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Transition Metal Complexes As Quantum Dots: Excellent alternatives to Organic Chromophores for Biological Imaging Applications

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***Abstract*—** In recent years, different types of inorganic nanoparticles (iNPs) with unique physicochemical properties have emerged.1−4 Among these, quantum dots (QDs) have proved to be very versatile,finding applications in electroluminescent displays, quantumcomputing, photovoltaics , solar cells,transistors,and biological imaging.For biological imaging applications, QDs are now excellent alternatives to organic chromophores.given that they can have similar sizes, shapes,and surface functional groups. A potentially prolific new direction in inorganic chemistry and nanochemistry could be to combine NPs with small metal complexes to seek synergistic and/or cooperative effects. In this context,combining QDs with coordination complexes is being explored as a new strategy to obtain cooperative systems with improved properties for applications in sensing, biological imaging, and molecular therapy. A prominent area of research in coordination chemistry is the development of metal complexes that can act as artificial nucleases. Overall, these synthetic DNA-cleaving reagents.

***Keywords*—** quantum dots, nano medicine,Coordination Compounds, Transition Metal Complexes.

I. INTRODUCTION

Quantum dots are tiny semiconductor particles a few nano meteres in size having optical and electronic particles that differ from large particles due to quantum mechanics.Their broad excitation bands are mainly helpful in their applications.Quantum dots are excellent alternatives to orgganic chromophores, given that they have similar sizes , shapes and surface functional groups.

II. DICUSSSION

In recent years, different types of inorganic nanoparticles (iNPs) with unique physicochemical properties have emerged.1−4 Among these, quantum dots (QDs) have proved to be very versatile,finding applications in electroluminescent displays, quantumcomputing, photovoltaics , solar cells,transistors,and biological imaging.

The useful physicochemical properties of QDs include ; their broad excitation bands with very high extinction coefficients and narrow emission bands that can be tuned across a region of the visible or near-infrared spectrum by varying the size and composition of the QD with high photostability. For biological imaging applications, QDs are now excellent alternatives to organic chromophores.

Given that they can have similar sizes, shapes,and surface functional groups. In this context, some studies have shown that nanosized QDs can be considered generic curved surfaces that DNA can wrap around. This is important because bending may open and close certain sites along the double helix, making certain regions of the DNA more or less accessible. Potentially, this could have widespread implications and applications because it could lead to artificial regulation of a wide array of cellular processes for therapeutic and biotechnological applications, much like protein−DNAinteractions do naturally.

In addition to enabling different applications, the effects of DNA−QD interactions need to be considered also from a toxicological point of view. However,there are contradictory reports concerning the ability of QDs to damage DNA in the absence and presence of light as well as their toxicity to cells.

A potentially prolific new direction in inorganic chemistry and nanochemistry could be to combine NPs with small metal complexes to seek synergistic and/or cooperative effects. In this context, combining QDs with coordination complexes is being explored as a new strategy to obtain cooperative systems with improved properties for applications in sensing, biological imaging, and molecular therapy. A prominent area of research in coordination chemistry is the development of metal complexes that can act as artificial nucleases. Overall, these synthetic DNA-cleaving reagents operate using one of two distinct mechanisms:

(I)oxidative scission of deoxyribose residues through redox chemistry and (ii) hydrolysis of the phosphodiester sugar backbone.

The most classical example of oxidative DNA-cleavage activity is exemplified by the Cu(II)-1,10-phenanthroline (1,10-phenantroline = phen) system, which has been utilized as a footprinting reagent for the evaluation of protein−DNA interactions as well as a probe for DNA and RNA secondary structure.In this intensively investigated system, [Cu(phen)2]+ generated in the presence of a reducing agent and molecular oxygen afford activated oxygen species for DNA cleavage, whereas the intercalation of phen into the DNA minor groove allows for DNA targeting.

Recently, we discovered that QDs cooperate and synergize with the Cu(II)-1,10-phenanthroline system for DNA cleavage, providing both the first example of cooperative DNA cleavage between NPs and a small-molecule-based synthetic metallonuclease and a potentially new approach to develop more efficient DNA-cleaving systems.

Many ligand systems and approaches have been tested with varying degrees of success to increase both the DNA scission capability and the affinity of copper metallonucleases for DNA.

A popular strategy is to use bimetallic agents because of the potential cooperative effects that can arise between the two metal centers. However, an emerging way to design more powerful synthetic catalysts for a wide range of transformations,including DNA cleavage, utilizes ligands with hydrogen bonding features resembling those found in the active sites of metalloenzymes.

We have combined the advantages of dinuclear copper catalysts with those of hydrogen-bonding ligands, and we exploit QDs as a redox-active protein-like nanostructure to activate strongly the copper catalysts for DNA cleavage.

***Two novel (μ-guanazole)-bridged binuclear copper(II) complexes with 1,10-phenanthroline (phen) or 2,2′-bipyridine (bipy), [Cu2(μ-N2,N4-Hdatrz)(phen)2(H2O)(NO3)4] (1) and [Cu2(μ-N1,N2-datrz)2(μ-OH2)(bipy)2]-(ClO4)2 (2) (Hdatrz = 3,5-diamino-1,2,4-triazole = guanazole), have been preparedand characterized by X-ray diffraction, spectroscopy, and susceptibility measurements.

Two mono(phen)-CuII fragments have been attached in the same compound by means of a single μ-triazole bridge using the ligand guanazole (guanazole= 3,5-diamino-1,2,4-triazole =Hdatrz,thus yielding two copper centers with labile coordination sites of facile substitution and a structure suitable for DNA intercalation.

The analogous bipy (2,2′-bipyridine = bipy) ternary compound was been prepared, resulting in a dinuclear compound that contains a bis(guanazolate) bridge. In addition to providing a bridge between the two copper centers, the X-ray crystal structures reveal that the guanazole/guanazolato provides N−H groups for hydrogen-bonding interactions with the DNA. To effect DNA cleavage efficiently, these new copper complexes are combined with water-soluble micelles filled with CdSe-ZnS core−shell QDs.

Synthesis of [Cu2(μ-Hdatrz)(phen)2(H2O)2(NO3)4] : An aqueous solution of Cu(NO3)2•2.5H2O (1.148 g, 5 mmol, 20 mL) was mixed with an aqueous solution of guanazole (0.248 g, 2.5 mmol, 5 mL). A green solution was formed to which an aqueous suspension of phenantroline•H2O (0.993 g, 5 mmol, 10 mL) was added dropwise. A dark turbidity was almost immediately observed. After 2 h of stirring, a black−green precipitate was filtered off, and the resulting dark green solution was allowed to stand at room temperature covered with Parafilm.

Within ca. 1 month, a few large black−green crystals, not suitable for X-ray, appeared; they were separated by filtration.

Synthesis of [Cu2(μ-datrz)2(μ-OH2)(bipy)2](ClO4)2:

An aqueous suspension of bipy (0.156 g, 1 mmol, 20 mL) was slowly added (drop by drop) to an equimolar aqueous solution of Cu(ClO4)2•6H2O (0.37 g, 1 mmol, 20 mL).

To this mixture, an aqueous solution of guanazole (0.02 g, 0.25 mmol, 5 mL) was slowly added. The reaction mixture was stirred for 2 h. A light blue precipitate was formed and filtered off. The remaining dark green solution was allowed to stand at room temperature. After 1 day, dark green single crystals of 2 appeared.

Synthesis of Micelles Filled with QDs and SPIONs.

Micelles core−shell CdSe-ZnS QD were synthesized, characterized, and purified as described previously.The CdSe-ZnS QD have an average diameter of 5.2 nm (4.0 nm CdSe core diameter and 0.6 nm ZnS shell thickness).

The MQDs were prepared by self-assembly process of PEGylated phospholipids around hydrophobic CdSe-ZnS core−shell QDs.The water-soluble micelles with encapsulated superparamagnetic iron oxide nanoparticles of 6 nm as core material were prepared in the same way.

DNA−Copper Complex Interaction Studies.

For QD−CT-DNA interaction studies, a working solution containing 600 nM QD in cacodylate buffer (0.1 M, pH 6.0) was prepared. The experiment entailed the addition of serial aliquots of a CT-DNA stock solution. After each addition, the samples were excited at 400 nm, and emission was recorded between 580 and 700 nm.

Samples were treated as described above in the presence of MPA. To test for the presence of reactive oxygen species (ROS) generated during strand scission in the presence of QD, various reactive oxygen intermediate scavengers were added to the reaction mixtures.

In addition, a chelating agent of copper(I), neocuproine (100 μM), was also assayed. Samples were treated as described above in the presence of MQD. All of the results are the average of experiments performed at least in triplicate.

DNA-Cleavage Experiments.

DNA-Binding and DNA-Cleavage Properties:

The study of the DNA-binding properties was carried out for 1 and 2 through a series of techniques: thermal denaturation, viscosimetry, and fluorescence-based assays.

Both complexes were prepared and isolated as solid products and then dissolved in water for the biological experiments. biological experiments.

The existence of the dinuclear unit of 1 and 2 in solution was tested by mass spectrometry.

DNA Binding Properties.

The shift in melting temperatures (ΔTm) resulting from the association of 1 and 2 with CT-DNA. The ΔTm produced by 1 is high and notably larger than by 2 (20 vs 5 °C), which implies that the stabilization of the DNA double strand produced by 1 is more pronounced.

DNA-Cleavage Activity

Mechanism of DNA Cleavage and Role of the QDs.

To clarify the mechanism (i.e., oxidative vs hydrolytic) of the nuclease activity of complexes 1 and 2, electrophoresis assays with ROS scavengers.

***These findings signal the minor groove of the DNA as the nuclease binding site, but in compound 1, this specificity is lower than, for instance, in [Cu(phen)2] 2+, presumably because 1 can interact through electrostatic interactions and hydrogen bonding when the minor groove is inaccessible (vide supra).

*** In the presence of MQDs (Figure 10B), a clear inhibitory effect was found for the superoxide scavenger Tiron, which indicates that the DNA damage produced under these conditions occurs by an oxidative mechanism.

To identify further the chemical processes involved in the QD-mediated DNA-cleavage process, we carried out X-ray photoelectron spectroscopy (XPS) studies.

FUTURE SCOPE AND AIMS:

DNA binding and cleavage studies reveal that Transition Metal Complexes/compounds can be used as efficient nucleases because of the cooperative effect of the two Cu(II) centers and the guanazole ligand, which in addition to providing a bridge between the two metals can participate in hydrogen-bonding interactions.

Of the two complexes, 1 shows the highest affinity for DNA and binds via intercalation of the phen ligands. In the presence of oxygen and micromolar concentrations of MPA or H2O2 as activators, only 1 is capable of causing DNA cleavage.

***However, in the presence of nanomolar concentrations of water-soluble QD-filled micelles, both systems are highly efficient at cleaving DNA.

III. PHOTOINDUCED ELECTRON TRANSFER FROM PBS QUANTUM DOTS TO COBALT(III) SCHIFF BASE COMPLEXES

Recent advances in the development of therapeutic antitumor and antiviral agents have focused on compounds that bind to the biological active site of an enzyme. Although these

reversibly bound drugs are susceptible to nonspecific and potentially undesirable reactions, the success of transition metal therapeutics, such as cisplatin, has refocused efforts aimed at investigating new complexes in this broad class.

This research has facilitated an improved understanding of the interactions between complex biological systems and inorganic coordination complexes.

Strategies for designing prodrugs, drugs that are administered as inactive compounds but are triggered by some controllable stimulus, have exploited differences in biological environments, such as pH, redox status, and protein expression,in achieving a higher level of specificity and efficacy.

***Photoreduction of Platinum(IV) is started by colloidal Quuantum dots...The electron transfer (PET) following photoexcitation at 615 nm.10 The wavelength of light that initiates PET and subsequent Pt(IV) reduction is within a favorable range (600−1300 nm) for maximal tissue depth penetration for in vivo applications.

***QDs possess highly tunable electrochemical and spectroscopic properties with excitonic transitions in the lowenergy visible and near-infrared (NIR) regions.

***QDs have high two-photon cross-sectional efficiencies that surpass those of traditional organic dyes.Properties such as water solubility, cellular uptake, and selective accumulation in malignant tumors have been tuned to achieve superior biocompatibility.

***These attributes make QDs favourable candidates as photosensitizers for accessing multiple redox states of metal-based therapeutics in prodrug designs.

Cobalt(III) Schiff base [Co(III)-SB] complexes of the equatorial tetradentate ligand bis(acetylacetone)-ethylenediimine (acacen) are known to be potent inhibitors of a wide array of zinc-dependent proteins, including thermolysin, α-thrombin.Modification of the acacen backbone to incorporate biomolecular targeting moieties (such as oligonucleotides) has been shown to selectively target zinc finger transcription factors.

***Evidence suggests the inhibition activity is due to disruption of the protein structure by coordination of Co(III) to active-site histidine residues.

***This coordination event occurs via a dissociative ligand exchange and is strongly dependent on the nature of the axial ligands present on the Co(III)-SB complex.

Selective enzyme inhibition is observed when the axial positions are occupied by

either sterically hindered 2-methylimidazole or labile amine ligands.In general, the coordination behavior of cobalt Schiff base complexes is dependent on the oxidation state of the metal ion.

***Because of the redox properties of cobalt, axial ligand coordination of Co(II)-SB complexes has an increased propensity for dissociation.

SYNTHESIS:

Several batches of NIR light-absorbing PbS were synthesized.We selected PbS QDs .

The Co(III)-SB complexes were synthesized and characterized according to literature.

To prepare the PbS QD/Co(III)-SB complex samples, we transferred a methanolic solution of

the Co(III)-SB complex into a scintillation vial and dried itunder nitrogen before adding 1.4 × 10−5 M PbS QDs in CHCl3. The vial was shaken until the Co(III)-SB complex was

dissolved, and the solution was allowed to equilibrate for 24 hbefore measurements were taken.

***In summary, It has been shown that that selective photoexcitation of PbS QDs within mixtures of the QDs and Co(acacen)(Im)2 increases the axial ligand reactivity of the Co(III)-SB complex.

It is proposed that the mechanism for this observation is electron transfer from the PbS QDs to Co(III), given that the dissociation of axial ligands is a documented and well-understood consequence of reduction of Co(III) to Co(II) in this Co(III)-SB complex, (ii) electron transfer from the bottom of the conduction band of PbS QDs of this size to Co(III) is energetically favorable by ∼100 meV while energy transfer is not ermodynamically possible,

(iii) addition of the Co(III)-SB complex to the QDs quenches their PL while exposure of the QDs to the acacen molecule without the redox-active Co(III) center does not quench their PL, and ***(iv) increasing the reduction potential of the Co(III) center within the Co(III)-SB complex by changing the axial ligand makes the Co(III)-SB complex a less efficient quencher of the PL of the QDs.

***We can improve the electronic coupling between the QD and the Co(III)-SB complex by using a QD coating that minimizes steric repulsion at the surface of the QD or by functionalizing the Co(III)-SB complex such that it can more closely approach the QD.

*** Results of such studies offer a unique route for light activation of a Co(III)-SB protein inhibitor via NIR excitation and suggestthat the development of inorganic therapeutic agents may be specifically coupled to a biologically active site by cooperative
redox binding ligation.

A new binding strategy of linking quantum dots (QDs) to magneticnanoparticles (MNPs) using DNA interaction with metal coordination bonding was developed.

Platinum was selected for binding QDs to DNA. This nanoconjugate acts as a new probe for diagnosis with its double modalities, fluorescence and magnetic property.

Owing to the high quantum yield and excellent photostability, QDs have been intensively investigated as a new probe for bioimaging and biosensor applications.

***The high potential medical utility of QDs in the areas of imaging and diagnosis has led to extensive exploration of methods to functionalize these nanoparticles. This effort has generated improvedmethods for surface modification and bioconjugation of QDs with various bioactive molecules, including small molecules,DNA and antibodies,and the mployment of the functionalized QDs for visualizing biological events in vitro and in vivo. In addition, because of the excellent optical properties of QDs, they have been utilized in approaches to create novel biologically interesting nanoconjugate probes.

***Various probes of this type, exemplified by gold nanoparticle (AuNP)–QD conjugates,have been devised to detect specific DNA, RNA,and peptides, as a part of new techniques for cancer diagnosis.

***Nanomaterials comprised of conjugates betweenMNPs and QDs,which have both fluorescent and magetic properties, have been investigated in the context of tools for the diagnosis of multiple cancers.

***A new strategy has been explored for binding QDs and MNPs taking advantage of metal coordination of DNA molecules as the key bonding component. This choice was inspired by platinum-based cisplatin, one of the most widely used anticancer drugs for the treatment of ovarian, testicular, and head and neck cancers.

The studies aimed at understanding the mode of action of this drug have shown that a cisplatin 1,2-intrastand d(GpG) cross link occurs on DNA to cause apoptosis of cancer cells. The central platinum (Pt)(II) of cisplatin, which possesses two ammonia and two chloride ligands coordinated with square planar geometry, forms stable coordination bonds with guanine N7 sites of DNA.

Studies have shown that the nanoconjugates are more stable in vivo than are simple Pt complexes.

Only a few approaches using strong coordination bonding character of the Pt(II) complex to generate nanoconjugates or nanoassemblies have been reported.

*** It is known that the nature and specificities of reactions of metal–ligand complexes depend greatly on the properties of both the metal and chelating ligands. As a result, the strength of the coordination bond and the related ease of ligand substitution can be easily tuned using specific ligands.

***Studies would be aimed at developing a new approach for conjugating QDs and MNPs.

Studies have used cis-dichlorobis-(dimethylsulfoxide)-platinum(II) (cis-PtII(DMSO)2Cl2), which contains a weakly bonded DMSO ligand that serves as a leaving group in a substitution reaction with a diamine, forming a (diamine)-QD–PtCl2 complex. The key proposal is that the N7 nitrogen of guanine bases in DNA linkedMNPs would then substitute for the chloride ligands in the resulting complex to form a QD–Pt(II)–MNP–DNA nanoconjugate.

Cd Se quantum dots are prepared by phosphorus-free methods using oleic acid as stabilizing surface ligand. Ligand exchange is monitored quantitatively by 1H NMR spectroscopy gives about 30 monodentate ligands per nanocrystal, with a ligand density of 1.8−2.3 nm−2.

***The arrangement of nanosized objects into more complex structures remains a challenging target.One of the preconditions for such higher level organization is the introduction of a limited number of attachment points. This offers the opportunity to link up spherical nanoparticles using covalent bonds or specific donor−acceptor interactions to generate, for example, divalent or trivalent nanoparticles that can be the building blocks for linear or branched arrangements.

***We are especially interested in the functionalization of colloidal semiconductor quantum dots (QDs), in particular CdSe, and were looking for ways of restricting the number of surface ligands by macrocyclic attachments, using coordination chemistry principles for the construction of stable nanocrystal−organic conjugates.

The successful functionalization of nanoparticles requires an understanding of their surface chemistry.Semiconductor nanocrystals are stabilized by surfactant-type surface

ligands which determine the growth rate, stability, and solubility of the nanocrystals..

While CdSe prepared using trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO) are frequently represented with a surface coverage of TOPO, it is now known that the surface is mainly covered by TOP oxidation products, alkyl phosphinates and phosphonates, which coordinate to metal surface sites as bridging ligands.

***Nanocrystals prepared under phosphorus-free conditions using long-chain carboxylic acids such as stearic or oleic acid are stabilized by a layer of metal carboxylate .

***The nature of ligandbinding has been explored mainly by NMR spectroscopy,as well as luminescence and isotopic labeling methods.

***Macrocyclic compounds with a rigid aromatic framework,such as phthalocyanines, subphthalocyanines, and porphyrins,are synthetically readily accessible and can easily be derivatized to give compounds with a predetermined number of anchorpoints (usually 0−4).

Hence we propose to synthesize such macrocyclic molecules.

***Such ligands are expected to bind to nanocrystal surface sites by substitution of the surfactant ligands present from the synthesis stage. For a given concentration, due to the chelate effect polydentate ligands should bind to the surface of a nanocrystal significantly more strongly than monodentate ligands.

***We would focus on ligands of type Subphthalocyanines possess a cup-shaped structure, which suggested a good geometric match with nanocrystals with diameters in the 2−3 nm range; in addition they contain a functional group perpendicular to the macrocyclic core which offers the possibility of connecting to other ligand-decorated nanoparticles by covalent bonds.

***We will focus on Porphyrins of this type which would be expected to act as monodentate ligands oriented perpendicular to the nanocrystal surface.

Although the central ring in porphyrins of this type is flattened, there is sufficient flexibility both in the ring and in the functionalized substituents X to allow these compounds to attach themselves to nanocrystals flat-on, i.e., parallel to the crystal surface.

***Alternatively these ligands may adopt a perpendicular orientation bridging between two adjacent quantum dots.

The structures and properties of the CdSe−macrocyclic constructs have been evaluated using a combination of 1HNMR, absorption, and fluorescence spectroscopies.

Relative Ligand Binding Strength.

Oleic acid (octadec-9-enoic acid) possesses a cis-vinylene moiety which acts as a convenient 1H NMR marker. Preliminary qualitative experiments would be performed to establish the relative binding preference of carboxylic acids, phosphonic acids, and thiols.

Number of Ligands per Nanoparticle. The number of oleate ligands per nanoparticle and hence the ligand density would be evaluated using a combination of UV−vis and 1H NMR

Spectroscopies

Reactions of Subphthalocyanines with CdSe QDs.

***In a series of initial reactions, the binding behavior of subphthalocyanine would be explored. This ligand is decorated with three meta-pyridyl substituents which give a bite angle that should be geometrically well-matched for binding to nanocrystals of 2−3 nm diameter, provided there are accessible Lewis acidic surface sites.

Treatment of a solution of CdSe QDs in chloroform gave a deeply colored solution. Quenching of the QDs fluorescence was observed. The nanoparticles were precipitated with methanol, isolated by centrifugation, and repeatedly washed with acetone until the washings were free of subphthalocyanine by UV/vis spectroscopy.

Reaction of Metal-Free Porphyrin Derivatives with CdSe Nanoparticles.

*** The interaction of pyridine-substitutedfree-base porphyrins as well as their metal complexes with CdSe and Cd/ZnS core−shell particles made by the TOPO route would be intensively studied, since we are interested in the interactions of carboxylate-typeCdSe nanocrystals with carboxylate-substitutedporphyrins.

***Preliminary studies were carried out with 5,10,15,20-tetra(4-decyloxyphenyl)porphyrin which carries only alkyl substituents but no surface-binding functional groups.

It quickly becomes evident that metal-free porphyrins become metalated in the presence of CdSe nanoparticles over time atroom temperature.

The insertion of Cd2+ into the ring system is evidenced by a visible color change; this was confirmed by the UV−vis spectra.

The conversion of the metal-free into 5,10,15,20-tetra(4-decyloxyphenyl)-porphyrinato cadmium as a typical example.

Monodentate Porphyrin Ligands and CdSe Nanoparticles. The binding of monodentate porphyrin ligands would be first examined.

Tetradentate Porphyrin Ligands and CdSe Nanoparticles.

While monodentate porphyrin ligands were readily able to substitute oleate ligands, their steric requirements are moderate since they bind to the nanoparticle surface in “upright” position. Polydentate ligands, on the other hand, have the potential of covering large sections of the nanocrystal surface by lying flat,

***and since we are interested in the question whether or not a nanoparticle could be essentially encapsulated by porphyrins and what binding mode would be adopted. The tetrasubstituted porphyrins are therefore synthesized.

WHY PORPHORINS WERE CHOSEN

Porphyrins were chosen due to the flexibility of the molecule.It is well-known that, in the case of tetraphenylporphyrins, the phenyl groups in the meso positions lie perpendicular to the tetrapyrrolic ring. Such a ligand is therefore able to bind parallelto the nanoparticle surface. Of course, the phenyl substituents are free to rotate, and suitable geometrieswill also be present.

Preparation of CdSe Nanocrystals.

CdSe nanocrystals were produced by a modification of a literature procedure. To cadmium oxide (300 mg, 2.34 mmol) in octadecene (20 mL) was added oleic acid (2 mL). The mixture was stirred under vacuum for 10 min, and then N2 was introduced. The mixture was heated to 250 °C and stirredat this temperature until a clear solution was obtained, which was then left to cool to ca. 120 °C. Selenium powder (100 mg, 1.27 mmol) was added and the mixture heated to 240 °C, causing the color to change from yellow to orange. Heating was stopped when the color of the solution was deemed the right shade of orange for the desired nanocrystal size (after various experimental trials). The solution was immediately cooled on an ice bath, and toluene (10 mL) was added.The solution was then transferred to two large centrifuge tubes withfiltration (syringe filter: 0.22 μL). The volume of both tubes was adjusted to 30 mL with toluene. The addition of acetone (20 mL)caused the formation of a white precipitate of unreacted starting material. The tubes were centrifuged (1400 rpm), the orange solutionswere collected, and the white precipitate was discarded. The solution (25 mL) was again placed in a large centrifuge tube. Methanol (25mL) was added, followed by centrifugation. An orange oil separated atthe bottom, which was collected; the clear top solvent layer wasdiscarded. This process was repeated until all the solution was processed in this way. The separated orange oils were combined and transferred to a smaller centrifuge tube with toluene (total volume 7 mL). Methanol (7 mL) was added and the tube centrifuged. Again the orange oil was decanted. The volume was made up to 7 mL in dichloromethane, and the same volume of ethanol was added, followed by centrifugation. This process was repeated until a thick orange oil or powder was obtained. Finally, acetone (14 mL) was added, and the tube was sonicated for several minutes and then centrifuged. This was sepeated five times. A free-flowing orange powder was finally obtainedafter drying under vacuum and stored under N2.

Encapsulation of zinc-rifampicincomplex into transferrinconjugatedsilverquantumdotsimproves its antimycobacterial activity and stability and facilitates drug delivery into macrophages in order to improve the chemotherapy of tuberculosis, there is an urgent requirement and hence synthesis of a novel antiTBdrugcomplex consisting of zinc and rifampicin (ZnRIF), and encapsulated it into transferrinconjugated silver quantumdots(ZnRIFTfQD) to improve delivery in macrophages.

Tuberculosis (TB), caused by Mycobacterium tuberculosis (M. tb), is one of the world's major health problems.

In combination chemotherapy,The "first line" therapy is of three or four drugs i.e. isoniazid, rifampicin, pyrazinamide and ethambutol, followed by the less efficacious and more expensive "second line" drugs, which include capreomycin, kanamycin, amikacin, paraaminosalicylicacid, ciprofloxacin, metal ions have many important physiological functions in the body. Transition metal complexes exhibit unique and interesting properties such as changing oxidation states and the ability to form specific interactions with other biomolecules . It was shown that some metaldrugcomplexes are more potent as compared to pure drug . Toward this end, the interactions of some antibiotics with transition metals have been studied . Among them, zinc is known to exhibit antibacterial activity . Zinc oxide (ZnO) nanoparticles are even more efficient along with reduced toxicity .Rifampicin (RIF), a broadspectrum antibiotic, is one of the most effective first line drugs against TB.

***Semiconductor nanocrystals, also known as quantum dots (QDs), have become an important tool in biomedical research, especially for quantitative and longtermfluorescence imaging and detection .

In view of this goal, we synthesized a zincrifampicin (ZnRIF) complex and checked its activity against non-pathogenic Mycobacterium smegmatis, and Mycobacterium bovisBCG, which behave like pathogenic M. tb. RIF is chosen since it coordinates with metal ions through chemical groups i.e. two phenolic and two aliphatic OH groups and ,the presence of additional nitrogen and oxygen donor atoms provide this compound interesting properities for studying its coordination behavior with transition metal ions.

Synthesis of ZnRIF complex and its conjugation to transferrin coupled QDs was studied by UVVisiblespectroscopy, transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), photoluminescence, Xraydiffraction (XRD), XrayFourier transform infrared spectroscopy (FTIR)UVVis Spectra analysis of the ZnRIF complex Photoluminescence analysis of the ZnRIFcomplex Xray photoelectron spectroscopy Xraypowder diffraction analysis of the ZnRIFcomplex Go to: photoelectron spectroscopy (XPS), and Nuclear magnetic resonance (NMR). We observed that conjugation of ZnRIFcomplex to transferrin coupled QDs.

IV. RESULTS

Fourier transformation infrared spectroscopy (FTIR).The structure of the ZnRIF complex (Fig. 1A) was confirmed by several physicochemical methods. The IR spectrum of ZnRIFwas compared with that of free RIF (Fig. 1B). In agreement with a previous report , the spectrum of free RIF showed characteristic peaks at3482 (OH), 2880,1726 (C=O), 1646(C=N), 1247(COC) and 808(CH) cm . A characteristic broad band at 3474 cm was observed with the Synthesis and Characterization of Zn-RIF Complex.

1. UV-Vis spectraanalysisof the Zn-RIF Complex UV-V
2. photoluminescence analysis of the Zn-RIF
3. Xrayphotoelectronspectroscopy

4. Xraydiffraction (XRD) analysis of ZnRIF COMPLEX

NMR spectroscopy1H NMR spectrum analysis of RIF and the ZnRIFcomplex was carried out in DMSO-D6using a Bruker AVANCE 400 NMR spectrometer. Tetramethylsilane (TMS) was used as an internal standard. Allpeaks arising from unbound RIF ligand were clearly

resolved (Fig. 2A) with the phenolic hydroxyl protonsappearing at 9–10 (2 H) ppm. In the ZnRIFspectrum these peaks were absent due to replacement with zinc. By comparing the splitting patterns, it can be concluded that the metal to ligand ratio is 1:1 .

Characterization of ZnRIFcomplex.

Transition metals can exhibit a wide variety of coordination properties, and reactivities, which can be used to form complex with drugs as ligands. Previously, several studies have reported improved therapeutic properties of several metal complexes against M. tb. RIF, which is considered the cornerstone in the shortcourseTB treatment regimen, exhibits detrimental side effects. To address these issues, we employed a strategy in which Zn was complexed with RIF to form a ZnRIFcomplex, which was subsequently encapsulated in transferrinconjugated silver QDs to yield the ZnRIFTfQD conjugate. Detailed physicochemical analyses confirmed the formation and encapsulation of ZnRIF complex in transferrincoupledQDs. We demonstrated that encapsulation of transferrin on the surface of quantum dots enhanced the binding efficiency of drug molecules. Then we showed thattransferrinconjugated and ZnRIFencapsulated silver QDs successfully targeted to the macrophages, remaining stable for up to 48 h and significantly reducing the bacterial burden inside the macrophages.

***Quantum dots (QDs) are nano scale semiconductor crystals with sizes of 1–10 nm. QDs provide excellent tools for sensing, imaging, drug delivery and therapy due to their optical properties, broad excitation range, welldefinedemission wavelengths and their ability to attain different shapes thus providing an excellent structure for coating with various biomolecules .

Development of nanoscale drug delivery system that ***allows a slow release of drug over prolonged periods of time is important to thus avoid burst effects.Our results show that ZnRIFTfQDsmeet these criteria.

For the synthesis of ZnRIFcomplex, 18 mg (0.095 mM) of hydrated zinc nitrate and 80 mg (0.097 mM) rifampicin in methanol and stirred for 3–4 h. The precipitate was dried, weighed and dissolved in dH O to do the experiments.. ZnRIFcomplex was characterized by Fourier transform infrared spectroscopy (FTIR) (Nicolet iS5, Thermo Scientific, India), UVVisible (Epoch, BioTek, Germany), Xrayphotoelectron spectroscopy (XPS) (S/N: 10001, Prevac, Poland), Powder Xraydiffraction (XRD) (Shimatzu6100, Japan) and Nuclear magnetic resonance (NMR) (AVANCE 400, Bruker, Switzerland). Silver QDs were synthesized as described previously . Briefly, silver QDs were synthesized by reduction of silver nitrate (1 mM) by addition of excess of ice cold sodium borohydride (2 mM) solution by vigorous stirring at roomtemperature. QDs were synthesized in less than a minute reaction time. Thestoichiometric ratio of silver nitrate tosodium borohydride is very critical for the synthesis of QDs.

To synthesize ZnRIFencapsulated transferrin conjugated silver QDs, 1 mg/ml of transferrin and 500 µg/ml of ZnRIFcomplex were added together with 1 mM concentration of silver nitrate. Then the reaction mixture was reduced by excess ice cold sodium borohydrate solution.

Characterization of ZnRIFencapsulated transferrin conjugated QDs (TfQDs)

Cytotoxicity assay

RAW 264.7 cells (1 × 10 cells/well) were grown in 24well plates for 24 h followed by treatment with different concentrations of drug complexes for another 24 h. Cell viability was determined by MTT assay as described previously.

V. CONCLUSION

FUTURE SCOPE AND AIMS:

DNA binding and cleavage studies reveal that Transition Metal Complexes/compounds can be used as efficient nucleases because of the cooperative effect of the two Cu(II) centers and the guanazole ligand, which in addition to providing a bridge between the two metals can participate in hydrogen-bonding interactions. Of the complexes, some show the highest affinity for DNA and binds via intercalation of the phen ligands. In the presence of oxygen and micromolar concentrations of MPA or H2O2 as activators, only some are capable of causing DNA cleavage. So A large amount of studies can be done using this Transition metal complexes As quantum dots.

VI. REFERENCES

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