Key Factor For Haemolysis

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ABSTRACT

Deep understanding of the nanomaterials toxicity dealing with the red blood cells, is of immense interest as red blood cells helps in the transportation of the oxygen via circulation. By using the semiconductor quantum dots , toxic mechanism have been approached along with following the infrared spectroscopy. It has been observed that MSA-QDs i.e. mercaptosuccinic acid-capped CdTe QDs is the reason for hem-agglutination and those which are medium sized induces the stomatocytes and echinocytes formation and the bigger ones results in the heavy hemolysis and leads to the formation of lot of ghost cells. The collected data have revealed that red light emitting quantum dots are responsible for the breaking of phosphodiester bond. Rapid development had been made in the field of mesoporous silica nanoparticles regarding their synthesis and applications in the field of biocomaptibility . Mesoporous silica nanoparticles having different size of the pores and shapes have been synthesized and their interaction with the red cells of blood along with protein are being studied . The study of the haemolysis assay provides the information on the size of pores and on about the morphology of the mesoporous silica nanoparticles and the morphology of the prepared particles have been found to be depressed by the protein corona formation.

Keywords: Nanomaterials, Haemolysis, biocompatibility, ghost cells, quantum dots.

INTRODUCTION

Nanoparticles are engineered and have expanded in various fields like those of technology, industry , and medicine and has increased the exposure of humans towards the use of nanoparticles in daily life. Nanoparticles can be made to enter the human body via different routes like gastrointestinal tract, respiratory system and the most common among these is through the intravenous injection. When nanoparticles reach the blood stream , then interaction takes place with the components of the blood. The protein conformation, and orientation are observed to be altered due to these interactions and this leads to functional disturbance. The protein which is absorbed modifies the nanoparticle surface and constitutes major part in identifying the biologica entity on nanoparticle surface.

Red blood cells haemolysis occur when these nanaoparticles interact with the the blood cells. Red blood cells have the ability to change the response of the defense system of the human body towards the nanoparticles that have been absorbed by the

system and results in increasing the invivo circulation time and reduction is observed in susceptibility of the particles to macrophages. For clarifying the interaction between the proteins, nanoparticles and the red blood cells there is a great need for understanding the designing and engineering of the nanoparticles having great biocompatibility. For reducing the nanotoxicity and improving the therapeutic applications of these nanoparticles , compatibility of blood evaluation is must. One among the various candidates derived via nanotechnology like mesoporus nanoparticles of silica are booming for bio-imaging, bio catalysis, bio sensing and in delivery of drugs.

In the recent years , many efforts had been made for developing effective and safe system for the delivery of drugs to the tumors (Langer ., 1998). One among the major limitation , involves the premature release of drug before approaching the site of target. A drug delivery system is said to be ideal if it delivers the drug to the specific site of the target without leakage of the drug on the way and is then internalized with the specific interaction among the receptors on the tumor cells and finally the drug is released at a rapid level on the targeted site according to the stimulus of the environment. For increasing the therapeutic efficacy and reducing the anti cancer side effects, the two main factors are stimuli release of drug and the active targeting.

They provide different properties like tunable scale of particles, having different kind of morphologies which range from spherical shape to rod like shape and these are also easy to synthesize. The mesopores are cylinderical in shape and have high surface area. Nanoparticles of mesoporous silica provide robust platform for different kinds of medical or biological applications. It has been found that , size of mesoporous nanoparticles , order of pores and integrity of pores on the hemolytic activity and founded that, hemolytic activity has been found to diminish to certain level of degree and decreases the particle size from 25 nm to 225 nm.

Mesoporous silica nanoparticles have been shown to posess low hemolysis as compared with non porous particles having same sizes. It has been found that , mesoporous nanoparticles having diameter 100 nm have low level of hemolysis as those with nanoparticles having 600 nanometer. Low hemolytic activity have been observed in case of spherical nanoparticles as compared with tubular counterparts. By modifying the surface, hemolytic activity can be reduced. The nanotoxic effect of these mesoporous nanoparticles should be properly evaluated before their therapeutic applications.cytotoxicity can be reduced when blood protein binds to the nanotubes of carbon . Biocompatibility of the nanoparticles have been observed via hemolysis. One of the example is when a plasma corona is formed on the surface then the hemolytic activity of mesoporous silica nanoparticles having different morphologies with different sizes are obtained.

It has been observed that large external available surface area and small size are observed for particles which show hemolysis and reduces the silanol groups and also reduces the steric or electrostatic repulsions taking place between red blood cells and particles . Mesoporous nanoparticles and rod shaped nanoparticles have been synthesized for evaluating the effect of morphology on the protein serum including the conformational changes and the adsorption kinetics. For comparing the effect of size of the pore , mesoporous silica nanoparticles having different pore sizes have

been synthesized. The figure 1 given below show the TEM images of the mesoporous synthesized nanoparticles having different sizes.

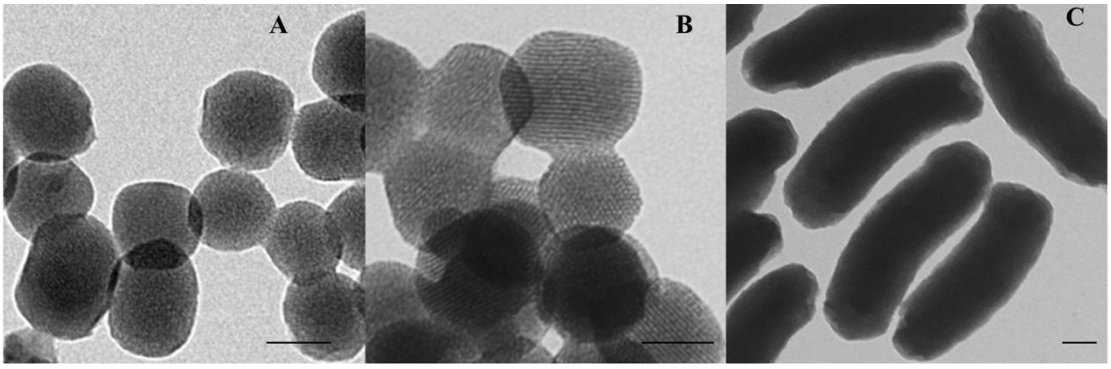


Figure 1. TEM images of (A) spherically shaped mesoporous silica nanoparticles with small pore size, (B) spherically shaped mesoporous silica nanoparticles with large pore size, (C) rod-shaped mesoporous silica nanoparticles (RPs). Scale bars in A−C = 50 nm.

CHARACTERIZATION AND FABRICATION OF VARIOUS MESOPOROUS NANOPARTICLES

A series of various mesoporous nanoparticles having different size of pores and the shapes have been prepared via cocondensation method using dilute tetraethylorthosilicate and surfactant with low concentration using aqueous ammonia as a base serving as a catalyst. This reaction has taken place via carrying out the reaction under controlled conditions and also by controlling the rate of reactions and stirring the reaction in appropriate conditions. By varying the ammonia's concentration, and also controlling the concentration of tetraethylorthosilicate , cetyltrimethylammoniumbromide, the width of the obtained mesoporous nanoparticles of silica is controlled. The pores are made to expand via introducing the swelling agent into the template which has been used in the preperation step . The surfactant is removed from the prepared mesoporous silica nanoparticles using the process of calcination. The morphology and size of the synthesized particles are characterized by using the technique of transmission electron microscope. It can be seen in above figure 1. It has been found that , two types of spheres having diameter of around 70 nanometer is obtained. It is evident that the mesoporous nanoparticles which are sphere shaped have diameter similar to those of rod like shaped particles. By studying the affect of hemolysis, the effect of size of pore and geometry has been studied.

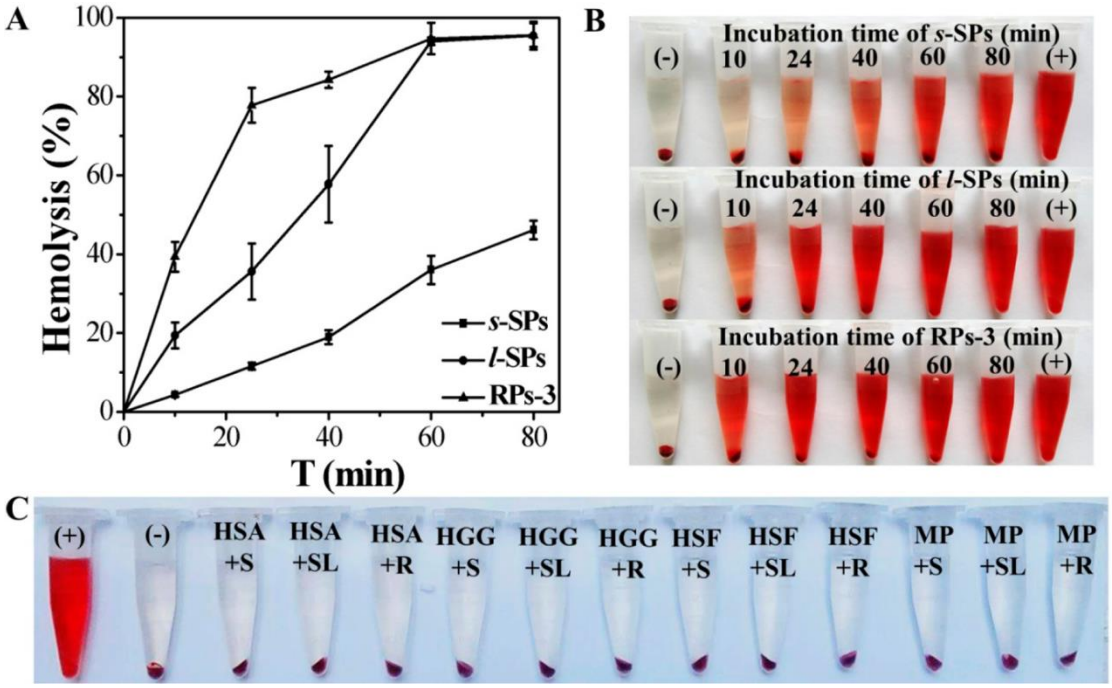


Figure 2. (A) Percentage hydrolysis of human RBCs when incubated with different mesoporous silica nanoparticles at a concentration of 500 µg/mL, plotted as a function with time. The value was obtained from two or three experiments, and the error bars indicated standard deviations from the mean. (B) images of red blood cells after exposure to different types of mesoporoyus particles at a concentration of 500 µg/mL for different times. (C) Images hemolysis assay of red blood cells after RBCs were mixed with either separated protein (HSA, HGG, and HSF) or mixed proteins (MPs) and three types of MSNs. In these experiments, I. water (+) and PBS (−) were used as positive and negative control, respectively.

The above taken figure 2(A)shows the study of hemolytic kinetics through exposing red blood cells to the sample with the 500 µg/mL concentration at ten degree celcius for 10, 24, 40, 60, and 80 min each. Figure 2(B) depicts the photos of red blood cells after exposing them to three different types of mesoporous silica nanoparticles for different period of time. It can be seen from figure 2 (A) and 2 (B) that the percentage of hemolysis for the red blood cells increases in a time dependant manner for three different types of mesoporous silica nanoparticles. It has been observed that , big size of the pores are responsible for the increase in the hemolytic activity of the mesoporous silica nanoparticles. The geometry is another aspect of increasing the hemolysis effect. The results shown in figure 2 shows that the hemolytic activity is dependant on the curvature of the surface and this results in increase of the hemolytic activity on the mesoporous silica nanoparticles. Low curvature of mesoporous silica nanoparticles show the conformational changes taking place in some proteins . Structural changes are involved when the low curvature mesoporous silica nanoparticles interacts with the red blood cells and this accelerates process of the red blood cells hemolysis.

It has been reported that , low content of hemolysis is observed as compared to those of the non porous silica particles. The same type of mesoporous silica nanoparticles

obtained are found to posses different size of the particles when prepared by following different types of methods, this is attributed to the variation in the scale of pores. Reason for the difference in hemolytic activity between the non porous and mesoporous silica particles is due to the difference in the particles zeta potential. In order to understand this deeply , two kind of stober nanoparticles with diameter 60 nm but posessing different zeta potential have been synthesized . Hemolytic activity has found to increase as an increase in zeta potential is observed. It can be deduced that , mesoporus silica nanoparticles hemolytic activity is dependent on the size of pores and also the hempolysis have been observed more for the mesoporus particles as compared to those of spherical particles.

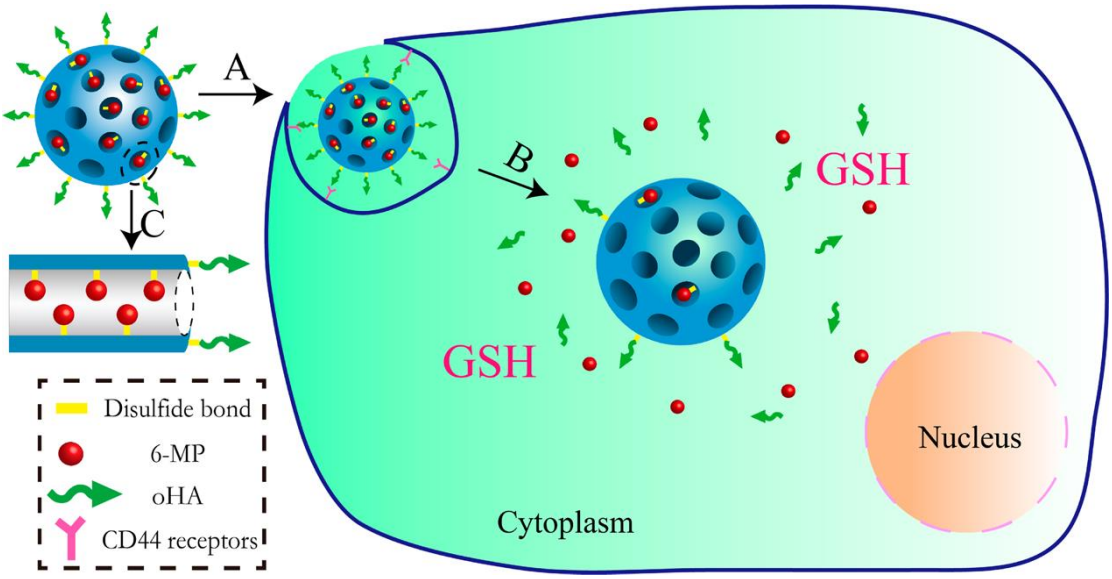
By studying the proteins adsorption kinetics, it can be easily understood that affinity for serum proteins on the surface of mesoporous silica nanoparticles are found tom be stronger than those for red blood cells. The given figure 2 (A) shows the red blood cells hemolysis of approximately 10 percent. It is quite necessary to evaluate the hemolysis of the mesoporous silica nanoparticles in serum proteins presence.three different types of mesoporous silica nanoparticles have been added and the temperature is maintained at 37 degree celcius. It is then incubated for three hours and this can been seen in figure 2(C). But in either of the conditions no hemolysis have been observed which can be seen in figure 2 (C). When mesoporous silica nanoparticles are added on the mixture of red blood cells with the proteins , then proteins are adsorbed on the surface of mesoporus silica nanoparticles first. The protein corona results in preventing the red blood cells. The protein serum has found to play an important role in understanding the biological behaviour of the nanoparticles which are injected intravenously .

DRUG RELEASE RESPONSE

A large attention has been made in response to the different external or internal stimuli like , change in the pH, enzymes, redox potential and temperature. The premature zero release has been achieved by using the strategies which are based on drug conjugation (Duan et al., 2013) .The bond of disulfide which is a redox responsive bond gets cleaved when comes under high glutathione concentration which is GSH and is a intracellular signal. This bond of covalent nature is quite stable in extracellular plasma and fluids and gets easily ruptured in the intracellular fluids because there is a concentration difference in extracellular and intracellular fluids. The concentration of cystolic glutathione is found to be three times higher than in the normal cells (Saito et al., 2003) . The nanoparticles of mesoporous silica have pore size which is quite tunable and also posess high surface area and posess excellent biocompatibility and also serves as ideal candidates for the responsive drug delivery system (Zhao et al., 2010) . Here , the usage of disulfide bond which is of redox responsive nature has been discussed . There are very few known drugs consisting of the mercapto group . There are few drugs of anticancer nature like doxorubicin, cisplatin which accepts mercapto group by following the modified process of grafting and is covalently linked to silica via the disulphide bond (Wang et al., 2013). Hyaluranon also known as hyaluronic acid is a large and linear polysaccharide, biodegradable , anionic and is made up of thousand to twenty five thousand repeating units of N acetylglucosamine and glucoronic acid and this acid is used for the drug delivery system because of the specific interaction with the receptors which are overexpressed on different cells. Previously , reports have been made showing

strategy of hyaluronic acid mesoporus silica nanoparticles for the targeting delivery and dispersity . Few limitations have been found while using the hyaluronic acid macromolecule as the drug or target carrier as it is rapidly cleared and destructed by the liver because it is less soluble in blood.

The macromolecule of hyluronic acid can be dissociated into the fragments known as oligosaccharides having a molecular weight less than ten thousand using hyaluronidase and also the acid can bind with the recepter in the competition leading to the HA replacement which were already bounded to the different receptors. There are some characterestics which are very attractive and these include long time of blood circulation and the removal from liver is quite slow when compared with the native hyluronic acid (Platt et al., 2008) . On the silica surface HA can be modified via disulfide bonds which can be easily cleaved inspite of the amino acid bonds. The main purpose is to increase drug delivery system's cellular uptake. But the groups which are targeting the surface may retard the drug release after the drug has been absorbed by the cells (Cui et al., 2012) .Till now there has been no reports which have published regarding the target moiety for increasing the cellular uptake in drugs. Presently , modified silica nanoparticles which are thiol functionalized posessing uniform size of particles of 80 nanometer have been synthesized to serve as nano drug carriers. A compond 6 - MP, I.e. 6- mercaptopurine which is considered as an antimetabolite has been chosen as model of drug which can be loaded in the silica pores via disulphide bond . The modified hyluronic acid has been grafted on the layers via disulfide bonds via selectively targetting the tumor cells and increasing bio compatibility and silica dispersibility. The cellular uptake and the targetted release of the drug has been shown in given scheme 1



Scheme 1. GSH-Responsive Drug Release in the Tumor Cells and Magnified Image of Pore Structurea

CD44 is a receptor and its specificity has been investigated in receptor positive which is HCT-116 and receptor negative which is NIH3T3 lines of the cell. The potent effect

has been found to be great in cells of HCT-116 as compared to 6MP or those in CMS-SSMP /oHA as found in 3T3 cells. This delivery system has a great potential for the target delivery of tumor and the drug release of the intracellular system. Condensation method has been followed to synthesize the CMS-SH , which involves the TEOS condensation and also the condensation MPTMS for mercapto group distribution on internal and external CMS carrier surface . The pore structure and morphology of the prepared nanoparticles have been characterized by TEM and SEM as shown in given figure 3A. it can be seen that the particles are of spherical shape having diameter 80 nm . The figure 3 B has shown the wormhole arrangement of mesopores.

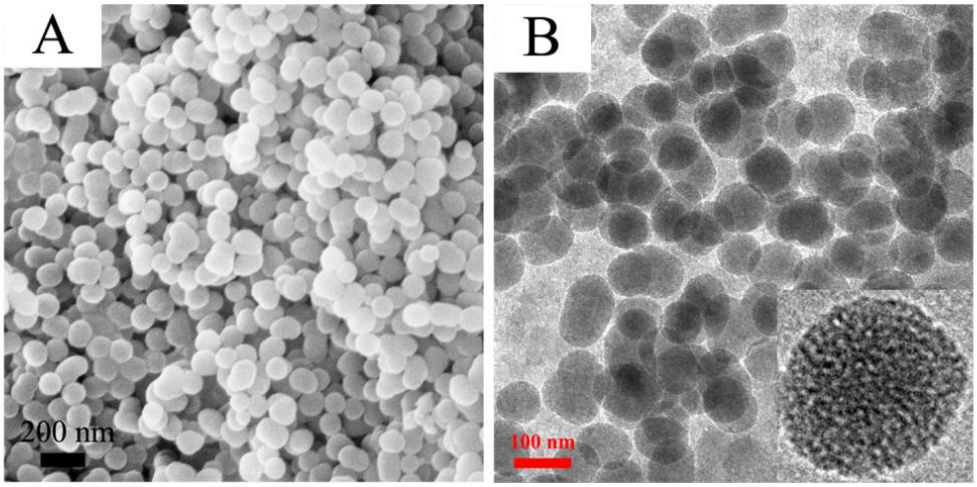
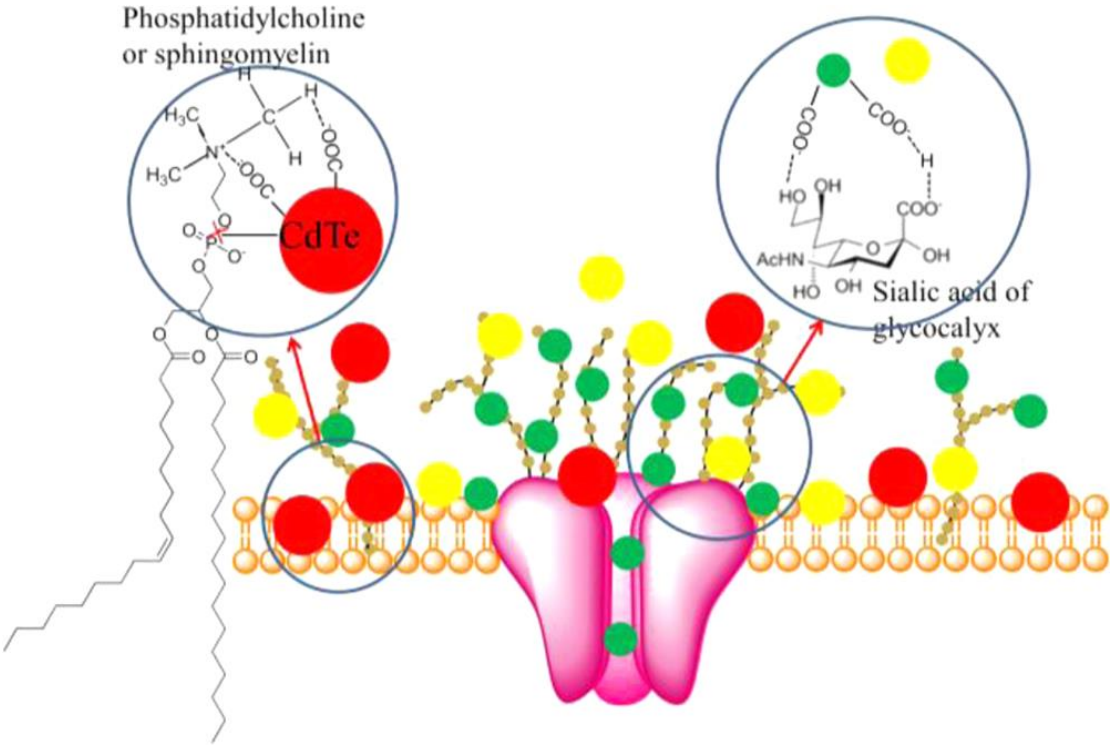


Figure 3. SEM (A) and TEM (B) of CMS-SH nanoparticles.

INDUCTION OF HAEMOLYSIS

Nanoparticles have great application in the field of drug delivery and the red blood cells are very much exposed to the nanomaterials which results in red blood cells hemolysis (Ashrani et al., 2010). The toxicity of nanomaterials has been investigated widely. Various factors which are responsible for inducing the hemolytic activity is the concentration, composition, geometery, size of particles, and the surrounding enviornment. The most important factor is the size factor which determines weather the particle can be washed away and recognised by phagocytic system which is mononuclear in nature. Nanoparticles are used in the nanomedicine field. Small nanoparticles having large surface area are found to be more hemolytic as compared with large ones. Zhao *et al* has founded that large nanoparticles exhibits great hemolysis as compared with small ones . For hemolysis mechanism , few researchers have observed the changes in the morphologies induced due to nanoparticles .It has been found that hemolysis damages the membrane of the cell like pores . The results were very conflicting and resulted in stress regarding the deep research on mechanism which control the hemolysis. The shape of red blood cells is controlled by content used for ATP and also depends on the bilayer structure of phospholipid occuring in spectrin . For studying the molecular order of membranes FT-IR spectroscopy is used as it helps in detecting biological information on global level. It is used in distinguishing the differences occuring in the population of cell. Till now, this spectroscopy has been used for studying the hemolytic phenomenon which have been

induced via nanomaterials. Here FT-IR spectroscopy has been used for studying the quantum dot interactions with the modular membranes which have been prepared on the solid supports and also the model membranes do not reflect the real cells also (Wang et al., 2012). In the in vitro probes, the semiconductor quantum dots are used in the biomedical applications , and is also used in invivo imaging and the vascular imaging. Due to the small size, these can easily overcome avoiding the biological barriers thus invading the living organisms. It has been found that , phosphodiester bond breaking is the major factor due to which hemolysis is induced.



Scheme 2:Schematic of Possible Interaction of MSA-QDs mercaptosuccinic acid-capped CdTe QDs (MSA-QDs) withRBCMembrane

This above shown scheme 2 shows the interaction of mercaptosuccinic acid-capped quantum dots with the red blood cells, via hydrogen bonding occuring between coo group at mercaptosuccinic acid-capped quantum dots surface and the lipid membrane is formed which is independent of the size. It has been found that , strong hydrogen bonds are formed when glycocalyx interacts with the hydrogen bond formation and a part of them enters between the gap occuring between the long chains of biopolymer and those of the outer protein membrane. The big red emitting quantum dots gets escaped from the glycocalyx capture and thus interacts with lipid. They posess strong ability of hydrogen bond formation and a big disturbance is caused in the lipids confirmers. The phosphate ester bond gets breaked up and a complex of phosphate

cadmium and a phosphoryl terminal group is formed. And this results in the pore formation in the red blood cells lipid membrane and also during the hemolysis. The confocal images of red blood cells when treated for three hours with different concentration of mercaptosuccinic acid-capped CdTe QDs . The figure 4 (A) shows confocal control red blood cell images , when red blood cells are exposed to green-emitting MSA-QDs then hemagglutination is induced.

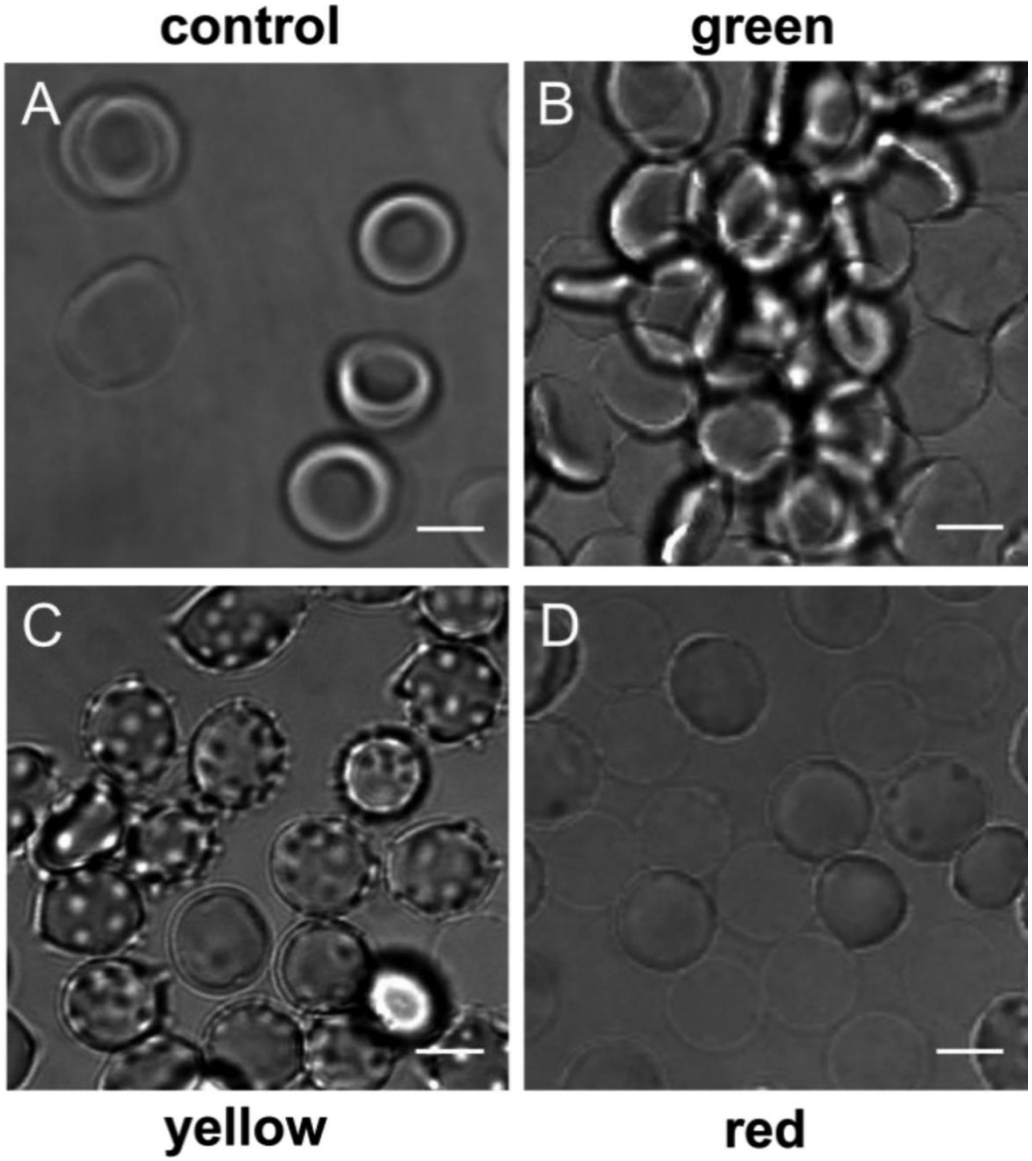


Figure 4. Confocal images of red blood cells incubated with (A) PBS, (B) the green-emitting, (C) yellow-emitting, and (D) red-emitting MSA-QDs at a concentration of 500 μg mL−1 for 3 h. Scale bar = 10 μm.

Figure 4(B) shows the aggregation of the red blood cells without undergoing any serious destructive effect. Figure 4(C) shows the destructive effect occurring in yellow and green emitting MSA-QDs. The results of hemolysis assay as depicted in

figure 4(D) that various ghost cells have been found after the incubation of red blood cells with the mercaptosuccinic acid-capped CdTe QDs. Many grooves have been found on the red blood cells surface when these are exposed to the silver nanoparticles. These pits might have been resulted because of the damage to the outer layer of membrane. Carrying out the experiments in controlled manner , it has been found that release of cadmium ions do not change the red blood cells morphology. Zhao et al. have reported that , the particle induced hemolysis is the reason for deformation of membrane and resulted in the transformation to the echinocytic shape (Zhao et al.,2011) .These deformed cells are unable to maintain their normal shape and leads to their destruction resulting in heavy hemolysis. The size of the nanoparticles affect the extent of hemolysis. The green emitting quantum dots induces hem-agglutination while the yellow emitting quantum dots induces hemolysis to slight extent and various echinocytes and stomatocytes have been formed but the red emitting quantum dots induces the ghost cell formation and these changes have been detected by using fourier transform infrared spectroscopy. Irrespective of the sizes, quantum dots have been found to interact with the red blood cells via the hydrogen bonding. Strong interactions between green and yellow quantum dots have been found with cellular glycocalyx whereas big changes in conformation of lipids are observed when red emitting quantum dots breaks the phosphate ester bond . and hemolysis extent depends on the damage done to this bond.

CONCLUSION

A redox responsive drug delivery system has been developed . the drug and the targetted ligand are made to conjugate together via a disulfide bond. Work has been done to increase the biocompatibility and stability under the suitable conditions. In the redox responsive drug delivery system, the drug is delivered to the respective target site without releasing any kind of drug and the drug is made to release to the target cytoplasm of the cells. Nanoparticles with different sizes induces the different level of toxicity of the red blood cells. Hemagglutination is induced by the green emitting quantum dots. Less amount of hemolysis is induced by the yellow emitting diodes. Red emitting diodes induces the heavy haemolysis and leads to the formation of various ghost cells.Quantum dots of mesoporous silica interacts with the red blood cells through the hydrogen bonding regardless of size. The yellow and green light emitting diode are found to have strong bonding with cellular glycocalyx . The red emitting quantum dots breaks the phosphate ester bond and causes the big changes of the lipid conformation. The process of hemolysis depends upon the damage to lipids phosphate ester bond and cytotoxicity has been found to reduce in the presence of proteins of serum.

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