Journal of Applied Pharmaceutical Science Vol. 8(08), pp 144-150, August, 2018
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2018.8820
ISSN 2231-3354
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Synthesis, Characterization of Novel PLGA Encapsulated Indole Nanoparticles and Study of its cytotoxic potential against A549 lung cancer cell line

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ARTICLE INFO

Article history:
Received on: 19/05/2018
Accepted on: 04/07/2018
Available online: 31/08/2018

Key words:
Nanoparticles; biomaterials; polymers; sustained release; cytotoxicity.

ABSTRACT

Objectives: Indole and its derivatives are gaining importance because of their anti-cancer activity. Here, we have reported the synthesis and characterization of novel polymeric poly D, L-lactide-co-glycolide (PLGA) indole nanoparticles, and investigated their cytotoxic potential against A549 lung cancer cells. **Materials and methods:** Nanoparticles were synthesized by solvent emulsion-diffusion-evaporation method. Size determination was done by Transmission Electron Microscopy (TEM), encapsulation efficiency using UV-Vis spectra, release kinetics using dialysis, measurement of drug-polymer interaction by Fourier Transform Infra Red Spectroscopy (FTIR) and surface charge by zeta potential. Cell viability of lung cancer cells (A549) was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and morphological analysis. **Results:** Nanoparticles were spherical in shape with an average diameter of 65 nm, encapsulation efficiency was found to be about 78% and zeta potential was −15.2mV. Drug-loaded nanoparticles showed sustained release kinetics fitting well in exponential Higuchi and Zero order Model. FTIR studies showed a broadening of the peak of PLGA indole nanoparticles at 2100-3400 cm^{-1} indicating the formation of drug-loaded nanoparticles. These nanoparticles showed about 95% cytotoxicity against A549 lung cancer cell lines. Results were supported by visible morphological changes in cells. **Conclusion:** PLGA encapsulated Indole nanoparticles were stable, having sustained release and good cytotoxic potential.

INTRODUCTION

Cancer has emerged as a debilitating complex disease over the last many decades. Although advances in research in some cancers have led to the successful prognosis of the disease, in most cases especially in advanced stages, it continues to remain incurable. Development of new generation of drugs with minimal side effects continues to be the mainstay of all research work undertaken to combat this deadly disease. The advent of nanotechnology has brought new hope in cancer treatment by targeted delivery, increased half-life, better stability and sustained release of the drug and hence helped in mitigating the problem of side effects (Hariharan *et al.*, 2006). There are reports of different classes of nanoparticles that might serve as potential anti-cancer agents themselves. The class of nanoparticles ranges from transition metal oxides (Pandey *et al.*, 2016; Tarnuzzer *et al.*, 2005; Sankar *et al.*, 2014), chitosan derivatives (El-Sayed *et al.*, 2017) to ceramics (which includes hydroxyapatite nanoparticles) (Kundu *et al.*, 2013). However, an alternate and popular approach in the field of nanomedicine is by either encapsulating the anti-cancer agent or adsorbing it in delivery vesicles i.e. nanoparticles (Mirza and Siddiqui). The agents that are used for encapsulating the drugs

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should be biocompatible in nature, and therefore, polymers have gained much importance. PLGA (Poly D, L-lactide-co-glycolide) is one such polymer widely used in drug delivery. These have been reported to have biocompatible nature, sustained release and non-immunogenic properties. Alternatively, other agents have also been experimentally proven to have efficient anti-cancer activities, however, the hydrophobic nature of the majority of such compounds restricts their bioavailability and lack of proper targeting reduces their efficacy and brings about undesirable side effects.

Natural products have served over the ages as potential anti-cancer agents like curcumin, quercetin, *Vitex agnus-castus* extract, etc. (Ghosh *et al.*, 2012, Ghosh *et al.*, 2010, Ibrahim *et al.*, 2017) in lieu of their potent antioxidant capabilities. Nitrogen, containing aromatic heterocycles, makes a major class of natural compounds that have medicinal applications. Among the aromatic nitrogenous heterocycles that serve as potential scaffolds for experimental anti-cancerous drug design, indole and its derivatives constitute a very important clan (Patel *et al.*, 2012; Chu *et al.*, 2011). Indole and its derivatives have shown promising anti-cancer activities rendering them of high interest to researchers (Patel *et al.*, 2012). Indole conjugated Gold nanoparticles have been reported to function as anticancer agents when used individually and in combination with therapeutic doses of ionizing radiation (Jain *et al.*, 2012). Indole has been found to regulate several microbial processes like motility, biofilm formation, quorum sensing, among others (Li and Young 2013; Lee *et al.*, 2009; Kim *et al.*, 2010).

Till now, experimental work has been primarily done with indole derivatives like Indole-3-acetic acid (IAA), Indole-3-carbinol for their putative anti-cancer properties. Indole and its derivatives are potent inhibitors of tubulin polymerization as well as DNA topoisomerases, leading to their screening as anti-cancer drugs. Indole-3-carbinol has been found to be effective against MCF-7 cell line, both *in vitro* and *in vivo* (Wang *et al.*, 2013). These derivatives have shown better results and fewer side effects compared to conventional chemotherapeutic drugs against various cancer cell lines (Wang *et al.*, 2013; Kim and Milner, 2005; Luo *et al.*, 2013).

However, reports suggest that Indole-3-carbinol have a tendency to oligomerize in acidic and even neutral conditions. This oligomerization reduces its bioavailability following oral administration, and finally in the gastric conditions of the stomach (Kim and Milner, 2005). To overcome this problem, chitosan nanoparticles of indole-3-carbinol have been prepared to improve the stability of the compound for better efficacy (Luo *et al.*, 2013). PLGA nanoparticles of Indole-3-Acetic acid have been reported to be effective against breast, colon, and prostate cancer cell lines (Shaikh *et al.*, 2009). Since indole derivatives have been shown to be effective against human cancers; indole also seems to be very promising for being an effective anti-cancer agent. However, keeping in mind the instability of indole and its derivatives, encapsulating indole in nanoparticles should work better in *in-vitro* and *in vivo* conditions.

Till now, there is no report available on the preparation of nanoparticles of indole itself. Here, we report an emulsion-diffusion-evaporation method for the preparation of PLGA encapsulated indole nanoparticles with controlled size and sustained release properties fitting well with exponential Higuchi model and zero order model of drug release kinetics (Gouda *et al.*, 2007; Thakkar *et al.*, 2009). These nanoparticles have shown efficient anti-cancer activities by increasing cell cytotoxicity against A549 lung cancer cell line.

MATERIALS AND METHODS

Materials

Polylactide-co-glycolide (PLGA) (Resomer RG 85:15H), Polyvinyl alcohol (PVA, MW:30000-70000), Indole were purchased from (Merck, India). A549 cell line used in the study was obtained from NCCS, Pune Dulbecco's modified Eagle's medium (DMEM), (GIBCO, USA) supplemented with 10% fetal bovine serum and 5 mg/ml ciprofloxacin (Sigma, USA) was used for cell culture. Cells were cultured in a humidified incubator at standard condition of 18% O_2 and 5% CO_2 at 37°C for an appropriate period of time using similar procedure (Pandey *et al.*, 2016).

Preparation of PLGA encapsulated indole nanoparticle

A modified emulsion-diffusion-evaporation method was used to make the nanoparticles using the similar procedure as described by Ghosh *et al.* (2010). In brief, 50 mg of PLGA was dissolved in 5ml of ethyl acetate at room temperature. Indole (5 mg) was dissolved in 2 ml of ethyl acetate. The organic solution of PLGA and drug in ethyl acetate was then emulsified with 10 ml of an aqueous phase containing 2% polyvinyl alcohol (PVA). The resulting o/w emulsion was stirred at room temperature for three hours before homogenizing at 15,000 rpm for 5 min with a high-speed homogenizer. The organic solvent was removed by constant stirring on a water bath set at room temperature. The suspension was centrifuged at 105,000 g in Sorval RC 5B Plus using the rotor sorval T-865 for one hour. Nano pellet was washed and resuspended in PBS buffer.

Physicochemical characterization of nanoparticles

Encapsulation efficiency

To estimate the encapsulated drug in the nanoparticles, the pellets were dissolved in ethyl acetate and kept for three days at 4°C. The O.D was measured at λ_{max} (Indole) 545 nm using a unicam UV-Vis spectrophotometer. The percentage of encapsulation was calculated from the absorbance value.

Particle size analysis

The nanoparticle suspension was sonicated properly, and then a drop was added to a carboncoated copper grid, dried and observed under Transmission Electron Microscopy (TEM) for particle size analysis. TEM was performed at an accelerating voltage of 300 kV. Photomicrographs were obtained using TEM (Tecnai G2 30, FEI, Netherlands) using the same procedure as described by Ghosh *et al.* (2010).

Release kinetics

This was carried out in a phosphate buffer saline (PBS) medium, according to the method of Shaikh *et al.* (2009). A small amount of nanoparticle suspension was kept in a dialysis bag and the bag was immersed in a sink (PBS solution) at 37°C with

moderate shaking. At different time periods, a known amount of the sink solution was withdrawn and the absorbance was taken at 280 nm using a UV-Vis spectrophotometer. The amount withdrawn was replaced by fresh medium. This experiment was performed in triplicate and the mean value was taken.

Mathematical model fitting of the release kinetics data

Different mathematical models were utilized to understand drug release kinetics of indole from PLGA nanoparticle. Here, the data was fitted to Zero order, First order and Higuchi model (both in linear and exponential mode) respectively (Dash *et al.*, 2010). For Zero order model, the working equation taken was $C_t = C_0 + K_0 t$ (Dash *et al.*, 2010)

C_t = amount of drug released at time t,

C_0 = initial concentration of drug at time $t = 0$,

K_0 = zero-order rate constant.

For the First order model, the equation used was $DC/dt = -K_1 C$.

K_1 is the first order rate constant, expressed in time^{-1} or per hour (Dash *et al.* 2010).

However, one of the most prominent models used in current day drug release kinetics is the Higuchi model which is based on both diffusion and dissolution of the drug (Subal, Patil *et al.* 2007). The classical basic Higuchi equation is represented by $Q = (D_\delta/\tau(2C - \delta.C_s)C_s.t)^{1/2}$

Where, Q = cumulative amount of drug released in time t/ unit area, C_O = initial drug concentration, C_S = drug solubility in the matrix and D = diffusion coefficient of the drug molecule in the matrix. In all of these aforementioned models, suitable parameters were plotted and from the value of $R^{2,}$ the fitting of the data to a particular model was analyzed.

Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared spectroscopy (FTIR) was used for detecting any possible drug-polymer interaction. For the FTIR analysis, the nanoparticle samples were dried and grounded with KBr pellets and analyzed on a Nicolet IR 200 (Thermo Electron Corp, US).

Surface charge

The nanoparticles were sonicated properly and their surface charges were measured using a zeta sizer (ZetasizerNanoZS; Malvern Instruments, Malvern, UK).

Cytotoxicity analysis

The cytotoxic potential of the PLGA-Indole nanoparticles was assessed using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) as described previously by (Pandey *et al.*, 2016). Wells containing complete medium, nanoparticles and MTT reagent without cells were used as blanks.

Cell morphology

About 3×10^4 cells were seeded in four-well chamber slide, and, after overnight culture, treated with PLGA indole nanoparticles (10 µg/ml, 30 µg/ml, 50 µg/ml) for 6 days at 37°C. Cells were then quickly washed with 1 ml PBS and visualized by a Zeiss Axiovert (Germany) 40 C microscope at a magnification of 40X.

Statistical analysis

The mean and standard error was calculated for cytotoxicity analysis and release kinetics data. Student's 't-test' was used for the statistical validation of cytotoxicity data. $P < 0.05$ was taken as the minimum level of significance.

RESULTS AND DISCUSSION

Physicochemical characterization

Transmission electron microscopy (TEM) analysis

The PLGA-indole nanoparticles formed were spherical in shape with an average diameter of 65 nm (Figure 1a). The nanoparticles formed did not exhibit any agglomeration. The small size of nanoparticles is crucial for increased cellular activity as the cellular uptake and biological functions of colloidal systems are largely controlled by particle size where a reduced size results in a significantly better uptake of these nanoparticles by the tumor cells and increased half-life in plasma (Murugan *et al.*, 2015).

Encapsulation efficiency

High encapsulation efficiency of the drug encapsulated nanoparticles is preferable for efficient use of the drugs used to prepare the nanoparticles. This is also important since it requires a lesser amount of nanoparticles to achieve a particular effective dose, thus, reducing the chances of any possible toxicity that might result from the agent used to encapsulate the nanoparticles. The encapsulation efficiency of the nanoparticles was found to be 78%, indicating better encapsulation of indole in PLGA as compared to the other reported nanoparticles of indole derivatives till date (Luo *et al.* 2013).

Release kinetics

Release kinetics is an important parameter of nanomedicine as it indicates its efficacy and stability. Effective anti-cancer treatment demands sustained release of the drugs. An early burst release is sometimes preferable, followed by a sustained release. The initial burst release gives maximum relief immediately and the following sustained release is desirable to avoid repeated administration (Preetha *et al.*, 2015). However, generally, the most preferable release pattern for a drug encapsulated in a nanoparticle is sustained release over a prolonged period of time. Here, in this work release pattern of the PLGA encapsulated indole nanoparticles showed a sustained release over a period of 20 days (Figure 1b). After 20 days, the cumulative release was 50%. The sustained release pattern is desirable for nanoparticles in treating cancer cells as the drug works much better than the nanoparticles showing only burst release kinetics. The chitosan nanoparticles of indole-3-carbinol & 3,3´-diindolemethane synthesized by Luo *et al.* (2013) showed a burst release within the first half-an-hour, followed by a sustained release, about 80% over a period of only seven hours. Indole nanoparticles showed a sustained release pattern persisting over a period of 20 days and without any burst release, confirming the much better stability of the nanoparticles as compared to previous reports till date (Luo *et al.*, 2013).

Mathematical model fitting of the release kinetics data

Release kinetics data of indole was fitted against Zero

order model, First order model, Higuchi model (linear and exponential mode) respectively (Figure 2). R^2 values of all them were analyzed, and it was found that the exponential Higuchi model and Zero order model show the best fit for the release kinetic data [$R^2 = 0.9853$ and $R^2 = 0.972$].

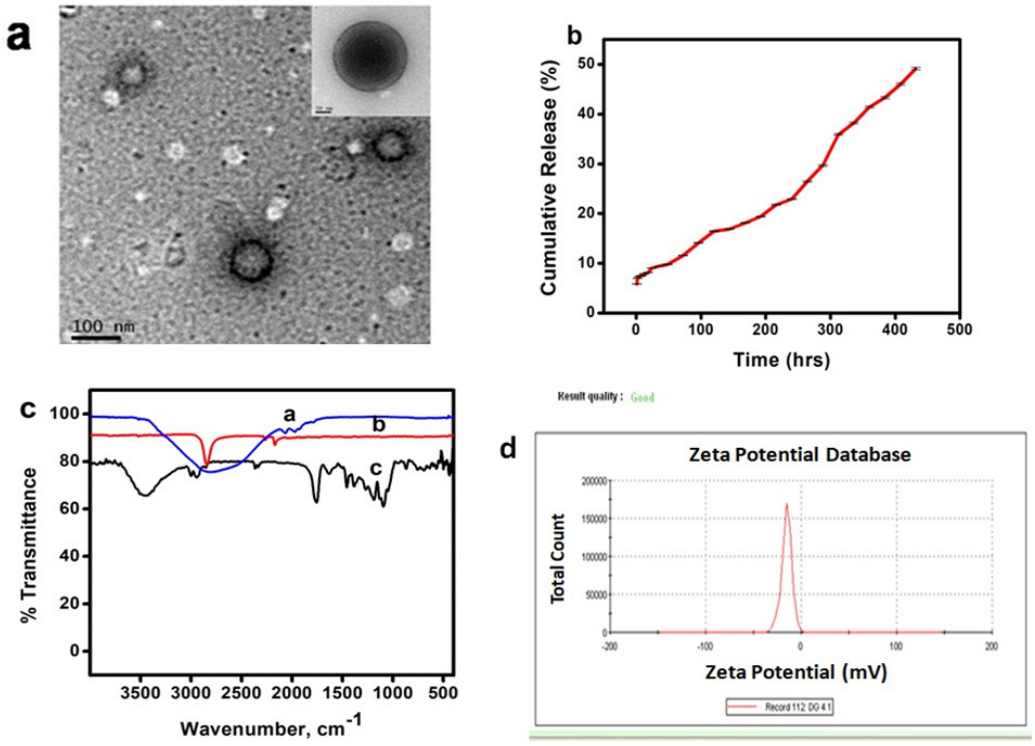

Fig. 1: Physicochemical Characterization of PLGA encapsulated indole nanoparticles showing Transmission Electron Micrographs (TEM) (a) release kinetics in *in vitro* conditions (PBS at 37°C), results are replicated in triplicates (b) and FTIR-spectrum (c) a) PLGA-Indole nanoparticles, b) Indole, c) PLGA.

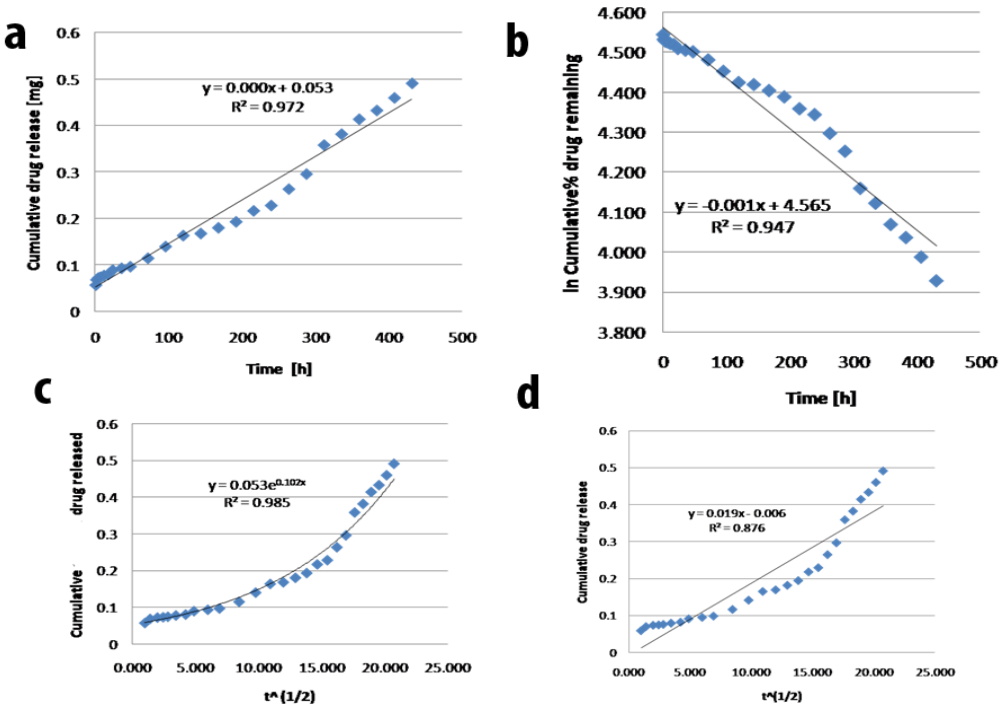

Fig. 2: The mathematical Model fit of release Kinetics data a) Zero Order Model b) First Order Model c) Higuchi Model (exponential) d) Higuchi Model (linear).

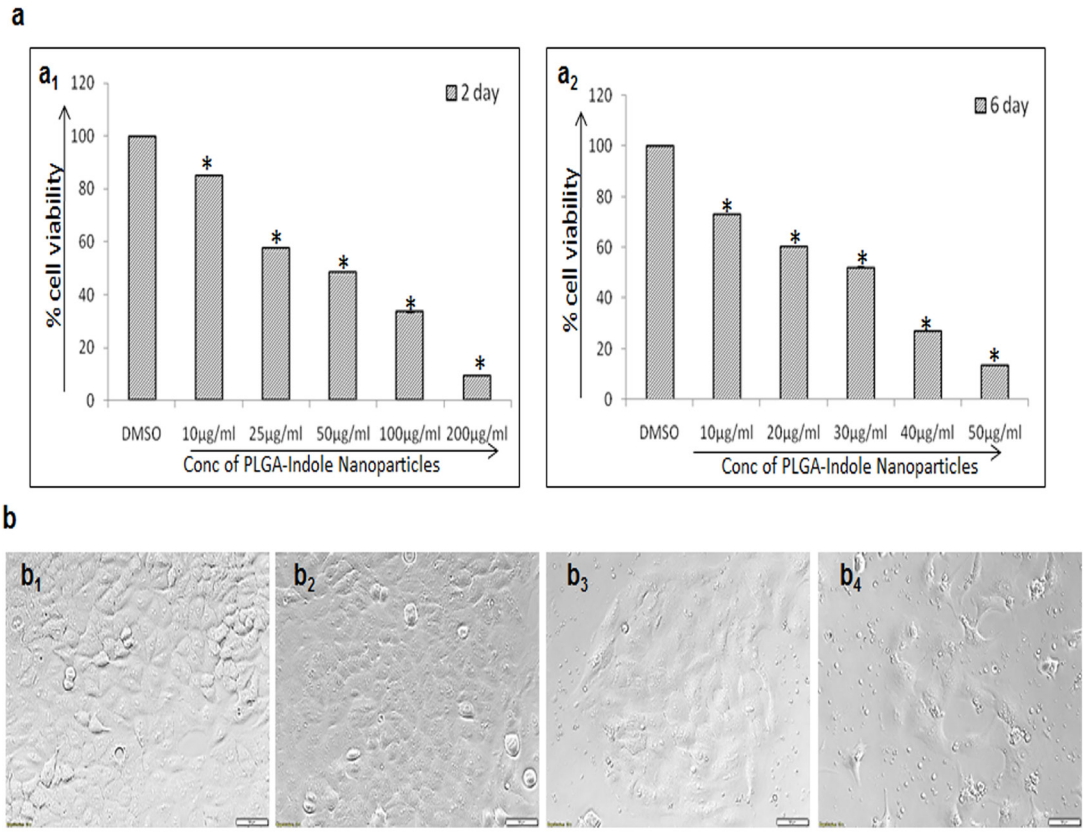

Fig. 3: (a) *In vitro* cell cytotoxicity (MTT Assay) exhibited by PLGA-Indole nanoparticles on A549 cells, after 2 days (a_1) and 6 days (a_2) incubation. (* = $P < 0.05$ vs. DMSO control). [Each bar represents a minimum of 18 data points from three biological replicates and six technical replicates analyzed of each biological replicate. The standard deviation is negligible in most cases.] (b) Representative microphotograph of DMSO treated control cells and PLGA-Indole nanoparticle-treated cells on A549 cells after 6 days of incubation at 60X magnification.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis is an important technique in determining any drug-polymer interaction. It is also helpful in judging the effective formation of drug encapsulated nanoparticles. A comparison of FTIR spectra of the free drug, polymer and the polymer encapsulated drug generally presents a good assessment of the extent of encapsulation through the peak shifting of functional groups. Here in this work, the FTIR analysis (Figure 1c) revealed no major shift as well as no loss of functional peaks of the spectra of drug-loaded PLGA nanoparticles compared to that of indole and native PLGA, indicating no major drug-polymer interactions that can change the effective nature of any functional group. A similar observation was reported by Ghosh *et al.* (2012) for PLGA encapsulated curcumin nanoparticles. The broadening of the peak of PLGA encapsulated indole nanoparticles at the region 2100-3400 cm^{-1} is indicative of interaction of secondary N-H group of indole with encapsulating PLGA moiety, suggesting the successful formation of PLGA encapsulated indole nanoparticles.

Surface charge

Zeta potential of the nanoparticles was found to be −15.2 mV (Figure 1d). Previous reports on A549 cells showed that these cells indicated mean ZP of −10.2 mV with a deviation of ±19.7 mV (Honary *et al.*, 2007). This suggests that these negatively charged nanoparticles bind to some specific cationic sites of the cell membrane in a cluster-wise manner (Patila *et al.*, 2007). High cellular uptake of negatively charged nanoparticles, therefore, seems to be a combination of non-specific adsorption and cluster formation of nanoparticle on cell membrane surface.

Cytotoxicity analysis

The cytotoxic potential of PLGA indole nanoparticles against non-small cell lung cancer cell lines was studied. The nanoparticles significantly decreased the viability of A549 cell lines in a dose-dependent manner (Figure 3) from 85% to 10% in two days and from 75% to 15% in 6 days. The IC_{50} value for PLGA indole nanoparticles in A549 was found to be ~50 µg/ml and ~30 µg/ml respectively for 48 hours (two days) and 144 hours (six days) treatment (Figure 3a). The prolonged incubation of A549 cells with 50 µg/ml of indole PLGA nanoparticles, showed an increase in cellular cytotoxicity from 50% to 95% suggesting a better effect of nanoparticle due to sustained release (Figure $3a_1$ and Figure $3a_2$). It was observed that indole PLGA nanoparticles caused appreciably higher cytotoxicity to A549 cells at all doses as compared to DMSO control, the results were statistically significant at $P < 0.05$.

Cell morphology

The photomicrographs of the A549 cells treated with different concentration of PLGA indole nanoparticles showed

changes in a cellular structure like reduced cell size, pyknosis, karyorrhexis, indicating the cytotoxic effect of nanoparticles. The decrease in a number of viable A549 cells with increasing concentrations of PLGA indole nanoparticles (10 µg/ml, 30 µg/ml, 50 µg/ml) as compared to untreated cells too suggested potent cytotoxicity as shown by the nanoparticles.

CONCLUSIONS

Stable and biocompatible PLGA encapsulated indole nanoparticles were successfully synthesized. The nanoparticles showed considerably small particle size with good encapsulation efficiency and sustained release properties fitting very well in exponential Higuchi model and Zero order model. TEM and FTIR analysis confirmed the formation of the drug-loaded nanoparticles. These nanoparticles showed about 95% cytotoxicity to A549 cancer cells at 50 µg/ml concentration after six days. Use of high concentration of anticancer drugs is seen to have significant side-effects in patients. Hence, there is a need to develop effective drug delivery systems containing low amounts of the anticancer drug with sustained release properties. The data obtained in our experiments suggest that effective cytotoxicity is achieved with a reduced concentration of the anti-cancer drug in the PLGA nanoparticles on increasing the duration of incubation of these nanoparticles with the cancer cells. This is a promising therapeutic intervention achieved with our formulation.

These highly stable PLGA encapsulated indole nanoparticles appear to be potentially promising anti-cancerous agents. Further *in vitro* and *in vivo* studies need to be performed to assess and characterize its function *in vitro* and *in vivo.*

ABBREVIATIONS

PLGA: poly D, L-lactide-co-glycolide;

TEM: Transmission Electron Microscopy;

FTIR: Fourier Transform Infra Red Spectroscopy;

MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide**.**

CONFLICT OF INTEREST

The authors do not have any financial, personal or another conflict of interest.

ACKNOWLEDGMENTS

We thank Dr. Biswanath Kundu of Central Glass and Ceramic Research Institute, Kolkata, for his help in TEM data collection. TS is thankful to UGC-SAP (BRS-III), DU-DST PURSE (Phase II) and DST-FIST (Level II). DG and SM acknowledge Amity University Haryana for their support.

CONTRIBUTION FROM AUTHORS

Sudip Majumder has done the synthesis of the drug-loaded nanoparticle and all the characterization like TEM, FTIR, and Zeta potential and analyzed the data and also contributed in writing the manuscript, Neha Sharma has performed the release kinetics and encapsulation efficiency and also contributed to the synthesis, Subhra Das has done the mathematical model fitting of the release kinetics data, Namita Pandey and Tapasya Srivastava have done the cytotoxicity analysis and cell morphology study. Debasree Ghosha has conceptualized the entire idea, optimized the formulation, written the manuscript and overall supervised the work.

REFERENCES

Chu W, Zere TR, Weber MM, Wood TK, Whiteley M, Romano E, Valenzuela BH, Mc Lean RJC. Microfluidic platform for rapid antibiotic susceptibility testing of polymicrobial communities. Appl and Environ Microbiol, 2011; 78:411-419.

Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm, 2010; 67:217-223.

El-Sayed ST, Omar NI, El-Sayed MES, Shousha WG. Evaluation Antioxidant and cytotoxic activities of novel chitooligosaccharides prepared from chitosan via enzymatic hydrolysis and ultrafiltration. Journal of App Pharm Sci, 2017; 7(11):050-055

Ghosh D, Choudhury ST, Ghosh S, Mandal AK, Sarkar S, Ghosh A, Saha KD, Das N. Nanocapsulated curcumin: Oral chemopreventive formulation against diethylnitrosamine induced hepatocellular carcinoma in rat. Chem Biol Interact, 2012; 195:206–214.

Ghosh, D, Ghosh S, Sarkar S, Ghosh A, Das N, Saha KD, Mandal AK. Quercetin in vesicular delivery systems: Evaluation in combating arsenic-induced acute liver toxicity associated gene expression in rat model. Chem Biol Interact, 2010; 186:61–71.

Gouda R, Vaishya H, Qing Z. Application of mathematical models in drug release kinetics of carbidopa and levodopa ER tablets. J Dev Drugs, 2007; 6(2):1-8.

Hariharan S, Bhardwaj V, Bala I, Sitterberg J, Bakowsky U, Ravi Kumar MNV. Design of Estradiol loaded PLGA Nanoparticulate formulations: a potent oral delivery system for Hormone therapy. Pharm. Res, 2006; 23(1):184–195.

Honary S and Zahir F. Effect of zeta potential on the properties of Nano-Drug Delivery Systems – A review (Part 1). Trop J Pharm Res, 2013; 12(2):255–264.

Ibrahim AY, El-Newary SA, Youness ER, Ibrahim AMM, El Kashak WA. Protective and therapeutic effect of *Vitex agnus-castus* against prostate cancer in rat. Journal of App Pharm Sci, 2017; 7(12):133-143.

Jain S, Hirst DG, Sullivan JMO. Gold nanoparticles as novel agents for cancer therapy. Br. J. Radiol, 2012; 85(1010):101-113.

Kim SY, Ryu JS, Li H, Park WJ, Yun HY, Baek KJ, Kwon NS, Sohn UD, Kim DS. UVB-activated indole-3-acetic acid induces apoptosis of PC-3 prostate cancer cells. Anticancer Res, 2010; 30 (11):4607-4612.

Kim YS, Milner JA. Indole-3-carbinol inhibits cell proliferation and induces apoptosis in Hep-2 laryngeal cancer cells. J Nutr Biochem, 2005; 16:65-73.

Kundu B, Ghosh D, Sinha MK, Sen PS, Balla, VK., Das N, Basu D. Doxorubicin-intercalated nano-hydroxyapatite drug-delivery system for liver cancer: An animal model. Ceramics International, 2013; 39(8):9557-9566.

Lee J, Maeda T, Hoong SH, Wood TK. Reconfiguring the quorum-sensing regulator SdiA of Escherichia coli to control biofilm formation via indole and N-acylhomoserine lactones. Appl Environ Microbiol, 2009; 75:1703-1716.

Li G, Young KD. Indole production by the tryptphanase Tna Ain Escherischia coli is determined by the amount of exogenous tryprophan. Microbiology, 2013; 159:402-410.

Luo Y, Wang TT, Teng Z, Chen P, Sun J, Wang Q. Encapsulation of indole-3-carbinol and 3,3'-diindolylmethane in zein/carboxymethyl chitosan nanoparticles with controlled release property and improved stability. Food Chem, 2013; 201:224-230.

Mao CG, Tao ZZ, Chen Z, Chen C, Chen SM, Wan LJ. Expression of TWIST an inducer of epithelial-mesenchymal transition, in nasopharyngeal carcinoma and its clinical significance. Exp Ther Med, 2014; 8(1):207-212.

Mirza AZ, Siddiqui FA. Nanomedicine and drug delivery: a mini review. Int Nano Lett, 2014; 4(94):1-7.

Murugan K, Choonara YE, Kumar P, Bijukumar D, Toit LC, Pillay V. parameters and characteristics governing cellular internalization and trans-barrier trafficking of nanostructures. Int. J. Nanomedicine, 2015; 10:2191-2206.

Pandey N, Dhiman S, Srivastava, T, Majumder S. Transition metal oxide nanoparticles are effective in inhibiting lung cancer cell survival in the hypoxic tumor microenvironment. Chem Biol Interact, 2016; 254(4):221-230.

Patel H, Darji N, Pillai J, Patel B. Recent advance in anti-cancer activity of indole derivatives. Int. J. Drug Res. Tech, 2012; 2(3):225-230.

Patila S, Sandberg A, Heckert E, Self W, Seal S. Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. Biomaterials, 2007; 28(31):4600-4607.

Preetha P, Srinivasa R, Pushpalatha P, Biphasic Drug Delivery in controlled release formulations-A review. Int. J Pharmacy Technology, 2015; 6(4)3046-3060.

Sankar R., Maheswari R., Karthik S, Shivashangari KS, Ravikumar V. Anticancer activity of Ficusreligiosa engineered copper oxide nanoparticles. Mater. Sci. Eng. C, 2014; 44:234-239.

Shaikh J, Ankhola DD, Beniwal V, Singh D, Ravi Kumar MNV. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur. J. Pharm. Sci, 2009; 37(3-4):223–230.

Subal CB. 2006. Modelling of Drug release: The Higuchi equation and its application. Pharmabiz.com.

Tarnuzzer RW, Colon J, Patil S, Seal S. Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. Nano Lett, 2005; 5:2573-2577.

Thakkar VT, Shah PA, Soni TG, Parmar MY, Gohel MC *et al*. Goodness-of-fit model-dependent approach for release kinetics of levofloxacin hemihydrates floating tablet. Dissolution Technologies, 2009; 16:35-39.

Wang YQ, Chen C, Chen Z, Xu Y, Wang Y, Xiao BK, Chen SM, Tao ZZ. Indole-3-carbinol inhibits cell proliferation and induces apoptosis in Hep-2 laryngeal cancer cells. Oncol. Rep, 2013; 30(1):227-233.

How to cite this article:
Majumder S, Sharma N, Das S, Pandey N, Srivastava T, Ghosh D. Synthesis, Characterization of Novel PLGA Encapsulated Indole Nanoparticles and Study of its cytotoxic potential against A549 lung cancer cell line. J App Pharm Sci, 2018; 8(08): 144-150.