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5.0 Bioactive polymers conjugated Nanoparticles

Bioactive diethylaminoethyl dextran chloride (DEAE-D) and carboxy methyl cellulose (CMC) (Figure 1) are versatile polymer with numerous industrial applications were used as a shape directing agents for the synthesis of gold (Au) nanoparticles (NPs) in a typical green chemistry synthetic route. Shape controlled growth was precisely directed by the quaternary amine functionalities of DEAE-D while its polycationic nature provided simultaneous colloidal stabilization. Mechanistic aspects were drawn from different sets of reactions in which DEAE-D was directly used as a reducing agent with respect to reaction time and temperature. DEAE-D coated NPs thus produced were subjected to hemolysis for their possible use as drug release vehicles in systemic circulation so as to explore their possible biomedical applications.

Figure 1 Molecular structures formula of DEAE-D and CMC.
Results and discussion

5.1 Direct reduction Method

5.1.1 Temperature effect

Aqueous solutions of DEAE-D do not show any absorbance in UV or visible range within 20 – 70 °C (Figure 2, red dotted line). However, in the presence of HAuCl₄ along with DEAE-D (Figure 2), two prominent peaks are observed around 220 and 290 nm in the UV region along with a very weak and much broader band around 550 nm in the visible region, due to AuCl₄⁻ ions, ligand to metal charge transfer (LMCT),¹⁻³ and SPR of NPs, respectively.⁴,⁵

![Figure 2. UV-visible scans of a reaction of 0.1 % DEAE-D and 0.25 mM HAuCl₄ with temperature from 20 – 70 °C. Red dotted line refers to blank (i.e. 0.1 % DEAE-D without gold salt) and blue dotted line represents the scan at 70 °C. Scans from top to bottom follow 20 – 70 °C temperature range. Inset show the variation in the normalized intensity of different peaks with temperature.](image)

Their normalized variation is depicted in inset of Figure 2, where first two peaks show a regular decrease with the increase in temperature along with a red shift of 20 nm, whereas the third peak shows a regular increase in intensity. A close inspection indicates that the absorbance of Au NPs due to SPR resonance (550 nm) starts becoming prominent around 56 °C where LMCT complex (290 nm) also starts converting into nucleating centers. From 40 to 56 °C, AuCl₄⁻ ions
complexation with oppositely charged DEAE-D increases as DEAE-D aggregates dismantle with temperature and hence peak at 220 nm shows a steep fall. At 56 °C, LMCT complex (290 nm) attains sufficient energy to convert into nucleating centers and thus leads to the formation of Au NPs. This temperature decreases linearly with the increase in the amount of DEAE-D as depicted in (Figure 3) which means that the reduction of Au(III) into Au(0) through Au(I) represented by LMCT complex is proportional to the amount of DEAE-D.

A 20 nm red shift in both peaks (i.e. 220 and 290 nm, Figure 2) indicates about the ongoing conformational changes in DEAE-D under the effect of temperature. As α-1,6 glycosidic linkages between glucose molecules constitute the backbone of DEAE-D (Figure 1), inter as well as intra-hydrogen bonding among them is expected to cause aggregation at low temperature which might restrict the approach of AuCl₄⁻ ions to electron donating ether oxygens. However, as the dismantling of the aggregates begins due to the breaking of the hydrogen bonds with temperature, more sites become accessible for AuCl₄⁻ ions for the electron transitions from

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**Figure 3.** Intensity versus temperature plot of different DEAE-D concentrations and 0.25 reaction mM HAuCl₄ with temperature from 20 – 70 °C and the inset depicts the linear variation in the reduction temperature with the amount of DEAE-D.
predominantly ligand 3e\textsubscript{u}(\pi) to metal 3b\textsubscript{1g}(\sigma^{*}).\textsuperscript{4} That in turn decreases the energy difference between the ligand and metal molecular orbitals and hence shifts the LMCT band to lower energy with a red shift of 20 nm.

Figure 4. UV-visible scans of a reaction of 0.1 % CMC and 0.25mM HAuCl\textsubscript{4} with temperature from 20 – 70 °C. Red dotted line refers to blank (i.e. 0.1 % CMC without gold salt) and blue dotted line represents the scan at 70 °C. Scans from top to bottom follow 20 – 70 °C temperature range. Inset show the same reaction at different CMC concentrations.

Although peaks at 220, 290, and 520 nm due to AuCl\textsubscript{4}^- ions, LMCT, and SPR of NPs, respectively, are observed in the case of CMC (Figure 4), no marked decrease in the intensity of 220 nm band is observed with temperature. Also, LMCT (290 nm) vanishes almost instantaneously in the beginning of the reaction which provides a greater reduction potential with the consequence 0.2 % CMC initiates the reduction at relatively much lower temperature (~ 30 °C) than 0.2 % DEAE-D (~ 50 °C, Figure 3) under identical reaction conditions. But the growth rate is much faster with much greater slope for 0.2% DEAE-D rather than CMC which means larger morphologies are expected in the former case. In fact, LMCT complex between the AuCl\textsubscript{4}^- ions and ether oxygens of the dextran backbone is facilitated by the quaternary amine groups which electrostatically attract AuCl\textsubscript{4}^- ions to DEAE-D backbone. Since this group is absent in
CMC therefore no stable LMCT complex is observed, and hence, AuCl$_4^-$ ions instantaneously convert into nucleating centres as soon as they come in contact with the ether oxygens of CMC.

In order to authenticate this proposed mechanism, we conducted the reaction for DEAE-D at pH = 9.5 (Figure 5) which reduces the possibility of electrostatic interactions between the quaternary amine groups and AuCl$_4^-$ ions to generate a stable LMCT complex. Remarkably, high pH has completely removed the peak due to LMCT complex rather generated much prominent peak due to SPR at 520 nm, the variation of which is depicted in the inset. Thus, high pH allows DEAE-D to initiate the reduction even at 20 °C or as soon as AuCl$_4^-$ ions come in contact because they are no longer entrapped by the quaternary amine groups to generate LMCT complex. Thus, a significant difference in the reduction mechanisms of DEAE-D and CMC is expected to have a marked effect on the crystal growth of Au NPs in the presence of respective polymers.

Figure 5. UV-visible scans of reaction 0.1 % DEAE-D and 0.25 mM HAuCl$_4$ with temperature from 20 – 70 °C carried out at pH = 9.5 . Red dotted line refers to blank (i.e. 0.1 % DEAE-D without gold salt) and blue dotted line represents the scan at 70 °C. Scans from top to bottom follow 20 – 70 °C temperature range. Peak intensity increases from 20 – 70 °C. Inset shows the variation in the Au NPs absorbance with temperature at different amounts of DEAE-D.
5.1.2 Time effect

UV-visible scans of a typical reaction of DEAE-D at constant 70 °C with reaction time (Figure 6) also show prominent peaks at 220, 290, and 550 nm due to AuCl₄⁻ ions, LMCT, and SPR of NPs, respectively, as noted previously. Variation in the intensity of all peaks with time is depicted in Figure 7 and 8, for 0.25 and 1 mM HAuCl₄, respectively. In the former case, the reduction is completed within 50 minutes of the reaction. Within this period (close up view, inset), the intensity of AuCl₄⁻ ions (220 nm) and LMCT (290 nm) decreases continuously due to the formation of tiny nucleating centres with insignificant absorbance at 550 nm up to 15 minutes (see dotted line).

![Figure 6 UV-visible scan Absorbance versus time of different peaks for a reaction with 0.1 % DEAE-D and 0.25 mM HAuCl₄. Red dotted line refers to blank (i.e. 0.1 % DEAE-D without gold salt) and green line represents the scan at 330 min.](image)

Thereafter, both 220 and 290 nm peaks disappear because fresh AuCl₄⁻ ions engage themselves in the autocatalytic reduction⁶⁻⁸ on the already created nucleating centres without going through LMCT and that causes an increase in the absorbance of Au NPs. As this process continues, NPs eventually grow into extraordinarily large size and settle at the bottom of the tube leaving a constant absorbance of Au NPs in aqueous phase.
On the other hand, when the concentration of gold salt is increased to 1 mM, the same reaction takes about 330 minutes (more than four folds) to complete (Figure 8). Interestingly, similar variation in the intensity of all peaks (except AuCl₄⁻ ions) is observed within 15 minutes (see inset) because the same amount of DEAE-D used with essentially same number of available ether oxygens to create the nucleating centres. However, greater amount of gold salt does not allow the intensity of AuCl₄⁻ ions (220 nm) to decrease even up to 100 minutes of the reaction due to a limited number of available ether oxygens. That is why LMCT still exists (even after 15 minutes) as more than one AuCl₄⁻ ions are expected to form LMCT complex with each reducing site as evident from the low energy absorbance (310 nm) in comparison to relatively high energy absorbance (290 nm) obtained for 0.25 mM HAuCl₄ (Figure 6). Importantly, intensity of both 220 and 310 nm peaks continuously decreases till 330 minutes whereas that of Au NPs tends to constant just after 15 minutes (like that of Figure 7). It means that the number density of suspended colloidal NPs practically remains constant in this reaction too after 15 minutes while the excess amount of AuCl₄⁻ ions involved in the autocatalytic process continuously replacing them with large shining NPs which settle at the bottom of the tube.

Figure 7. Intensity versus time plot of reaction 0.05 % DEAE-D with 0.25 mM HAuCl₄ at 70°C.
In the case of CMC, time effect does not produce any LMCT band as observed for temperature effect (Figure 4) while a clear absorbance due to SPR of Au NPs around 520 nm is observed (Figure 9). It remains quite weak up to 100 minutes thereafter it increases with a red shift of about 50 nm and bifurcates to produce even much stronger absorbance at longer wavelength of 850 nm. This variation has been depicted in Figure 10 and divided into three parts especially for a reaction of 1 mM HAuCl₄. Weak absorbance in the first part can be attributed to the presence of tiny NPs which may coalesce in the second part as indicated by a red shift of 50 nm, and then produce large NPs or arrange themselves in nanowires with prominent absorbance at 850 nm. Usually, rod or nanowires produce simultaneously two absorbances; one at shorter wavelength (520 nm) is due to the short axis (traversed SPR) and other at longer wavelength (850 nm) is due to the long axis (longitudinal SPR, depends on the length). Reaction with 0.25 mM HAuCl₄ does not show this behavior and growth process tends to constant after sometime. Thus, a comparison between the results of Figures 7 and 8 with that of Figures 9 and 10 indicates that DEAE-D has a precise control over the growth kinetics of Au NPs while this is not so when CMC is used because the profiles of growth kinetics of Figure 10 of different gold salt concentrations indicate the formation of different morphologies.
Figure 9. Plot of absorbance versus wavelength of a reaction with 0.05 % CMC and 0.5 mM HAuCl$_4$ with time at 70 °C. Black dotted line shows the 50nm shift in the absorbance wavelength with time in 240 min and which shift to 850nm in 330 min.

Figure 10. Plots of Intensity versus time for reactions with 0.25 and 0.5 mM HAuCl$_4$. Red and blue circles belong to the same plot, they just represent different regions.
5.2 Indirect reduction method

5.2.1 S – G method (Temperature effect)

By using this reaction protocol, we want to see how both DEAE-D and CMC control the growth of seeds to develop shape controlled morphologies. Figures 11 and 12 show two UV-visible scans of reactions conducted with a constant amount of 7 µL of the Au seed solution along with 0.25 and 1 mM HAuCl₄, respectively, in the presence of DEAE-D. In both cases, broad absorbance of Au NPs is initially located around 540 nm which increases with the increase in temperature and also red shifts (indicated by the arrows). We call this as peak 1. It gets broader in Figure 11 but splits into two in Figure 12 to produce another peak (peak 2) at lower wavelength. A much greater amount of HAuCl₄ (1 mM) in the latter case in comparison to the former (0.25 mM) causes a shape transformation and responsible for the appearance of peak 2 because four folds higher amount of gold atoms are now accommodated on a constant amount of seed (i.e. 7 µL).

Figure 11. UV-visible scans of a reaction of 0.05 % DEAE-D and 7 µL of seed solution with 0.25 mM HAuCl₄. Solid arrows in figure represent peak 1 with marked red shift.
A variation in the wavelength of both peaks (i.e. peak 1 and peak 2 of Fig 12) is depicted in Fig 13 where filled circles (for 7 μL) shows a huge red shift of ~180 nm in peak 1 which bifurcates to produce peak 2 (empty circles) around 55 °C. The shaded area refers to the region where two peaks coexist. Almost double amount of the seed (15 μL, diamonds) reduces the red shift to ~100 nm and produces peak 2 at even higher temperature of 66 °C, while 50 μL of the seed (triangles)

![Image](image.png)

**Figure 12.** UV-visible scans of a reaction of 0.05 % DEAE-D and 7 μL of seed solution with 1.0 mM HAuCl₄. Solid and empty arrows in figure represent peak 1 and 2 respectively.

![Image](image.png)

**Figure 13.** Show the plots of red shift in the absorbance of Au NPs with temperature of reaction 0.05 % DEAE-D and 7 μL of seed solution with 0.25 mM HAuCl₄.
further reduces the red shift to just 40 nm without the appearance of peak 2. This sequence points to the fact that entire growth process is precisely controlled and directed by DEAE-D to happen only on the existing number of seeds. That is why the shape transformation vanishes with increasing amount of seed.

5.2.2 S – G method (Time effect)

Same reactions when conducted at constant 70 °C with time produce a similar variation in the wavelength of Au NPs absorbance (Figure 14). Absorbance appears around 540 nm (peak 1) and demonstrates a continuous red shift with time. Within 50 minutes of the reaction, it bifurcates to generate peak 2 at lower wavelength (as noticed in Figure 13) which ultimately survives while peak 1 disappears. The only difference between the two reactions is that one can see a large red shift of ~300 nm when 7 µL of the seed is used in comparison to ~200 nm for 15 µL of seed due to a greater growth in the former case that leads to the formation of much larger morphologies. The time period in respective reactions where two peaks coexist i.e. the respective shaded area of the phase diagram, is in fact represents the growth kinetics of shape transition in growing NPs.

![Figure 14](image)

*Figure 14. Plots show the red shift in the absorbance of Au NPs with time of reaction 0.05 % DEAE-D and 7 µL of seed solution with 0.25 mM HAuCl₄.*
It means that in the beginning of the reaction (i.e. before the shaded area) peak 1 produces only one kind of shape that continuously grows with prominent red shift while within 50 minutes of the reaction another shape appears that absorbs at shorter wavelength and is represented by peak 2. Meanwhile the reaction mixture also shows the presence of two kinds of particles. Large orange colored particles settled at the bottom while smaller ones in suspension (inset). Peak 1 in fact belongs to large particles which settle at the bottom and hence it disappears as particles grow to certain size and cannot support themselves in colloidal suspension. Reaction completes when peak 1 disappears because peak 2 remains more or less constant with time. Thus, Figure 13 and 14 are of the same reactions with temperature and time, respectively, and again demonstrates a precise control of DEAE-D on the growth of Au NPs, because the reaction is precisely directed on the number of available seeds in the solution. Whereas, this is not the case with CMC where we do not see any substantial growth in seeds and behavior is more or less the same as is depicted in Figure 4 and 9, which means that CMC is not directing the growth on already available seeds as DEAE-D can do.

5.3 Microscopic studies

5.3.1 Au NPs – DEAE-D samples

Microscopic studies help us to understand the difference in the reduction and stabilizing behaviors of DEAE-D and CMC, that altogether results in different shapes and structures of Au NPs. The presence of DEAE-D produces several micrometer sized triangular and hexagonal thin plates\(^9,10\) (Figure 15) carrying aggregates of small faceted Au NPs (Figure 16) in all samples.

*Figure 15. (a) SEM image of several microplates carrying groups of small Au NPs synthesized with 0.05% DEAE-D and 0.25 mM HAuCl\(_4\) at 70 °C.*
Some of the microplates even reach the size of 20 µm with average size varies between 5 to 15 µm which is huge in comparison to that of NPs (~150 nm). EDX analysis of NPs (Figure 17) shows maximum amount of Au followed by C, Cl, and O coming from the DEAE-D coating. The golden – yellow shining particles settled at the bottom of sample tubes are in fact large microplates. They start settling at the bottom of the tube after 15 minutes of the reaction according to Figure 7 and 8, because thereafter the absorbance of colloidal Au NPs (shown in Figure 16) in the solution becomes constant.

**Figure 16. Close up image of a group of faceted Au NPs of Figure 15.**

**Figure 17. EDX analysis of TEM image of Figure 15.**
These microplates seem to generate from the merger of the smaller ones. Figure 18 provides direct evidence where one can see a single large somewhat hexagonal plate being derived from smaller ones whose merged boundaries are still visible (indicated by the white arrows). Similarly Figure 19 shows another image of a large plate undergoing the same process along with the presence of other smaller plates with wavy margins (indicated by empty block arrows) ready to merge to produce larger plates. This information is further substantiated with TEM images.

![Figure 18. SEM image of hexagonal plates made from the merger of smaller ones through their lateral planes shown by the white arrows.](image)

EDS analysis of single plate confirms the presence of Au (Figure 20) along with the DEAE-D coating. TEM image of a single hexagonal microplate is shown in Figure 21 along with the corresponding SAED pattern (Figure 22) of hexagonal symmetry indicating the fact that the microplate is a single crystal with preferential growth along the \{111\} crystal planes of fcc geometry which is also supplemented by the corresponding XRD patterns with single prominent peak at 38.2° (Figure 23). Increase in the amount of gold salt from 0.25 to 1 mM converts some plates into large sheets (Figure 24) as demonstrated by Figure 8 over a time period of 330
minutes. Each sheet bears surface patterns originating from the regular interference fringes and the bending contours produced by its extraordinary thin nature.\textsuperscript{11,12}

**Figure 19.** SEM image of hexagonal plates made from the merger of smaller ones through their lateral planes shown by the white arrows.

**Figure 20.** EDX analysis of TEM Figure 18.
Figure 21. TEM image of single micro plate.

Figure 22. Selected area electron diffraction pattern (SAED) image.
Figure 23. XRD patterns of the samples prepared with different amounts (0.05%, 0.1%, 0.2%) of DEAE-D.

Figure 24. High resolution TEM image of large Au sheet.
We get similar thin micro plates (Figures 25, 26, 27) carrying faceted NPs (Figure 28) when we increase the amount of DEAE-D from 0.05 to 0.1 %.

Figure 25. SEM image of several thin microplates stacked one above the other synthesized with 0.1 % DEAE-D and 0.25 mM HAuCl₄ at 70 °C.

Figure 26. SEM image of several thin microplates stacked one above the other synthesized with 0.1 % DEAE-D and 0.25 mM HAuCl₄ at 70 °C.
Figure 27. TEM images of several thin microplates stacked one above the other synthesized with 0.1 % DEAE-D and 0.25 mM HAuCl₄ at 70 °C.

Figure 28. TEM image of a group of faceted NPs of the same sample.
But further increase in the amount up to 0.2 % generates more NPs and places them in large quantities on the microplates (Figures 29, 30). Some of the plates are completely loaded with NPs and nanorods (Figure 31) which are stacked together in an ordered fashion (Figure 32).

**Figure 29.** SEM image of the sample prepared with 0.2 % DEAE-D and 0.25 mM HAuCl₄ at 70 °C.

**Figure 30.** SEM image show self assembled NPs of different shapes on large microplates.
A side wise as well as end to end stacking of the nanorods indicates that DEAE-D apart from stabilizing the NPs by surface coating also helps in their self-aggregation. DEAE-D is well known flocculating agent\textsuperscript{13-16,17,18} which helps in the self aggregation of colloidal particles.

Figure 31. SEM image show small self assembled NPs loaded on large microplates.

Figure 32. SEM image of self assembled rod like NPs on large microplates.
Figures 30 and 31 are the best examples of accommodation of NPs of different shapes on large microplates due to flocculating behavior of DEAE-D which can be explained on the basis of DLVO theory.\textsuperscript{19,20} Potential energy of interacting colloidal particles is the sum of potential energy due to electrostatic ($V_R$) and van der Waals ($V_A$) interactions. $V_R$ establishes the Columbic repulsions and provides colloidal stability but flocculation happens when $V_A$ predominates $V_R$. Electric charge on the colloidal particle surface can be neutralized by changing pH or adding salt and that in turn allows $V_A$ to predominate $V_R$.\textsuperscript{21} However, herein the neutralization of the charge happens through the hydrogen bonding\textsuperscript{22} between the sugar backbone of the capping layers of different NPs when they approach each other. This association reduces the magnitude of $V_R$ and induces flocculation. NPs surface adsorption of DEAE-D is expected to take place through the quaternary amine head groups\textsuperscript{23} just like that of cetyltrimethyl ammonium bromide (CTAB)\textsuperscript{24-26,27} a well known stabilizer and shape directing agent. This leaves sugar backbone in the aqueous phase for hydrogen bonding with similar moieties from other NPs (Figure 33). Hence, hydrogen bonding seems to be the driving force for the flocculating behavior of DEAE-D coated NPs and their accommodation on large microplates.

Figure 33. Schematic representation of DEAE-D capped Au NPs.
One can clearly see the glue type DEAE-D coating responsible for the self aggregation of NPs in Figure 34 (indicated by white arrows). Interestingly, if we drastically increase the amount of DEAE-D from 0.2 to 0.8 %, we get fine core–shell morphologies with single NP forming the core and thick coating of DEAE-D constituting the shell (Figure 35). The thickness of the shell remains constant around ~75 nm though the size of the NP decreases from ~450 nm (Figure 35 a) to ~75 nm (Figure 35 d), which indicates that the coating is DEAE-D concentration specific because when the amount of DEAE-D is 0.2 %, then the thickness of coating is hardly visible (Figure 34). This thick coating in the form of a shell suggests a high degree of association among the capping layers of DEAE-D.

5.3.2 Microplate thickness measurements

AFM studies help us in quantification of the thickness of the microplates and size distribution of NPs. Figures 36 a and b show the deflection and height images of two microplates. Thickness of the plates was measured by performing the line analysis on both plates separately. Blue line passing through plate “A” allows us to determine the thickness of this plate from points “1” and “2”. The corresponding scan is illustrated in the upper frame of Figure 36c along with the thickness in adjoining table.

Figure 34. Self aggregated faceted Au NPs due to DEAE-D capping.
Figure 35. (a) – (d) TEM images of single Au NP coated with thick layer of DEAE-D in a typical core-shell morphology prepared with 0.8 % DEAE-D and 0.25 mM HAuCl₄ at 70 °C.
Both points measure the thickness around 47 nm which suggest a fairly flat plate. Likewise, red line passing through plate “B” gives an average thickness of 45 nm. Figure 36d shows another image of two plates lying one above the other. Line analysis simultaneously measures the thickness of both plates. Points “1” and “4” allow to measure the thickness of the lower larger plate which is about 41 nm but the thickness of the upper triangular plate (points “2” and “3”) is 80 nm which is almost double than that of the lower one. These microplates are quite thin in comparison to those already reported in the literature.9,10,23,28

Figures 36 f and g show self-assembled NPs on the microplates as previously depicted in TEM images (Figures 30, 31). Line analysis (Figure 36h) measures the average size of NP close to 154 nm which is in good agreement with that of TEM analysis (Figure 16). Increase in the amount of DX to 1.6 % produces very large plates of about 30 μm in size. One such large plate is shown in Figure 36i with interesting features which are not demonstrated by other images. This plate has a large variation in the thickness and the line scan is presented in Figure 36j along with the thickness data in the adjoining table. Left edge of the plate provides thickness close to 1 μm which continuously decreases towards the opposite edge (see thickness data in the table) and final thickness of most of the plate is 38 nm which is close to that for plates from the previous images. It seems that as if the left vertex (indicated by an arrow) of this hexagon is further involved in the growth process and the growth spans slowly to cover rest of the plate resulting in a much thicker plate similar to the one shown in Figures 36 f and g bearing large NPs. This is basically triggered by the active sites on the lattice plane which are created by the dislocation and imperfections in the atomic arrangement and usually prevalent on the vertices, edges, or grain boundaries as previously shown in Figures 18, 19. Usually, a typical crystallization process proceeds through a layer-by-layer growth with an approximate thickness of 1 nm. It is carried out
by freshly produced atomic species or nucleating centres in the solution, which approach the surface and look for the active sites with greater surface energy over rest of the crystal planes. The active sites then attract them favorably and allow them to integrate into the lattice plane. Figure 36i shows this process clearly as it proceeds from left to right in a typical layer-by-layer fashion and that is why the thickness decreases from 1043 nm to 38 nm as growth proceeds from left to right. Since the growth process is spanning over an area of several μm, therefore some of the imperfections survive during the growth process and lead to the formation of large NPs. On the contrary, if there are little imperfections as in the case of large plate shown in Figure 36a,b,d,k, the thickness remains same throughout the microplate (note the average thickness of ~54 nm, Figure 36 l). The presence of surface wrinkles on the plate of Figure 36k is mainly caused by extraordinarily thin nature in comparison to a surface area of several μm.

5.3.3 Au NPs – CMC samples

CMC on the other hand produces entirely different morphologies from that of DEAE-D as indicated by the UV-visible behavior (Figures 9, 10). No microplate formation happens in the presence of CMC (Figure 37) and instead Au NPs of polyhedral shapes of 43 ± 9 nm are produced which are arranged in chain like arrangements due to the CMC coating.

![Figure 37. TEM image of polyhedral Au NPs prepared with 0.05 % CMC and 0.25 mM HAuCl₄ at 70 °C.](image-url)
As the amount of gold salt increases from 0.25 to 0.5 mM, it converts the chain like arrangement into continuous nanowires of ~10 nm thick (Figures 38, 39). Polyhedral morphologies are usually bound with high energy {111} crystal planes.

Figure 38. Low magnification TEM image of polyhedral Au NPs prepared with 0.05 % CMC and 0.25 mM HAuCl₄ at 70°C.

Figure 39. High magnification TEM image of polyhedral Au NPs prepared with 0.05 % CMC and 0.25 mM HAuCl₄ at 70°C.
Increase in the amount of gold salt preferentially involves them in the growth process to convert the chain-like arrangement into continuous nanowires which is facilitated by the CMC coating. XRD patterns (Figure 40) further support this inference where one can find single prominent peak at 38.2° representing the substantial growth along the {111} crystal planes of fcc Au. Similar behavior is reported for phospholipids stabilized Au NPs.29

The morphologies of the Au NPs generated by the S–G method are not much different from that we have discussed above. S–G method clearly depicts the formation two kinds of morphologies for reactions with DEAE-D from UV-visible behavior which are fully supported by the TEM images. Small NPs represented by peak 2 of Figures 13, 14 and large microplates represented by peak 1 (because plates grow and then settle at the bottom) with both 7 and 15 μL of the seed solution. However, plate formation can be dramatically reduced if 50 μL of the seed solution is used, which generates fine spherical NPs. It happens because fixed amount of gold salt is distributed over a large number of seeds which don’t get chance to grow into microplates. In contrast, CMC still produces polyhedral morphologies even in the presence of seeds with no sign of shape controlled growth or nanowire formation. Thus, the contrasting difference in the shape, size, and arrangement of Au morphologies produced in the presence of DEAE-D and
CMC can simply be related to the entirely different course of the reaction mechanism which we will discuss in the discussion section.

5.4 Mechanism

Above results explains that DEAE-D can very well be employed for the synthesis of desired morphologies of Au NPs. For example, large plate like morphologies can be obtained when DEAE-D is used in a simple one step reaction, while S – G method helps in achieving predominantly facets or roughly spherical small NPs without the formation of nanoplates. A remarkable difference in the shape controlling behaviors of DEAE-D and CMC can primarily be related to the presence and absence of quaternary amine functional groups, respectively. Since both polymers initiate the reduction of Au (III) to Au (0) mainly due to ether oxygens (Figure 41a-f) and generate nucleating centres (Figures 41b and g), thereafter difference in their stabilizing behaviors mainly governs the overall shape and size of the final Au morphologies. DEAE-D produces nucleating centres through stable LMCT whereas CMC produces nucleating centres without LMCT band. LMCT forms by the charge donation of ether oxygens to electropositive metal centre of AuCl₄⁻ ions when they come in their vicinity due to electrostatic interactions with quaternary amine groups. This decreases the possibility of instant reduction of Au (III) into Au (0) and produce nucleating centres only through LMCT. In other words, DEAE-D enroutes the nucleation through restricted number of nucleating centres which further accommodate rest of the Au to produce large microplates as depicted in the UV-visible behavior (Figure 7 and 8). Contrary to this, instant reduction without going through LMCT by CMC generates several nucleating centres which later on grow into polyhedral morphologies as demonstrated by Figure 10. Finally, the mode of surface adsorption of individual polymer matters and directs the crystal growth in a particular direction. DEAE-D instantaneously blocks the high energy {111} crystal planes of small nucleating centres due to its polycationic nature that leaves low energy {110}/{100} planes for participation in the growth process (Figures 41c and d). Several factors contribute to this preferential adsorption. Surface reconstruction is the most important factor among them. The upper most layer of the atoms on a particular crystal plane is highly thermodynamically unstable because it is balanced only from one side of the plane. It is true for all crystal planes of fcc geometry but is more complex and energetically unfavorable in the case of {111} rather than {110}/{100} due to its greater atomic density which
Figure 41. A schematic representation of the various steps involved in the synthesis of Au microplates (a – e) and nanowires (f – j) in the presence of DEAE-D and CMC, respectively. Steps (a) and (f) represent the reduction reaction, (b) and (g) nucleation, and (c – e) and (h – j) growth processes of respective cases.
continuously tries to attain equilibrium because of strains and energetic reasons. Under such circumstances, the growth process proceeds in that direction which allows it to attain lower total energy. Adsorption of DEAE-D on {111} crystal planes effectively reduces the surface free energy through electrostatic as well as van der waals interactions and completely passivates it. Structural factors of DEAE-D further participate in the effective passivation due to polyelectrolyte nature which allows DEAE-D to attain rigid and expanded state best suited for a complete surface coverage. It promotes nucleation on {110}/{100} planes of already created nucleating centres (as observed in Figure 7,8) or seeds (Figure 13,14) rather producing new nucleating centres which simultaneously expands {111} surface that is rapidly passivated by the adsorption of fresh DEAE-D. This process continues till the precursor supply goes on and takes about 330 minutes to complete when 1 mM of HAuCl₄ is used (Figure 9) and hence leads to the formation of large plates bound with thin edges of {110}/{100} crystal planes. Since the lateral planes of large plates are now actively involved in the nucleation which is clearly evident from the wavy margins (Figure 19, see empty block arrows), therefore they even merge with each other (Figure 18,19, see solid white arrows) to lower the overall surface energy and lead to the formation of micro plates (Figure 41e). That is why all reactions generate mainly microplates whether we use 0.25 mM or 1 mM HAuCl₄ because once the nucleating centres are created within 15 minutes of the reaction (Figure 7,8), they are directed into plate like morphologies by DEAE-D. However, almost similar situation exists when 7 μL of the seed is used (Figure 13,14) but 50 μL of seed almost eliminates the possibility of microplate formation since no peak 2 appears for this reaction in Figure 13. This is because now total amount of the gold salt is distributed over a large number of seeds thereby reducing the possibility of a substantial growth to generate microplates.

On the other hand, lack of quaternary amine functional groups devoid CMC from controlled nucleation thereby producing nucleating centres proportional to the overall reduction potential of the polymer. In such a situation, the growth is solely controlled by the capping and stabilizing behavior of CMC which prefers to adsorb on low energy {110}/{100} crystal planes due to its polyanionic nature just like that of any other anionic or neutral amphiphilic molecule leaving {111} for active participation in the growth process. This behavior alters the direction of growth process from that of DEAE-D and destined for the formation of polyhedral morphologies (Figure 41g) predominantly bound with {111} crystal planes (Figure 37) which...
later on convert into nanowires (Figure 41i,j) as the amount of gold salt increases. Thus, the polymer functionality and polyelectrolyte behavior are the most important characteristic features which simultaneously work in DEAE-D to control the crystal growth.

5.5 Hemolysis

The DEAE-D coated NPs thus produced are further subjected to hemolysis to broaden their applicability in the field of bio-nanotechnology. Usually uncoated NPs are highly toxic and show strong hemolysis when their surface interacts with the cell membrane of red blood cells (RBCs).\textsuperscript{21,35-38} NP surface charge is the most important parameter in deciding the extent of hemolysis. We employ microplates of Figure 15 and 25, as well as core – shell NPs of Figure 35 for hemolysis and the results are presented in Figures 42 and 43.

![UV-visible absorbance of hemoglobin due to hemolysis of few concentrations of microplate sample of Figure 15 along with the positive and negative controls.](image)

Both samples of microplates show hemolysis which increases with the increase in the amount whereas a little hemolysis is observed for the core – shell type NPs initially which
becomes only prominent with 25 μg/ml of the sample. Overall, microplates induce much greater extent of hemolysis in comparison to core – shell NPs. Thus, a lower degree of hemolysis in the latter case can be attributed to an effective coating by a thick layer of DEAE-D which prevents the RBCs membrane deformation or penetration. In contrast, a much larger size of microplates provides sufficient surface area for RBCs to interact with the gold surface with greater possibility of hemolysis. Free metal surface is considered to interact with the RBC membrane through specific interactions which are effectively screened by DEAE-D coating in core – shell NPs thereby leading to a less degree of hemolysis. These findings are quite interesting in view of intravenous drug delivery applications of DEAE-D coated NPs where they exhibit lower affinity for RBCs and could prove to be better vehicles for drug release. This is especially important because DEAE-D is frequently used as adjuvant in vaccines and gene therapy\textsuperscript{[39-41]}, therefore DEAE-D coated NPs might provide better advantages to carry a load of specific drug for the target delivery.

![Graph showing percentage hemolysis induced by microplates of sample of Figure 15 (filled circles), sample of Figure 25 (empty circles), and sample of Figure 35 (filled diamonds).](image)

\textit{Figure 43. Percentage hemolysis induced by microplates of sample of Figure 15 (filled circles), sample of Figure 25 (empty circles), and sample of Figure 35 (filled diamonds).}
5.6 Conclusions

Results conclude that biopolymeric species with specific functionality and effective surface adsorption behavior can be employed for a precise shape control environmental benign synthesis of Au NPs with important implications in nanotechnology. DEAE-D possesses these properties due to quaternary amine functionality and polycationic behavior and thus effectively uses them in shape control synthesis of Au NPs. Quaternary amine groups attract precursor gold ions due to electrostatic interactions and supply them to the reducing sites on polymer chain which control their nucleation and subsequent growth by effective surface passivation. Whereas CMC simply lacks this specific functionality and hence cannot control the nucleation while its polyanionic nature is not that effective in surface passivation of Au NPs to generate shape controlled morphologies. This has been tested and authenticated by using two different reaction protocols in which present polymers are involved in direct reduction reaction and S – G method. DEAE-D thus produces extraordinarily thin large microplates and core – shell NPs which have been tested for hemolysis in view of their biomedical applications. Core – shell NPs with a thick DEAE-D coating are considered to be the best candidates for the drug release vehicles in systemic circulation in view of their little interactions with RBCs that further expands the applications of industrial important DEAE-D in bio-nanotechnology.
5.7 References

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