European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

DESIGN AND EVALUATION OF STIMULI-RESPONSIVE CHITOSAN-BASED POLYMERIC NANOPARTICLES FOR TARGETED DRUG DELIVERY

Article History:

Name of Author:
[1*]Arpan Debnath, [2]Debjit Teri, , [3]Arpan Bera ,[4]Ayush Mohanty, [5] Rupesh Soni , [6]Aishik Paul, , [7] Ishita De

Affiliation: *[1*]Assistant Professor Department Of Pharmacology, B.R Nahata College Of Pharmacy, Faculty Of Pharmacy, Mandsaur University*
[2]Assistant Professor, Department Of Pharmacology, Mazidia Academy Of Pharmacy
[3] Assistant Professor, Department Of Pharmacaceutics , Rangamati College Of Pharmacy
[4]Assistant Professor, Department Of Quality Assurance , B.R Nahata College Of Pharmacy, Faculty Of Pharmacy, Mandsaur University
[5] Professor Department Of Pharmacology, B.R Nahata College Of Pharmacy, Faculty Of Pharmacy, Mandsaur University
[6]Assistant Professor Department Of Pharmacology, Apc College Of Pharmacy
[7]Assistant Professor Department Of Pharmaceutics, Gandhari College (School Of Pharmacy)

Corresponding Author:
Arpan Debnath

Received: 11-11-2025
Revised: 25-11-2025
Accepted: 11-12-2025
Published: 31-12-2025

Abstract: The efficacy and safety of drug delivery technologies of life-threatening ailments is still a significant challenge to be addressed because of extreme side effects, systemic toxicity, and development of drug resistance in relation to the conventional treatment. Polymeric nanoparticles have considerable advantages over alternative nanocarrier-based drug delivery systems like polymeric nanoparticles, including reduced off-target toxicity, increased bioavailability and increased accumulation at diseased sites due to the enhanced permeation and retention (EPR) effect. The current research work is dedicated to inflammation and lung cancer, which are the conditions where cellular microenvironment is distorted in contrast to usual tissues. This research aimed to prepare and assess the stimuli-reactive copolymers that are able to unleash the therapeutic agents in a spatial and controlled time at the diseased locations. The structural characterization was conducted by FTIR, the use of the method of colorimetric, XRD, and the use of the method of NMR. Anti-inflammatory and anticancer (dexamethasone) and anticancer (doxorubicin) were drug-loaded into the copolymerized polymers to create polymeric nanoparticles (PNPs). The PNPs were also surface-functionalized and atrial natriuretic peptide (ANP) to be targeted to natriuretic peptide receptor-A (NPRA), a receptor overexpressed in cancer cells. Drug loading, encapsulation efficiency, particle size, morphology and zeta potential were determined. The cellular uptake, cytotoxicity, and anti-inflammatory activities were evaluated with the cell lines of pulmonary epithelia and macrophage that revealed better selectivity and lower toxicity to normal cells. Anti-inflammatory investigation in BALB/c mice in vivo further proved better therapeutic response of the stimuli-responsive PNPs, in comparison to free drug. In sum, the paper has shown that stimuli-responsive polymeric nanoparticles in the form of surface-modified nanoparticles offer potential to be safe and efficient, precise, and targeted carriers of drugs.

Keywords: Nanoparticle, Anti-Inflammatory and Anticancer, Enhanced Permeation and Retention (EPR) Effect.

INTRODUCTION

The Nanotechnology has transformed contemporary pharmaceutics through the creation of nanosized drug delivery systems (NDDS) that enhance the safety, efficacy as well as targeting of the therapeutic agent. NDDS usually employ carriers at nanometer scale (1 1000 nm) that are required to increase drug solubility, stability, bioavailability, and controlled release with minimum systemic toxicity.[1,2] Such systems have been particularly useful with drugs that

are poorly soluble in aqueous solutions, drugs with short half-life, or drugs with a therapeutic index that is narrow. Depending on the composition and shape, nano drug delivery systems can be divided into a number of groups which include polymeric nanoparticles, liposomes, solid lipid nanoparticles, nanostructured lipid carriers, dendrimers, micelles, nanogels, and polymer-drug conjugates.[3,4] All of the classes possess distinct benefits in terms of drug loading capacity, release behaviour and targeting potential. Polymeric nanoparticles (PNP) are one of them, and they have attracted considerable attention because of their biodegradability, biocompatibility, and tunable physicochemical characteristics.[5,6] Development of nanosized drug carriers is done in solvent evaporation, nanoprecipitation, dialysis, ionic gelation, emulsification-diffusion and high-pressure homogenization. Particularly, nanoparticle types of polymeric particles are usually made through nanoprecipitation, emulsion solvent evaporation, or ionic crosslinking and it is feasible to accurately regulate particle size, surface charge and drug encapsulation efficiency.[7,8] Some of the common polymers used include chitosan, PLGA and PEG-based copolymer. The more advanced version of NDDS is the stimuli-responsive drug delivery system (SRDD) that emits drugs to detect certain internal or external stimuli like pH, temperature, redox potential, enzymes or light.[9,10] SRDD works through a mechanism of action based on alterations in the polymer structure or cleavage of bonds provoked by the disease microenvironment that allows the release of drugs in specific locations and can be controlled.[11,12] The method will cause therapeutic accuracy and minimize off-target effects. Specificity is further enhanced by targeted drug delivery systems that use ligands like peptides, antibodies or small molecules which identify receptors that are over expressed on diseased cells enabling receptor-mediated uptake.[13,14] These systems are evaluated by cytotoxicity research to determine biocompatibility and safety using pertinent cell lines and also by anti-inflammatory ability research to establish therapeutic efficacy. Combined, these approaches are a bright perspective in the creation of the next-generation, safe, and effective drug delivery platforms.[15,16]

2. MATERIAL AND METHODS

2.1 MATERIALS

Any chemicals and polymers employed in the research were of the analytical grade and obtained in the reputable chemical suppliers. Cell lines were obtained, L132, A549, and RAW 264.7 of NCCS, Pune, India. IL-4, IL-5, and IL-13 ELISA kits were bought at the RayBiotech Inc., USA. The experiments were done using deionized water.

2.2 METHODS

2.2.1 Characterizations of Synthesized Copolymer

FTIR spectral studies were carried out with a spectrometer (JASCO 6300 series) in the range between 4000 and 400 cm-1 with a resolution of 4 cm-1. All the samples were analyzed by KBr pellet method. 1H NMR spectra were recorded on a Bruker (500 MHz) spectrometer after dissolving the samples in DMSO-D6.

2.2.2 Preparation of CH-Hz-mPEG PNPs

The copolymer's B-PNPs were made using a dialysis technique. At room temperature, 40 mg of copolymer was dissolved in 5 mL of DMSO under shifting circumstances. Additionally, the outcome was transferred into a dialysis bag (MWCO 12,000–14,000 Da, HiMedia, Mumbai, India) and dialysed against distilled water for the entire night. A 0.45 µm microfiltration membrane was used to filter the obtained B-PNPs. Additionally, 10 mg of prednisone was added to the copolymer to create P-PNPs using the same method. A UV/Vis spectrophotometer was used to measure the absorbance at 242 nm in order to quantify the attention of prednisone. The following formulas were used to compute medication loading and ruse efficacy.

:

$$\textbf{Medicine lading (\%)} = \frac{\text{Weight of medicine entangled within nanoparticles}}{\text{Total weight of nanoparticles}} \times 100$$

$$\text{Ruse effectiveness (\%)} = \frac{\text{Weight of medicine entangled within nanoparticles}}{\text{Total weight of drug}} \times 100$$

2.2.3 Surface Functionalization of PNPs with ANP

ANP had been surface adsorbed onto polymeric nanoparticles (P-PNPs) through a non-covalent adsorption process. In short, the P-PNPs were spread in PBS (7.4, 0.6 mg/mL), and the mixture was prepared with ANP solution (1 mg/mL), and the volume was adjusted to 1500 µL. The incubation was carried out at 4 o C and 24 h to permit adsorption and centrifugation at 10,000 rpm in 15 min to filtrate ANP-P-PNPs. The pellet was rinsed and reshaken in PBS. In the case of fluorescence studies, the conjugation of FITC was done under the dark environment and the free FITC was eliminated through dialysis.

2.2.4 Characterization of PNPs

Particle sizes of PNPs were measured at 25°C using a Zetasizer Nano-ZS, Malvern Instruments. PNPs were dispersed in deionized water (1 mg/mL) to give optimum signal intensity. TEM (Tecnai 10-Philips) images of PNPs define the morphology of PNPs. A drop of the sample containing PNPs was placed on a carbon-coated copper grid and the images were acquired after the sample was completely dried.

2.2.5 Measurement of Weight Loss of B-PNPs

The loss of weight of blank polymeric nanoparticles (B-PNPs) was measured at different pH of 5.0 and 7.4 in buffers. In a nut shell, B-PNPs were put in a

dialysis membrane and left to soak in 50 mL of buffer at the temperature of 37C and swirled gently. Samples collected at fixed time intervals were dried under lyophilization and the percentage weight loss was determined against their starting weight.

Weight loss (%)= $\frac{weight\ feed - weight\ found}{weight\ feed} \times 100$

2.2.6 In vitro Drug Release Cytotoxicity Studies

ANP-P-PNPs were assessed in the presence of acidic (pH 5.0) and neutral (pH 7.4) conditions at 37^0C to evaluate hydrolytic degradation of ANP-functionalized polymeric nanoparticles using a dialysis bag (MWCO 12,000-14,000 Da). In summary, 2 mL of P-PNPs were put in the dialysis membrane and put in the corresponding buffer solutions. The 0.5 mL samples were taken at pre-defined time intervals and substituted with a new buffer. The release of prednisone was determined spectrophotometrically at 242 nm. Replication was done in all experiments (in triplicate).

2.2.7 Cytotoxicity Studies

Normal cell biocompatibility of polymeric nanoparticles (PNPs) was tested using the L132 cell line whereas cytotoxicity cancer cells were tested in the A549 cell line using MTT assay. Cells were plated at 1x 10^4 cells/well, and incubated with serial concentrations of B-PNPs, ANP-P-PNPs and the same doses of free prednisone over 24 h. MTT was incorporated and developed crystals of formazan were dissolved in DMSO. The absorbance was recorded at 570 nm and cell viability was determined as a factor of untreated control.

2.2.8 Morphological Analysis

L132 and A549 cells were inoculated in 96-well plates in 10% heat-inactivated FBS DMEM and left to grow at 37 o C in a humid environment at 5 per cent CO 2. The medium was changed after 24 h to B-PNPs or ANP-P-PNPs (100 µg/mL) and free prednisone (25 µg/mL) was used as a control. Control cells were untreated ones. After the treatment, PBS (pH 7.4) was used to wash the cells, and morphological changes were observed under an inverted phase-contrast microscope with a magnification of 40x.

2.2.9 In vitro Anti-inflammatory Activity of PNPs

The RAW 264.7 murine macrophages were used to measure the in vitro anti-inflammatory activity of polymeric nanoparticles (PNPs). To determine the biocompatibility of prednisone, blank PNPs (B-PNPs), and drug-loaded P-PNPs, MTT assay was used to determine cell viability before inflammation studies. There was quantification of cells treated with graded concentrations and viability was measured spectrophotometrically. The nitric oxide (NO) and intracellular reactive oxygen species (ROS) levels were used to test the activity of anti-inflammatory in lipopolysaccharide (LPS)-stimulated macrophages. The Griess reagent assay method was used to quantify NO production whereas DCFH 2 -DA fluorescence imaging was used to assess the production of ROS. P-PNPs considerably lowered the levels of LPS-induced NO and ROS when compared to the free drug, which showed an increased anti-inflammatory effect.

2.2.10 *In vivo* Studies

The anti-inflammatory effect of pH-responsive polymeric nanoparticles (PNPs) was tested in vivo on six seven-week-old female BALB/c mice in a model of ovalbumin (OVA)-induced airway inflammation. Animals were kept in pathogen-free conditions where they have free access to normal diet and water, and all the protocols of the experiment were accepted by the Institutional Animal Ethics Committee (IAEC). There were five groups (n=4) of mice randomly selected. Group I was the unsuspecting control and was treated with no saline and OVA sensitization. To induce inflammation, OVA as an antigen was intraperitoneally (i.p.) sensitized on 1 and 8 days and natrally (i.n.) challenged on the 20th and 21st days to induce inflammation in Group II. Group III was OVA-sensitized and caused with prednisone (1 mg/kg) during days 1619 before OVA challenge. On days 16-19, cells IV, and V were OVA-sensitized and intranasally administered with a pH-responsive blank PNPs (B-PNPs) and drug-loaded P-PNPs (3mg/kg), respectively, prior to OVA challenge. In the 22nd day, animals were euthanized and bronchoalveolar lavage (BAL) fluid was removed by tracheal cannulation with sterile PBS. The total and different cell counts were established (eosinophils and lymphocytes). ELISA was used to analyze BAL supernatants in terms of Th2 cytokines (IL-4, IL-5, and IL-13). At the end of the study, oxidative stress parameters were determined through the measurement of reactive oxygen species (ROS) using DCFH 2 -DA fluorescence reaction, and levels of nitric oxide (NO) using a Griess reagent-based colorimetric reaction. To assess them histopathological, lung tissues were fixed, sectioned and stained with hematoxylin and eosin (H&E). The integrated analyses allowed the overall evaluation of inflammation, immune modulation, oxidative stress, and tissue architecture, which proved the therapeutic value of PNP-based therapy in allergic airway inflammation.

RESULTS AND DISCUSSION

3.1 Characterization of CH-Hz-mPEG

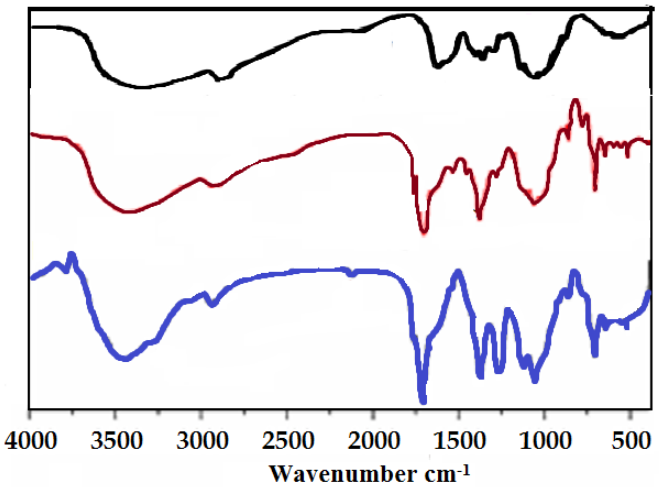

Figure 1. FTIR spectra of (a) chitosan (b) N-phthaloyl-chitosan (c) N-phthaloyl-O-propargyl-chitosan

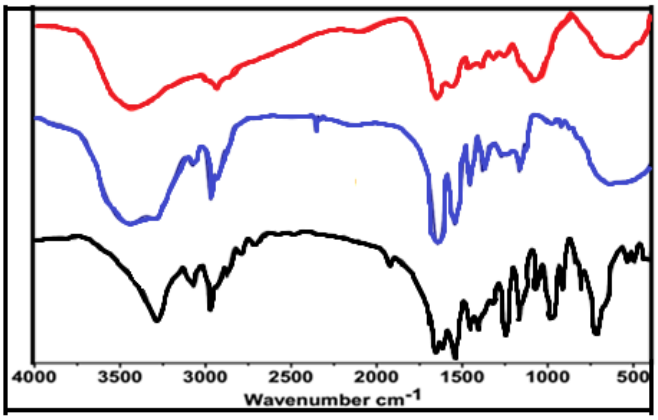

Figure 2. FTIR spectrum of (a) NIPAm (b) PNIPAm-NH2 (c) andPNIPAm-g-OCMC

3.2 Preparation and Physiochemical Characterizations of PNPs

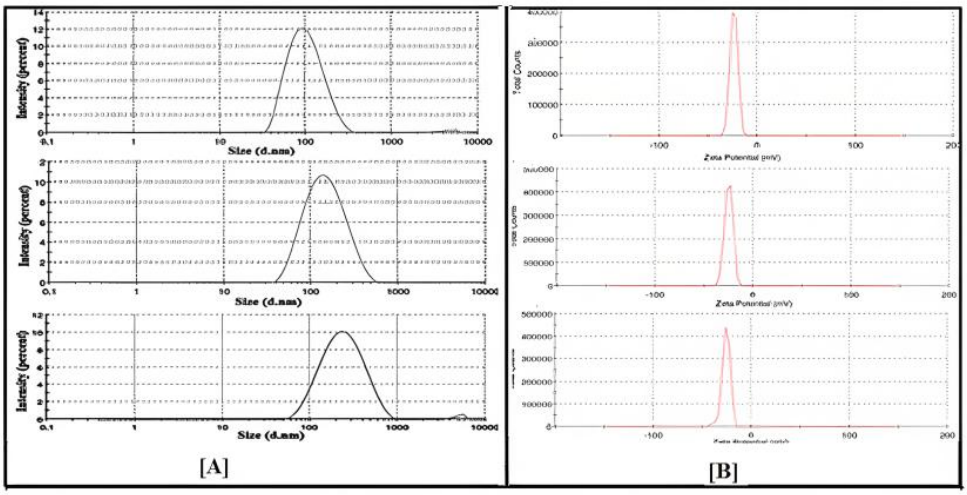

Figure 3. Particle size of (a) B-PNPs (b) P-PNPs and (c) ANP-P PNPs. [B] Zeta potential of (a) B-PNPs (b) P-PNPs and (c) ANP-P-PNP

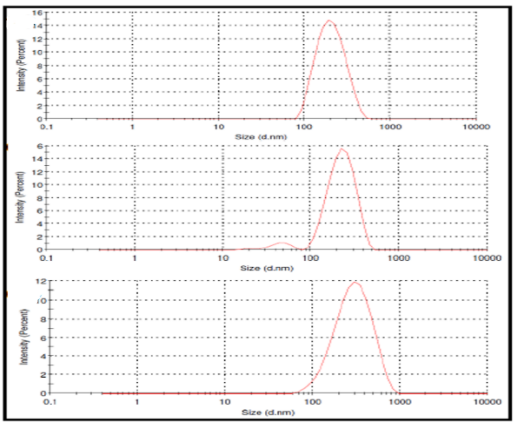

Figure 4. Particle size of (a) B-PNPs (b) D-PNPs and (c) ANP-D-PNPs

3.3 TEM analysis

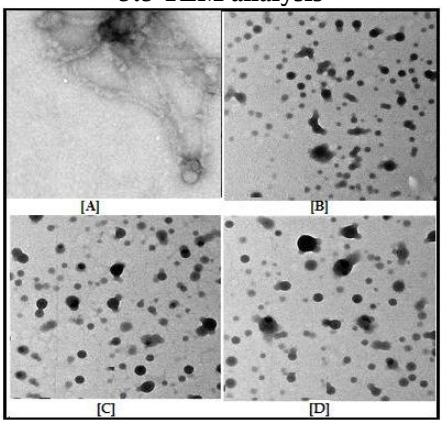

Figure 5. TEM micrographs of [A] ANP [B] B-PNPs [C] P-PNPs and [D] ANP-P-PNPs

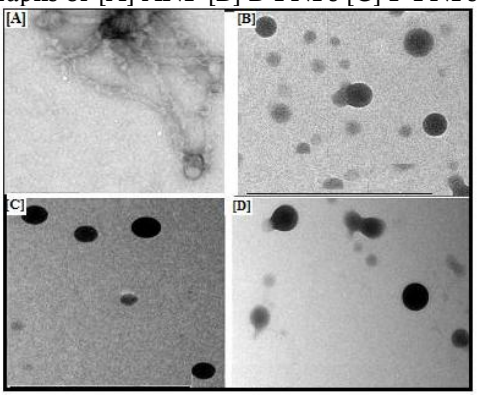

Figure 6. TEM images of [A] ANP [B] B-PNPs [C] D-PNPs and [D] ANP-D-PNPs

3.4 pH-Dependent Degradation of B-PNPs: Weight Loss Measurement

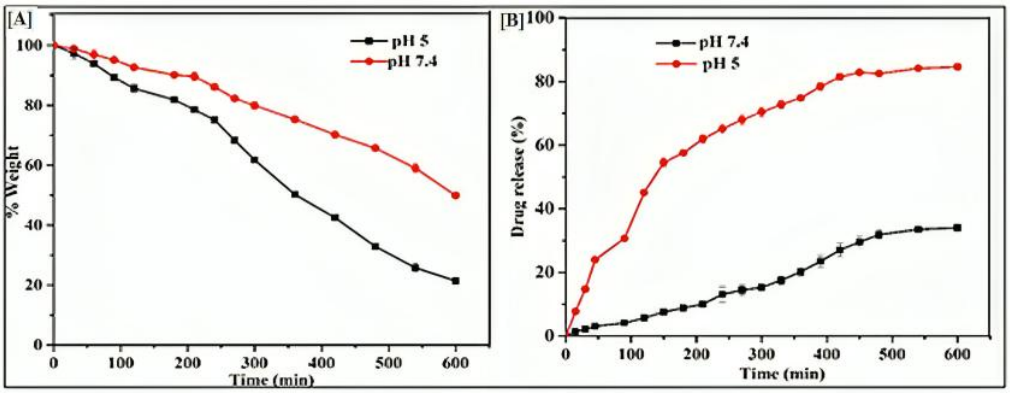

Figure 7. [A] In vitro pH-dependent degradation studies of B-PNPs. [B] In vitro drug release studies of ANP-PPNPs

3.5 Drug Loading and Release Studies

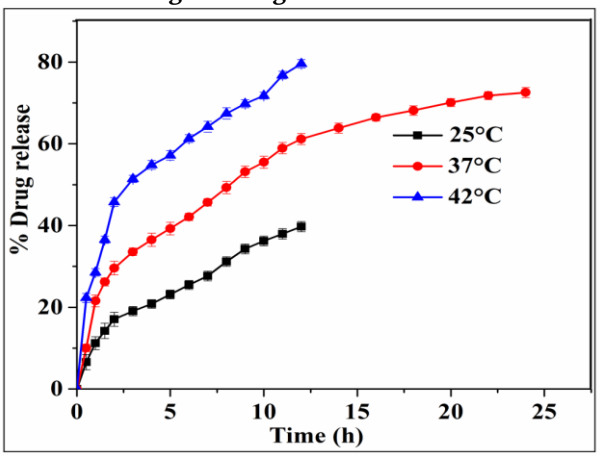

Figure 8. Release of Doxorubicin from ANP-D-PNPs at different temperatures

3.6 Effect of PNPs on Cytotoxicity and Cellular Morphology

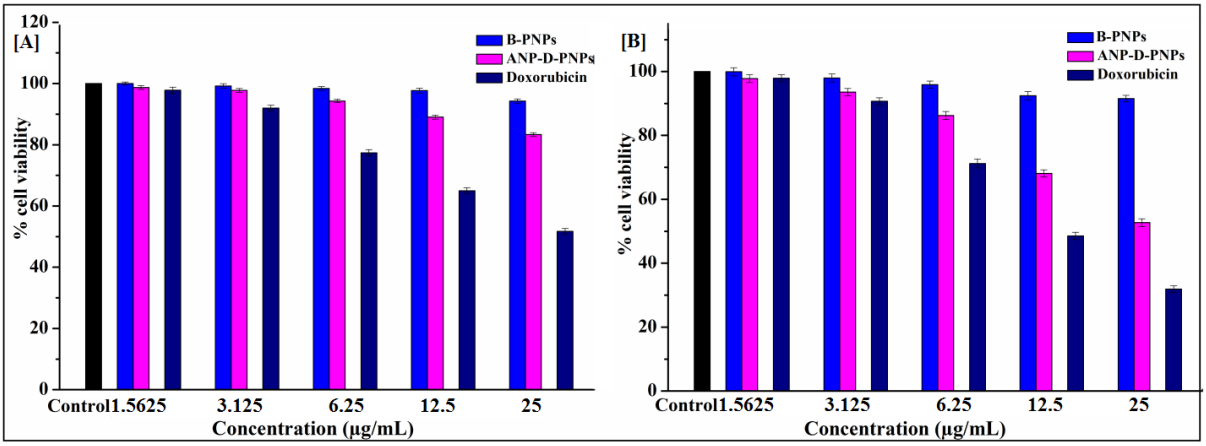

Figure 9. In vitro cytotoxic effect of PNPs at an equivalent doxorubicin dose respectively in [A] L132 and [B] A549 by MTT assay

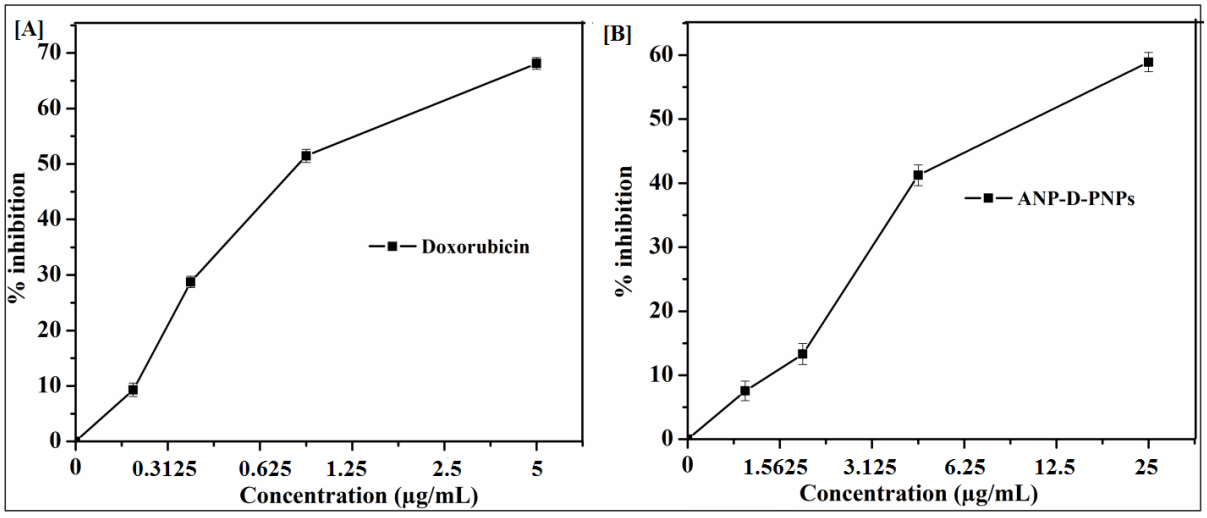

Figure 10. Dose-response effect of [A]doxorubicin and [B] ANP-DPNPs in A549 cancer cells with concentration vs. percentage inhibition

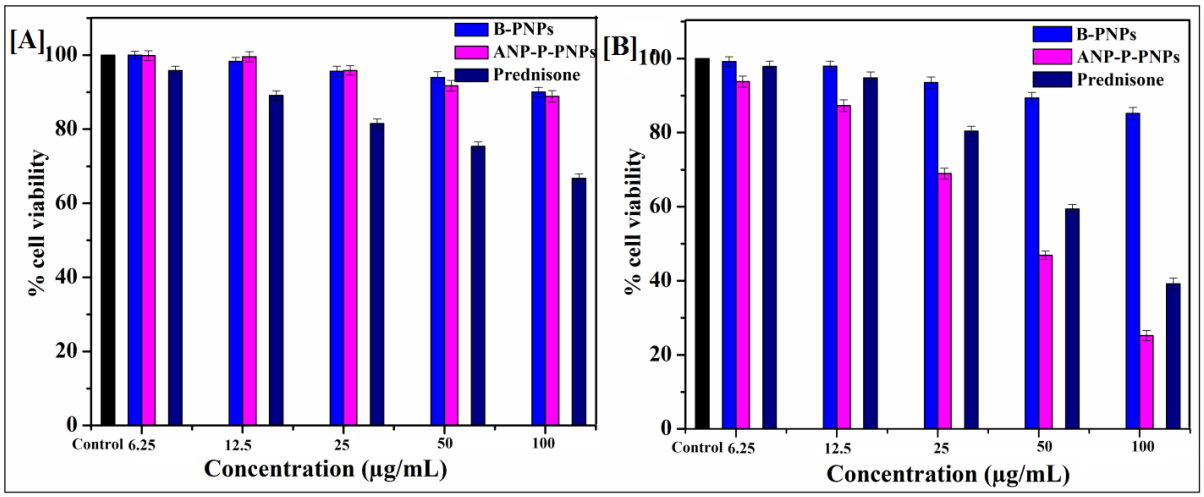

Figure 11. *In vitro* cytotoxic effect of PNPs at an equivalent prednisone dose in [A] L132 and [B] A549 by MTT assay

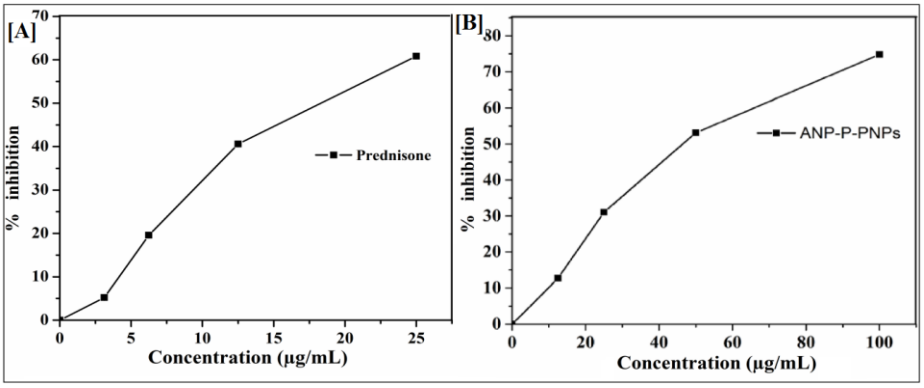

Figure 12. Dose-response effect of [A] prednisone and [B] ANP-P-PNPs in A549 cancer cells with concentration vs. percentage inhibition

3.7 Morphological examination

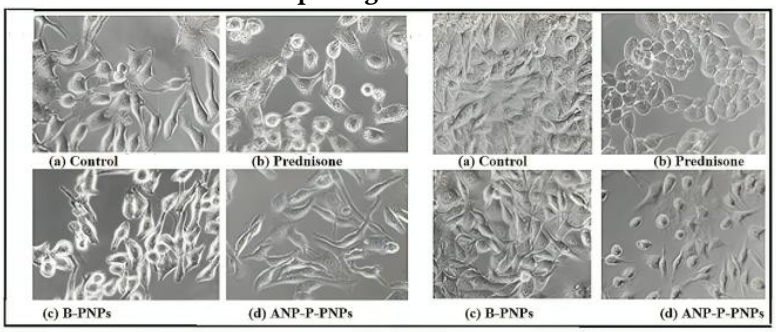

Figure 13. Morphological examination of PNPs (100 µg/mL) treated [A] L132 and [B] A549 cells at an equivalent prednisone dose (25 µg/mL) after 24 h incubation

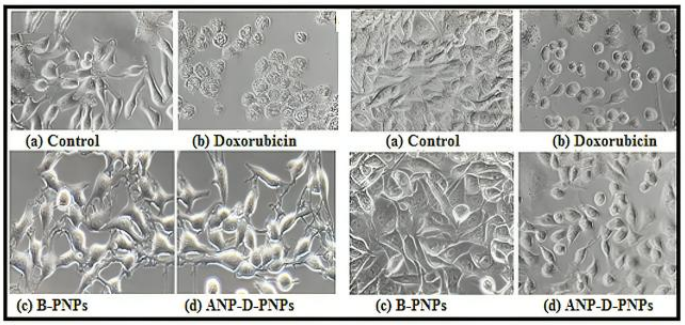

Figure 14. Morphological examination of PNPs (25 µg/mL) treated [A] L132 and [B] A549 cells at an equivalent doxorubicin dose (5 µg/mL) after 24 h incubation.

3.8 Stimuli Responsive PNPs Ameliorate OVA-induced Lung Inflammation in BALB/c Mice Model

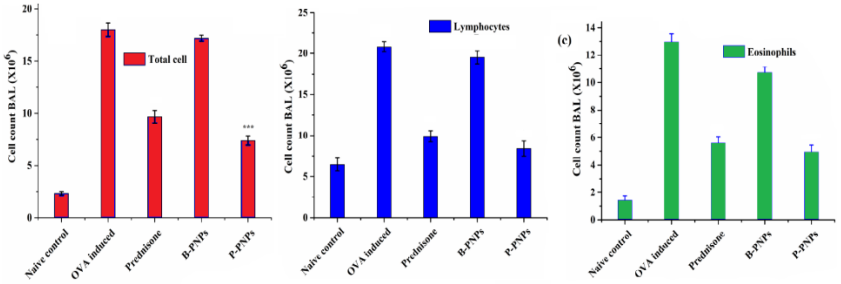

Figure 15. Total and differential BAL fluid cell count in different animal groups. The total inflammatory cell count in BAL fluid. (b&c) The differential cell counts are shown as percentages of the total cell count in all animal groups.

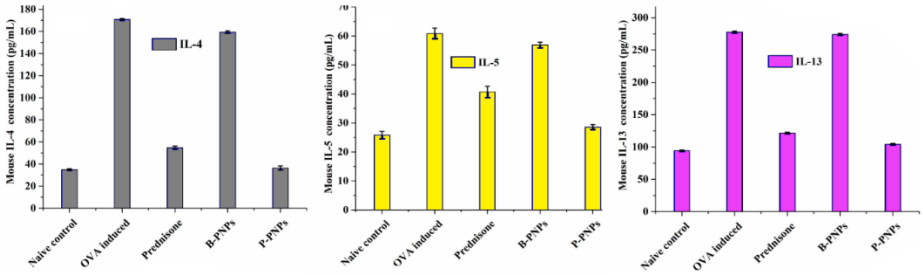

Figure 16. Effects of pH-responsive PNPs on the production of IL-4, IL-5 and IL-13 in BAL fluid.

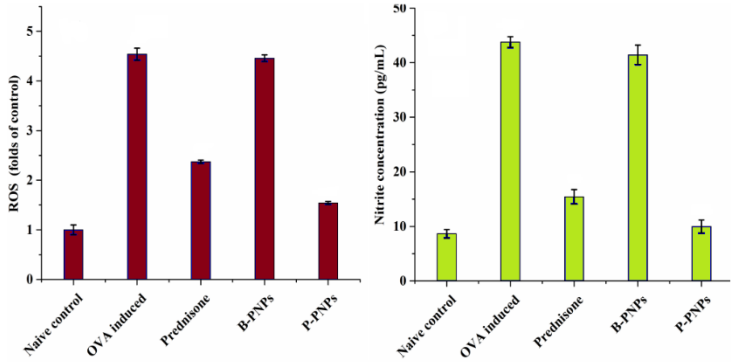

Figure 17. Effects of pH -responsive PNPs on the generation of ROS and NO in BAL fluid.

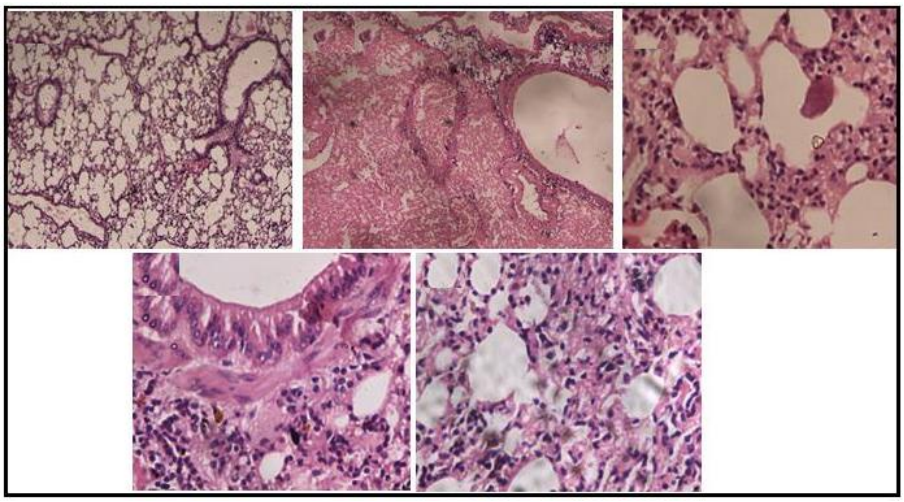

Figure 18. Histopathological evaluations of lungs. The lung tissue was fixed, embedded, cut into slices, and stained with H&E solution (100X). NC group, OVA induced group, prednisone treated group, pH-responsive B-PNPs treated group and pH-responsive P-PNPs treated group

3.9 Fluorescence microscopic of doxorubicin

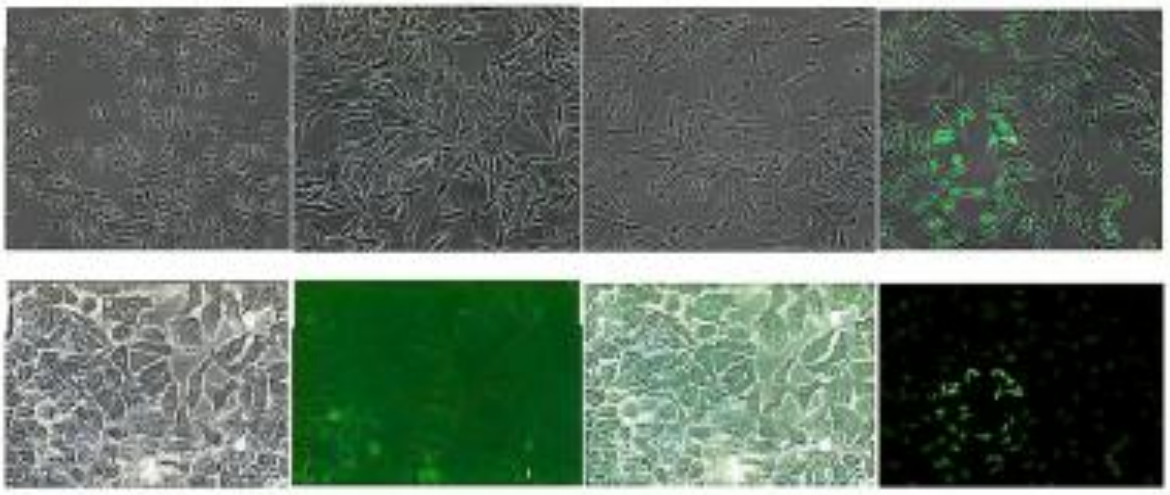

Figure 19. Fluorescence microscopic images and bright field image of cells incubated with FITC labelled PNPs (100 µg/mL) for 4 h. fluorescence image of FITC treated A549 cells fluorescence image of FITC-ANP treated A549 cells, fluorescence image of cells treated with FITC labelled DPNPs, fluorescence image and of cells treated with FITC labelled ANP-D-PNPs.

CONCLUSION

This research portrays how intelligent, stimuli-responsive polymeric nanoparticle (PNP) systems can be designed and evaluated successfully to deliver drugs to specific areas in inflammatory and cancer-based situations and be controlled. An azide-alkyne click chemistry based the production of a novel pH-sensitive chitosan-hydrazone-mPEG (CH-Hz-mPEG) copolymer that was deeply characterized by both FTIR and 1H NMR spectroscopy. The diameter of the nanoscale particles in prednisone-laden PNPs prepared through the dialysis technique was uniform with nanoscale particle sizes and ANP surface functionalization increased the particle size and targeting ability. The pH-controlled release of the drug via acid-cleavable hydrazone bond demonstrated site-specific delivery, and the results supported the potential of hydrazones to deliver the drug to the inflammatory site. In vitro cytotoxicity analyses demonstrated that blank PNPs were very biocompatible and that drug-loaded PNPs and ANP-functionalized PNPs demonstrated the increase in the therapeutic activity, especially A549 cancer cells. Targeting using ANP was very effective in enhancing cellular uptake in diseased cells than normal cells. The anti-inflammatory effect was confirmed in RAW 264.7 macrophage, where P-PNPs significantly decreased the production of NO and ROS by means of the pH-responsive processes. An OVA-induced model of allergic disease in the lungs was used to evaluate the pH-responsive PNPs in vivo to confirm its high anti-inflammatory effect, which could help prevent disease progression caused by inflammation. Also, PNIPAm-g-OCMC thermo-responsive copolymer could be synthesized and used to prepare doxorubicin-loaded D-PNPs through ionic gelation. Such systems were found to show temperature-dependent size change, an appropriate LCST (c. 38 o C), and increased drug release at the temperature above a physiological temperature. ANP-functionalized thermo-responsive D-PNPs had enhanced uptake and doxorubicin release by cancer cells and mediated by temperature-induced changes in the structure. Altogether, this paper confirms that the ANP-functionalized, pH- and thermo-responsive PNPs have a strong potential of being a safe, targeted, and efficient tool of intracellular drug delivery. The potential of these intelligent nanocarriers is high in the treatment of inflammatory driven disorders and cancer with better therapeutic effects and lesser systemic toxicity.

5.REFERENCES

1. Abulateefeh, SR, Spain, SG, Thurecht, KJ, Aylott, JW, Chan, WC, Garnetta, MC & Alexander, C 2013, 'Enhanced uptake of nanoparticle drug carriers via a thermoresponsive shell enhances cytotoxicity in a cancer cell line', Biomaterials Science, vol. 1, pp. 434-442.
2. Allen, DD, Caviedes, R, Cárdenas, AM, Shimahara, T, Aguilar, JS & Caviedes, PA 2005, 'Cell lines as in vitro models for drug screening and toxicity studies', Drug Development and Industrial Pharmacy, vol. 31, no. 8, pp. 757-768.
3. Allen, LV 2008, 'Dosage Form Design and Development', Clinical Therapeutics, vol. 30, no. 11, pp. 2102-2111.
4. Aryal, S, Hu, CMJ & Zhang, L 2010, 'Polymer-cisplatin conjugate nanoparticles for acid-responsive drug delivery', ACS Nano, vol.4, no. 1, pp. 251-258.
5. Asai, T, Tsuzuku, T, Takahashi, S, Okamoto, A,

Dewa, T, Nango, M, Hyodo, K, Ishihara, H, Kikuchi, H & Oku, N 2014, 'Cell-penetrating peptide-conjugated lipid nanoparticles for siRNA delivery', Biochemical and Biophysical Research Communications, vol.444, pp. 599-604.

6. Aykul, S & Hackert, EM 2016, 'Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis 'Analytical Biochemistry, vol. 508, pp. 97-103.
7. Bae, YH & Park, K 2011, 'Targeted drug delivery to tumors: Myths, reality and possibility' Journal of Controlled Release, vol. 153, no. 3, pp. 198-205.
8. Banerjee, SS & Chen, DH 2008, 'Multifunctional pH-sensitive magnetic nanoparticles for simultaneous imaging, sensing and targeted intracellular anticancer drug delivery', Nanotechnology, vol.19, no. 50, pp. 505104.
9. Banik, BL, Fattahi, P & Brown, JL 2016, 'Polymeric nanoparticles: the future of nanomedicine', Nanomed Nanobiotechnol, vol.8, pp. 271 299.
10. Bao, H, Lin, L, Leong, WC & Gan, LH 2010, 'Thermo-responsive association of chitosan-graft-poly (N-isopropylacrylamide) in aqueous solutions', The Journal of Physical Chemistry B, vol.114, no.32, pp. 10666-10673.
11. Barua, S & Mitragotri, S, 2014, 'Challenges associated with Penetration of Nanoparticles across Cell and Tissue Barriers: A Review of Current Status and Future Prospects' Nano Today, vol. 9, no. 2, pp. 223-243.
12. Bazak, R, Houri, M, ElAchy, S, Hussein, W & Refaat, T 2014, 'Passive targeting of nanoparticles to cancer: A comprehensive review of the literature', Molecular and Clinical Oncology, vol.2, pp. 904-908.
13. Belabassi, Y, Moreau, J, Gheran, V, Henoumont, C, Robert, A, Callewaert, M, Rigaux, G, Cadiou, C, Elst, LV, Laurent, S, Muller, RN, Dinischiotu, A, Voicu, SN & Chuburu, F 2017, 'Synthesis and characterization of PEGylated and fluorinated chitosans. Application to the synthesis of targeted nanoparticles for drug delivery', Biomacromolecules, vol. 18, no. 9, pp. 2756-2766.
14. Binauld, S & Scarano, W & Stenzel, MH 2013, 'pH-Triggered release of platinum drugs conjugated to micelles via an acid-cleavable linker', Macromolecules, vol. 45, no. 17, pp. 6989-6999.
15. Blanco, E, Shen, H & Ferrari, M 2015, 'Principles of nanoparticle design for overcoming biological barriers to drug delivery', Nature Biotechnology, vol. 33, no. 9, pp. 941-951.
16. Cabane, E, Zhang, X, Langowska, K, Palivan, CG & Meier, W 2012,'Stimuli-Responsive Polymers and Their Applications in Nanomedicine', Biointerphases, vol.7, no.9, pp. 2-27.

