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SURFACE MODIFIED LEAD SULPHIDE QUANTUM DOTS FOR *IN VITRO* IMAGING OF BREAST CANCER CELLS ADOPTING CONFOCAL RAMAN SPECTROSCOPY

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ABSTRACT

Nanoparticles are potentially used for early cancer detection, accurate diagnosis, and cancer treatment. Due to their small direct band gap and large excitation Bohr radius, lead sulphide quantum dots are important semiconductor material. The red shifted emission band, coupled with the small particle size, is facilitating clearance of imaging. Lead sulphide quantum dots bio-conjugates are promising candidates for targeted infrared molecular imaging and future infrared tissue imaging applications. Because of these many characters, in this paper, we tagged cancer cells with quantum dots for better imaging in IR region. We synthesized lead sulphide quantum dots using Hines and Scholes method. Surface modification of quantum dots is carried out using L-cysteine. Surface modification makes semiconductor quantum dots water soluble. The amino groups present in L-cysteine will bind to the cancer cells and the thiol group will bind to the quantum dots. Confocal Raman spectroscopy is used to image the cancer cells tagged with modified lead sulphide quantum dots.

Key words: Confocal raman spectroscopy, Breast cancer cell, Lead sulphide, Quantum dots, Bio-imaging, L-Cysteine.

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INTRODUCTION

Nonradioactive tagging technology has gained deep interest in biology for the exploration of the complex spatiotemporal interchange of biomolecules, since it provides safer and more stable alternatives. Up to now, several types of tagging materials have been developed. Fluorescence-based materials have been used most extensively in biological applications[1-3] however; they have intrinsic problems, such as photobleaching, narrow excitation with broad emission profiles, and peak overlapping in multiplex experiments. Semiconductor quantum dots have been under development as a tagging material for both *in vivo* cellular imaging and *in vitro* bioassay[4-6]. Despite their potential impact, the practical application of quantum dots is limited by some key problems of surface modification and safety issues associated with semiconductor surface chemistry[7]. Recently, Raman scattering has attracted considerable interest for its potential applications in sensitive optical detection and spectroscopy[8,9]. To address many problems with fluorophores and quantum dots, Raman tagging materials have been developed by several groups[10-12]. These materials have many advantages that they can generate large numbers of different Raman tagging signatures for high throughput screening of various biomolecules; can be excited at any wavelength; do not photobleach; and have narrow peak widths, avoiding spectral overlapping. Nevertheless, the previous Raman tagging materials based on individual gold and silver nanoparticles produced relatively low Raman signatures for small organic compounds used as a label. According to the theoretical calculations, a molecule at nanocrystal junctions can produce several higher orders of magnitude in the intensity of Raman scattering than one on the surface of a single spherical nanoparticle[13-15]. Therefore, aggregates of silver or gold nanoparticles have emerged as effective Raman spectrum substrates[16-19]. Very recently, silver clusters directly coalesced from a destabilized single nanoparticle with organic Raman labels are also developed as a Raman tag[20]. However, precise and reproducible control of the structure of silver clusters has experienced difficulties, which induce a large in homogeneity in their local structure and the enhancement of Raman scattering. In this paper we report the use of surface modified lead sulphide quantum dots for the better imaging of the cancer cells.

EXPERIMENTAL

Synthesis of lead sulphide quantum dots

PbS (lead sulfide) quantum dots are synthesised by one pot reaction method. All chemicals are purchased from Sigma-Aldrich and used as received. The synthesized method is summarized here. In this synthesis we used PbO (Lead oxide) and bis(trimethylsilyl)sulphide (TMS) as precursors of lead and sulphur respectively. Lead oxide (PbO) (446 mg, 2 m mol) is dissolved in a mixture of 2 mL oleic acid and 20 mL octadecene,

which is then heated to 90^{o}C in the presence of nitrogen in three necked flask. Octadecene is a non-coordinating agent, which does not allow any other chemical change to occur once Quntum dots (QDs) has been formed and oleic acid is used to cap QDs and it provides uniform heat to RB flask, since boiling point of oleic acid is 360^{o}C. Lead oxide is converted into lead oleate, which is indicated by a colour change from yellow to clear. The temperature of the solution is raised to 120^{o}C. Then 0.2 mL of bis (tri methylsilyl) sulphide (TMS) dissolved in 10 mL octadecene is rapidly injected into the lead oleate solution. TMS is highly reactive chemical used to increase the yield of QDs in shorter time. The colour changes from colourless to deep brown indicates the formation of QDs. The reaction is allowed to continue for 2 min so that PbS nanocrystals are completely formed. The aliquote is withdrawn from the reaction mixture and dispersed in 10 mL of toluene. The approximate concentration of the quantum dots solution is 0.018 mM. The yield of quantum dot solution is 90%. It is then re-precipitated with methanol and acetone, followed by centrifugation and re-dispersed in toluene. Argon gas is purged into the recovered QDs to give inert condition. To analyze the QDs, absorption spectra, photoluminescence spectra and TEM images are taken.

Surface modification of synthesized PbS Quantum dots using L-cysteine

We modified the surface of the QDs with L-Cysteine ligand to make the QDs water soluble. For this, 5 mL PBS buffer solution is dissolved along with 200 mg of L-cysteine in a beaker. Then 5 mL QDs solution is taken from stoke and added to the above solution. The solution is kept stirring for 6 hrs. After complete mixing the quantum dots from the organic phase is transferred to the aqueous phase and both phase appears as separate layers. The upper organic layer obtained after complete stirring is separated from the lower aqueous layer by filtration. To the aqueous layer, acetone is added to precipitate the QDs. Then the precipitate is centrifuged at 6000 rpm for 5 min. The precipitate is isolated from the solution by adding PBS buffer until it is dissolved in PBS and a clear solution is obtained. Then 2 mL, 1 M mercaptoethanol is added to the solution. Mercaptoethanol is added to prevent the dimerisation of sulphur and thereby making the solution stable. Then using vacuum evaporator the powder form of this solution is obtained. To confirm whether QDs is coated with cysteine, FTIR spectrum is taken and analyzed.

Attaching the cancerous cell with modified PbS QDs

MDA-MB-231 Breast cancer cell lines and 3T3LI normal fibroblast cells are initially cultured in DMEM media, by treating with 100 microlitres of 0.018 mM lead sulphide quantum dots. PBS buffer solution is used as control. After 4 hrs of treatment media is removed and dishes are washed with PBS. Then it is fixed on a glass slide with 4% paraformaldehyde for 1 hr at room temperature. After fixation, the dishes are washed with PBS, 3 times. The cover slip is fixed on to a slide with a mounting agent. The same

procedure is followed for attaching cancer cells to lead sulphide QDs. Then images of both samples are obtained using Confocal Raman imaging.

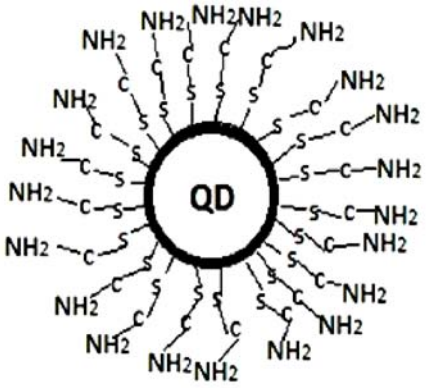


Fig. 1: A schematic diagram of surface modified PbS quantum dots

RESULTS AND DISCUSSION

Absorption and photoluminescence spectrum analysis of the synthesised PbS quantum dots

The absorption spectra of the synthesised PbS quantum dots shows a lowest absorption peak at 730 nm. It is evident that this peak corresponds to an energy gap of 1.7 eV. It is shown in Fig. 2. The particle size corresponding to this absorption is about 5 nm. The peak of the PL is at 860 nm. Such a large Stokes shift is commonly observed with small PbS QDs.

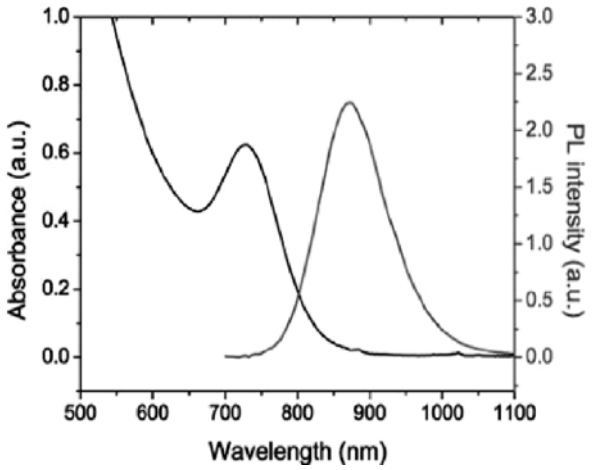


Fig. 2: The absorption and PL spectra of the synthesized PbS quantum dots

TEM analysis of the synthesized PbS quantum dots

The TEM observation for oleic acid capped PbS QDs is shown in Fig. 3(a). The resolution of this image is taken at the scale of 10 nm. From the micrograph, it is seen that the size distribution of the synthesized PbS QDs are uniform. The TEM micrograph clearly shows that these particles are unaggregated in the entire film. The average size of the PbS QDs, observed from TEM is 5 nm, which are in uniform size distribution. The morphology of the OA capped PbS QDs are spherical. From the TEM micrograph, it is clearly observed that the average size of the OA capped PbS nanoparticles is smaller than the exciton bohr radius (18 nm) of bulk PbS.

Fig. 3 (b) shows the HRTEM image of the OA capped PbS QDs. The resolution of this image is taken at the scale of 2 nm. The existence of lattice planes from the HRTEM image further confirms the crystallinity of synthesized PbS QDs. It can be clearly seen that the morphology of the OA capped PbS QDs are exactly spherical and all the particles are easily distinguishable. It demonstrates that all the particles are free from agglomeration.

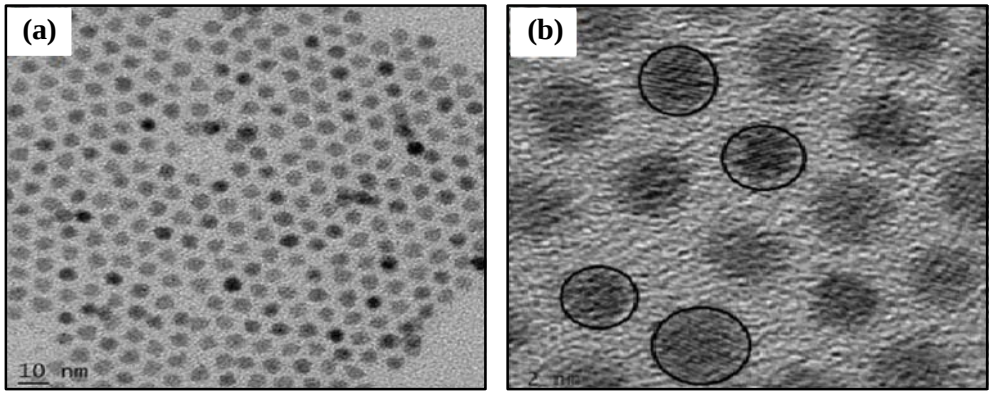


Fig. 3: The HRTEM images for oleic acid capped PbS quantum dots (a) and shows crystal planes (b)

FTIR Studies of the surface modified PbS quantum dots

Fig. 4 represents the FTIR spectra of L-cysteine powder. It has peaks in following wavelengths. 3374 nm (N-H), 2879 nm (C-H), 1738 nm (C = O), 1516 nm, 1399 nm, 1218 nm (C-O).

Fig. 5 represents the FTIR spectra of surface modified QDs treated with L-cysteine. The spectra of L-cysteine has vibrations in the following wavelengths-3345 nm (N-H), 2910 nm (C-H), 1734 nm (C=C), 1477 nm (C=O), 1044 nm. Both the FTIR spectra are

initially analysed. The FTIR spectra of QDs treated with L-cysteine contain IR peaks in similar range to that of L-cysteine. This confirms the presence of cysteine in QDs.

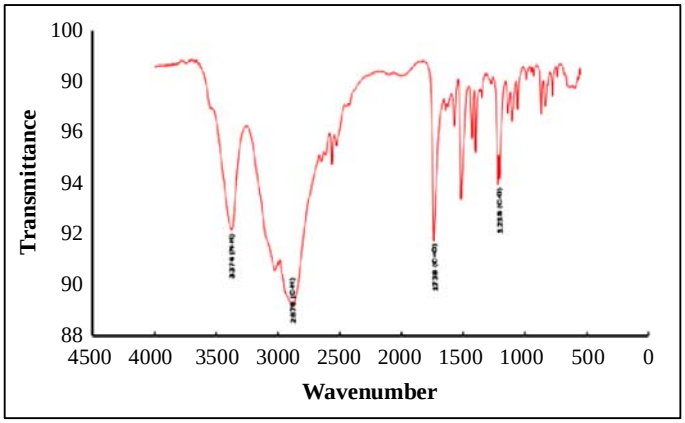


Fig. 4: FTIR spectrum of powdered L cysteine

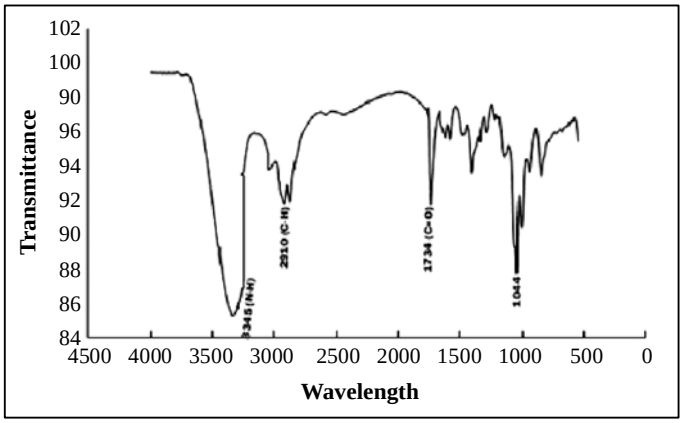


Fig. 5: FTIR spectra of surface modified QDs

Confocal Raman imaging of breast cancer cells attached with surface modified QDs

Fig. 6 corresponds to the Raman spectrum of PbS QDs. Raman peaks are obtained at 210 nm, 451 nm, 613 nm, 966 nm and 1605 nm. Raman spectrum plots the intensity of the scattered light (y-axis) for each energy (frequency) of light (x-axis).

Fig. 7 (a) represents Raman image of normal cells and separate spectrum of red and blue regions are obtained and are shown in Fig. 7 (b) and (c). With the purpose of attaching QDs, normal cells are treated with PbS QDs and corresponding Raman imaging and Raman spectra are obtained. Fig. 8 (a) represents bio-imaging of normal cells, which are treated

with surface modified QDs. The corresponding Raman spectra are also obtained (Fig. 8 (b), and Fig. 8 (c)). The spectra obtained donot contain any Raman peaks corresponding to QDs.

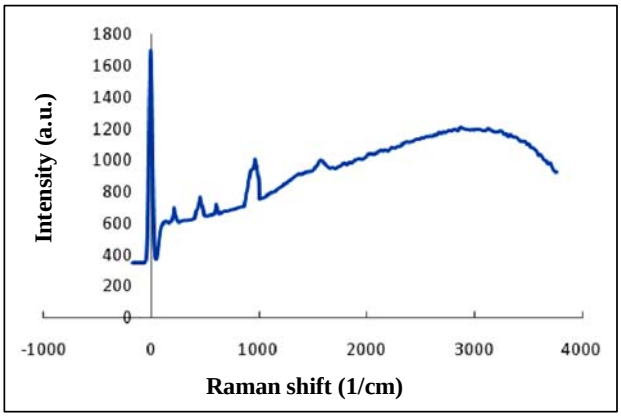


Fig. 6: Raman spectrum of lead sulphide QDs

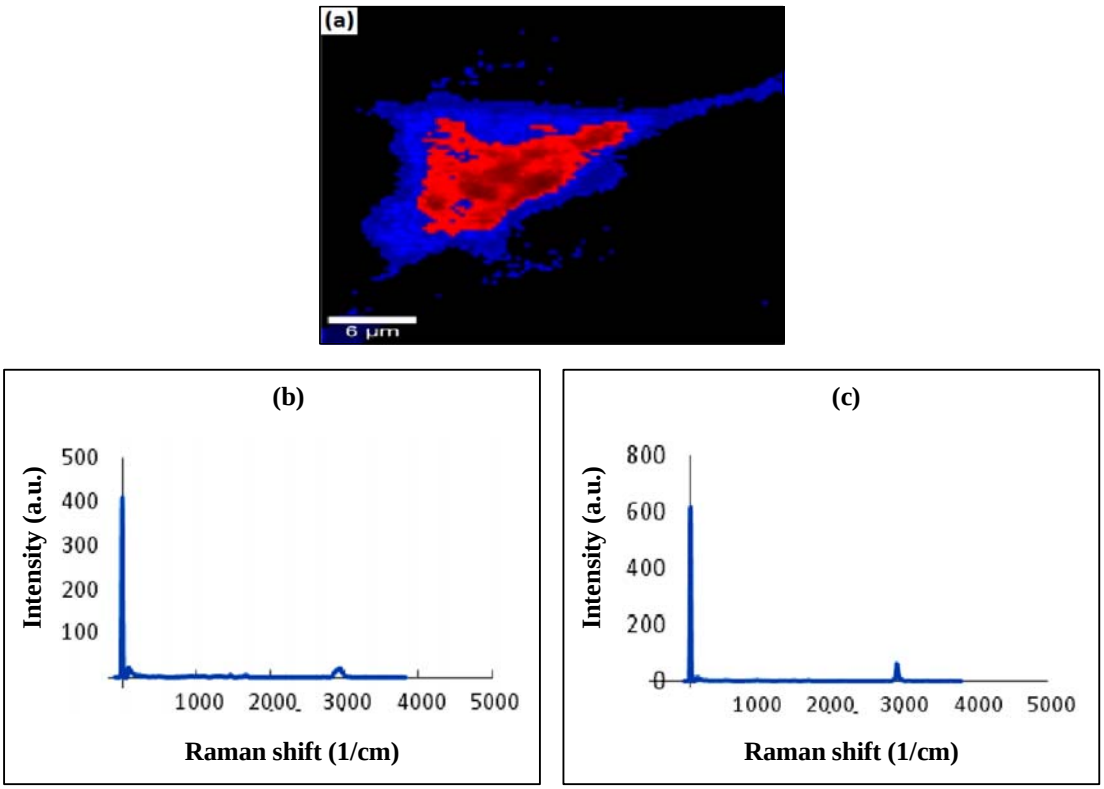


Fig. 7: (a) Raman imaging of normal cells. (b) Raman spectra of red region in normal cells (c) Raman spectra of blue region in normal cells

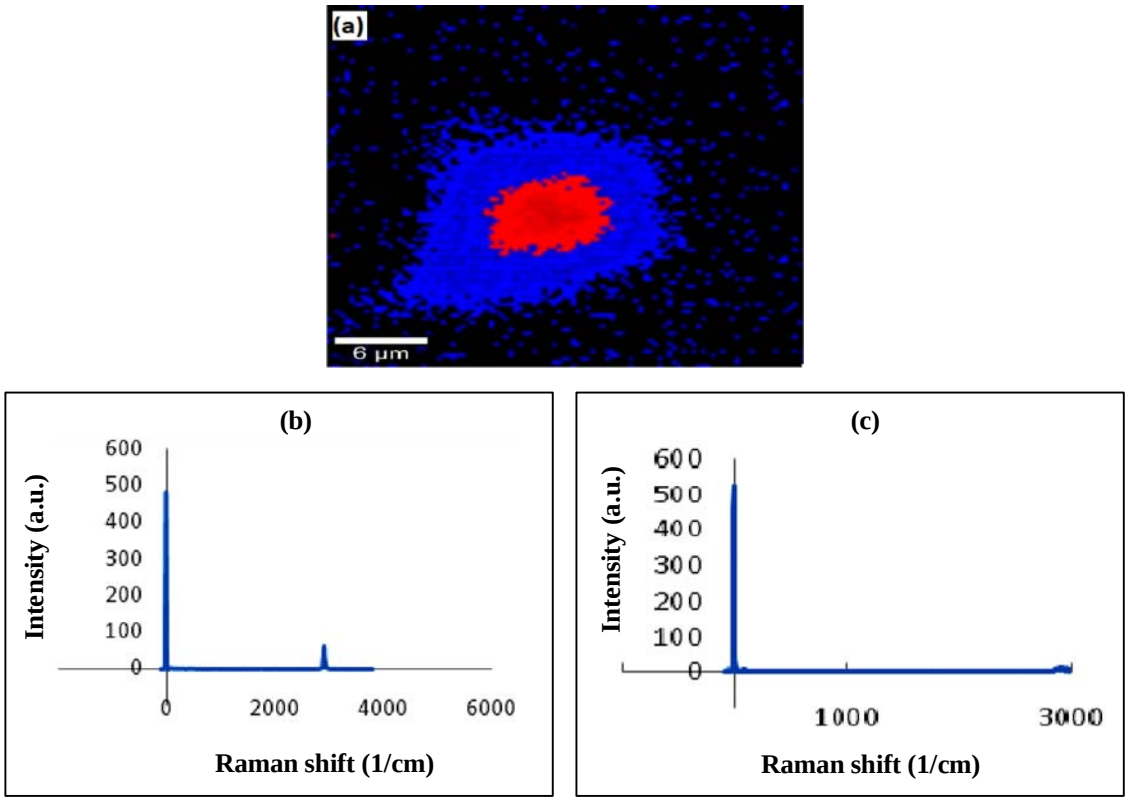
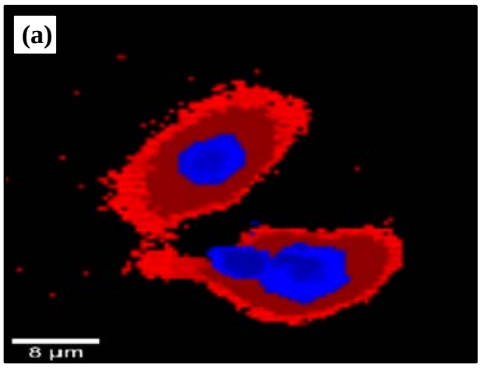


Fig. 8: (a) Raman image of normal cells treated with PbS QDs (b) Raman spectra of blue region in normal cells treated with PbS QDs (c) Raman spectra of red region in normal cells treated with PbS QDs

MDA-MB-231 Breast cancer cell lines are used as control and Raman image and Raman spectra of the breast cancer cell lines are obtained (Fig. 9 (a), (b) and (c)).



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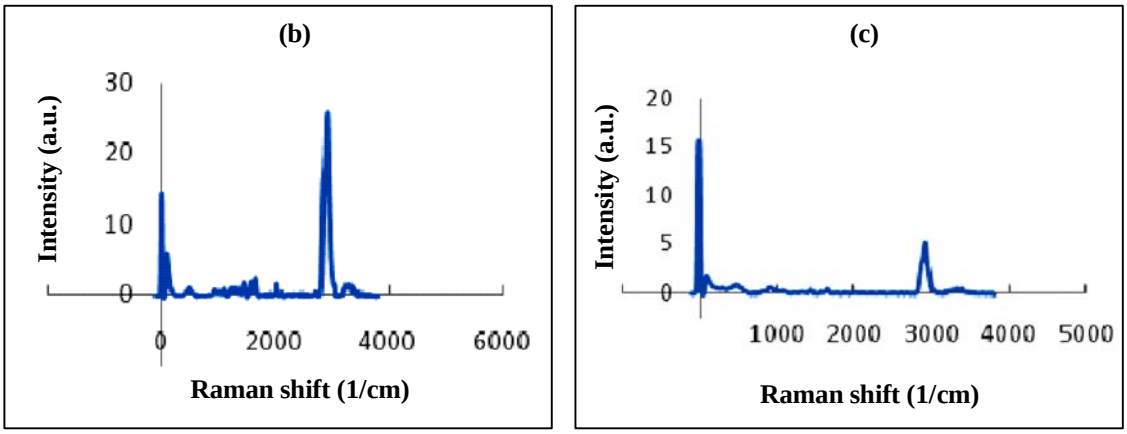

Fig. 9: (a) Raman image of breast cancer cells (b) Raman spectra of blue region in breast cancer cells (c) Raman spectra of red region in breast cancer cells

Raman peaks corresponding to PbS QDs are seen in spectrum of cancer cells attached with QDs (210 nm, 966 nm).

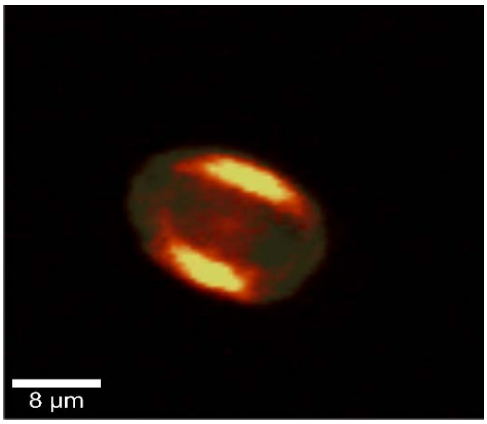

Fig. 10: Raman image of breast cancer cells treated with surface modified QDs

All the assignment of Raman spectrum measured from breast cancer cells treated with surface modified PbS QDs are summarized in Table 1. All the main peaks are analyzed and summarized in the table. For carrying out Raman spectroscopic analysis, four samples are used – Normal cells, normal cells treated with QDs, MDA-MB-231 breast cancer cells, MDA-MB-231 breast cancer cells treated with QDs. Initially spectroscopic analysis of QDs is carried out. Then spectra of normal cells and normal cells treated QDs are compared. Raman peaks of QDs are not found in Normal cells treated with QDs. Also no major differences are found between the Raman images of both samples. Analysis of spectral and

imaging data confirmed that QDs are not able to attach to normal cells. In the second case, MDA-MB-231 breast cancer cells are used as control. Then MDA-MB-231 breast cancer cells are treated with QDs and Raman spectroscopic analysis are carried out. Raman peaks are found in breast cancer cells treated with QDs. Major vibrations are found at 2933 nm, which corresponds to C-H bond. C=C vibrations are found in 1666 nm and C-S vibrations at 1080 nm. Vibrations are found at 210 nm and 966 nm too. This vibrations in 210 nm and 966 nm corresponds to Raman peak of QDs. The presence of corresponding Raman peaks in breast cancer cells treated with QDs confirms the internalization of qdots.

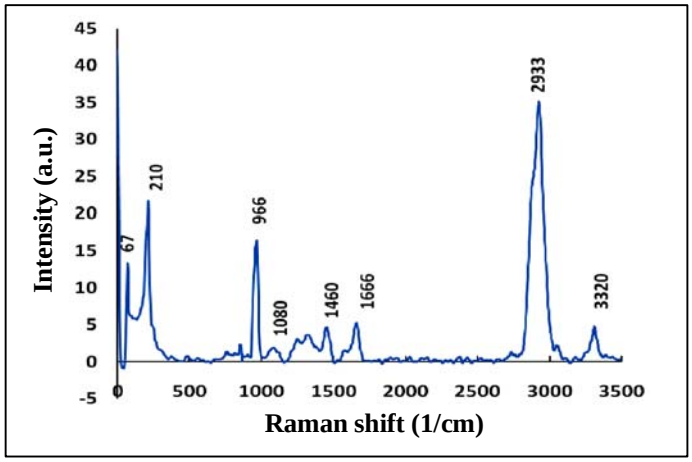

Fig. 11: Raman spectra of breast cells treated with surface modified QDs

Table 1: Assignment of Raman spectrum measured from breast cancer cells treated with surface modified PbS QDs

Bands (cm^{-1})	Tentative assignment
67	Lattice vibrations in crystals
210	PbS QDs
966	PbS QDs
1080	C-S
1460	CH_3
1666	C=C
2933	C-H
3320	N-H

QDs show absorption at 730 nm. Emission wavelength is at 890 nm. QDs emit fluorescence, which can be used to produce better quality imaging of samples using normal techniques. QDs are semiconducting nanoparticles. PbS QDts are spherical in size with diameter of 5 nm. In comparison to other fluorescent dyes, QDs are very small in size. Thus they have high penetrating capacity and can be used for specifically targeting source. All these properties including high penetration, fluorescence and targeting can result in the production of better quality images of samples attached with QDs in comparison to samples without QDs.

CONCLUSION

Present work includes the synthesis of lead sulphide quantum dots using Hines and Scholes method. Surface modification of quantum dots is carried out using L-cysteine. Surface modification makes semiconductor quantum dots water soluble. The amino groups present in L-cysteine will bind to the cancer cells and the thiol group will bind to the quantum dots. Confocal Raman spectroscopy is used to image the cancer cells tagged with modified lead sulphide quantum dots. Quantum dots can be used extensively for efficient bioimaging with better quality. QDs contains amino group. This amino group acts as a ligand, which attach to cells. Further modification of the amino group can be done by tagging it with ligands. This modification includes tagging with antigens, lectins, functional groups, receptor binding proteins and other molecules, which are specific. Using this modification, we can specifically target the complimentary molecules of ligand and thereby, yield better images.

Thus, a lot of modifications can be made based on the attachment of ligand groups. Further modifications can be carried out by tagging QDs with silanes, lead, selenium, zinc sulphide, cadmium selenide, and cadmium sulphide. Modifications can be made by changing size of QDs, less is the size, more is the penetration.

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