INTERACTION OF JACALIN WITH CADMIUM SULFIDE QUANTUM DOTS FOR SELECTIVE LABELLING OF CANCEROUS CELLS
2.1. Summary

Distinguishing healthy and cancer cells were the major challenge in the cancer diagnostics and therapeutics. Cadmium sulfide quantum dots (CdS QDs) surface functionalized with jacalin was explored for selective labelling of cancerous cells. Surface functionalized QDs are expected to improve their optoelectronic properties and may advance their targeting nature, which is highly warranted for application in molecular imaging and biomedical diagnostics. Therefore, understanding the interaction between surface functionalization agents and QDs is a prerequisite for their application. In this chapter, the interaction between cadmium sulfide (CdS) QDs and dietary T-antigen binding lectin, jacalin isolated from Indian jack fruit seeds were evaluated. Fluorescence spectroscopy study showed that CdS QDs effectively quench the intrinsic fluorescence of jacalin. Analysis of fluorescence quenching at different temperature indicated that the mechanism of interaction is static and a non-radiation energy transfer occurred within the molecules. The obtained binding constant, $K_a$ value in the order of $10^4$ M$^{-1}$ at the tested temperature range suggested that the binding affinity between jacalin and CdS QDs is in the same range as those obtained for the interaction of lectin with carbohydrate. Hemagglutination activity of jacalin is well preserved even after binding to CdS QDs, indicating that the jacalin-CdS QDs complex can recognize T-antigens of the malignant tissues. To support the claim, the selective fluorescence labelling of chronic myeloid leukaemia cells, K562 with jacalin-CdS QDs complex was demonstrated.

2.2. Introduction

Targeted therapies are highly attractive to lower the overall dosage of administered drug without compromising the anticancer efficacy (Sachs et al., 2016). Active
targeting of a drug can be accomplished through labelling with antibodies, carbohydrates, folic acids, peptides, aptamers, a monoclonal antibody of specific surface receptor expressed by cancer cells and so forth (Liu et al., 2015; Cerchia et al., 2010; Dash et al., 2015). Thomsen-Friedenreich disaccharide antigen (T-antigen) is one of the appealing molecular targets for cancer therapies. T-antigen disaccharide is 2-acetamido,2-deoxy,3-O-β-D galactopyranosyl-α-D-galactopyranoside(Galβ1-3GalNAc- O-serine/threonine), which is over-expressed in more than 85% of primary human carcinomas, but is usually concealed in healthy cells (Springer et al., 1984). As mentioned in chapter 1, jacalin bind specifically to the T-antigen and has high potential to encapsulate molecules other than carbohydrates.

Semiconductor quantum dots have attracted researchers for their interesting optical and electronic properties (Michalet et al., 2005; Alsharif et al., 2009). It has been widely used as luminescent probes, sensor, optical filters, solar cells, electrochemical devices and microelectronics. QDs emerged as a better fluorescent probe than the traditional organic fluorophores used in biological labelling. The advantages of QDs are excellent photoluminescence, greater photostability, continuous excitation spectrum, size-tuneable and narrow emission bands (Lakowicz et al., 1999; Raevskaya et al., 2005). In general, for the preparation of QDs, stabilizing agents such as trioctyl phosphine/trioctyl phosphine oxide, thiols, thioacids and amides was used. The most challenging part of using QDs in biological environment is their toxicity that arises due to the decomposition and release of the metal ions or that from the capping agents (Yong et al., 2013). Derfus et al, have reported the degradation of CdSe QDs under UV irradiation which led to the release of cadmium ions and induce cell death (Derfus et al., 2004). Clift et al., showed that
the different surface coated QDs can cause oxidative stress and affect cell signalling (Clift et al., 2010). Therefore, it is imperative to prepare water-soluble, non-toxic, and biocompatible QDs. The possible method to synthesize biocompatible QDs is to use non toxic chemicals and environmental benign solvents (Zhou et al., 2015). In order to improve its practical applications, researchers started to modify the large surface area of QDs with bio recognition molecules, such as proteins, peptides and antibodies (Shao et al., 2011; Chan et al., 2002). Huang et al., reported that the binding between QDs and proteins are significantly influenced by the capping agents. Thus, understanding the interaction of QDs and capping agents is important to determine the diagnostic effectiveness of the QDs.

In this chapter, a convenient method to synthesize stable CdS QDs using the biomolecule, pectin as a templating agent and taurine as an antioxidant was reported. Taurine and pectin were chosen because it is benign and more importantly, taurine plays a significant role in protecting the cells against oxidative damage (Messina et al., 2000). Pectin is a biopolymer that consists of a linear backbone of (1-4) linked α-D-galacturonic acid residue with a varying degree of methylesterified carboxyl group. Pectin is widely used as gelling, thickening and stabilizing agents in the food industry (Colin, 1990). The mucoadhesive natures of pectin have been exploited for in vivo delivery (Sriamornsak, 2011). Jacalin binds strongly with carbohydrates derivatives bearing a hydrophobic group over other simple saccharides, indicating the existence of some hydrophobic site near the sugar-binding site (Sastry et al., 1986; Jeyaprakash et al., 2005). More importantly, jacalin bind specifically to the T-antigen expressed on the surface of HT-29 cells with a dissociation constant ($K_d$) of 500 ± 50 nM (Yu et al., 2001; Jeyaprakash et al., 2002; Sastry et al., 1986). These properties have been
previously investigated to prepare jacalin-phthalocyanine conjugate to find application in photodynamic therapy (Obaid et al., 2012). Therefore, it is possible for jacalin to bind to highly luminescent CdS QDs, which may find application in biological labelling. Binding experiments performed at different temperature revealed that the interaction between jacalin and CdS QDs was governed by enthalpic forces with negative entropy contributions. Synchronous fluorescence spectroscopy study showed that the microenvironment of tryptophan residues was altered upon binding to CdS QDs. Hemagglutination assay revealed that the CdS QDs binds to jacalin at a site that is different from the carbohydrate binding site. As a proof of concept, an application of jacalin-CdS QDs in selective imaging of cancer cells has been demonstrated. The results obtained from this study suggest that the judicious use of lectin nanoconjugates would create wonder in targeted drug delivery.

2.3. Materials and methods

2.3.1. Materials

Cadmium chloride, sodium sulfide, citrus pectin, sodium phosphate dibasic and monobasic, sodium chloride and epichlorohydrin were purchased from Merck. Taurine, acrylamide, bis-acrylamide and sodium dodecyl sulfate was purchased from Sigma. Jackfruit (Artocarpus integrifolia) seeds were obtained from local seed vendors. Fetal bovine serum (FBS), genatmycin, streptomycin, penicillin, was obtained from Sigma, India. RPMI 1640 medium was purchased from Himedia. Guar gum was obtained from Loba. All other chemicals and solvents used in this work are of analytical grade obtained from the local supplier. Double distilled water was used throughout the work.
2.3.2. Synthesis of pectin stabilized cadmium sulfide quantum dots

CdS QDs were synthesized by wet chemical method using analar grade sodium sulfide, Na$_2$S.9H$_2$O as a source for S$^{2-}$ ions and cadmium chloride as the source of Cd$^{2+}$ ions, with pectin as the capping agent and taurine as the antioxidant. About 50 mg of pectin, 2 mM of cadmium chloride, and 10 mM of taurine was dissolved in 50 ml distilled water. The resultant solution was heated for about 1 h at 80 °C till all the components was dissolved. After 1 h, freshly prepared Na$_2$S was injected (2 mM final concentration) under vigorous stirring and then, the reaction mixture was refluxed for 8 h (Fig. 2.1). The obtained yellow colour solution showed bright blue fluorescence when illuminated by UV lamp at 360 nm.

![Fig. 2.1: Schematic representation of synthesis of cadmium sulfide quantum dots](image-url)
2.3.3. Characterization of cadmium sulfide quantum dots

The synthesized QDs were characterized by high resolution transmission electron microscopy (HR-TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), UV-Visible spectroscopy and fluorescence spectroscopy.

The transmission electron micrographs of the synthesized QDs were measured on JEM 1011, JEOL, Japan. A drop of the QDs was placed on a carbon-coated copper grid and dried prior to the measurement. SEM-EDAX was measured on JEOL-JSM 6701F, Japan. The samples were stuck onto a double-face conducting carbon tape mounted on a brass stub. Prior to imaging the samples were coated with a thin layer of platinum in an auto fine coater. The dried samples for XRD and FTIR were prepared by lyophilization. XRD measurements of the QDs were done on a XRD-Bruker D8 Advance X-ray diffractometer using monochromatic Cu Ka radiation. FTIR spectroscopic measurements were carried out on a Perkin Elmer spectrum-one instrument in the diffuse reflectance mode at a resolution of 1 cm$^{-1}$ in KBr pellet. The optical absorbance of the synthesized QDs was monitored by UV-Vis spectrophotometer (Thermo Scientific Evolution 201) between wavelengths of 200 to 800 nm at a resolution of 1 nm. Fluorescence measurements were carried out on a JASCO spectrofluorometer FP 8200 with a 1.0 cm quartz cell. The fluorescence of QDs was recorded in 370 – 600 nm at an excitation wavelength of 360 nm and the slit widths of both excitation and emission were set at 5 nm.

2.3.4. Preparation of guar gum column

Typically, alkaline epichlorohydrin solution was prepared by dissolving 1.5 mL of epichlorohydrin in 25 mL of 3 N sodium hydroxide. To this mixture, 5 g of guar gum
powder was added slowly and mixed vigorously using a clean glass rod. The resultant thick paste was then kept in a water bath maintained at 40°C for 24 h. After 24 h, the cross-linked guar gum was removed and cut into small pieces and placed in hot air oven at 80 °C for 8 h for complete drying. The dried guar gum pieces was soaked in 100 mL of 20 mM phosphate buffer saline (PBS) containing 150 mM sodium chloride, pH 7.4 and washed thrice before packing into a glass column.

2.3.5. Isolation of jacalin

About 50 g of dry jackfruit seeds were deshelled and cut in small pieces and ground to a fine powder using blender. The fine powder was soaked in 200 mL of 20 mM PBS containing 150 mM sodium chloride, pH 7.4 and stirred in magnetic stirrer for 12 h at 4°C. After 12 h, the solution was filtered using cheese cloth and subjected to centrifugation at 6000 rpm for 30 minutes at 4°C. The obtained supernatant was treated with 40% of ammonium sulphate and stirred for 1 hr at 4°C. After 1 h, the solution was centrifuged at 6000 rpm for 30 minutes at 4°C and the pellet was collected. The obtained pellet was dissolved in 10 mL of PBS buffer, pH 7.4 and subjected to dialysis using a dialysis membrane with cut-off of 10 kDa. The sample was dialysed for 6 times. Each dialysis was performed for 6 h and PBS buffer was changed after each dialysis. After dialysis, the residual precipitate was removed by centrifugation and the supernatant was loaded to a guar-gum column, which is pre-equilibrated with PBS buffer. After 6 h of incubation at 4°C, the column was washed thoroughly with PBS buffer until the optical density at 280 nm reaches zero. Then, the bound protein was eluted with 100 mM galactose. In order to free from the galactose, the purified jacalin was thoroughly dialyzed against 10 mM PBS buffer, pH 7.4, containing 150 mM NaCl. The purity of the protein was assessed by 10 %
polyacrylamide gel electrophoresis in the absence as well as in the presence of sodium dodecylsulfate. Staining was carried out with 0.1% (w/v) coomassie brilliant blue (CBB) and images were recorded in UVi-Tec gel documentation system. The concentration of jacalin was determined by Lowry assay using bovine serum albumin as the standard. The column was washed with 0.1% SDS followed by PBS buffer and stored at 4°C for future use (Fig. 2.2).

![Fig. 2.2: A schematic representation of jacalin purification from jackfruit seeds](image)

2.3.6. Lectin activity assay

The activity of jacalin was determined using haemagglutination and haemagglutination inhibition assays. Typically, 5 mL of blood was collected from a healthy volunteer by a trained professional using Alsevar anticoagulant. The blood
was initially stored at 4°C for 1 h. After 1 h blood sample was centrifuged at 1200 rpm for 15 minutes at 4°C. Yellowish colour plasma in the supernatant was separated and the pellet containing RBC was washed 2-3 times with PBS buffer to ensure complete removal of plasma from the sample. The RBC was stored at 4°C for further use. 4 % RBC solution in PBS was used for the experiment. PBS with RBC was used as control while jacalin with RBC was used as the test. The availability of sugar binding site in jacalin was also assessed by incubating jacalin with 100 mM galactose. To determine whether CdS QDs binding altered the sugar-binding activity of the lectin, the haemagglutination experiments were conducted by preincubating jacalin with a high concentration of CdS QDs as used in the fluorescence studies. Hemagglutination assay was performed by adding 100 µL of samples to the round bottom microtiter plate contains 100 µL of 4 % RBC solution. The microtiter plate was incubated for 3 h at 4°C and the agglutination activity was evaluated by naked-eye.

2.3.7. Binding of CdS QDs to jacalin

The interaction between jacalin and cadmium sulfide quantum dots was measured in JASCO spectrofluorometer FP 8200 with a 1.0 cm quartz cell. The binding study was performed using the intrinsic fluorescence of jacalin. The intrinsic fluorescence spectra of jacalin were recorded between 300–400 nm at an excitation wavelength of 280 nm. A fixed volume of jacalin solution (3.0 mL, 5 µM) was titrated by adding small aliquots of the CdS QDs from a concentrated stock solution (1 mM) and the fluorescence intensity was recorded after an equilibration period of 2 min. To determine the binding of CdS QDs interferes with the natural saccharide binding characteristic of jacalin, we performed QDs interaction studies by preincubating
jacalin with a high concentration (50 mM) of galactose. All binding experiments were performed in PBS buffer. All titrations were repeated at least three times to arrive at average values. Fluorescence intensities were corrected for volume changes before further analysis.

2.3.8. Fluorescence imaging

A fresh confluent culture of K562 cells was used as model cells of chronic myeloid leukemia (CML) and human peripheral blood mononuclear cell (PBMC) were used as normal control cells. 1 × 10^4 cells were seeded in 8 well cover glass slide (Genetix) in complete culture medium [RPMI1640, supplemented with 10 % FBS and 1X pentrep (Gibco)]. The overnight culture of the seeded cells was washed with PBS three times and fixed with 4 % paraformaldehyde (freshly prepared in 0.1 M phosphate buffer) followed by a wash with PBS to remove the paraformaldehyde. After that, 50 μL of stock jacalin-CdS QDs complex (1:20) was added to the cover slide in dark. After 30 min of incubation at room temperature, the QDs solution was removed and washed the cell with PBS two times. Cells were observed under phase contrast fluorescence microscope (Nikon) at excitation/emission 350/470.

3. Results and Discussions:

3.1. Synthesis and characterisation of cadmium sulfide quantum dots (CdS QDs)

Cadmium sulfide quantum dots (CdS QDs) were synthesized by using the biomolecules, pectin and taurine. Fig. 2.3A shows scanning electron microscope image showed zero dimensional particle nature of the CdS QDs. Fig. 2.3B shows a typical TEM image of the pectin capped CdS QDs. The image shows the presence of nearly mono dispersed CdS QDs with the average size of 4.7 ± 0.5 nm. The appearance of regular and clear lattice fringe patterns in the HRTEM (Fig 2.3C) and
selected area electron diffraction (SAED) pattern confirms the crystalline nature of the synthesized QDs (Fig. 2.3D). The elemental analysis was performed by EDAX analysis. The EDAX spectrum of CdS QDs showed the presence of Cd and S elements (Fig. 2.4A). The powdered XRD pattern showed three distinct diffraction peaks situated at 26.82, 43.97, and 52.09°, corresponding to the crystal faces of (1 1 1), (2 2 0) and (3 1 1) planes, respectively (Fig. 2.4B). The diffraction peaks in this pattern are in agreement with a cubic structure of CdS QDs (JCPDS 75-0581) and are consistent with the literature (Guo et al., 2012; Giribabu et al., 2012; Park et al., 2011). In order to examine the interaction between pectin and QDs, FTIR spectra of pectin and pectin stabilized CdS are compared (Fig. 2.4C). IR peaks at 1730 cm\(^{-1}\) and 3400 cm\(^{-1}\), which are characteristics of carbonyl and hydroxyl groups, respectively, are present in both pectin and pectin stabilized CdS QDs (Fig. 2.4C), indicating the presence of pectin around the particles. Noteworthy, the broadening of peak at 3000 – 3500 cm\(^{-1}\), that corresponds to the stretching vibrations of the hydroxyl group, suggests the strong interaction between the hydroxyl group and CdS QDs. This also indicates the presence of an intermolecular hydrogen-bonded network on the surface of the nanocrystallite. These results suggest that the pectin networks could effectively prevent the particles from further growing and agglomeration and can also prevent from fluorescence self quenching.
Fig. 2.3: Electron micrographs for CdS QDs. (A) SEM image, (B) TEM image (C) HR TEM image, and (D) SAED pattern.

Fig. 2.4: Characterisation of CdS QDs (A) EDAX spectrum of CdS QDs, (B) X-ray diffraction pattern of CdS QDs, and (C) FTIR spectra of Pectin (dotted line) and pectin capped CdS (solid line).
The synthesized pectin capped CdS QDs was optically characterized by UV-visible and fluorescence spectroscopy (Fig. 2.5). Inset of Fig. 2.5A shows the blue luminescence when illuminated with UV lamp at 360 nm. The synthesized CdS QDs showed a clear blue shift of the absorbance edge in the colloidal particles (490 nm) from bulk CdS (515 nm) due to quantum confinement effect (Fig. 2.5). The band gap computed from the absorption edge for CdS QDs was 2.53 eV, which suggests that these QDs can be used in the visible region, since the range of the band gap in the visible region is 1.5 – 3.0 eV (Cody et al., 1982). The observed increase in the band gap as compared to bulk were due to the size effect and are consistent with the previous report on semiconductor CdS QDs (De Azevedo et al., 2012). The absorbance onset can also be used to estimate the size (2R) by using Henglein empirical formula (Weller et al., 1986).

\[
2R_{CdS} = \frac{0.1}{(0.1338 - 0.0002345 \lambda_{exc})}
\]

where \(\lambda_{exc}\) is the wavelength value of the first excitonic transition. The size estimated by equ. 1 is 5.29 nm, which is in close agreement with the size determined from TEM. CdS QDs at excitation wavelength 360 nm showed an emission maximum centred at 450 nm (Fig. 2.5B), which is the typical luminescence of CdS QDs (De Azevedo et al., 2012). When the emission peak is set at 450 nm, the excitation peak was observed at 363 nm (Fig. 2.5B). Fig. 2.5C shows that the excitation wavelength 360 nm exhibits high emission intensity of CdS QDs. It is observed that synthesized QDs was very clear and stable for more than 30 days without any aggregation, however, there is a small change in the fluorescence intensity over a period of storage.
**Fig. 2.5:** UV and fluorescence characterisation of CdS QDs. (A) UV-visible spectrum of CdS QDs. Inset corresponds to the photograph of CdS in normal light (i) and UV-light (ii), (B) Fluorescence excitation and emission spectrum. Emission spectra were measured by exciting at 360 nm. Excitation spectra were measured by fixing the emission at 450 nm, and (C) Emission spectra of CdS at different excitation wavelength. Exciting at 360 nm shows strong fluorescence with emission maxima at 450 nm.

3.2. **Purification and characterization of jacalin**

Jacalin was purified from the jackfruit seeds as described in the material method. About 16 mg of pure jacalin was obtained from 50 g of jackfruit seeds. The purity of the protein was assessed by SDS-PAGE and native PAGE.
Fig. 2.6: Electrophoresis. (A) Native PAGE and (B) SDS-PAGE of jacalin. Arrow indicates jacalin.

As noted from Fig. 2.6A, a single 66 kDa band corresponds to jacalin was observed in the native PAGE, while denatured SDS-PAGE showed two bands at 16 kDa and 12 kDa accounting for α and α’ chain of jacalin (Kabir et al.). The absence of additional band indicates that the isolated jacalin is free from other proteins.

The activity of jacalin was judged by haemagglutination assay. It is one of common technique used to determine the presence of lectin (Ponnuswamy et al., 2012). Lectins tend to bind to RBC (Sung et al., 1985) and crosslink the RBC through binding to cell surface glycan and prevent the RBC from precipitation. As noted from Fig. 2.7, the addition of PBS buffer precipitates the RBC (Lane A), while addition of jacalin displays the haemagglutination of RBC. Further, the addition of jacalin pre-incubated with galactose showed precipitation (Lane D), suggest that the glycan
binding site of jacalin was masked with specific sugar, galactose. These results confirm that the isolated jacalin is pure and functionally active.

3.3. Interaction of jacalin with CdS QDs

The interaction of jacalin with CdS QDs was elucidated by following the change in fluorescence. The intrinsic fluorophores of jacalin are deeply buried in the hydrophobic core and showed characteristic fluorescence emission maximum at 330 nm (Komath et al., 2000). Tryptophan and tyrosine are the main fluorophores responsible for the protein fluorescence, whose microenvironments may undergo modification upon drug binding as a result fluorescence quenching can be observed (Tang et al., 2014). Titration of jacalin with CdS QDs resulted in quenching in the fluorescence intensity in the wavelength range of ca. 300-400 nm, with the maximum
change in fluorescence intensity around 330 nm, suggesting an interaction between jacalin and CdS QDs (Fig. 2.8A).

![Graph](image)

**Fig. 2.8:** Interaction of jacalin with CdS QDs. (A) Jacalin fluorescence emission spectra monitored after addition of increasing concentrations of CdS QDs (0 – 100 µM)., (B) Stern-Volmer plot for jacalin binding with CdS QDs

In general, protein fluorescence can be quenched either by static or dynamic (collisional) mechanism (Eftink et al., 1981; Lackowicz J.R. 1999). Static quenching involves the formation of a protein-quencher complex, while collisional quenching is solely caused by free diffusion. As a result of free diffusion, the rate of collisional quenching increase with an increase in temperature (Eftink et al., 1981; Lakowicz J.R. 1999). Interestingly, the percent quenching decreases with the increase in the
temperature (Table 2.1). The fluorescence quenching data was analyzed with Stern-Volmer equation:

\[ \frac{F_0}{F_c} = 1 + K_{sv} [Q] \]  

(1)

where \( F_0 \) and \( F_c \) are the relative fluorescence intensities of jacalin at 330 nm in the absence and presence of the drug, respectively, \( K_{sv} \) is the Stern-Volmer fluorescence quenching constant, \([Q]\) is the concentration of the CdS QDs. Stern-Volmer plot showed good linearity (Fig. 2.8B) and the quenching constant, \( K_{sv} \) decreases with the increase in the temperature (Table 2.1). These results support that the probable quenching mechanism of the interaction between jacalin and CdS QDs were initiated through complex formation rather than dynamic collision. Zeta potential of CdS QDs upon binding to jacalin increases significantly from -22.0 mV to -33.3 mV, suggesting that the electrical boundaries of the QDs are well-separated because of an interaction between the QDs and jacalin. The particle size analysis reveals that the hydrodynamic size of CdS QDs increases from 77.5 nm to 121.7 nm, indicating that an additional layer of protein was formed on the surface of QDs due to the formation of CdS QDs-jacalin complex.
Fig. 2.9: Determination of binding constant for jacalin-CdS QDs. (A) Representative binding curve for the interaction of CdS QDs with jacalin. The change in fluorescence at 330 nm resulting from the addition of CdS QDs to the lectin is plotted as a function of the total drug concentration. Inset: plot of $F_0/\Delta F$ as a function of the reciprocal total CdS QDs concentration. The reciprocal of the Y-intercept of this plot gave the value of $\Delta F_\infty$, the change in fluorescence intensity, when all the lectin molecules are bound by the drug. (B) A plot of $\log \left( \frac{F_0 - F_c}{F_c - F_\infty} \right)$ against $\log$ [CdS QDs].

A typical binding curve for the interaction of CdS QDs with jacalin was shown in Fig. 2.9A. The binding curve showed that the change in fluorescence intensity ($\Delta F$) increases with increasing CdS QDs concentration initially, but displays saturation behaviour at higher concentration. In order to obtain the binding constant ($K_a$), the binding data were analyzed according to the following expression (Zheng et al., 2009; Huang et al., 2011),

$$\log (\Delta F/F_c - F_\infty) = \log K_a + n \log \text{[QDs]} \tag{2}$$

where $\Delta F$ is the change in fluorescence at any point of the titration, $F_c$ is the corresponding fluorescence intensity of the protein, $F_\infty$ is the fluorescence intensity of the protein that is fully saturated with the QDs, which can be determined from the ordinate intercept of the plot of $F_0/\Delta F_\infty$ vs $1/[\text{QDs}]$ (inset of Fig. 2.9A). A double
logarithmic plot for the interaction of CdS QDs with jacalin was given in Fig. 2.9B. According to equation 2, the X-intercept of a plot of log (\(\Delta F/F_c - F_\infty\)) versus log [QDs] will yield \(pK_a\) for the association of QDs with jacalin. From the abscissa intercept of this plot, the \(K_a\) value characterizing the binding of CdS QDs to jacalin was obtained as \(3.92 (± 0.86) \times 10^4\) M\(^{-1}\) at 25\(^\circ\)C. The obtained binding constant are comparable to those observed generally for lectin-monosaccharide complexes as well as those obtained for the interaction of lectin with other ligands (Komath et al., 2006).

Thermodynamic forces govern the interaction between CdS QDs and jacalin were estimated by performing binding titrations at different temperature. From the binding constant, the changes in the Gibbs free energies (\(\Delta G^0\)) at different temperature have been calculated using eq. 3 and reported in Table 2.1.

<table>
<thead>
<tr>
<th>T (K)</th>
<th>% quenching</th>
<th>(K_a) (\times 10^{-3}) (M(^{-1}))</th>
<th>(n)</th>
<th>(K_a) (\times 10^{-4}) (M(^{-1}))</th>
<th>(\Delta G) (kJ.mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>46.58 ± 0.78</td>
<td>10.31 ± 0.47</td>
<td>1.04</td>
<td>6.96 ± 0.83</td>
<td>26.70</td>
</tr>
<tr>
<td>303</td>
<td>44.15 ± 0.98</td>
<td>9.63 ± 0.35</td>
<td>0.98</td>
<td>4.86 ± 0.81</td>
<td>26.29</td>
</tr>
<tr>
<td>308</td>
<td>40.90 ± 0.76</td>
<td>8.17 ± 0.70</td>
<td>1.11</td>
<td>3.92 ± 0.87</td>
<td>26.21</td>
</tr>
<tr>
<td>313</td>
<td>38.01 ± 0.12</td>
<td>7.39 ± 0.12</td>
<td>1.07</td>
<td>2.79 ± 0.88</td>
<td>25.79</td>
</tr>
<tr>
<td>318</td>
<td>35.18 ± 1.38</td>
<td>6.28 ± 0.40</td>
<td>1.05</td>
<td>2.09 ± 0.86</td>
<td>25.48</td>
</tr>
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Using van’t Hoff plot (Fig. 2.10), the change in the enthalpy of binding ($\Delta H^0$) and the change in entropy of binding ($\Delta S^0$) associated with the interaction of CdS QDs and jacalin were determined from the eq. (4):

\[
\Delta G^0 = -RT\ln K_{aT} \quad (3)
\]
\[
\ln K_{aT} = (-\Delta H^0/RT) + (\Delta S^0/R) \quad (4)
\]

where, $K_{aT}$ is the association constant at the corresponding temperature, R is the gas constant. The van’t Hoff plot shows a linear relationship between $\ln K_{aT}$ versus $1/T$ (Fig. 2.10). The change in enthalpy and change in entropy of binding for CdS QDs were obtained as $\Delta H^0 = -44.24 \pm 1.21 \text{ kJ mol}^{-1}$ and $\Delta S^0 = -60.92 \pm 4.10 \text{ J mol}^{-1} \text{ K}^{-1}$. The negative value of $\Delta G^0$ suggests that the interaction between jacalin and the surface of CdS QDs was a spontaneous process (Table 2.1). The negative $\Delta S^0$ suggested that hydrophobic interaction was not predominant because hydrophobic interaction generally results in a positive entropy change. The negative $\Delta S^0$ can be attributed to hydrogen bond and van der Waals interaction (Tian et al., 2010; Ross et al., 1981). The negative $\Delta S^0$ and $\Delta H^0$ also suggested that the interaction is governed by enthalpic forces, most likely through electrostatic interaction similar to the one observed for the interaction between bovine serum albumin and QDs (Tian et al., 2010).
It has been reported previously that the interaction between cadmium telluride QDs and Chymotrypsin (Jung et al., 2002) and the binding between CdS QDs and haemoglobin also governed by enthalpy forces (Shen et al., 2007). Based on these results it has been suggested that the polar interactions are the major contributor for the interaction between CdS QDs and jacalin, as observed in the Concanavalin A-porphyrin complex (Goel et al., 2001) and *Momordica charantia* lectin-porphyrin complex (Sultan et al., 2004). The driving forces for the binding was largely from $\Delta H^0$, as a result, the $\Delta G^0$ of binding is still negative.

Synchronous fluorescence spectroscopy was widely applied to infer the microenvironment changes of the protein due to binding reactions (Lloyd, 1971). Synchronous fluorescence experiments were performed by keeping the wavelength difference between excitation and emission, $\Delta \lambda$ value at 15 nm for the tyrosine
residues and Δλ value at 80 nm, for the tryptophan residues. Fig. 2.11 shows that the intensity of Trp or Tyr residues decreases in the presence of CdS QDs.

Fig. 2.11: Synchronous spectra of jacalin-CdS QDs system. (A) Δλ = 80 nm, (B) Δλ = 15 nm, and (C) Far-UV circular dichorism spectra of Jacalin (solid line) and jacalin + CdS QDs (dotted line). The concentration of Jacalin and CdS QDs is 12 µM and 0.25 mM CdS QDs, respectively. No significant changes in the jacalin secondary structure upon binding to QDs.

However, only the Trp emission peak position showed a significant red shift from 350 nm to 353 nm whereas Tyr residues did not show a significant shift. These results indicate that the polarity around Tyr residues are unchangeable, but the environment of Trp is altered upon binding to CdS QDs and moved towards a more polar environment, as a result protein fluorescence was quenched. Far-UV circular dichroism spectra of jacalin were not affected upon QDs binding, indicating that the secondary structure of the lectin remains intact after binding to QDs (Fig. 2.11C).

The functional form of the lectin upon QDs binding was evaluated through the well-known hemagglutination and the hemagglutination inhibition activity assay (Fig.
As noted from Fig. 2.12A, the presence of CdS QDs does not affect the erythrocyte-agglutination activity of jacalin. However, an addition of galactose to jacalin-CdS QDs complex inhibits agglutination. These results suggest that the sugar recognition site of jacalin remains active upon QDs binding and retaining the QDs fluorescence, which is a good sign for developing jacalin-CdS QDs complex for active targeting the sugar epitome of cancer cells.

**Fig. 2.12**: Determination of jacalin- Cds Qds sugar binding activity. (A) Hemagglutination assays with human erythrocytes. (a) PBS buffer, (b) Jacalin, (c) galactose, (d) jacalin-galactose complex, (e) CdS QDs (f) Jacalin-CdS QDs complex and (g) Jacalin-galactose- CdS QDs complex. About 100 µL of 4% human erythrocytes are used in this experiment. Jacalin agglutinates erythrocytes whereas the presence of galactose inhibits the agglutination. As inferred from this data, agglutination was only inhibited by the specific sugar galactose (d) and not by CdS QDs (e), suggesting that CdS QDs and galactose bind at different site. Experiments were performed in triplicate. (B) Fluorescence spectra of CdS QDs and jacalin-CdS QDs complex. Excitation – 360 nm. Inset showed that both CdS QDs and jacalin-CdS QDs complex exhibit blue colour fluorescence when illuminated with UV lamp, 360 nm.
At 25°C, the binding constant obtained here for the jacalin-CdS QDs complex in the presence of 50 mM galactose is $3.03 (\pm 1.19) \times 10^4 \text{ M}^{-1}$, which is comparable to the binding constant obtained without the specific sugar (Komath et al., 2006). These results suggest that the monosaccharide binding site is different from the CdS QDs binding site, augmenting the hemagglutination assay (Fig. 2.12A), however, the binding strength is comparable to lectin-monosaccharide complexes (Komath et al., 2006). Noteworthy, the fluorescence of jacalin is quenched while binding to CdS QDs, but the fluorescence properties of CdS QDs is well preserved even after binding to the protein (Fig. 2.12B). Considering the strong interaction between jacalin and QDs, it is possible that the maximum percentage of CdS QDs is expected to be in the lectin bound state and it can be used as a vehicle for targeting CdS QDs to T-antigens of cancer cells.

3.4. Fluorescence microscopy study

To validate our claim, an attempt was made to label healthy human peripheral lymphocytes (PBMC) and human chronic myeloid leukaemia (K562) cells with CdS QDs and jacalin-CdS QDs complex. Strikingly, CdS QDs showed blue fluorescence with both PBMC and K562 cells, indicating no selectivity (Fig. 2.13A and Fig. 2.13B). At the same time, jacalin-CdS QDs complex could not stain PBMC, but showed strong blue fluorescence with K562 cells (Fig. 2.13C and Fig. 2.13D).
These results suggest that the jacalin-QDs complex can selectively recognize the cancer cells while leaving the healthy tissues under the current experimental conditions. Similar observation was made by Marangoni et al., with AuNPs-jacalin-FITC nanoconjugates. The reported specific recognition of cancer cells by jacalin is highly useful for the future theranostics application.