Original Article

Synthesis and *in vitro* evaluation of silica-based formulations

Hem Raj Heer*, Vishav Prabhjot Kaur, Raj Kumar Narang, Charan Singh

Department of Pharmacology, ISF College of Pharmacy, Moga, Punjab (Affiliated to IK Gujral Punjab Technical University, Jalandhar, Punjab), India

Correspondence:
Hemraj Heer, Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab, India.
E-mail: hemraj.pharma@gmail.com

How to cite this article: Heer HR, Kaur VP, Narang RK, Singh C. Synthesis and *in vitro* evaluation of silica-based formulations. Pharmaspire 2019;11(4):124-127.

Source of Support: Nil,
Conflicts of Interest: None declared.

ABSTRACT

Background: The cytotoxicity of the silica-based nanoformulations was investigated using non small cell human lung cancer cell line and RAW 264.7 macrophages cell line. **Method:** The formulations were exposed for 24 h at different dosage levels. 3-[4, 5-dimethylthiazol2-yl]-2, 5-diphenyl tetrazolium bromide assay was used to assess the cell response to the each formulation. **Results:** The formulations were found to have non-toxic in nature. **Conclusion:** These nanoformulations could be potentially used for delivering therapeutics for improved biopharmaceutical attributes.

Keywords: Cell line, cell viability, chitosan, cytotoxicity, porous aggregated nanoparticles

INTRODUCTION

Recently, to avoid the use of animals for testing toxicity aspects of newly developed nanoformulations various *in vitro* studies is performed.[1] Consequently, *in vitro* cytotoxic methods have gain tremendous attraction for assessing the toxicity of environmental, occupational health risks, and nanodrug delivery.[2] The analysis of cytotoxicity is needed in various biological fields, including toxicology, drug delivery, and ecotoxicology to determine the toxic effects of the chemicals, new drugs, or the environmental samples.[3] *In vitro* evaluation is easy to handle and to keep under a controlled environment. These assays are also faster and cheaper to conduct therefore suitable for testing different compounds simultaneously and screening for chemical toxicity can be performed at a lower cost than in animal models.[4] Moreover, they are helpful in predicting acute toxicity and determining the toxic conditions *in vivo*. It identifies the starting dose for the *in vivo* study and reduces the number of necessary animals for such determinations.[5] These assays in 96 well tissue culture plates allow rapid sample handling for multi-conditional experiments.[6] Furthermore, human cell cultures eliminate, to some extent, the complexity and variability of human metabolisms and biochemical phenomena. *In vitro,* evaluation of cytotoxic compounds includes various traditional methods such as counting of viable cells after staining with dye, measurement of radioisotope incorporation as measurement of DNA synthesis, automated cell counter, and measurement of ATP elevation level.[7] However, 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) assay and neutral red assay have become the most popular methods to determine the cytotoxicity of various compounds.[8] Chitosan (CHT) has been investigated extensively as a potential drug carrier because of its biocompatible properties.[9] Some studies have suggested using CHT to coat nanoparticles (NPs) made of other materials to reduce their impact on the body and increase their bioavailability. Several research groups have studied the properties of CHT NPs with a view to using them as a drug delivery agent. The biocompatibility and non-toxicity of the material makes it attractive as a neutral agent for delivery of active agents.[10] Due to the positive charge of CHT, it can bind with the negatively charged mucus. Thus, CHT can act as an excellent carrier for mucoadhesive drugs. CHT -based NPs have been used to deliver drugs to the lungs, with CHT helping to attach to the lung mucosa. Moreover, CHT NPs also used as antibacterial agents, nasal drug delivery; wound infection, gene delivery vectors, and carriers for protein release and drugs.[11] Large porous NP systems can be made of diverse materials, prepared in a variety of different conditions, and designed to deliver drugs to specific sites of the body using NPs with diameters ranging from 25 nm to 700 nm.[12] They appear to be robust drug delivery systems that may be useful for encapsulating drugs of varying chemistry and molecular weight into biodegradable NPs.[13]

Access this article online	
Website: www.isfcppharmaspire.com	**P-ISSN:** 2321-4732 **E-ISSN:** XXXX-XXXX

The aim of this study was to synthesize polyaniline nanoparticles (PANPs) and to investigate their cytocompatibility on murine macrophage cell line (RAW264.7) and non-small cell lung cancer (A-549) cell lines.

MATERIALS AND METHODS

Materials

CHT was obtained from Sigma-Aldrich, India. MTT was obtained from Sigma-Aldrich, India. Dimethyl sulfoxide (DMSO) was obtained from HiMedia, New Delhi, India. All other chemicals used were of analytical grade.

Methods

Synthesis of porous NP aggregate systems (porous nanoparticle-aggregate particles [PNAPs]) with CHT

The synthesis of PNAPs involved two steps. First, CHT solution was prepared by dissolving accurately a weighed amount of CHT in 1% w/v of acetic acid (100 ml). Thereafter, the pH of solution was maintained ~5 using 0.1 N NaOH. The solution was kept on stirring for homogenous mixing overnight. Second, sodium acetate solution (0.05 M) was prepared by dissolving 0.6805 g of sodium acetate in PBS pH 7.4 (100 ml). Finally, sodium silicate was dissolved in 0.05 M sodium acetate solution to prepare a sodium silicate solution. A known volume of sodium silicate solution was agitated for 30 min on the magnetic stirrer and then, CHT solution ($1/10^{th}$ of volume of silica solution) was added dropwise under constant stirring.

Synthesis of PNAPs with Tween 20 and Span 20

By adopting the same procedure in the previous section, two more formulations of the PNAPs were prepared using 0.1 ml of Tween 20 and Span 20 (0.1% v/v each), respectively.

Particles size and zeta potential measurement

The different NPs were analyzed by Zetasizer for their size, polydispersity index, and Zeta potential (Delsa Nano C-Particle Analyzer, Beckman Coulter,).

Surface morphology studies

The surface characteristics were studied by scanning electron microscope (JEOL, Japan). The powder sample was sprinkled onto the carbon tape affixed on aluminum stubs. Thereafter, aluminum stubs were placed in the vacuum chamber and observed for morphological characterization.

Cell-based assays

For conducting cell-line studies, A-549 non-small cell lung cancer and Raw 264.7 cell lines were obtained from NCCS Pune, India. They were maintained in nutrient mixture F-12 Hem, Kaighn's and Dulbecco modified Eagle Medium supplemented with 10% inactivated fetal bovine serum, 100 U/ml penicillin, and 100ul/ml streptomycin incubated at 37°C with 5% CO_2 (HiMedia). For this, 1 mg/ml stock solution was prepared for different formulations of PNAPs.

MTT assay

The cytotoxicity of silica nanopartical was determined by tetrazolium-based colorimetric assay (MTT assay). Cells were plated in 96 – well plates at 1.5×10^3 per 200 µl per well with density determined on the basis of growth characteristics of each cell line and incubated overnight. Cells were treated in triplicate with varying concentration of silica nanoparticals ranging from 1 to 1000 µg/ml and incubated for 24 h. After 1 day, treatment medium was replaced with 5 mg/ml MTT solution (HiMedia) and cells were incubated for 3 h. Formazan crystals were dissolved in DMSO. The relative percentage of metabolically active cells compared with untreated controls was then determine on the basis of mitochondrial conversion of MTT to formazan crystals that were dissolved in DMSO. Formazan crystals were dissolved in DMSO. Spectrophotometric absorbance of treated cells was determined by microplate reader (BIORAD) at 570/630 nm. Concentrations of NPs showing 50% reduction in cell viability (i.e., IC_{50} values) were then calculated.

RESULTS AND DISCUSSION

Particle size and zeta potential

The particle size and zeta potential of developed PNAPs ranges from 370 nm to 446 nm and 20 mV to 22 mV, respectively. All the developed nanoformulations showed good dispersibility index, i.e., <0.4; which shows their monodisperse nature. The obtained nanosize range could be due to continuous stirring at ambient temperature. Results are compiled in Table 1.

Surface morphology studies

Figure 1 presents the scanning electron microscopy results. It could be clearly seen that PNAPs exhibited almost spherical shape. Figure 1a depicts CHT coated PNAPs/CHT; however, on the other hand, Figure 1b and c displays PNAPs-Tween 20 and PNAPs-Span 20, respectively.

Cell line studies

We studied the effect of PNAPs in different concentrations on cell viability. As expected, we find that the cell toxicity is material composition and concentration dependent but not much cell type variation in their cytotoxic effect in both cell lines. The viability of A-549 and RAW 264.7 is shown in Figures 2 and 3, respectively. Cells vary in dose-dependent manner as the concentration of formulations increase from 1 ug/ml to 1000 ug/ml. The findings of MTT assay indicated that cell viability was more than 75% in A-549 and it was around 70% in RAW 264.7 as the dose increase from 1 ug/ml to 100 ug/ml. The findings of the cell viability studies confirmed the non-toxic nature of the nanoformulations. However, cell viability reduces to approximately 30% as the dose increase from 100 ug/ml to 1 mg/ml which quite high dose.

Table 1: Particle size analysis

Formulations	Particle size	PDI	Zeta potential
PNAP/CHT	446 nm	0.194	22.20 mV
PNAP+Tween20	383 nm	0.210	20.11 mV
PNAP+Span20	372 nm	0.299	21.63 mV

PNAP: Porous nanoparticle-aggregate particles, CHT: Chitosan

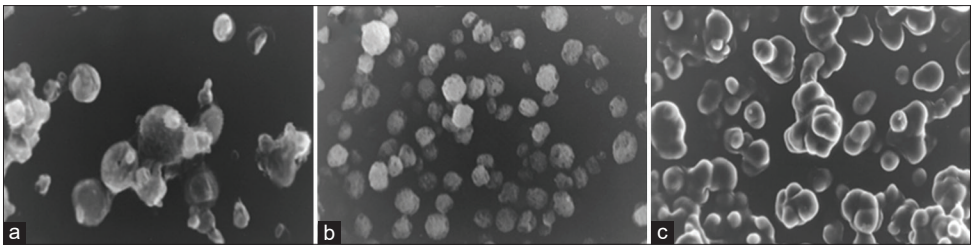

Figure 1: Surface morphology analysis (a) Porous nanoparticle-aggregate particles (PNAP)/chitosan (b) PNAP-Tween 20 and (c) PNAP-Span 20

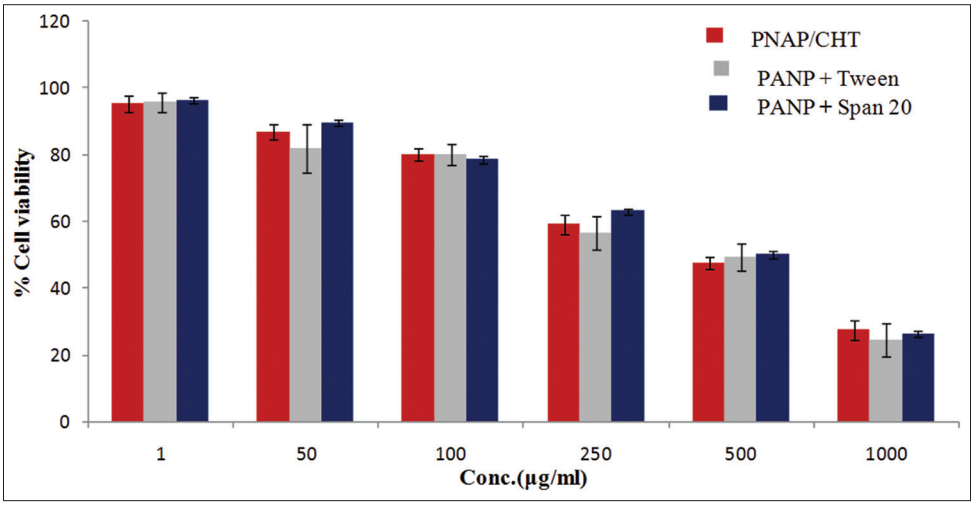

Figure 2: Cell viability data of A-549 non-small cell cancer cell lines

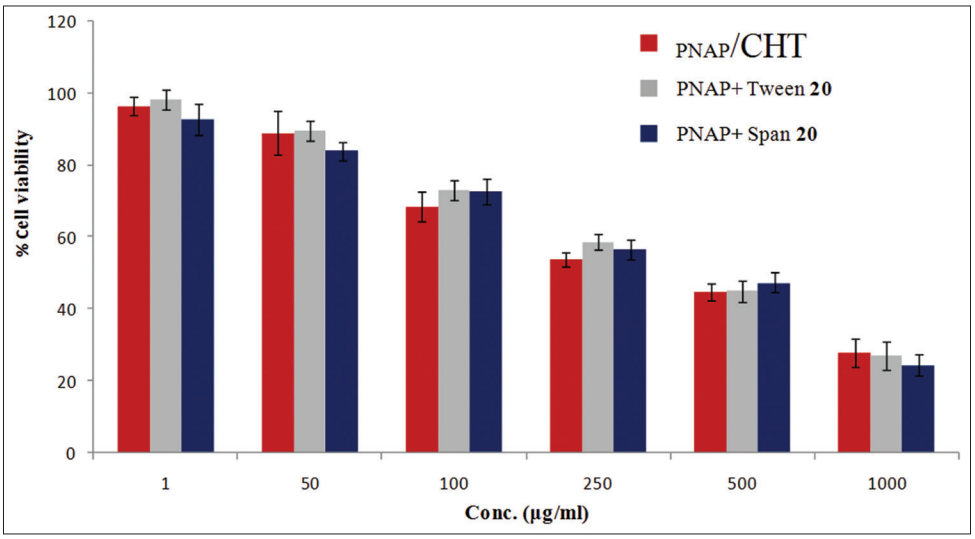

Figure 3: Cell viability data of RAW 264.7 macrophage cell lines

CONCLUSION

The PNAPs were developed systematically and characterized in terms of particles size and zeta potential. Surface morphology studies evident the spherical nature of the formulations. In addition, cytotoxicity evaluation of formulations using non-small cell lung cancer and RAW 264.7 macrophages cell line showed negligible cell death up to a dose of 100 µg/ml in both the cell lines. Finally, these PANPs could be loaded with therapeutics for delivery of diverse biomedical applications.

ACKNOWLEDGMENT

The authors are thankful to NCCS, Pune, for providing us A549 and Raw 264.7 cell lines and Punjab University, Chandigarh, for providing the facility of scanning electron microscope.

REFERENCES

1. Jones CF, David WG. *In vitro* assessments of nanomaterial toxicity. Adv Drug Deliv Rev 2009;61:438-56.
2. Vinay K, Neha S, Maitra SS. *In vitro* and *in vivo* toxicity assessment of nanoparticles. Int Nano Lett 2017;7:243-56.
3. Daniel K, Daniel A, Melvin A, Henry A, Johen CB, Kim B, *et al*. Toxicity testing in the 21st century: A vision and a strategy. J Toxicol Environ Health B Crit Rev 2010;13:51-138.
4. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother 2011;2:74-9.
5. Gregory N, Paul SK, Dordick JS, Kwon SJ. Cell-based assay design for high-content screening of drug candidates. J Microbiol Biotechnol 2016;26:213-25.
6. Kristine MG, Karla FS, Lawrence EM. Cytotoxicity and reactive oxygen species generation from aggregated carbon and carbonaceous nanoparticulate materials. Int J Nanomedicine 2008;3:83-94.
7. Keshawa EM, Hemamala TK, Ranganath SS. *In vitro* assays and techniques utilized in anticancer drug discovery. J Appl Toxicol 2019;39:38-71.
8. Ashutosh B, Imran K, Vivek KB, Chul KS. MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J Pharmacol 2017;12:115-8.
9. Mukred SR, Isra D, Mutasem OT. Stable chitosan-based nanoparticles using polyphosphoric acid or hexametaphosphate for tandem ionotropic/covalent crosslinking and subsequent investigation as novel vehicles for drug delivery. Front Bioeng Biotechnol 2020;l8:1-21.
10. Divya K, Jisha MS. Chitosan nanoparticles preparation and applications. Environ Chem Lett 2018;16:101-2.
11. Antonio R, Massimiliano B, Blasi P, Barbara P, Attilio CB. Chitosan nanoparticles: Preparation, size evolution and stability. Int J Pharm 2013;455:219-28.
12. Saeed AM, Najma S. Porous nanoparticles in drug delivery systems. Pak J Pharm Sci 2006;19:155-8.
13. Valentin V, Lubomira T. Porous nanosized particles: Preparation, properties, and applications. Chem Rev 2013;113:6734-60.