PNIPAM microgels loaded gold nanorods for cancer therapy

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INTRODUCTION

Nanotechnology is poised to make significant contributions to the fight against cancer, both in diagnostics and in novel treatment technologies. The gold nanorods strongly absorb near-infrared radiation and produce localized cytotoxic heat upon NIR irradiation. Hyperthermia has diverse therapeutic effects even at the cellular level. Even an increase in the temperature of 5 degrees may result in denaturation of the proteins and the disruption of the assemblies of the nucleus and the cytoskeletal systems. In addition hyperthermia can also lead to production of the heat shock proteins and other immunostimulants and trigger the onset of apoptosis or acidosis within the cancer cells. The combination of heat and chemotherapeutic agents is an especially encouraging approach to optimize cancer therapy. Hyperthermia combined with certain chemotherapeutic drugs, such as alkylating agents, often results in synergistic effects.

Tissue is optically transparent from 700–1100 nm, allowing efficient penetration of light at these wavelengths that selectively heats only nanoparticle-containing regions. The main objective of the work is to develop PNIPAM microgels loaded gold nanorods. When wavelengths in the NIR region are incident on such a system, the temperature would increase due to the presence of the gold nanorods, which will act as a switch for the release of the chemotherapeutic drug from the thermosensitive carrier. As a result, we can target the cancer cell using the synergism of hyperthermia and chemotherapy.

MATERIALS AND METHODS

Materials for the synthesis of gold nanorods

Hydrogen tetrachloroaurate(III) trihydrate (HAuCl4.3H2O, 99.9+%), trisodium citrate dihydrate (99%), sodium borohydride (NaBH4, 99%), and L-ascorbic acid (99+%) were all purchased from Sigma Aldrich. All chemicals were reagent grade and used as received.

Materials for the synthesis of PNIPAM microgels

N-Isopropylacrylamide (NIPAm), *N*-isopropylmethacrylamide (NIPMAm), a cross-linking agent *N*-*N*-methylene-bis-acrylamide (BIS), and an initiator potassium persulfate (KPS) were purchased from Aldrich and used as received.

Instrumentation

Gold nanorods were characterized for particle size, surface analysis, absorption spectra and zeta potential using Transmission electron microscope (CM200, Philips) and Fourier transform infrared analysis (Nicolet Instruments Corporation, USA), Absorption spectra (U2900, HITACHI) Zeta plus (Brookhaven Instruments, USA). PNIPAM microgels were characterized using FTIR and Atomic Force Microscope

Method for the preparation of the Gold nanorods

Preparation of the seed solution

Seed solution is prepared by mixing CTAB solution (5.0 mL, 0.20 M) with 5.0 mL of 0.00050 M HAuCl4. To the stirred solution, 0.60 mL of ice-cold 0.010 M NaBH4 is added.Vigorous stirring of the seed solution was continued for 2 min, then the solution was stirred, it was kept at 25 °C without further stirring.

Preparation of the growth solution

CTAB (5, 6, 7, 8, 9 ml, 0.20 M) is added to 0.10ml of 0.0040 M AgNO3 solution at 25 °C. To this solution, 5.0 mL of 0.0010 M HAuCl4 is added, and after gentle mixing of the solution 70 *micro* L of 0.0788 M ascorbic acid was added.The final step is the addition of 12 *micro*L of the seed solution to the growth solution at 27-30 °C. The final solution was centrifuged twice at a speed of 16000 rpm for 30 min and the supernatant was removed and then redispersed in 5ml of deionized water in order to remove the excess of surfactant.

Method for the preparation of the PNIPAM Microgels

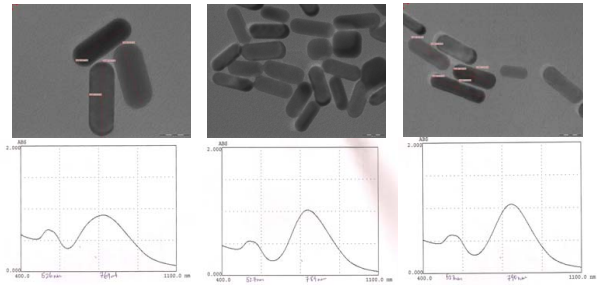
In the precipitation polymerization method the primary monomer, NIPAm, and the cross-linker, BIS are dissolved in 90mL of deionized water, together with comonomers and stabilizing surfactant at a concentration below the critical micelle concentration (CMC). The solution is added to a three-necked, jacketed round bottomed flask connected to a circulating water bath and equipped with a mechanical stirrer, nitrogen inlet and condenser. The reaction mixture is purged with nitrogen for one hour to remove any dissolved oxygen.The solution is then heated to 70 °C, under a gentle stream of nitrogen gas. The initiator, potassium persulfate (KPS), is dissolved in 10mL of deionized water and added to the heated solution. The reaction is allowed to proceed for at least four hours under continuous mechanical stirring at 300 rpm for the duration of the polymerization. At the end of the reaction the solution is cooled and filtered.

RESULTS AND DISCUSSION

Gold Nanorods

Particle size analysis and Absorption spectra

The length of the NRs was controlled by changing the concentration of surfactant added to the growth solution and Figure 1 shows the variation in lengths and widths of nanorods with increase in surfactant. Table 1 shows the corresponding shifting of the longer wavelength as the size increases.



(a) (b) (c)

Fig 1: TEM images and Absorption spectra of the gold nanorods with increasing volume of surfactant added; (a) 6ml (b) 7ml (c) 8ml

Table 1: Red shift of obtained gold nanorods with increasing surfactant volume

SAMPLE	Volume of surfactant added(0.20 M, CTAB)	Short wavelength peak	Long Wavelength peak	Red Shift	Aspect Ratio
1	5 ml	531nm	740nm	--------	--------
2	6 ml	526nm	769nm	29nm	2.86
3	7 ml	527nm	784nm	15nm	3.69
4	8 ml	527nm	790nm	6nm	4.15
5	9 ml	528nm	802nm	12nm	--------

Zeta Potential

Zeta potential of the gold nanorods was found to be 33.72mv before centrifugation which reduced to 6.11mv after the centrifugation which confirms the removal of excess of the surfactant from the solution which might be toxic.

FTIR Analysis

FTIR spectroscopy of NR-CTAB (Figure 3) showed peak at 965 cm-1 due to the quarternary amine stretch of CTAB and strong C-H vibrating region between 2850cm-1 to 3000cm-1 due to the presence of the cetyl group. This confirms the presence of the surfactant on the surface of the gold nanorods whose positive charge can be utilized for its loading into the negatively charged PNIPAM microgels.

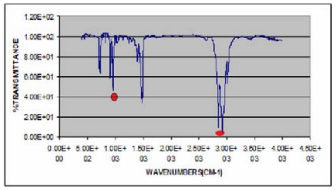


Figure 3: FTIR spectrum of coated nanorods

PNIPAM Microgels

PNIPAM microgels have been fabricated and characterized using DLS and AFM. The size of the microgels was found to be less than 300nm. AFM images obtained also demonstrated the size uniformity of the prepared particles (Figure 4).

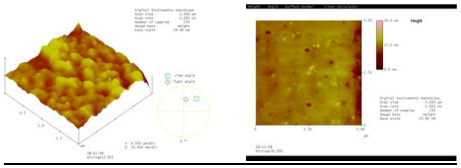


Figure 4: AFM images of prepared microgels

CONCLUSION

Addition of the seed solution to the growth solution results in the formation of the gold nanorods whose aspect ratio can be varied with different concentrations of the surfactant. Further the removal of excess surfactant from the solution was confirmed from a drop in the zeta potential. The presence of the surfactant on the surface of gold nanorods was confirmed using FTIR analysis. PNIPAM microgels have been prepared and characterized. Experiments are underway to test the system for photothermal therapy.

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