Synthesis, docking, and preliminary in vitro/in vivo evaluation of MPP-dithiocarbamate-capped silver nanoparticle as dual-imaging agent for 5HT$_{1A}$

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Abstract: Methoxyphenyl piperazine is a versatile pharmacophore and has been exploited for targeting 5HT$_{1A}$ receptors. In the present study, silver nanoparticles were conjugated (capped) with methoxyphenyl piperazine-dithiocarbamate for application as targeted optical imaging agent at extremely low detection limits. Our results demonstrate an easy synthesis of the ligand methoxyphenyl piperazine-dithiocarbamate and silver nanoparticles and their conjugation was free from extraneous impurities.

Keywords: receptor imaging, optical imaging, targeted nanoparticles

Introduction

Neuroreceptor imaging is indispensable for understanding the pathophysiology of neuropsychiatric disorders and neurocognitive pathways. Selective and specific targeting of receptors in the central neural system enhances receptor imaging capability. Existing literature reports the specific and selective targeting of 5HT$_{1A}$ neuroreceptors using methoxyphenyl piperazine (MPP).

MPP is a versatile pharmacophore that can be modified for enhanced targeting and conjugation with other ligands for applications, such as imaging. In this study, MPP scaffold has been functionalized as dithiocarbamate (MPP-DTC) because DTC ligands are capable of complexation with a gamut of metal ions, which can be used as a signaling component for imaging. MPP-DTC and Ag nanoparticles (NPs) are conjugated for application as targeted optical imaging agent at extremely low detection limits. An added advantage of the DTC functionalization is the ligand ability to cross the blood–brain barrier because the lipophilicity requirements are not compromised.

Materials and methods

The reagents were purchased from Sigma Chemical Co. Ltd. (St Louis, MO, USA). The HEK cell line used for the experiments was procured from National Centre for Cell Science (NCCS; Pune, India), which is under the Government of India. NCCS distributes cell lines only to registered organizations, which have qualified investigators to use cell lines in their research.

Synthesis

MPP was DTC functionalized in a single step using carbon disulfide. Briefly, to a stirred solution of MPP (1 eq, 0.2 mmol) in dichloromethane (5 mL), triethylamine (3 eq, 0.6 mmol) was added. The solution was cooled in an ice-salt bath, and carbon disulfide was added dropwise. The reaction mixture was stirred for 24 hours at room temperature. The reaction was monitored by thin-layer chromatography (TLC), and the reaction was quenched by the addition of water. The resulting mixture was extracted with ethyl acetate, and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by column chromatography using silica gel and dichloromethane as eluent. The pure product was obtained as a white solid in 80% yield.
disulfide (1 eq, 0.2 mmol) was added dropwise to the solution with continuous stirring over a period of 30 min. The reaction mixture was then brought to room temperature and stirred overnight. The pale yellow solution was evaporated, and the crude product was recrystallized from ethanol/diethyl ether. MPP-DTC was characterized using mass spectrometry and ultraviolet (UV) spectroscopy. Ag-NPs were synthesized by electro-explosion of wire (EEW technique). Briefly, a thin Ag wire is exploded in a thin Ag plate by passing a current density of $-10^6$ A/m$^2$, in a time of $10^{-5}$ s. The Ag-NPs were then conjugated to MPP-DTC in the presence of sodium acetate in water:methanol (1:1) solvent at room temperature.

**Particle characterization**

The physicochemical parameters of the NPs were evaluated using X-ray diffraction (XRD), transmission electron microscopy (TEM), fluorescence spectroscopy, ultraviolet–visible (UV-vis) spectra, and infrared (IR) spectroscopy before and after conjugation.

**Cytotoxicity**

In vitro cellular studies include MTT assay for cytotoxicity assessment on HEK cells. Exponentially growing cells were seeded (3,000 cells/well for HEK) in a 96-well microtiter plate. After 24 h, media was replenished for all wells and cells were incubated with varying concentrations of the capped Ag-NPs at different time intervals (2, 6, 18, 24, and 48 h). After the specified time, standard MTT assay was performed. Optical density was measured at 570 nm with continuous stirring over a period of 30 min. The reaction mixture was then brought to room temperature and stirred overnight. The pale yellow solution was evaporated, and the crude product was recrystallized from ethanol/diethyl ether. The UV spectra of MPP-DTC showed a mass peak at 255 and 285 nm corresponding to $\pi-\pi^*$ and $n-\pi^*$ transitions. The contaminating Na$_2$CS$_2$ was not found at 330 nm. Ag-NPs were formed through EEW technique. This technique is a novel, physical, top-down approach, wherein the flow of the current through the Ag wire plate leads to heating at the point of contact, followed by melting. The melted Ag metal at the point of contact is further heated increasing the current density that leads to the evaporation of Ag metal and subsequent plasma formation. This plasma is contained by the self-induced magnetic field. When the vapor pressure of the plasma overwhelms the self-induced magnetic field, explosion occurs and the plasma products are dispersed in the medium with high speed. Synthesized Ag-NPs are free from extraneous impurities as no chemicals have been used. Ag-NPs and MPP-DTC conjugation was carried out in a single-step reaction. MPP-DTC when docked on the 5HT$_{1A}$ receptor homology model retained its binding with a glide score of −4.94.

The physicochemical parameters are depicted in Figure 2. XRD pattern (Figure 2A) of the Ag-NPs corresponds to that of face centered cubic. On conjugation with MPP-DTC, only a slight distortion was observed. The capped MPP-DTC–Ag-NPs reflect peaks corresponding to (111), (200), (220), (311), and (222). Peaks in the region (0<20<35) match with the standard XRD data of Ag$_2$CO$_3$, the formation of which is attributed to carbon-dioxide in atmosphere. The UV-vis spectra (Figure 3A) showed a peak at ~400 nm characteristic of Ag-NPs and assigned to surface plasmon resonance (SPR). This peak, due to the SPR, is dependent on medium’s refractive index, size and shape of NPs, and absorption substance at the surface of the NPs. UV-vis spectra of MPP-DTC–Ag-NPs (Figure 3B) show two resonant peak absorption at ~250 and 300 nm (characteristic peaks of DTC), indicating conjugation although the peak at 400 nm got compromised because of capping. The broadening of the peak in 410–430 nm is due to the presence of DTC that acts as electron donor and changes the bonding pattern of the Ag-NPs. The fluorescence spectra of the Ag-NPs dispersed in water exhibit a single fluorescence emission at 300 nm when it is excited in the range of either 215–235 or 255–280 nm ($\lambda_{ex}$), whereas the MPP-DTC–Ag-NPs fluorescence with a singular emission at 425 nm for $\lambda_{ex}$ is excited in the range of 230–250 nm (Figure 4A). The fluorescence emission peak intensity is maximum at 425 nm at $\lambda_{ex}$ 235 nm and...
decreases thereafter as $\lambda_{ex}$ increases. This indicates that resonant absorption/maximum transition probability is at $\lambda_{ex} \sim 235$ nm for capped Ag-NPs. The reason for the red shift could be attributed to the change in the environment of the MPP-DTC–Ag-NPs.

The IR spectra (Figure 4B) indicated the formation of silver-sulfide bonds by the disappearance of 2,550–2,600 cm$^{-1}$ peak. The typical DTCs frequencies were 1) peak at 1,460 cm$^{-1}$ associated primarily with the “thioureide”, 2) peak at 1,503 cm$^{-1}$ indicating the polar structure of DTC, and 3) peak at 1,016 cm$^{-1}$ indicating the symmetrical binding mode of DTC. The size of the synthesized Ag-NPs in TEM images was 10–20 nm. Conjugation with MPP-DTC resulted in NPs with similar size, but exact size could not be determined due to clustering. The cellular toxicity studies performed on HEK cell lines showed that the MPP-DTC–Ag-NPs were nontoxic up to a dose of 1 mM.

**Conclusion**

Our findings establish 1) easy synthesis of the ligand MPP-DTC, 2) easy synthesis of Ag-NPs free from extraneous impurities, 3) conjugation of Ag-NPs with MPP-DTC, and 4) evaluation of physicochemical parameters. Further work should concentrate on exact size and polydispersity index determination of the MPP-DTC–Ag-NPs and their application for optical imaging.

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**Figure 2** X-ray diffraction patterns of (A) Ag-NPs and (B) MPP-DTC–Ag-NPs.

**Figure 3** UV-vis spectra of (A) Ag-NPs and (B) MPP-DTC–Ag-NPs.

**Abbreviations:** au, arbitrary unit; MPP-DTC, methoxyphenyl piperazine–dithiocarbamate; NPs, nanoparticles; UV-vis, ultraviolet–visible.
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Disclosure

The authors report no conflicts of interest in this work.

References

1. Yang W, Lin Y, Zhang X, Zhang J, Wang X. Synthesis of several MPP derivatives for 99mTc-labelling and evaluated as potential 5-HT1A receptor imaging agents. Sci China Chem. 2011;54(7):1148–1154.
2. Chaturvedi S, Kaul A, Yadav N, Singh B, Mishra AK. Synthesis, docking and preliminary in vivo evaluation of serotonin dithiocarbamate as novel SPECT neuroimaging agent. MedChemComm. 2013;4(6):1006–1014.
3. Sen P, Ghosh J, Abdullah A, Kumar P. Preparation of Cu, Ag, Fe and Al nanoparticles by the exploding wire technique. J Chem Sci. 2003;115(5-6):499–508.
4. Shankaranarayana ML, Patel CC. The electronic spectra of some derivatives of xanthic, dithiocarbamic and trithiocarbonic acids. Acta Chem Stand. 1965;19:1113–1119.
5. Liu C, Yang X, Yuan H, Zhou Z, Xiao D. Preparation of silver nanoparticle and its application to the determination of ct-DNA. Sensors. 2007;7(5):708–718.
6. Yeshchenko OA, Dmitruk IM, Alexeenko AA, Kotko AV, Verdal J, Pinchuk AO. Size and temperature dependence of the surface plasmon resonance in silver nanoparticles. Ukr J Phys. 2012;57:266.
7. Mankad V, Kumar RK, Jha PK. Investigation of blue-shifted plasmon resonance: an optical properties study of silver nanoparticles. Nanosci Nanotechnol Lett. 2013;5(8):889–894.
8. Mogensen KB, Kneipp K. Size-dependent shifts of plasmon resonance in silver nanoparticle films using controlled dissolution: monitoring the onset of surface screening effects. J Phys Chem C. 2014;118(48):28075–28083.
9. Mathew EI, Studies on Some Metal Complexes of Dithio Ligands [doctoral thesis]. Cochin, India: Cochin University of Science and Technology; 1990.
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