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Resorcinarene-Embedded Stable Silver Nanoparticles: A Fluorescent Nanoprobe for Pb(II) in Water

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Abstract*: Calix[4] resorcinarene tetrahydrazide capped silver nanoparticles (CRTH-AgNps) were synthesized using a simple chemical reduction method. The silver nanoparticles (AgNps) obtained were used without further modification for the detection of lead and were characterized using surface plasmon resonance (SPR), transmission electron microscopy (TEM) and energy dispersive x-ray analysis (EDX). The stability of the CRTH-AgNps was examined at different pH values and temperatures. The interaction of the CRTH-AgNps with different metal ions was investigated using UV-visible and fluorescence spectroscopy. Their response was found to be highly selective for Pb(II), as the absorption and emission spectra were found to be uninterrupted by the presence of various metal ions, such as Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II). The emission spectra showed that CRTH-AgNps are highly selective and sensitive for Pb(II) by means of fluorescence quenching. The minimum detection limit was found in the range of 1 nM to 0.980 µM. The plausible mechanism is discussed where the carbonyl oxygen and amide nitrogen atoms of CRTH not only participate in the binding with Pb(II) but also behave as a PET acceptor. The synthesized nano-probe was successfully used for the detection of Pb(II) in industrial waste-water samples.
Keywords: Calix[4] resorcinarene, Silver nanoparticles, Fluorescence study, Colorimetric sensor, Water samples

I. INTRODUCTION

Recently, nanoscience and nanotechnology have emerged as revolutionary developments in science and technology. These areas of research are involved in the physical and chemical sciences, biological sciences, health sciences and other interdisciplinary fields [1]. Owing to the development of nanotechniques, silver nanoparticles (AgNps) have been used as excellent light-scattering reagents to detect small molecules based on their unique optical properties in the visible spectral region, which originate from the excitation of the collective oscillations of conducting electrons, known as surface plasmon resonance (SPR) [2]. Among all other metal nanoparticles that have SPR properties, AgNps are probably the most important because they exhibit strong and well-defined colors, and the color changes between individual and well-spaced nanoparticles, compared to aggregate ones, are easily visualized as a change from yellow to brown [3]. As a general physical process, the light-scattering signals can be easily measured with a common spectrofluorimeter, and intensive efforts have been made to develop AgNps-based UV–visible spectrometric and spectroflurometric analytical methods for the sensitive detection of a wide variety of molecules including metals, drugs and biomolecules. Dispersed AgNps have shown high affinity in their interaction with trace levels of target species at a minimum volume of sample [2, 4]. In the past decade, the rapid industrialization and excessive use of chemicals have intensified metal contamination in the environment, which affects soil and the aquatic ecosystem. Water plays a pivotal role in all ecosystems on earth, but water pollution by carcinogenic chemicals, harmful metals and pathogenic bacteria has become a serious problem [5]. Pollution with heavy metals has been recognized as a major environmental concern, leading many to become interested in addressing this issue [6]. The use of optical sensors based on SPR is one of the most sensitive, simple, inexpensive and fast methods for the detection of heavy metals in water. In the past years, a number of nanoparticle based sensors have been reported [7]. Large numbers of colorimetric sensors have been utilized for the detection of various species including metal ions like Co(II), Cr(III), Hg(II), Cu(II), Pb(II), proteins, oligonucleotides, organic molecules and biological applications [8, 9].

Among various metal ions which may prove harmful at aberrant levels, the detection of lead ions is crucial owing to its detrimental effects on the environment. Due to its wide distribution and use in batteries, gasoline and pigments, lead is the second most abundant element in the environment (from the list of toxic substances in the environment). Even a low-level exposure to lead can cause neurological, reproductive, cardiovascular and developmental disorders [10]. In 2011, the World Health Organization

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guideline for drinking water stipulated that the maximum permissible quantity of lead in drinking water is 10 μgL^{-1}. Different traditional methods for Pb(II) analysis include atomic absorption spectrometry (AAS), atomic emission spectrometry and inductively coupled plasma mass spectrometry (ICP-MS) [11-13]. However, many of these systems present limitations, such as being time-consuming or expensive and/or requiring sophisticated equipment and complicated processing. In response to these shortcomings, a cost-effective and simple detection method is required for rapid detection of lead ions. Recent years have led to great progress in the development of procedures for the detection of lead ions by optical and electrochemical techniques [14] using chromophores [15], DNAzyme [16], functional spherical gold nanoparticles [17] and quantum dots [18]. However, some of these procedures lack sensitivity and ease and are expensive. Thus, a simple, sensitive and economical procedure for the detection of lead is still required. The fluorescence method of detection avoids the use of long procedural steps and provides trace level detection of analytes [19].

The application of various calixarene platforms as well as conjugation of calix with nanomaterials as sensors has become an emerging topic of interest [20-31]. The present work represents a rapid, sensitive, low cost and convenient assay for Pb(II) detection in water using calix[4]resorcinarene tetrahydrazide [32]. Furthermore, the selectivity of Pb(II) detection system is excellent when tested against a series of heavy metal ions, such as Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Hg(II) in both aqueous solutions and mixtures of these ions.

II. MATERIALS AND METHODS

A. *Materials and general methods*

All metal salts including silver nitrate were purchased from Sigma-Aldrich. Other reagents and solvents of analytical grade were purchased from commercial sources and used without further purification. All aqueous solutions were prepared from Millipore water (resistivity 18 MΩ.cm at 25°C; Millipore Systems). Fluorescence-active TLC plates (F-2009) were procured from Merck.

The melting points (uncorrected) were obtained using a VEEGO (Model; VMP-DS) melting point apparatus. Fourier-transform infrared (FT-IR) spectra were recorded on a Bruker Tensor 27 infrared spectrometer with samples prepared as KBr pellets. A JY 2000-2 inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) was used to check the absence of Ag in the centrifugate. Absorption spectra were studied at room temperature using a Jasco V-570 UV-Vis recording spectrophotometer with a variable wavelength between 200 and 800 nm using a quartz cuvette with a 1.0-cm optical path. The pH of the solutions was measured using an LI 614-Elico pH analyzer. Samples were sonicated by using a PCI 1.5L (H) sonicator. The Malvern Zetasizer (ZEN3600) was used for the zeta potential measurements using laser Doppler electrophoresis without dilution. Transmission electron microscopy (TEM) images were recorded in MACK/*model* JEOL JEM 2100 at an accelerated voltage of 200 kV. The TEM specimens were prepared by depositing a drop of dilute solution of an appropriate amount of CRTH-AgNps in water on carbon-coated copper grids, were dried in a vacuum and were directly observed in the TEM. Fluorescence spectra were recorded on a Jasco FP-6500 spectrofluorometer.

B. *Synthesis of calix[4]resorcinarene tetrahydrazide protected silver nanoparticles (CRTH-AgNps)*

Silver nanoparticles were prepared by the reduction of $AgNO_3$ in the presence of calix[4]resorcinarene tetrahydrazide (CRTH)[32]. A series of experiments were performed varying the concentration of silver nitrate and CRTH to obtain highly stable AgNps. In a typical procedure, 50 mL of a 1.0 mM solution of CRTH was rapidly added to a 50 mL boiling solution of 1.0 mM $AgNO_3$. The mixture was heated on a distillation apparatus for 24 hrs, and the solution was stirred further at room temperature for an hour. The solution changed from colorless to orange, indicating the successful formation of CRTH-AgNps. The resulting colloidal solution (0.0054 %) was then subjected to centrifugation at REMI (R-8C) 14000 RPM. The centrifugate was checked for the absence of Ag^+ and CRTH by ICP-AES and UV-visible spectrophotometry, respectively. The residue was washed with copious amounts of deionized water to remove any unbound molecules and then redispersed in deionized water to yield a 0.0054 % solution of CRTH-AgNps for further studies. The particle size, morphology and composition of the nanoparticles were determined using TEM.

C. *General procedure for the UV-visible and fluorescence measurements*

A stock solution of the CRTH-AgNps (0.0054 %) and nitrate salts (200 µM for UV-visible measurements and 2 µM for spectrofluorimetric measurements) of various cations, including Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II), were prepared in deionized water. Then, 2.5 mL of CRTH-AgNps and 2.5 mL of each cation were poured into a 5 mL volumetric flask to provide an effective concentration of the metal ions of 1 µM. UV-visible and fluorescence spectra of the

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resulting solutions were compared with the SPR band (426 nm) and emission spectra (531 nm) of the CRTH-AgNps**.** Silver nitrate and CRTH individually do not absorb or emit in the visible region.

III. RESULTS AND DISCUSSION

A. Synthesis and mechanism of formation of CRTH-AgNps

In this study, water-dispersible CRTH, a hydrazide derivative of calix[4]resorcinarene with the enhanced properties of hydrazide, acts as a reducing agent. In a simple process, $AgNO_3$ is reduced by CRTH via oxidation of the amino group by the transfer of electrons from the amine of CRTH to Ag^+. The hydrazide (-NH-NH_2)-coordinated metallic silver nucleates to form stable AgNps electrostatically as follows [33-35]:

$$\mathbf{AgNO_3 + RNHNH_2 \longrightarrow Ag^0 + RNHNH_2^+ + NO_3^-}$$

CRTH also holds the web-type structure of calix with an inherent hollow cavity, which makes it a very effective stabilizing agent for the formation of water-dispersible stable nanoparticles, such as CRTH-AgNps. CRTH passivates the surface of the AgNps and stabilizes the nanoparticles through the coordination of the nitrogen atoms of the hydrazide (-NH-NH_2) group with the Ag atoms at the surface of the AgNps. This robust, one-step synthetic route is simple, highly reproducible and does not require careful control of experimental conditions.

B. Characterization of CRTH-AgNps

A drop of a dilute solution of aqueous nanoparticles was placed on carbon-coated copper grids, which were dried in a vacuum and directly observed using TEM. The morphology of the CRTH-AgNps reveals that the particles are roughly spherical in shape and well dispersed with an average particles size of 15±5 nm **(Fig. 1)**

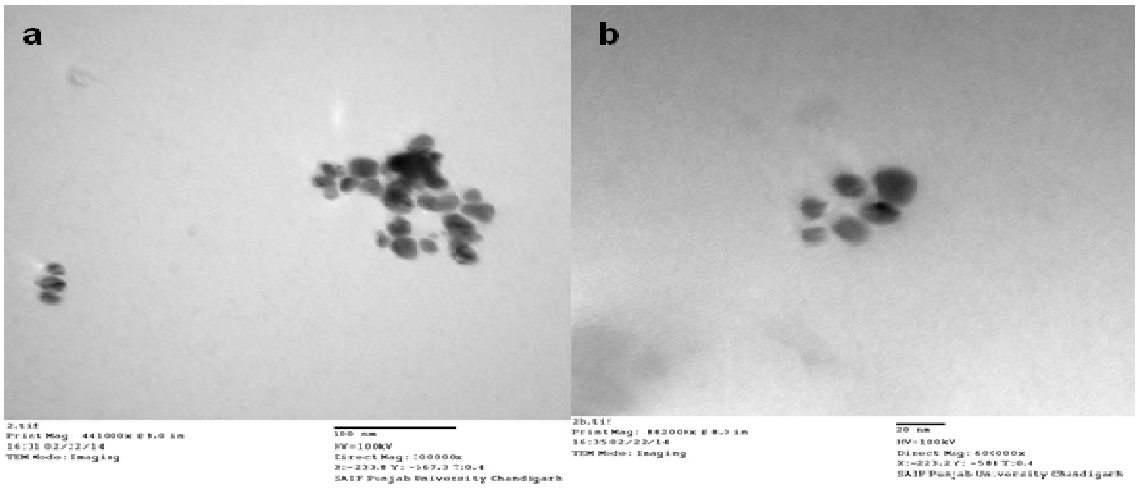


Fig. 1 a) TEM image of CRTH-AgNps, b) A high-magnification image of CRTH-AgNps

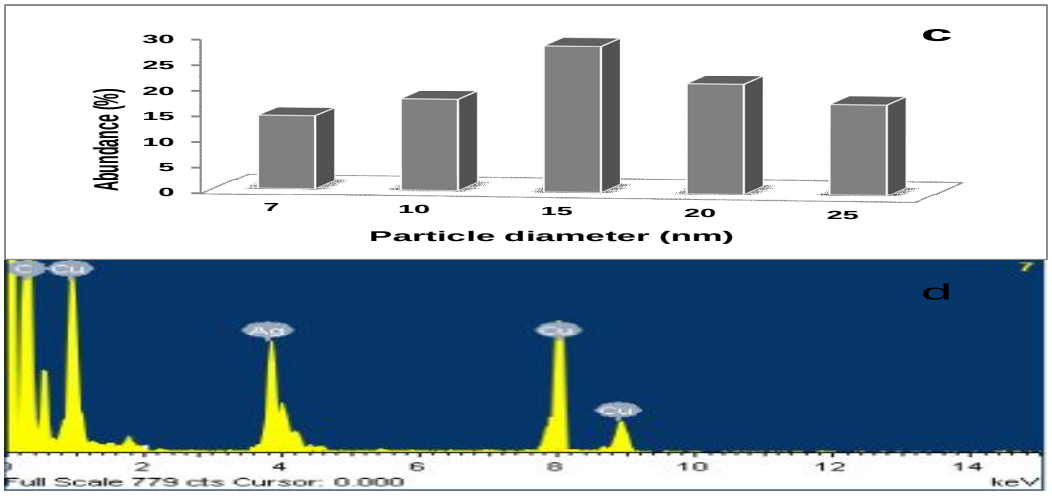


Fig. 1 c) Particle size distribution graph of CRTH-AgNps d) EDX of CRTH-AgNps

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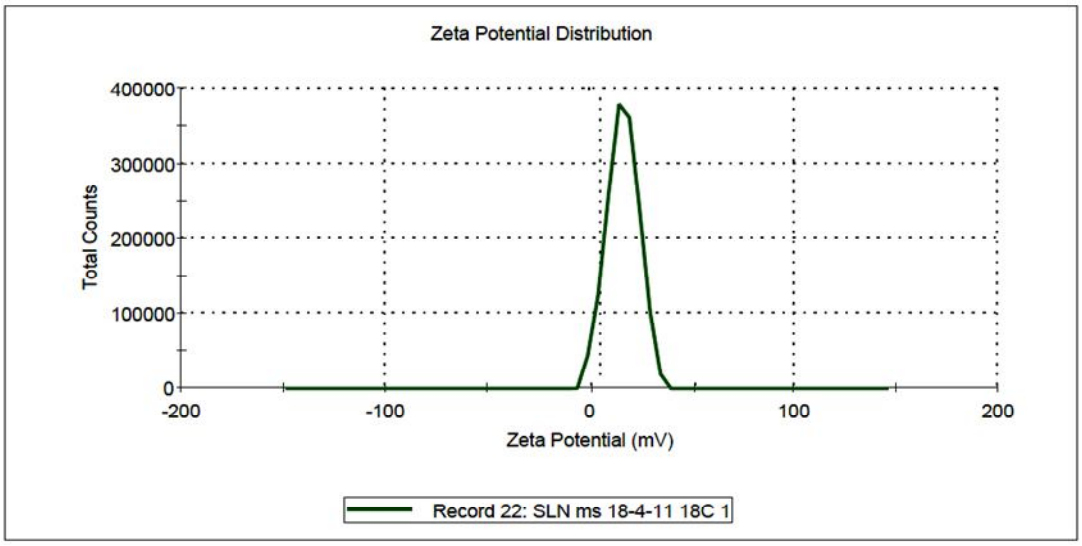


Fig. 2 Zeta potential distribution graph of CRTH-AgNps

The stability was also studied by measuring the zeta potential value, which was found to be 14.3 ± 2 MeV (Fig. 2). Here, the positive zeta potential of the synthesized AgNps was due the capping of the particles by CRTH. The zeta potential value of the CRTH-AgNps was found to be sufficient to prevent particle aggregation and maintain their stability. In addition, a positive value also suggests that the hydrazide groups were successfully introduced onto the surface of the nanoparticles to facilitate the reduction and capping.

C. Effect of pH and time on the stability of CRTH-AgNps

Due to SPR, a strong absorption of electromagnetic waves is exhibited by metal nanoparticles in the visible range and thus can be measured using a UV–visible spectrophotometer [36]. SPR is sensitive to the size and shape of a nanoparticle; thus, it is an important factor to ascertain the stability of nanoparticles. The stability of the nanoparticles was investigated at different pH values (4-10) and was evaluated by the changes in their SPR bands and fluorescence intensity **(Fig. 3)**.

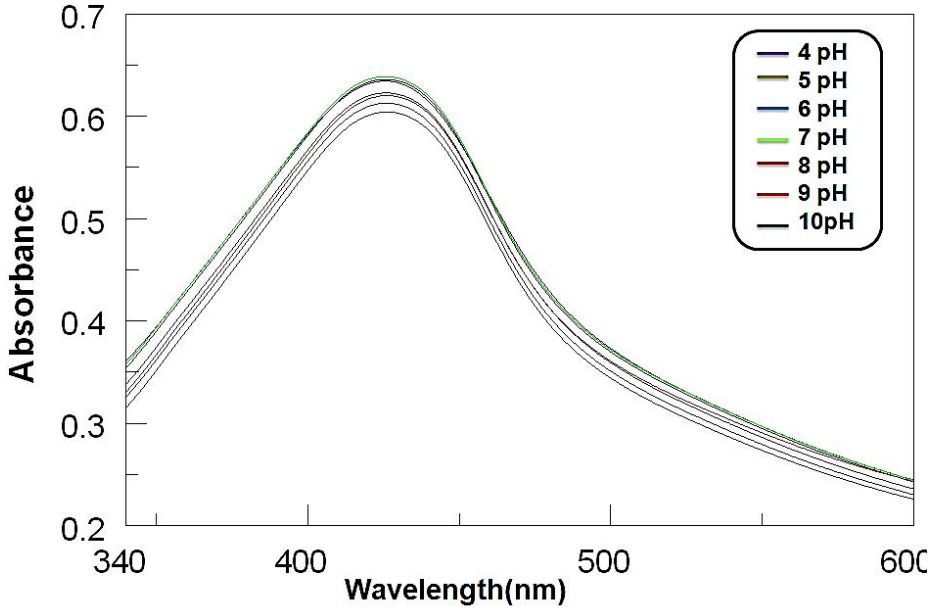


Fig.3 Effect of pH on surface plasmon resonance of CRTH-AgNps

The SPR band of the CRTH-AgNps shows a negligible change at pH 7.0 for 90 days, but the CRTH-AgNps tend to agglomerate after approximately 90 days. No change in the SPR band and absorbance of the CRTH-AgNps at pH 7.0 was recorded up to 90 days (Fig. 4).

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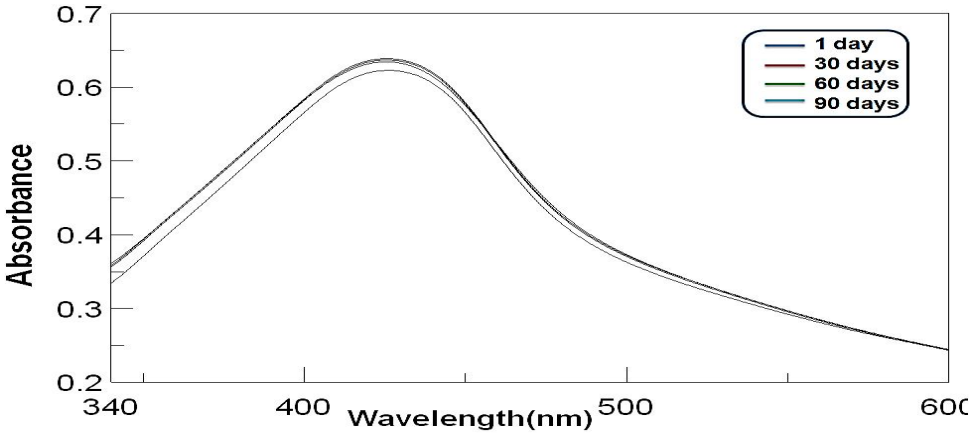


Fig.4 Stability of (CRTH-AgNps) with respect to time on wavelength/absorbance

However, at pH values other than 7.0, a slight decrease in absorbance is observed. Similarly, the fluorescence intensity (λ_{exc} = 560 nm, λ_{em} = 580 nm) of the CRTH-AgNps decreases slightly at pH values other than 7.0 without any change in the emission wavelength (Fig. 5).

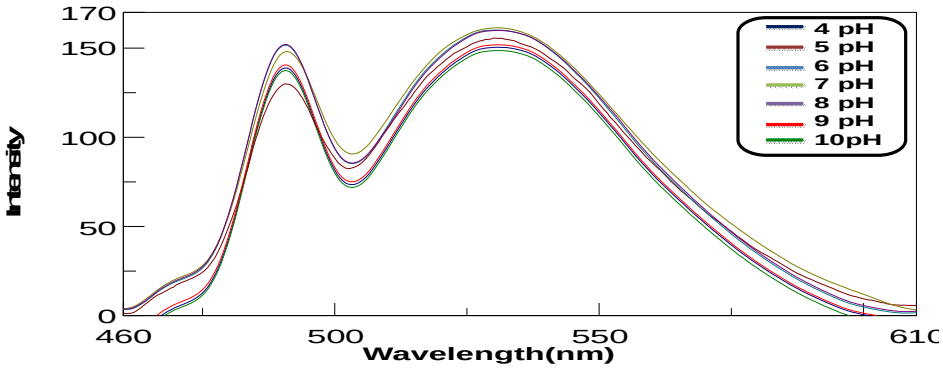


Fig. 5 Effect of pH on fluorescence intensity/wavelength of CRTH-AgNps

The CRTH-AgNps were observed to remain stable only between 10°C-40°C, as evidenced by negligible changes in absorbance in that temperature range (Fig. 6).

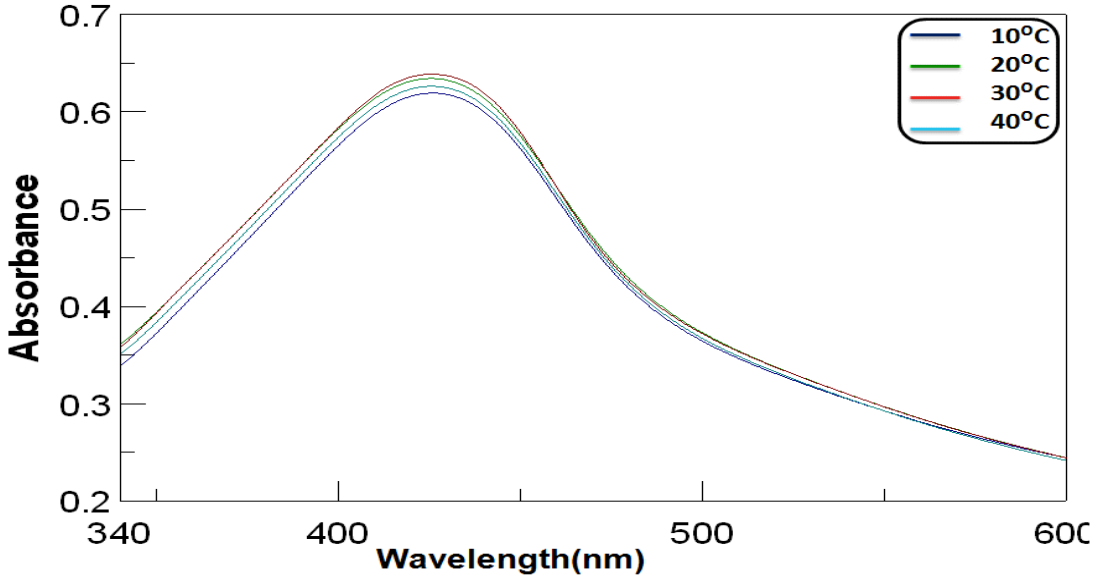


Fig. 6 Effect of temperature on SPR of CRTH-AgNps

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Beyond this range, the particles tend to agglomerate. Therefore, 30ºC and pH 7.0 were selected as conditions to use in all experiments with the CRTH-AgNps because they show maximum stability and fluorescence intensity at pH 7.0.

D. Colorimetric detection of Pb(II) using CRTH-AgNps

Theoretical and experimental studies have shown that the plasmon oscillations of metal nanoparticles couple to each other when they are brought in proximity and exhibit different colors [17, 37]. When 2 mL of CRTH-AgNps (0.0054 %), which are orange in color, was mixed with 2 mL (2 µM) of each metal ion (Pb(II), Cd(II), Mn(II), Fe(III), Ni(II), Zn(II), Hg(II), Co(II) and Cu(II)) separately, there was no visible change in color with all the metal ions except Pb(II) where the color changed instantly from orange to brown (Fig. 7).

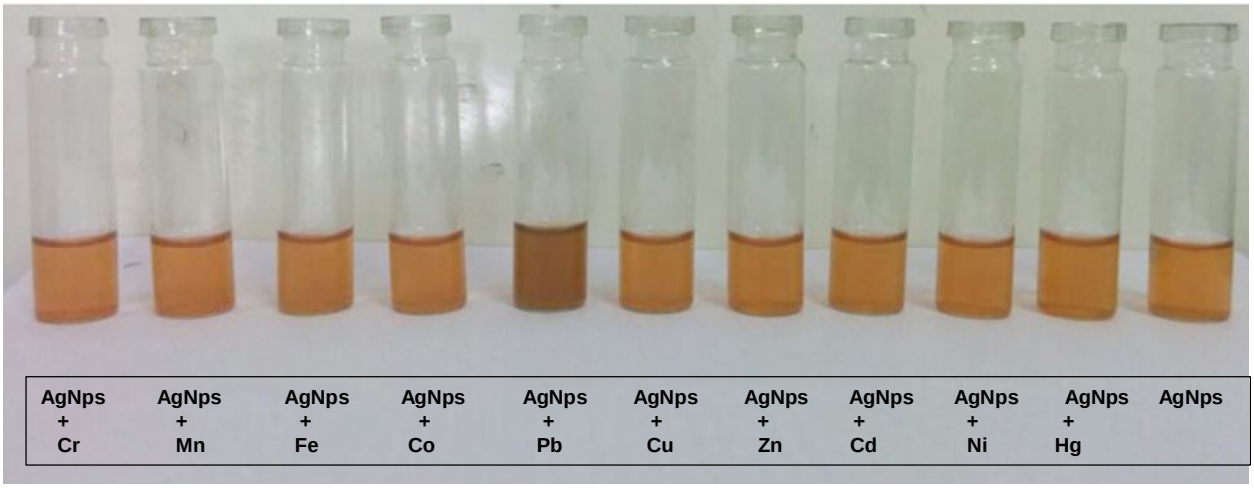


Fig. 7 Visual colour change of CRTH-AgNps upon addition of different metal ions (200 µM) at pH 7.0

The repeated observation confirms that this method is simple, sensitive and selective for the colorimetric detection of Pb(II) in aqueous media. Various studies have reported the colorimetric detection of Pb(II) [38, 39]. In this work, CRTH-AgNPs have been shown to be unique in their performance for the selective and sensitive naked-eye detection of Pb(II) in aqueous samples. The reason for the selective colorimetric changes with only Pb^{2+} is not clear but may be due to the interactions of Pb^{2+} with the nanoparticles.

E. Interaction of Pb(II) with CRTH-AgNps using UV-visible and fluorescence spectroscopy measurements

Spectrophotometric methods have been extensively used for the determination of the interactions of different analytes (cations) with ligands. The colloidal solution of the CRTH-AgNps shows an SPR band at 426 nm (Fig. 8).

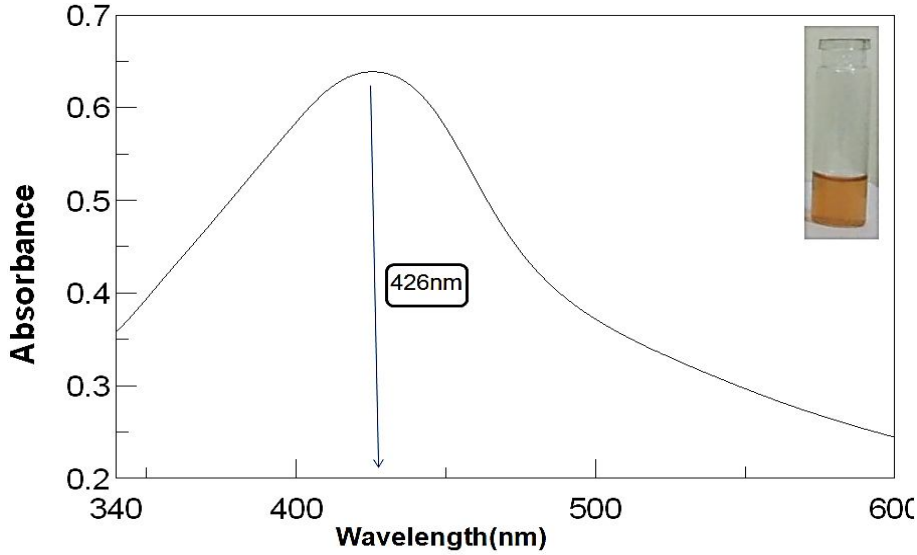


Fig. 8 UV-Vis spectra of CRTH-AgNps upon addition of different metal ions (200 µM)

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The absorption spectra of the CRTH-AgNps in the presence of different metal ions, including Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II), was studied **(Fig. 9)**.

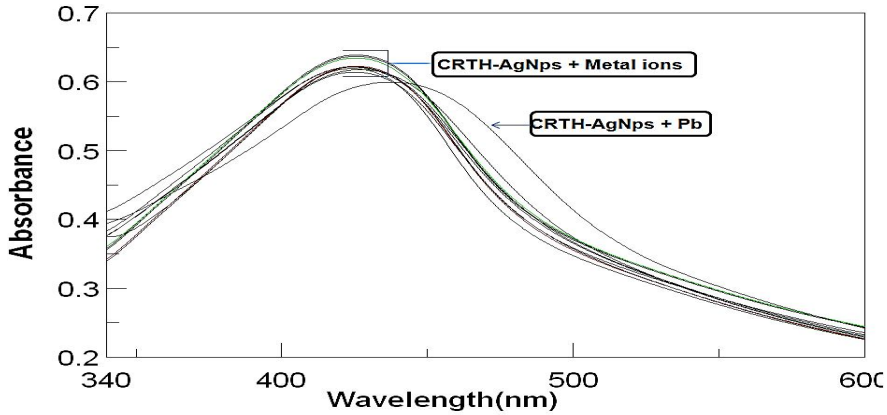


Fig. 9 UV-Vis spectra of CRTH-AgNps after the addition of various metal ions (200 µM)

A noticeable change in the SPR band of the CRTH-AgNps was only observed with Pb(II) among all the metal ions tested. The absorbance of the SPR band at 426 nm decreased dramatically and shifted toward a higher wavelength (440 nm). The red shift in the SPR band correlates to the near-field coupling that occurs when the inter-particle distance decreases. These results indicate that the AgNps were aggregated[40, 41]. The emission spectrum of the CRTH-AgNps shows the emission maximum at 531 nm, where excitation was carried out at 427 nm **(Fig. 10)**.

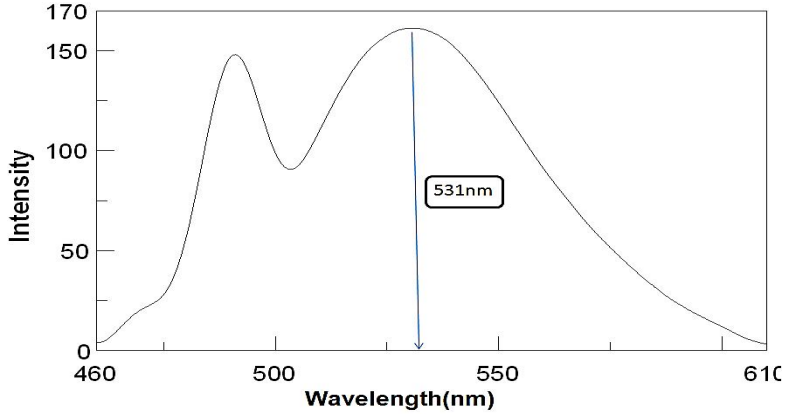


Fig. 10 Fluorescence spectra of CRTH-AgNps (λmax at 427 nm)

The interaction of different metal ions, including Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II), with the CRTH-AgNps was further studied using spectro flurometric studies **(Fig. 11)**.

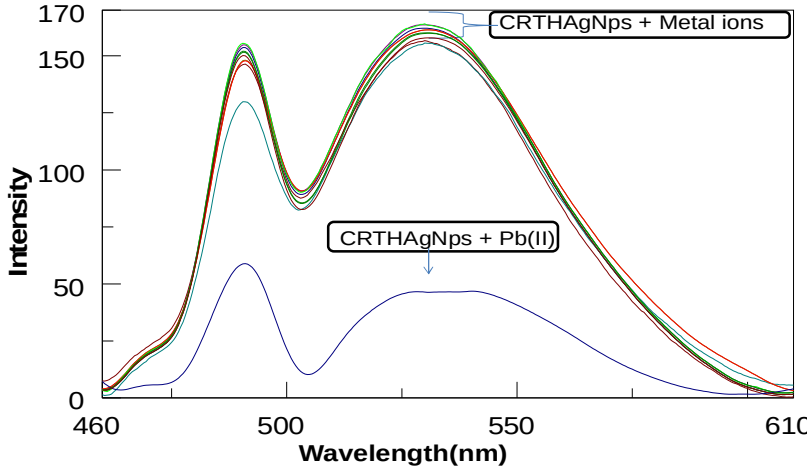


Fig. 11 Fluorescence spectra of CRTH-AgNps on addition of various metal ions (2 µM)

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Interestingly, quenching was observed with Pb(II) only, and no distinct changes in emission spectra were observed with the other metal ions (Fig. 12).

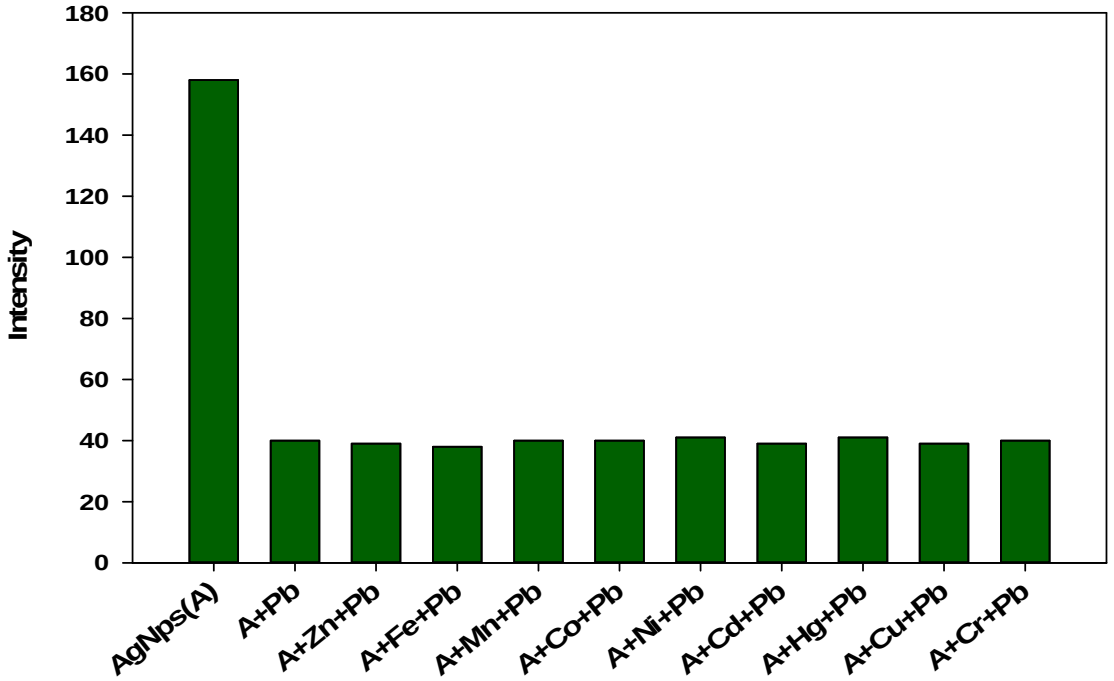


Fig.12 Effect of various metal ions interference on the detection of Pb(II) ions

The linear range of detection of Pb(II) (100 nM and 980 nM) was determined by means of fluorescence titration of the CRTH-AgNps (0.0054%) with an increasing concentration of Pb(II). It was noted that the fluorescence intensity, at 531 nm, gradually decreases with an increase in the concentration of Pb(II). The minimum and maximum fluorescence quenching was observed at 1 nM and 0.98 µM concentration of Pb(II), respectively, which is considered to be the minimum and maximum detection limit of CRTH-AgNps (Fig. 13).

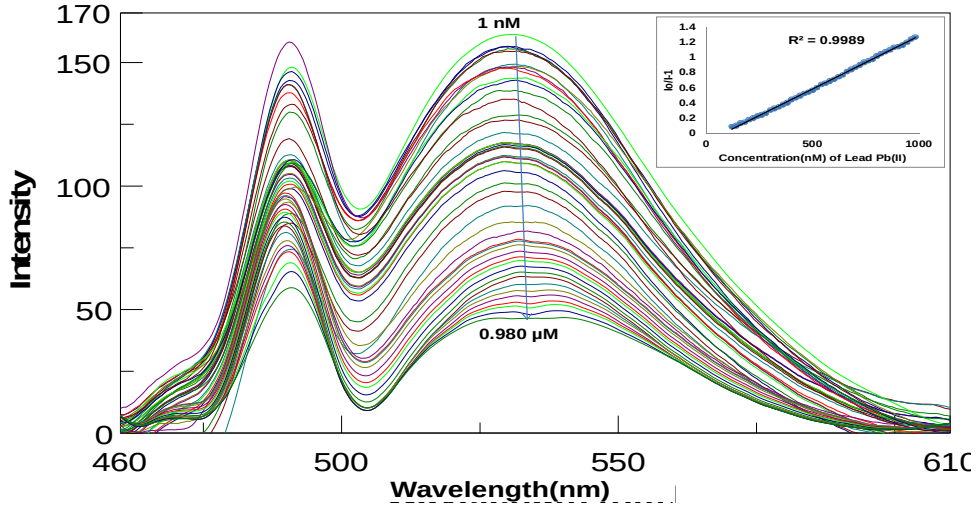


Fig. 13 Fluorescence spectra of CRTH-AgNps on addition of various metal ions (2 µM)

F. Plausible sensing mechanism

The sensing mechanism is proposed to occur through the reverse photo electron transfer (PET)[14, 42] between Pb(II) and CRTH. CRTH-AgNps are an actual fluoroionophore with CRTH, which is an ionophore consisting of carbonyl and hydrazide groups. According to the Lewis "hard and soft acids and bases rules" [43, 44], the highly selective recognition of CRTH-AgNps for lead ions can be attributed to two factors. First, the carbonyl oxygen and Pb^{2+} are typical "hard base" and "soft borderline acid", respectively, and the very strong affinity between them is natural. Second, the nitrogen atom properties may play an important role

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in the affinity of nitrogen to the heavy-metal ion. It is well-known in coordination chemistry that the complexation of lead with a ligand containing at least three nitrogen donor atoms is favored by the activation of the inert pair on the Pb(II) leading to a shortening of the Pb–N bond length [45]. In this system, the carbonyl oxygen and amide nitrogen atoms not only participate in the binding with Pb(II) but also behave as a PET acceptor. This type of interaction remarkably restricts the existing PET mechanism (reverse PET) and so induces quenching in the fluorescence intensity of fluorescent nanoparticles. Therefore it is deduced that CRTH-AgNps can be used as a selective and sensitive sensor for Pb(II). The developed sensor has been successfully employed for the determination of trace lead in water samples. This CRTH-AgNps optode shows good stability and sensitive response for analytical applications.

G. Analytical application

To investigate the practical application of our Pb(II) detection system in real water samples (waste water), different concentrations of Pb(II) were added to real water samples, which is known as the standard addition method, and detected by using the calibration curve at room temperature. The Pb(II) aqueous solutions were prepared using MilliQ water and waste-water, respectively. The waste-water sample was filtered and used directly without further purification. The waste-water sample was detected using ICP-AES and then different concentrations of Pb^{2+} were added.

Because the linear range of detection of Pb(II) by CRTH-AgNps is 100 nM to 980 nM, we spiked the waste-water sample with 200, 400, 600 and 800 nM of a standard sample of Pb(II). Quenching of intensity is linear with the successive addition of analyte ions. The difference in fluorescence intensity was evaluated using a Stern-Volmer plot to determine the Pb^{2+} concentration in the waste-water sample **(Fig. 14).** Finally, these water samples were also detected using ICP-AES. The observed results using the calibration curve and ICP-AES are given in Table 1. The observed Pb(II) concentrations by our calibration curve are very close to those added and those observed using ICP-AES.

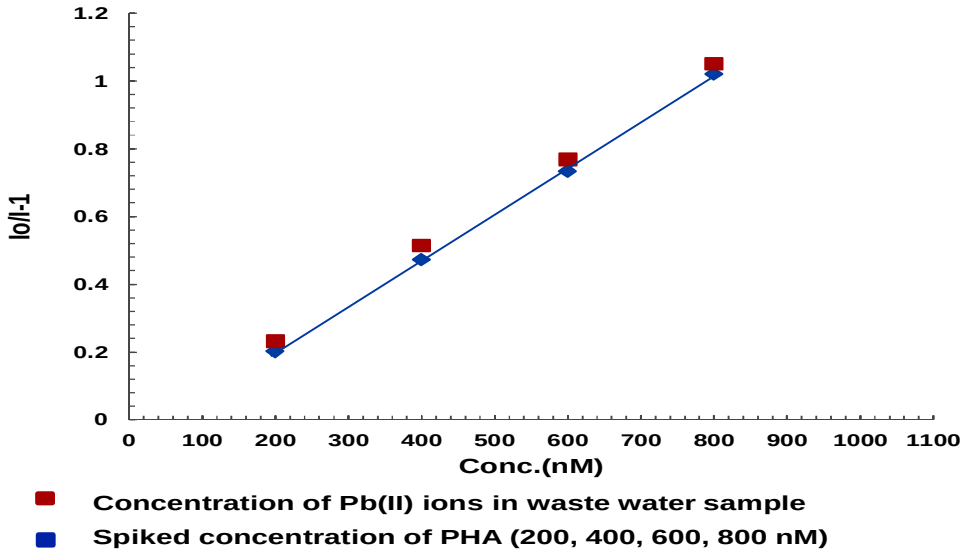


Fig. 14 Graph showing the concentration of Pb(II) ions found in waste water samples

TABLE 1: DETERMINATION OF PB(II) IONS IN WASTE WATER SAMPLE

Waste water sample	Conc. of Pb(II) (nM) obtained from ICP-AES in sample solution	Spiked Concentration of Pb(II) (nM)	Conc. of Pb(II) (nM) obtained From Fig. 14	Conc. of Pb(II) (nM) in sample solution	Average Conc. Of Pb(II) (nM)	Conc. of Cu(II) (nM) obtained from ICP-AES in sample solution after spiking
1	23.42	200	224.52	24.52	24.08	223.44
2	23.42	400	423.61	23.61		423.47
3	23.42	600	623.65	23.65		623.51
4	23.42	800	824.56	24.56		823.47

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IV. CONCLUSION

Stable water-dispersible AgNps were synthesized using CRTH, which acts as both reducing as well as stabilizing agent. CRTH-AgNps were found to be stable over 90 days at varied pH values. CRTH-AgNps can be used as a selective and sensitive sensor for colorimetric and spectrofluorometric determination of Pb(II) in waste-water samples. Micromolar concentration of Pb(II) can be detected by the naked eye, as the color of CRTH-AgNps changes from orange to brown. The minimum and maximum of the linear range of detection of Pb(II) ions by spectrofluorometry are 100 nM to 0.98 µM, respectively. The method was successfully applied for the determination of Pb(II) in industrial waste-water samples. The CRTH-AgNps can also be explored for their use in the detection of Pb(II) in environmental and biological samples.

V. ACKNOWLEDGEMENT

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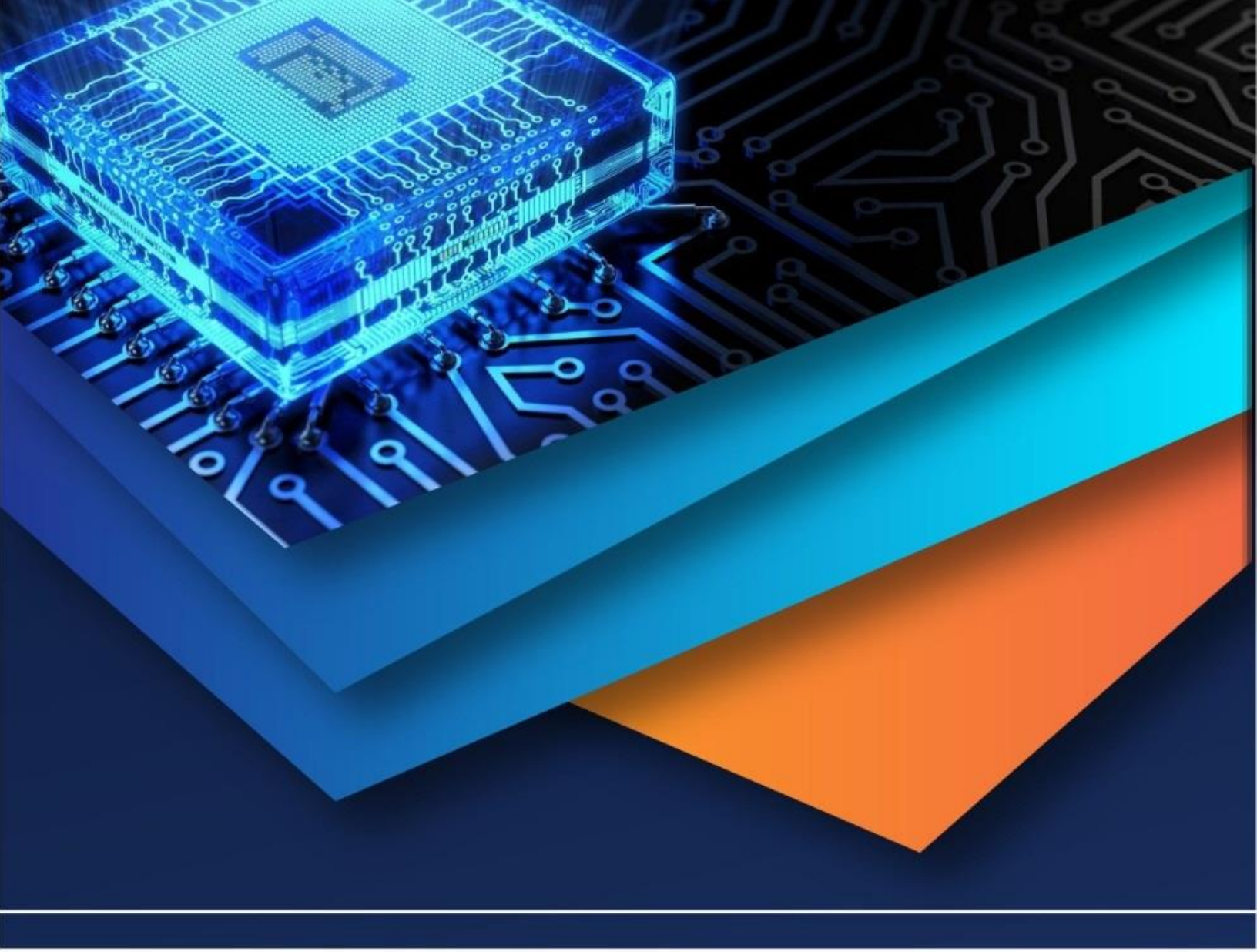
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