O8-4

MULTICOMPONENT NANOPARTICLE FOR DUAL DRUG DELIVERY AND HYPERTHERMIA TREATMENT OF CANCER

Shanavas A. [1#] **Sonara D.** [1] **Aslam M.** [2] **Bahadur D.** [3] **and Srivastava R.** [1*]
Departments of [1] BSBE, [2] Physics, [3] MEMS, Indian Institute of Technology-Bombay, India
* Supervisor # Contact email: asifkhan@iitb.ac.in

INTRODUCTION AND OBJECTIVES

Nanomedicines are vital entities of research interest in recent times, for not just increasing bioavailability of hydrophobic drugs to an *in vivo* target site, but also to evaluate their potential to explore multi modal therapy regimes. The present work proposes such a system, consisting of a core containing a hydrophobic drug plus oleic acid coated magnetic nanoparticles co-encapsulated together and a shell containing a hydrophilic drug.

The core and shell of the nanoparticles comprises of oppositely charged PLGA and Chitosan respectively, which are FDA approved polymers and widely used drug carriers.

Magnetic nanoparticles have gained increasing attention as agents for MR Imaging, targeting and hyperthermia applications related to cancer therapy (Hergt R, 2006). SPIONs, of size less than 50nm exhibit paramagnetism under an external field and can give raise to a temperature of up to 45°C, under an AC field. Cancer cells are sensitive to hyperthermia and thus SPIONs are being exploited along with chemodrugs to give a strong impact in treating the disorder (N K Prasad 2007).

As model drugs for this system, we have chosen highly hydrophobic prostate cancer drug Bicalutamide and a hydrophilic anti-inflammatory drug, Diclophenac sodium salt.

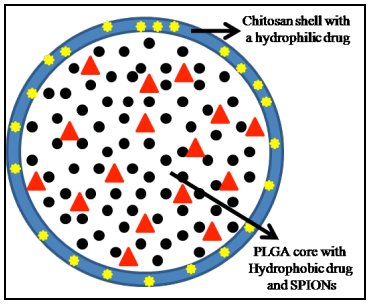

Figure1: Schematic of the Multicomponent NP

The aim of the study was to develop and characterize a multicomponent nanomedicine, which can act as a dual drug carrier and a depot for SPIONs for magnetic hyperthermia.

MATERIALS AND METHODS

PLGA (lactide to glycolide ratio 50:50) and chitosan (medium molecular weight) were obtained from Sigma Aldrich. PVA (M_w 15000-20000), Ferrous chloride tetra hydrate and Ferric chloride hexa hydrate were obtained from the S.D. fine chem Ltd.

Synthesis of magnetic nanoparticles

SPIONs synthesized using co-precipitation method (Racuciu M, 2009). In brief, aqueous solutions of 4.16 g $FeCl_2.4H_2O$ and respectively 10.44 g $FeCl_3.6H_2O$ in 380 ml deionized water, were heated at 80°C and mixed under vigorous and continuous stirring with 40 ml of 25% NH_4OH as precipitant agent and the temperature was raised to 90°C under continuous stirring for 60 min.

Preparation of uncoated and chitosan coated Bicalutamide–magnetic PLGA nanoparticles (CBMP NP)

BMP NPs synthesized by emulsification - solvent evaporation method (Yang J, 2007). In brief, PLGA (30 mg), MNPs (30 mg), Bicalutamide (3 mg) were dissolved in 5 mL of dichloromethane. The organic phase was added to 10 mL of an aqueous phase containing 3% PVA as a stabilizer. The mixture was emulsified for 10 min with an ultrasonicator. After solvent evaporation, the products were purified with three cycles of centrifugation at 20000 rpm. Chitosan coated Bicalutamide –Magnetic PLGA nanoparticles were prepared by a simple mixing: A batch of BMPNP thus prepared was mixed with desired amount of chitosan (0-10 mg) and stirred for 2 hours and centrifuged at 15000 rpm for purification. For loading of hydrophilic drug, diclofenac sodium was added during the coating process.

Characterization

The SPIONs and CBMP NPs were analyzed for size and content by DLS, FEG-SEM, HRTEM and EDAX respectively. Vibrating sample magnetometry (VSM) was used in evaluating the magnetic property of the particles. Specific Absorption Rate (SAR) for measuring the heating rate of bare SPIONs and CBMP NPs for suitability as hyperthermia agents. Finally, *in vitro* drug release pattern of Bicalutamide and Diclophenac sodium was evaluated.

RESULTS AND DISCUSSION

The polycrystalline SPIONs were found to have a size range from 10 to 20 nm under HRTEM and had XRD peaks corresponding to iron oxide crystal structure as shown in Fig-2. The SPION loaded PLGA nanoparticles were found to be in size range of 200 to 300nm in HRTEM. With chitosan coating the size increased upto 350nm with a coat thickness of approx. 20nm (Fig3).

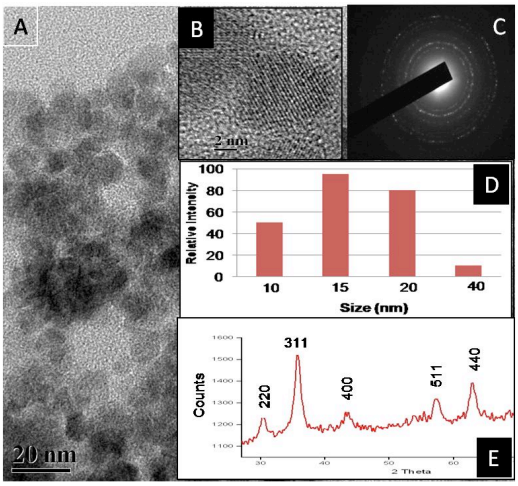

Figure 2: TEM (A), HRTEM images (B), SAD pattern (C), size distribution (D) and XRD pattern (E) of SPIONs.

In DLS, the hydrodynamic diameter was high after chitosan coating due to hydrophilic nature of the polymer.

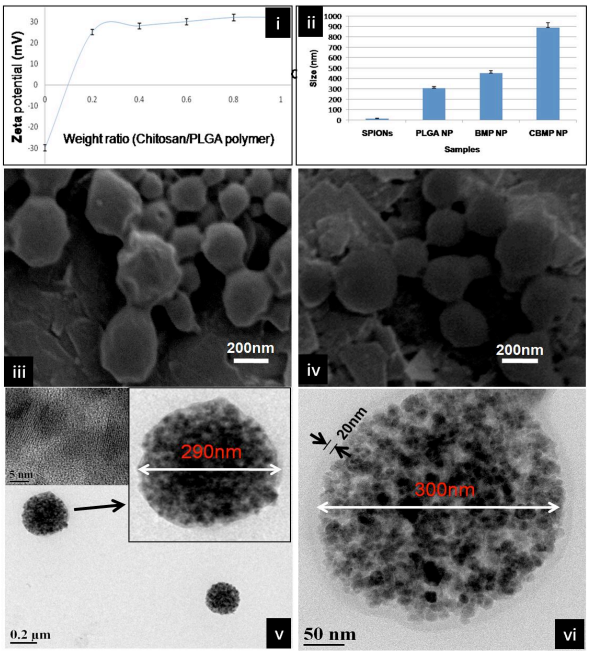

Figure 3: Zeta potential of BMP NPs with varying C/P weight ratio (i), Hydrodynamic diameter of SPIONs, PLGA, BMP and CBMP NPs from DLS (ii), FEG-SEM and HRTEM images of BMP NPs (iii,v) and CBMP NPs, (iv,vi).

The amount of SPIONs loaded in PLGA NP was analyzed by Inductive Coupled Plasma (ICP), which showed near to 50% encapsulation efficiency (E.E) (Tab-1).

Table 1 : Inductive coupled plasma analysis result of bare SPIONs and Magnetic PLGA NPs

Sr No	Name of sample	PPM	Fe_3O_4	Encapsulation efficiency
1	SPIONs	15.44	7.14 mg /ml	-
2	Magnetic PLGA NP	7.14	12.735 mg /ml	44 %

The loading was also confirmed by FTIR analysis (Fig4a), which showed Fe-O bond at 585cm^{-1} in SPIONs, which was present in SPION loaded PLGA NPs. A peak at 1750cm^{-1} and 1650cm^{-1} are related to ester group and N-H stretching in PLGA and Chitosan respectively.

The magnetic property of the CBMPs was checked using VSM, which gave an M-H hysteresis curve typical of a superparamagnetic material (Fig4b). As expected, in SAR analysis, CBMPs showed a raise in temperature up to 43^{o}C, which is sufficient for hyperthermia applications (Fig4c).

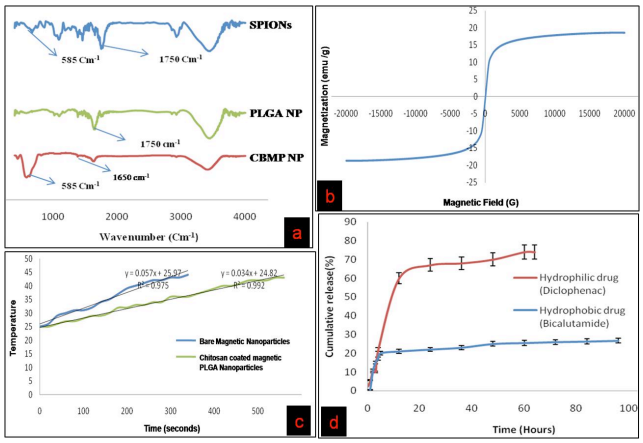

Figure 4: FTIR spectra (a), Hysteresis curve (b) and SAR (c) of CBMP NPs. (d) shows release kinetics of hydrophilic and phobic drugs from the nanostructure.

The E.E. of Bicalutamide and Diclofenac were of 80% and 60% respectively. Bicalutamide was released slowly up to 15days due to hydrophobic interactions with PLGA and retarded diffusion due to hydrophilic chitosan coating. The Diclofenac release happened up to 72 hrs.

CONCLUSIONS

We are reporting a multicomponent nanoparticle which can give simultaneous sustained release of both hydrophilic and phobic drugs while may effectively generate hyperthermia for enhanced tumor cell killing.

REFERENCES

- Hergt R et al. (2006) *Magnetic particle hyperthermia: nanoparticles magnetism and materials development for cancer therapy*. Journal of physics,18, 2919–2934.
- N. K. Prasad. et al (2007) *Mechanism of cell death induced by magnetic hyperthermia with nano particles of γ-$Mn_xFe_{2-x}O_3$ synthesized by a single step process*, J. Mater. chem. 17, 5042-5051.
- Racuciu M. (2009) *Synthesis protocol influence on aqueous magnetic fluid properties*, Current Applied Physics, 9, 1062–1066
- Yang J et al., (2007) *Antibody conjugated magnetic PLGA nanoparticles for diagnosis and treatment of breast cancer*, J. Mater. Chem. 17, 2695–2699.

We like to acknowledge DST INDIA for funding.