10 nM) capped AgNPs (5HT-Ag).

Raman spectra of 5HT in solid and aqueous solution. The geometry optimization was also performed for the possible 5HT-Ag complexes with B3LYP/LANL2DZ for Ag atom. The computed vibrations at the optimized geometries were then compared with the SERS spectrum of 5HT-AgNPs and 5HT-NSCFs.

Results & Discussion

The UV-vis absorption spectrum of NSCF and 5HT-NSCF with 5HT concentration of 10 nM is shown in Fig. 1 (A). In NSCF, a broad band appears with maximum at 420 nm, which is attributed to the bulk-like surface plasmon resonance (BL-SPR) band [4,15,16]. It is known that the BL-SPR band of metal NPs strongly depends on the shape, size, extent of aggregation and dielectric constant of the medium [17]. The absorption spectrum of 5HT-NSCF (Fig.1(A)) displays reduced absorbance in the BL-SPR band which is also red-shifted to 430 nm. In addition, a lower energy band with maxima around 618 nm is observed, which is attributed to the surface-like surface plasmon resonance (SL-SPR) peak [4,15,16]. The SL-SPR band arises due to the dipole-dipole interactions of the higher aggregated particles. The UV-vis absorption spectrum of AgNPs and 5HT-Ag with 5HT concentration of 10 nM is shown in Fig.1 (B). A single sharp peak at 394 nm attributed to the BL-SPR band is observed for the AgNPs. In case of 5HT-Ag, the spectrum is broad with reduced absorbance at 394 nm along with the appearance of a shoulder around 450-600 nm. The red-shifted band is attributed to the SL-SPR band that appears due to 5HT induced aggregation of AgNPs.

The optimized (B3LYP/aug-cc-pVDZ) molecular structures of cationic, neutral, zwitterionic and anionic conformers of 5HT are shown in Fig.2 (I-IV). In order to know the relative stability of the conformers, the minimum energies at their respective optimized geometries were compared. The relative energies of the 5HT conformers computed at the B3LYP/aug-cc-pVDZ level of theory indicated that the cationic conformer is the most stable followed by neutral, zwitterionic and anionic forms. The relative energies of the cationic, neutral, zwitterionic and anionic conformers are 0, 10.46, 11.17 and 23.17 kcal mol⁻¹, respectively. The Raman vibrations for all the conformers of 5HT were computed at their optimized geometries and compared with the normal Raman spectrum in solid and aqueous solution. The acid dissociation constant (pKa) of 5HT is known to be 9.97 and 10.73 [18]. Thus, 5HT may exist in solid and aqueous solution in either of the possible conformation, viz. cation, neutral, zwitterion or may get deprotonated (anion) in solution and on the surface of AgNPs and NSCFs.

The Raman spectrum of 5HT in solid is shown in Fig.2 (A). The Raman bands are assigned based on comparison of the experimental spectrum with the theoretically computed (B3LYP/aug-cc-pVDZ) Raman vibrations of the cationic, neutral, zwitterionic and anionic forms of 5HT. The Raman spectrum (Fig.2 (A)) exhibits strong and medium intensity peaks at 1540, 1428, 1353, 1307, 1238, 941, 842, 773, 754 and 462 cm⁻¹, which are assigned to indole ring C=C stretch, NH in-plane (ip) bend, indole ring C-C stretch combined with OH ip bend, CH ip bend, indole ring distortion combined with CH₂ rock, C-NH₂ stretch, CH out-of-plane (oop) bend, indole ring breathing and indole ring bend, respectively. The experimental and theoretical results clearly suggest that 5HT in solid remains predominantly in the neutral form although slight contributions from other conformers cannot be completely ignored. The Raman spectrum of aqueous solution of 5HT (10⁻¹ M) as shown in Fig.2 (B) displays intense peaks at 1554, 1438, 1349, 1241, 939, 826 and 762 cm⁻¹ that are assigned to indole ring C=C stretch, NH in-plane (ip) bend, indole ring distortion combined with CH₂ rock, indole ring C-C stretch combined with CH ip bend, C-NH₂ stretch, CH out-of-plane (oop) bend and indole ring breathing, respectively. The Raman spectrum of 5HT in aqueous solution when compared with the computed Raman vibrations indicated the predominance of the zwitterionic conformer in solution.

The SERS spectrum of 5HT-NSCF and 5HT-Ag with 10 nM concentration of 5HT at neutral pH is shown in Fig.3 (A) and

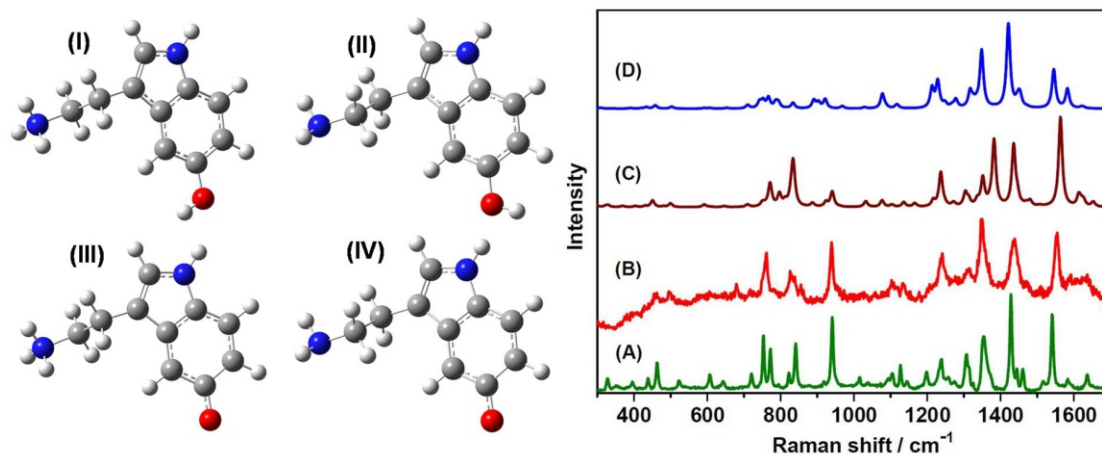


Fig.2: Optimized molecular structures of (I) cationic, (II) neutral, (III) zwitterionic and (IV) anionic conformers of 5HT. The colour codes used to identify the atoms are N (blue), O (red), C (grey) and H (white). Normal Raman spectrum of 5HT in (A) solid, (B) aqueous solution and simulated Raman spectrum of (C) neutral and (D) zwitterionic conformers.

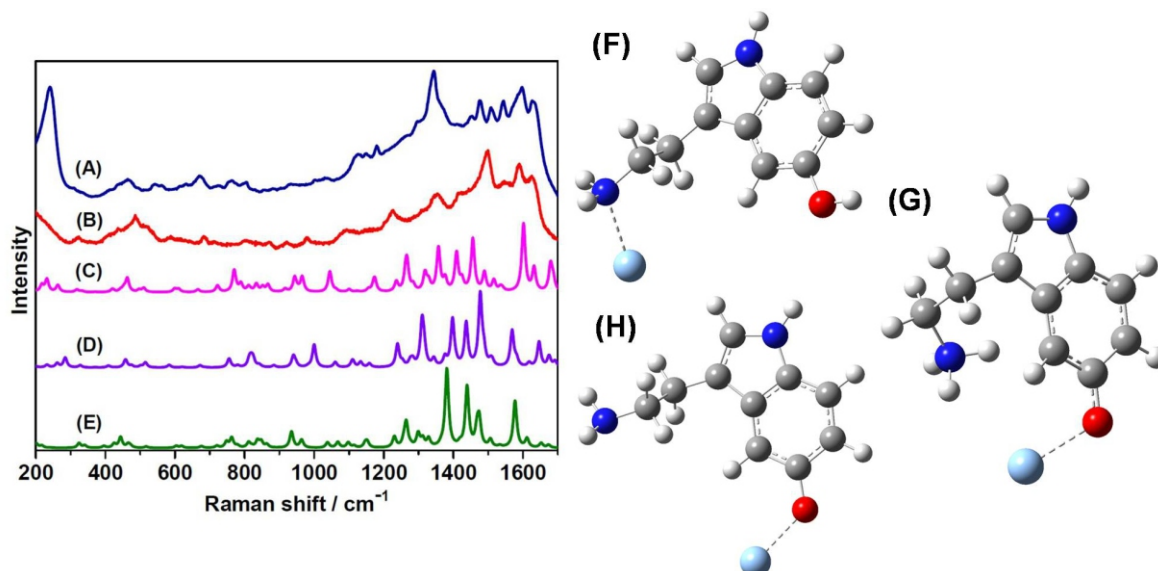


Fig. 3: SERS spectrum of (A) 5HT-NSCF, (B) 5HT-Ag and simulated Raman spectrum of (C) 5HT (neutral)-Ag, (D) 5HT (zwitterion)-Ag and (E) 5HT (anion)-Ag. Optimized molecular structures of (F) 5HT (neutral)-Ag, (G) 5HT (zwitterion)-Ag and (H) 5HT (anion)-Ag. The colour codes used to identify the atoms are N (blue), O (red), C (grey), H (white) and Ag (sky blue).

3 (B), respectively. The SERS spectrum shows good signal to noise (S/N) ratio. The SERS spectrum recorded for 1 nM concentration of 5HT (figure not shown) also showed reasonable S/N ratio. In case of 5HT-NSCF, intense SERS bands were observed at 1597, 1343 and 242 cm^{-1} that are assigned to indole ring C=C stretch, indole ring distortion combined with CH_2 rock and Ag-O stretch, respectively. Medium intensity peaks observed at 1626, 1544, 1508, 1475, 669 and 464 cm^{-1} are assigned to indole ring C=C asymmetric stretch, CH_2 twist, CH_2 twist combined with CH and NH ip bend, NH oop bend, indole ring breathing and indole ring CH bend, respectively. Similarly, the SERS spectrum of 5HT-Ag as shown in Fig.3 (B) displays strong and medium intensity peaks at 1589 (indole ring C=C stretch combined with C-N stretch), 1499 (CH_2 twist), 1353 (indole ring distortion combined with CH_2 rock), 1226 (indole ring distortion combined with CH_2 rock) and 485 (indole ring CH ip bend) cm^{-1} , respectively. The SERS spectrum of 5HT-Ag and 5HT-NSCF were compared with the computed (B3LYP/LANL2DZ) Raman spectrum of the zwitterion, neutral and deprotonated neutral (anion) conformer of 5HT-Ag complex as shown in Fig.3 (F-H). The computed relative energies suggest that the 5HT (neutral)-Ag is most stable, followed by 5HT (zwitterion)-Ag and the 5HT (anion)-Ag is least stable and their relative energies are 0, 1.01 and 10.78 kcal mol^{-1} , respectively. Thus, any of the three conformers, viz. neutral, zwitterion and the deprotonated neutral (anion) may be adsorbed on the surface of AgNPs and NSCFs. Comparison of the SERS spectrum and the computed Raman spectrum of the Ag-complexes indicate the presence of the analyte predominantly in its reduced neutral (anion) form on the silver nanosurface with probable adsorption through the oxygen/hydroxyl site.

Conclusions

In this study, a detailed investigation of serotonin (5HT) detection using AgNPs and NSCFs as the SERS nanosubstrate is presented. The results suggested that trace concentrations of 5HT (1-10 nM) could be easily detected using SERS, which may be useful for the prediction of various disorders that arise due to the variation in serotonin levels in the body under physiological conditions. In addition to the detection of 5HT, the agreement between normal Raman and computed

(B3LYP/aug-cc-pVDZ) Raman spectrum indicated the presence of serotonin in the neutral and zwitterionic conformers in solid and aqueous solution, respectively. The SERS and DFT results also revealed the presence of the analyte predominantly in its reduced neutral (anion) form on the silver nanosurface with the probable adsorption through the oxygen/hydroxyl site.

Acknowledgements

The authors gratefully acknowledge the encouragement and constant support provided by Dr. A. K. Tyagi, Director, Chemistry Group, BARC and Dr. Awadhesh Kumar, Head, Radiation & Photochemistry Division (RPCD), BARC, Trombay, India.

References

- [1] M. Berger, J.A. Gray, B.L. Roth, The Expanded Biology of Serotonin, *Annual Review of Medicine*, 60 (2009) 355.
- [2] P. De Deurwaerdère, G. Di Giovanni, Serotonin in Health and Disease, *International Journal of Molecular Sciences*, 21 (2020) 3500.
- [3] J. Guo, Y. Liu, H. Ju, G. Lu, From lab to field: Surface-enhanced Raman scattering-based sensing strategies for on-site analysis, *TrAC Trends in Analytical Chemistry*, 146 (2022) 116488.
- [4] R. Chadha, A. Das, J. Lobo, V.O. Meenu, A. Paul, A. Ballal, et al., γ -Cyclodextrin capped silver and gold nanoparticles as colorimetric and Raman sensor for detecting traces of pesticide "Chlorpyrifos" in fruits and vegetables, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 641 (2022) 128558.
- [5] A. Das, R. Chadha, B. Chalke, N. Maiti, Gold nanoparticle based colorimetric and Raman "turn-off" sensing of melamine in milk, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 651 (2022) 129717.
- [6] R. Chadha, A. Das, A.K. Debnath, S. Kapoor, N. Maiti, 2-thiazoline-2-thiol functionalized gold nanoparticles for detection of heavy metals, Hg(II) and Pb(II) and probing their competitive surface reactivity: A colorimetric, surface enhanced Raman scattering (SERS) and x-ray photoelectron spectroscopic (XPS) study, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 615 (2021) 126279.

- [7] S. SenGupta, N. Maiti, R. Chadha, S. Kapoor, Conformational analysis of morpholine studied using Raman spectroscopy and density functional theoretical calculations, *Chemical Physics Letters*, 639 (2015) 1.
- [8] N. Maiti, R. Chadha, A. Das, S. Kapoor, Surface selective binding of 2,5-dimercapto-1,3,4-thiadiazole (DMTD) on silver and gold nanoparticles: a Raman and DFT study, *RSC Advances*, 6 (2016) 62529.
- [9] N. Biswas, S. Thomas, S. Kapoor, A. Mishra, S. Wategaonkar, T. Mukherjee, Studies on adsorption of mono- and multi-chromophoric hemicyanine dyes on silver nanoparticles by surface-enhanced resonance raman and theoretical calculations, *The Journal of Chemical Physics*, 129 (2008) 184702.
- [10] N. Maiti, R. Chadha, A. Das, S. Kapoor, Adsorption and sub-nanomolar sensing of thioflavin T on colloidal gold nanoparticles, silver nanoparticles and silver-coated films studied using surface-enhanced Raman scattering, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 149 (2015) 949.
- [11] N. Maiti, Mukherjee, S. Kapoor, Tulsı Mukherjee, Surface-enhanced Raman Scattering (SERS) Spectroscopy For Trace Level Detection Of Chlorogenic Acid, *Advanced Materials Letters*, 4 (2013) 502.
- [12] A. Sarkar, R. Chadha, N. Biswas, T. Mukherjee, S. Kapoor, Phase-transfer and film formation of silver nanoparticles, *Journal of Colloid and Interface Science*, 332 (2009) 224.
- [13] M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, et al., *Gaussian 09 (Revision A02)*, Gaussian Inc. Wallingford CT, (2009).
- [14] A.D. Becke, A new mixing of Hartree-Fock and local density-functional theories, *The Journal of Chemical Physics*, 98 (1993) 1372.
- [15] E.C. Le Ru, P.G. Etchegoin, Single-Molecule Surface-Enhanced Raman Spectroscopy, *Annual Review of Physical Chemistry*, 63 (2012) 65.
- [16] R. Chadha, A. Das, S. Kapoor, N. Maiti, Surface-induced dimerization of 2-thiazoline-2-thiol on silver and gold nanoparticles: A surface enhanced Raman scattering (SERS) and density functional theoretical (DFT) study, *Journal of Molecular Liquids*, 322 (2021) 114536.
- [17] J. Jana, M. Ganguly, T. Pal, Enlightening surface plasmon resonance effect of metal nanoparticles for practical spectroscopic application, *RSC Advances*, 6 (2016) 86174.
- [18] J. Pratuangdejkul, W. Nosoongnoen, G.-A. Guérin, S. Loric, M. Conti, J.-M. Launay, et al., Conformational dependence of serotonin theoretical pKa prediction, *Chemical Physics Letters*, 420 (2006) 538.