INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 4.295
(Volume 5, Issue 2)
Available online at: www.ijariit.com

Chitosan nanocarrier system for tumour targeting

Aarthye Pandian
aarthye@gmail.com
Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu

K. Jeyasubramanian
kjeyasubramanian@yahoo.co.in
Mepco Schlenk Engineering college, Sivakasi, Tamil Nadu

M. Sureshkumar
msknano@gmail.com
Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu

ABSTRACT

The aim of this paper was to develop and characterize chitosan nanoparticle as a carrier for adriamycin delivery as chitosan nanoparticles are the potential delivery system for hydrophilic drugs due to its outstanding physicochemical and biological properties. Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. Chitosan nanoparticles containing the anticancer drug adriamycin was prepared by the ionic gelation technique and characterized by various techniques such as particle size analysis, FT-IR and Atomic Force Microscopy. Results showed that chitosan nanoparticles prepared are of uniform size with an average diameter of 90 nm and zeta potential 62.5mV with a high encapsulation efficiency of 91.9%. Adriamycin loading efficiencies were determined and release studies were performed in PBS at room temperature. The drug release rate of Adriamycin from the chitosan nanoparticles was approximately 60% showing the potential of chitosan nanoparticles as a sustained drug delivery system. Cytotoxicity tests showed that the Adriamycin-loaded chitosan had higher cell toxicity by delivering the drug to the tumour tissues than adriamycin alone.

Keywords— *Chitosan, Nanocarrier, Adriamycin, Drug delivery, Tumor therapy*

1. INTRODUCTION

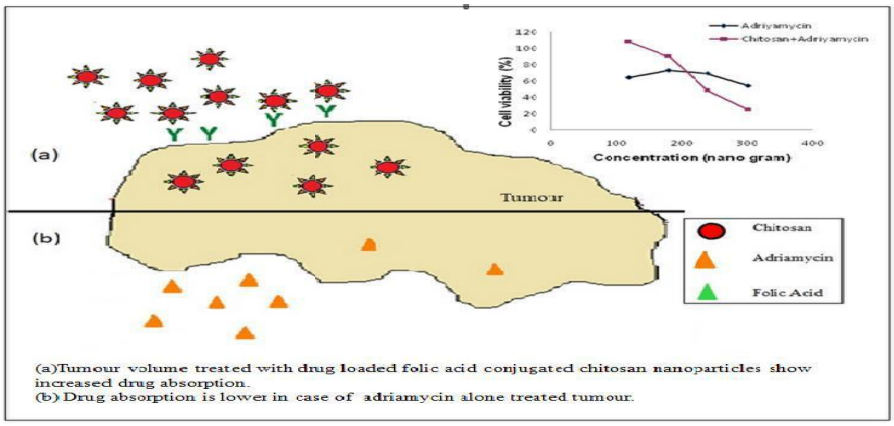
Adriamycin, hydrochloride of doxorubicin (14- hydroxydaunomycin) is a cytotoxic, anthracycline antibiotic used in antimitotic chemotherapy. Adriamycin has a positive charge which binds it to cell and mitochondrial surfaces having a negative charge at physiological pH, particularly to phospholipids. The undesirable side effects of the drug such as accumulative cardiac toxicity and myelosuppression lead to low therapeutic index. After intravenous administration, the drug rapidly disappears from the blood as it has a high affinity for living tissues [1-2]. Therefore, there is a little chance of the drug's being taken up by tumor tissue [3]. To overcome this critical issue, the development of targeted drug delivery systems is an effective approach that releases the drugs at the desired site of action which increases the therapeutic efficacy of pharmaceutical agents through bio-distribution and improved pharmacokinetics [4-7]. For the efficient delivery of Adriamycin to tumour relies on the use of nanocarriers functionalized to encapsulate such drugs and then deliver them to the target site, a promising approach to potentially reduce undesired side effects [8-9].

Nanoparticles have a special role in targeted drug delivery due to their long shelf life and entrapment efficiency and their nanoscale particle size have embraced the site-specific targeting and tend to permeate deeply [10-12]. Presently, polymer nanoparticles from biodegradable and biocompatible polymers are being widely investigated as a carrier for drug delivery [13]. Polymeric NPs have attracted prominent interest as a novel drug carrier because of their biodegradable property and high biocompatibility, low toxicity, higher drug entrapment efficiency, longer half-life and versatile chemical and physical properties [14-16]. Such systems control the rate of drug administration that prolongs the duration of the therapeutic effect and also deliver the drug to specific sites. Furthermore, nanocarriers regulate the drug release profile, while allowing intimate contact between molecules and mucosal barriers, contributing to their epithelial permeation [17-18]. The potential use of polymeric nanoparticles as drug carriers has led to the development of many different colloidal delivery vehicles [19-20]. Various synthetic and natural polymers like alginate, chitosan and polyesters have been used for entrapping and delivering drugs [21].

Chitosan is a cationic polysaccharide obtained by partial deacetylation of chitin, is a hydrophilic polymer with a positive charge that comes from weak basic groups, give it special characteristics as a biomaterial and as a pharmaceutical excipient for drug delivery [22-23]. Chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable and nontoxic [24]. Further, it possesses positive charge that targets the chitosan carriers to the negatively charged cell membrane and has mucoadhesive properties to prolong the retention time of chitosan in the targeted locations enhancing the absorption effect of

chitosan that renders chitosan a very attractive material as a drug delivery carrier. Furthermore, chitosan is a linear polyamine containing a number of free amino groups which are available for cross-linkages, and its cationic nature also allows for ionic cross-linking with multivalent anions [25-27].

In light of the considerations above, we envisioned that chitosan a cationic biopolymer would be more favorable for therapeutic applications based on targeting-specificity, bio-distribution, as well as cellular internalization profiles [28-29]. In the present study, we aimed to design and evaluate the applicability of chitosan as a drug carrier, to deliver the potential anti-cancer compound Adriamycin with increased efficacy over free Adriamycin to cancer cells.



Graphical Abstract

2. MATERIALS AND METHODS

2.1 Materials

Chitosan (medium molecular weight) with deacetylation of 92%, acryloyl chloride, potassium persulfate (KPS) and monomethoxy poly (ethylene glycol) (MPEG, Mn = 2000) were purchased from Sigma–Aldrich (USA). Ultrapure water from a Milli-Q water system was used to prepare the aqueous solutions. All other chemicals used in this work were analytical grade.

2.2 Method

Chitosan nanoparticles were prepared by inotropic gelation of chitosan with TPP anions according to the procedure developed by Calvo et al [30]. The Chitosan nanocarrier encapsulated with Adriamycin and the drug loaded chitosan nanocarrier were scrutinized using Fourier transform infrared spectroscopy (FT-IR, Bruker optics GmbH-Alpha T spectrometer, Germany). The surface charge (zeta potential) of the Chitosan nanocarrier, drug loaded chitosan nanocarrier and drug loaded chitosan nanocarrier were measured using Zeta sizer (Malvern instrument, UK) and the surface morphology of the nanocarrier were examined using atomic force microscopy in non-contact mode (XE 70, SPM, Park system, South Korea). UV-Vis. spectroscopy of the chitosan nanocarrier and the drug loaded chitosan nanocarrier were measured in Perkin-Elmer Lambda-25 spectrophotometer.

2.3 Preparation of Chitosan Nanoparticles

Chitosan was dissolved in acetic acid aqueous solution, 1.75 times higher than that of chitosan. Under magnetic stirring at room temperature, a variable volume of 0.5% (w/v) TPP aqueous solution was added into 10mL of 0.1% (w/v) chitosan solution, respectively. Three kinds of phenomena were observed: solution, aggregates and opalescent suspension. The zone of opalescent suspension corresponds to a suspension of very small particles. The nanoparticles formed spontaneously were then concentrated by centrifugation (13,000 rpm, 30 min room temperature). The supernatants were discarded, and Chitosan Nanoparticles were re-suspended in Milli-Q water for further characterization [31-32].

2.4 Encapsulation of Adriamycin loaded Chitosan Nanoparticles

Varying amount of Adriamycin was added to 10 ml chitosan solution (0.1% w/v) under magnetic stirring. To this solution, TPP was added (4 ml) leading to the controlled gelation of chitosan. The nanoparticles were isolated by centrifugation (13000g, 30 min, and room temperature) and then re-suspended in 0.5 ml of Milli-Q water.

2.5 Determination of yield of the nanoparticle

5 ml of the prepared chitosan solution was centrifuged at 13000g for 30 min. The supernatant was discarded and the pellet was collected. After drying the sample overnight at 50ºC, a constant weight of dried nanoparticle was obtained. 5 ml of the drug loaded chitosan nanocarrier was centrifuged at 13000 g for 30 min. The amount of unbound drug was determined from the supernatant solution and the production yield was calculated comparing the actual weight with the theoretical weight of the nanocarriers.

2.6 The Adriamycin loading ratio and encapsulation efficiency

The Adriamycin loaded chitosan nanocarrier was suspended in an equal volume of buffer solution and absorbance was measured using UV-Vis spectrophotometer. From the absorbance of the drug, the concentration of encapsulated Adriamycin has been determined from a standard graph. The Drug Loading (DL) [33] and Encapsulation Efficiencies (EE) [34] were calculated using the following formulae:

$$Drug\ loading\ =\ (W1/W2)\ x\ 100\% \quad (1)$$

$$Encapsulation\ efficiency\ =\ [(W3-W4)/W3]\ x\ 100\% \quad (2)$$

Where,
W1 is the weight of the drug encapsulated;
W2 is the gross weight of the chitosan;
W3 is the total weight of the drug added initially;
W4 is the weight of the drug released in the liquid medium.

The *in vitro* drug release experiment was carried out using the dialysis tube diffusion technique. The *in vitro* experiments were performed in phosphate buffer saline (PBS) at 37 °C under sink conditions. Drug loaded chitosan nanocarrier of 1 mg mL–1 (5 mL) was taken in a dialysis bag (Himedia, India) with a 12000-14000 Da (~2.4 nm) cut-off pore size and immersed in 25 mL of PBS and dialyzed against the phosphate buffer saline and incubated at 37ºC under constant stirring. Samples were withdrawn periodically and replaced with fresh PBS and the amount of the drug was quantified spectrophotometrically from the standard graph. The released drug was calculated in percentage relative to the initial concentration taken for dialysis [35].

2.8 Cytotoxicity analysis: MTT ASSAY

Dalton Lymphoma Ascites (DLA) cells were seeded into 96-well plates at a density of approximately 2.5×10^4 cells per well in RPMI 1640 media and incubated for 12 h. Following the incubation, various concentrations of free and nanoencapsulated adriamycin (120 ng, 180 ng, 240ng, 300 ng and 360 ng) were added to the wells. Then MTT (3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyl-tetrazolium bromide) cell viability assay solution was added to each well in a concentration of 5 mg mL^{-1}, and the plates were incubated at 37 °C for 4 h. The supernatant was aspirated and the resulting formazan crystals were washed with 100 µL of isopropanol. Plates were read at 570 nm (MR 700 Dynatech, UK) and the mean cell survival for free Adriamycin and nano encapsulated Adriamycin treated cells were quantized for each concentration and expressed as a percentage of control (cells not treated) cell survival values [36]. Cell viability was calculated by the following formula:

$$Cell\ viability\ (\%) = \frac{\text{Average absorbance of treated group}}{\text{Average absorbance of the control group}} * 100\% \quad (3)$$

3. RESULTS AND DISCUSSION

3.1 Particle Size and Zeta Potential Analysis

The size of chitosan nanoparticles ranges from 78.3 to 91nm, an average particle size of chitosan nanoparticles was 90.27 nm (figure 1. a) [37]. Whereas, the zeta potential of chitosan nanoparticles have an overall positive surface charge of about 62.5mV (figure 1.b) which may be due to the free amine groups of chitosan. The zeta potential values of chitosan nanoparticles have a positive charge which shows that the nanoparticles can cross the cell membrane easily.

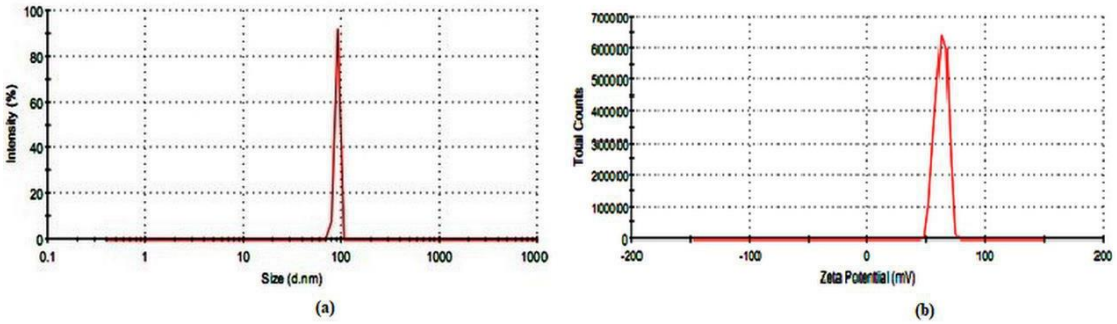


Fig. 1: Particle size (a) and Zeta potential (b) distribution for chitosan nanoparticles

The mean diameter of the drug loaded chitosan nanoparticles was found to be 439.1 nm. At the low TPP concentration (0.05%), combined drug loaded chitosan nanoparticles are in the size range from 412 nm to 553 nm (figure 2.a). The mean diameter and the size of the drug loaded chitosan nanoparticles was found to be increased compared to the chitosan nanoparticles alone. This may be due to the crosslinking of the Adriamycin molecules and free acid groups present in the particles. The observed drug loaded nanoparticles size was larger than the hydrodynamic diameter of the chitosan nanoparticles from the DLS experiment. The zeta potential of Adriamycin loaded chitosan nanoparticles decreased, with a surface charge between -9.63 mV to 14.6mV. (figure2.b)[38].

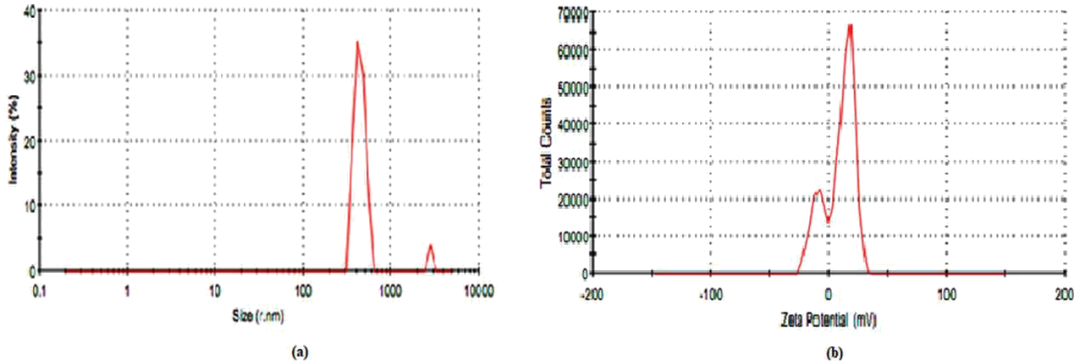


Fig. 2: Particle size (a) and Zeta potential (b) distribution for drug loaded chitosan nanoparticles

The adriamycin loaded chitosan nanoparticles have a positive charge and a negative charge which makes the nanocarriers to diffuse easily and deliver the drugs deep into the tissues.

Nanoparticles effectively deliver drugs deep inside tumour tissue if the particles are positively electrically charged because a positive charge allows better uptake of the nanoparticles across the cell membrane which controls delivery to the proliferating cells. The negative charge of the nanoparticles diffuses faster into the tissues and delivers drugs deeply [39].

3.2 FT-IR analysis

FTIR studies of chitosan, chitosan nanoparticles and drug-loaded chitosan nanoparticles were performed to characterize the chemical structure of nanoparticles (figure 3). [40-42]

There are three characteristic peaks of chitosan at 3449 cm^{-1}, 1271 cm^{-1} and 1739 cm^{-1}. A band at 3449 cm^{-1} corresponds to the combined peaks of the NH_2 and OH group stretching vibration in chitosan. The band at 1739 cm^{-1} is attributed to the $CONH_2$ group. The 1540cm^{-1} peak corresponds to the C (NH_2) group.

A shift from 3314 to 3345 cm^{-1} is shown, and the peak is wider in the chitosan nanoparticles, which indicates that the hydrogen bonding is enhanced. The intensities of ($CONH_2$) band at 1739 cm^{-1} and (NH_2) band at 1540cm^{-1}, which can be observed clearly in pure chitosan, decrease dramatically, and two new sorption bands at 1638 and 1550 cm-1 appear which shows that the ammonium groups are cross-linked with tripolyphosphate molecules. Thus it is postulated that polyphosphoric groups of sodium polyphosphate interact with the ammonium groups of chitosan, which serves to enhance both the inter- and intra-molecular interaction in chitosan nanoparticles.

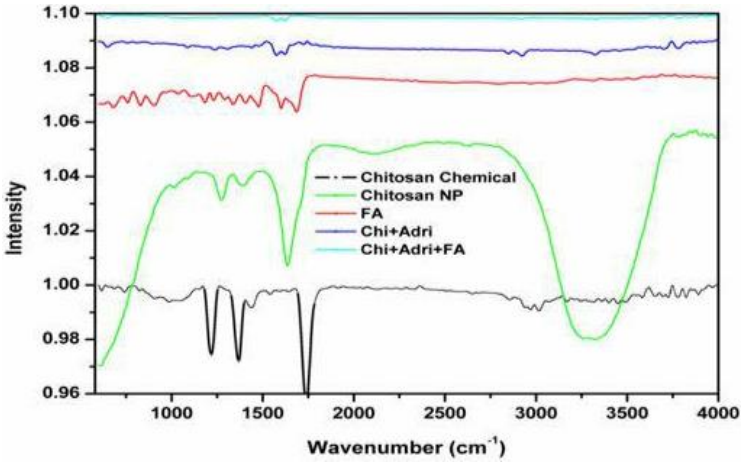


Fig. 3: FT- IR spectrum of chitosan, chitosan nanoparticles, adriamycin loaded chitosan Nanoparticles

The FT-IR spectra of drug loaded chitosan nanocarrier attributed to the linkage between phosphoric and ammonium ions concluding that the di polyphosphate groups of TPP are linked with ammonium groups of chitosan. Moreover, the emergence of the prominent peaks in adriamycin loaded chitosan nanoparticles were quite similar with nanoparticles without the drug, suggesting the possibility of the conjugation of the drug with chitosan nanoparticles.

The compatibility between the drug loaded chitosan nanoparticles and the drug loaded chitosan nanoparticle was evaluated using FTIR peak matching method which shows the characteristic peaks of chitosan nanoparticles, adriamycin and drug loaded chitosan nanoparticles.

3.3 AFM analysis

Figure.4 (a) and figure. 4(b) represents the 3D image and histogram of chitosan nanoparticles and gives information about the particle size distribution. The nanoparticles in the scanned region possess near-spherical shape or ellipsoidal shape with an average particle size of 7.5 nm.

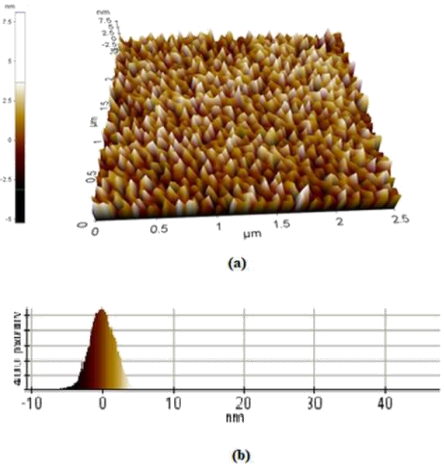


Fig. 4: (a) 3D image of chitosan nanoparticles and (b) Histogram of chitosan nanoparticles.

The histogram curve figure 4(b) of the chitosan nanoparticles, the mean particle size of the chitosan nanoparticles was 7.5 nm.

After adriamycin encapsulation (figure. 5. a) the particles size increases and have a spherical morphology. The increase in size may be due to the crosslinking of the amine group of the adriamycin molecules and free acid groups present in the particles. The observed nanoparticles size was smaller than the hydrodynamic diameter obtained from the DLS experiment.

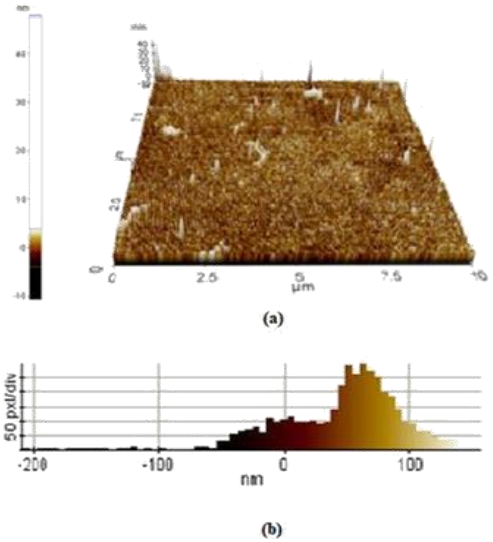


Fig. 5: (a) 3D image of adriamycin chitosan nanoparticles and (b) Histogram of adriamycin chitosan nanoparticles.

From the histogram curve figure 5(b) the mean particle size of the drug loaded chitosan nanoparticles were 100 nm.

3.4 Drug Loading Efficiency and Encapsulation Efficiency

The maximum quantity of the drug loading capacity of the nanocarrier has been evaluated from the drug loading studies which were followed spectrometrically (figure 6). From the standard graph, the drug Encapsulation Efficiency (EE) and the Drug Loading Capability (DL) of the chitosan nanocarrier was calculated to be 91.9 % and 0.59 % respectively [40]

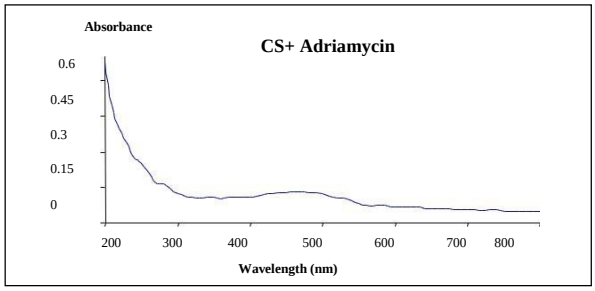


Fig. 6: UV spectra of Drug loaded chitosan nanoparticles

3.5 In Vitro Release

The controlled agitation of the drug loaded chitosan nanocarrier released the drug in a controlled manner into the PBS medium through the cut off pore size of the dialysis membrane. The quantity of drug released with time has been analyzed spectrophotometric ally. Figure .7 shows the *in vitro* drug release profile of drug loaded chitosan nanocarrier in PBS in the first 60 h. It is clear from the figure that the release of the drug started with a burst initially during the first 10 h. The drug release from the medium is further enhanced to 60% within the next 48h. More or less the same quantity of the drug has been released from the system in a sustained manner, once it crosses the initial 10 h time.

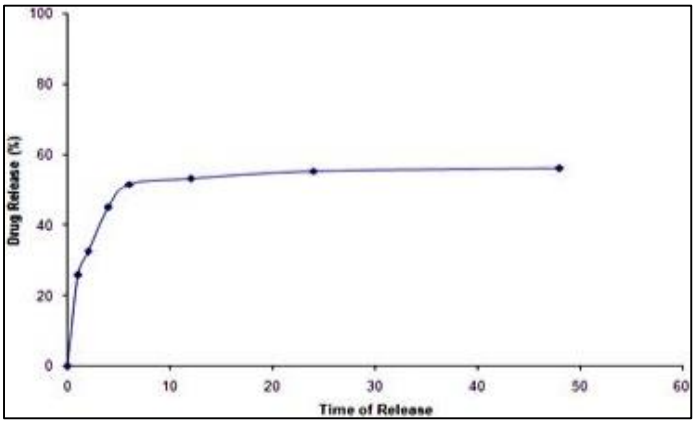


Fig. 7: Release profile of adriamycin loaded chitosan nanoparticles.

3.6 Cytotoxicity Analysis: MTT Assay

The cytotoxicity of the Adriamycin and the Adriamycin-loaded chitosan nanocarrier were treated with DLA cells *in vitro*. The percentage of cell survival with respect to untreated control cells is shown in Table1. A significant reduction in the DLA cells viability was observed when the cells containing the same concentration of the direct administration of the Adriamycin were exposed to the Adriamycin-loaded chitosan nanoparticles. A gradual decrease in the viability of the DLA cells was observed as 64.48% at 120 ng and 51.49% at 300 ng of Adriamycin, respectively. The results demonstrate a concentration-dependent response of Adriamycin-loaded chitosan nanoparticles. In contrast, the viability of the cells was 107.92%, 90.25%, 48.57%, and 25.32% for cells administered with adriamycin loaded chitosan nanoparticles at 120ng, 180ng, 240ng and 300ng respectively. Adriamycin-loaded chitosan nanoparticles were found to have higher cell toxicity than free Adriamycin. The graphical representation (Fig.8) shows that Adriamycin loaded chitosan nanoparticles exhibited considerable anticancer effects than free Adriamycin [43].

Table 1: % of cell viability for various concentrations of Adriamycin and adriamycin Chitosan treated cell lines

Concentration In ng	Cell viability(%) Adriamycin treated	Cell viability(%) Chitosan +Adriamycin treated
120	64.48	107.92
180	72.98	90.25
240	69.48	48.57
300	51.49	25.32

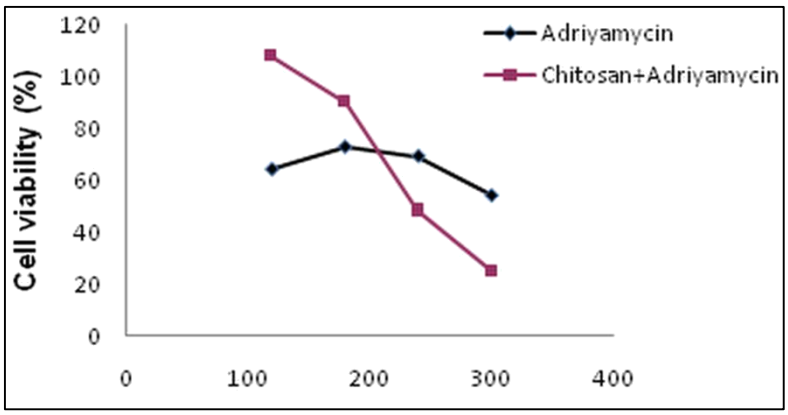


Fig. 8: Graphical representation showing the cell viability for various concentrations of free drug and drug loaded chitosan nanocarrier

4. CONCLUSION

In conclusion, this work showed the feasibility of using chitosan nanoparticles as colloidal carriers for delivery of the small, cationic anthracycline drug, adriamycin. Specifically, to eliminate the side effects of adriamycin, the Adriamycin-loaded degradable chitosan nanoparticles were developed in this study. The nanocarrier imparts excellent stability in an aqueous medium with reasonably good hydrodynamic size. After adriamycin loading in the nanoparticles, their size increases. Adriamycin loaded chitosan nanoparticle has a spherical shape and its particle sizes were around 412–553 nm. Release behaviors of adriamycin from nanoparticles showed a sustained release pattern. The nano-particle cytotoxicity to the cells was measured by MTT assay. There was a significant difference in cell viability between cells treated with and without nanoparticles. It was observed that survival in cancer cells treated with adriamycin loaded nanoparticles was lower than that of a normal cell in similar concentrations. The adriamycin loaded chitosan nanoparticles showed enhanced cytotoxicity. In this study, a simple process has been developed to synthesize chitosan–adriamycin conjugate nanoparticles and due to high specific intracellular uptake, and surface positive charge, this drug loaded chitosan system may further be explored for its applications for targeted delivery of the drug. The data presented here suggest that further in vivo studies are warranted to define the therapeutic index of this polymeric carrier for tumor targetting and will constitute the basis for the next generation of drug delivery devices.

5. ACKNOWLEDGEMENTS

The authors wish to thank Mepco Schlenk Engineering College, Sivakasi, TN, India for providing support and the necessary facilities to carry out this work successfully.

6. DATA AVAILABILITY

The research data used to support the findings of this study are included in the article.

7. REFERENCES

[1] Yagi et al, Patent No. 4756910.
[2] https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/062921s022lbl.pdf.
[3] Saesoo, S., Bunthot, S., Sajomsang, W., Gonil, P., Phunpee, S., Songkhum, P., Saengkrit, N., Phospholipid-chitosan hybrid nanoliposomes promoting cell entry for drug delivery against cervical cancer, J. Colloid and Interface Science, 480 (2016), 240- 248. doi:10.1016/j.jcis.2016.06.071.

[4] Ghaz-Jahanian, M. A., Abbaspour-Aghdam, F., Anarjan, N., Berenjian, A., & Jafarizadeh-
[5] Malmiri, H., Application of Chitosan-Based Nanocarriers in Tumor-Targeted Drug Delivery, Molecular Biotechnology, 57(3) (2014), 201–218. doi:10.1007/s12033-014-9816-3.
[6] Sun, T., Zhang, Y. S., Pang, B., Hyun, D. C., Yang, M., & Xia, Y., Engineered Nanoparticles for Drug Delivery in Cancer Therapy, Angewandte Chemie International Edition, n/a–n/a (2014). doi:10.1002/anie.201403036.
[7] Srinivasan, M., Rajabi, M., & Mousa, S., Multifunctional Nanomaterials and Their Applications in Drug Delivery and Cancer Therapy, Nanomaterials, 5(4) (2015), 1690– 1703.doi:10.3390/nano5041690.
[8] Manish P. Patel, Ravi R. Patel, Jayvadan K. Patel, Chitosan Mediated Targeted Drug Delivery System: A Review, J Pharm PharmaceutSci.13(3) (2010), 536 – 557. https://pdfs.semanticscholar.org/5c62/3d3e425da7c259c81ae5c1ce507047830fa6.pdf.
[9] Fu, S., Xia, J., & Wu, J., Functional Chitosan Nanoparticles in Cancer Treatment, Journal of Biomedical Nanotechnology, 12(8) (2016), 1585–1603. doi:10.1166/jbn.2016.2228.
[10] Lakshmanan, V.-K., Therapeutic efficacy of nanomedicines for prostate cancer: An update. Investigative and Clinical Urology, 57(1) (2016), 21. doi:10.4111/icu.2016.57.1.21.
[11] S.H. Bang, I.C. Hwang, Y.M. Yu, H.R. Kwon, D.H. Kim & H.J. Park, Influence of chitosan the coating on the liposomal surface on physicochemical properties and the release profile of nanocarrier systems, J. Microencapsulation, 28(7) (2011), 595-604, doi: 10.3109/02652048.2011.557748.
[12] Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R., Biodegradable long-circulating polymeric Nanospheres, Science, 263 (1994), 1600–1603. https://www.ncbi.nlm.nih.gov/pubmed/8128245.
[13] Yan Wu, Wuli Yang, Changchun Wang, Jianhua Hu, Shoukuan Fu, Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate, Int. J. of Pharmaceutics. 295 (2005), 235–245. doi:10.1016/j.ijpharm.2005.01.042.
[14] Fonseca, A. C., Serra, A. C., & Coelho, J. F., Bioabsorbable polymers in cancer therapy: latest developments, The EPMA Journal, 6(2015), 22. doi:10.1186/s13167-015-0045-z.
[15] Cao, L., Zhang, H., Cao, C., Zhang, J., Li, F., & Huang, Q., Quaternized Chitosan-Capped Mesoporous Silica Nanoparticles as Nanocarriers for Controlled Pesticide Release, Nanomaterials (Basel, Switzerland), 6(7) (2016), 126. doi:10.3390/nano6070126.
[16] Najafi, S., Pazhouhnia, Z., Ahmadi, O., Berenjian, A., & Jafarizadeh-Malmiri, H. (2014). "Chitosan Nanoparticles and their Applications in Drug Delivery: A Review", Current Research in Drug Discovery, 1(1), 17–25. doi:10.3844/crddsp.2014.17.25.
[17] Ordikhani, F., Erdem Arslan, M., Marcelo, R., Sahin, I., Grigsby, P., Schwarz, J., & Azab, A. (2016). "Drug Delivery Approaches for the Treatment of Cervical Cancer. Pharmaceutics", 8(3), 23.doi:10.3390/pharmaceutics8030023
[18] Mikael Larsson, Annika Borde, Emma Mattisson1, Dean-Mo Liu and Anette Larsson (2013), "Evaluation of Carboxymethyl-Hexanoyl Chitosan as a Protein Nanocarrier", J. Nanomaterials and Nanotechnology, Vol. 3 Art. 7.
[19] Pérez-Herrero, E., & Fernández-Medarde, A. (2015), "Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy", European Journal of Pharmaceutics and
[20] Estanqueiro, M., Amaral, M. H., Conceição, J., & Sousa Lobo, J. M. (2015), "Nanotechnological carriers for cancer chemotherapy: The state of the art", Colloids and Surfaces B: Biointerfaces, 126, 631–648.doi:10.1016/j.colsurfb.2014.12.041
[21] Vieira, D. B., & Gamarra, L. F. (2016), "Advances in the use of nanocarriers for cancer diagnosis and treatment", Einstein (São Paulo), 14(1), 99–103.doi:10.1590/s1679-45082016rb3475
[22] Karra, N., & Benita, S. (2012), "The Ligand Nanoparticle Conjugation Approach for Targeted Cancer Therapy", Current Drug Metabolism, 13(1), 22– 41.doi:10.2174/138920012798356899
[23] Saikia, C., & Gogoi, P. (2015), "Chitosan: A Promising Biopolymer in Drug Delivery Applications", Journal of Molecular and Genetic Medicine, s4.doi:10.4172/1747-0862.s4-006
[24] Bao, H., Pan, Y., Ping, Y., Sahoo, N. G., Wu, T., Li, L., Gan, L. H. (2011), "Chitosan-Functionalized Graphene Oxide as a Nanocarrier for Drug and Gene Delivery", Small, 7(11), 1569–1578. doi:10.1002/smll.201100191.
[25] Li Li and Kang Moo Huh (2014), "Polymeric nanocarrier systems for Photodynamic Therapy", J.Biomaterials Research, 19(1), 1-14. doi:10.1186/2055-7124-18-19.
[26] WareeTiyaboonchai (2003), "Chitosan Nanoparticles: A Promising System for Drug Delivery", Naresuan University Journal, 11(3) 51-66. doi: 10.1.1.460.1550.
[27] Sundar, S., Kundu, J., & Kundu, S. C. (2010), "Biopolymeric nanoparticles", Science and Technology of Advanced Materials, 11(1), 014104. doi:10.1088/1468-6996/11/1/014104.
[28] Elgadir, M. A., Uddin, M. S., Ferdosh, S., Adam, A., Chowdhury, A. J. K., & Sarker, M. Z. I. (2015), "Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: A review", Journal of Food and Drug Analysis, 23(4), 619 –629. doi:10.1016/j.jfda.2014.10.008.
[29] Solaro, R., Chiellini, F., & Battisti, A. (2010), "Targeted Delivery of Protein Drugs by Nanocarriers", Materials, 3(3), 1928–1980.doi:10.3390/ma3031928.
[30] Senthilkumar, R., Karaman, D. Ş., Paul, P., Björk, E. M., Odén, M., Eriksson, J. E., & Rosenholm, J. M. (2015), "Targeted delivery of a novel anticancer compound anisomelic acid using chitosan-coated porous silica nanorods for enhancing the apoptotic effect.", Biomaterials Science, 3(1), 103–111. doi:10.1039/c4bm00278d.
[31] Calvo, P., Remuñan-López, C., Vila-Jato, J. L., & Alonso, M. J. (1997). "Chitosan and Chitosan/Ethylene Oxide-Propylene Oxide Block Copolymer Nanoparticles as Novel Carriers for Proteins and Vaccines" Pharmaceutical Research, 14(10), 1431–1436. doi:10.1023/a:1012128907225.
[32] Sanpui, P., Chattopadhyay, A., & Ghosh, S. S. (2011). Induction of Apoptosis in Cancer Cells at Low Silver Nanoparticle Concentrations using Chitosan Nanocarrier. ACS Applied Materials & Interfaces, 3(2), 218–228. doi:10.1021/am100840c.
[33] Li-Ming Zhao, Lu-E Shi, Zhi-Liang Zhang, Jian-Min Chen, Dong-Dong Shi, Jie Yang and Zhen-Xing Tang, Preparation and application of chitosan nanoparticles and nanofibers, Brazilian Journal of Chemical Engineering, 28(03) (2011),353 – 362. http://www.scielo.br/pdf/bjce/v28n3/v28n3a01.

[34] Mariana da Silva Barbi, Flávia Chiva Carvalho, Charlene Priscila Kiill, Hernane da Silva Barud, Preparation and Characterization of Chitosan Nanoparticles for Zidovudine Nasal Delivery, J. of Nanoscience and Nanotechnology, 15 (2015), 865–874. https://www.ncbi.nlm.nih.gov/pubmed/26328451.

[35] Rashi Rajput, Sachin Kumar, Payal Nag, Manisha Singh, Fabrication and Characterization of chitosan-based polymeric Escitalopram nanoparticles, J. of Applied Pharmaceutical Science, 6 (07) (2016), 171-177. doi: 10.7324/japs.2016.60725

[36] Junise V, R. Saraswathi, Development and Characterisation of chitosan Nanoparticles with Isoniazid, J. of Pharmaceutical and scientific innovation, 4(3) (2015), 190- 195.doi: 10.7897/2277-4572.04342.

[37] Venkatesan, B., Tumala, A., Subramanian, V., & Vellaichamy, E., Data on synthesis and characterization of chitosan nanoparticles for in vivo delivery of siRNA-Npr3: Targeting NPR-C expression in the heart, Data in brief, 8 (2016), 441-7. doi:10.1016/j.dib.2016.05.074.

[38] Ali, S. W., Joshi, M., & Rajendran, S., Synthesis and Characterization of Chitosan Nanoparticles with Enhanced Antimicrobial Activity. International Journal of Nanoscience, 10(04n05) (2011), 979–984. doi:10.1142/S0219581x1100868x.

[39] Mohammadpour Dounighi, N., Mehrabi, M., Avadi, M.R., Zolfagharian, H., Rezayat, Preparation, characterization and stability investigation of chitosan nanoparticles loaded with the Echiscarinatussnake venom as a novel delivery system, J. Archives of Razi Institute, 70(4) (2015), 269-277. doi: 10.7508/ari.2015.04.007

[40] Kim, B., Han, G., Toley, B. J., Kim, C. K., Rotello, V. M., & Forbes, N. S. Tuning payload delivery in tumour cylindroids using gold nanoparticles. Nature Nanotechnology, 5(6) (2010), 465-72. doi: 10.1038/nnano.2010.58.

[41] Venkatesan, J., Alam, M. S., Hong, E. J., Kim, S.-K., & Shim, M. S. Preparation of piperlongumine-loaded chitosan nanoparticles for safe and efficient cancer therapy. RSC Advances, 6(83) (2016). , 79307–79316.doi:10.1039/c6ra12699e.

[42] DongzhiHou, RuyiGui, Sheng Hu1, Yi Huang, ZuyongFeng, Qineng Ping, Preparation and Characterization of Novel Drug-Inserted-Montmorillonite Chitosan Carriers for Ocular Drug Delivery, J. Advances in Nanoparticles 4(3) (2015), 14 pages, doi:10.4236/anp.2015.43009.

[43] Dinesh.Wanule, J.V. Balkhande, P.U. Ratnakar, A.N. Kulkarni and C.S. Bhowate, "Extraction and FTIR Analysis of Chitosan from American cockroach, Periplaneta Americana", Int. J.of Engineering Science and Innovative Technology, 3(3) (2014), 299-304. http://www.ijesit.com/Volume%203/Issue%203/IJESIT201403_39.pdf

[44] Anusha, J. R., & Fleming, A. T., Synthesis and Characterization of Chitosan Nanoaggregates from Gladius of Uroteuthis duvauceli, Int.journal of biomaterials, 2016 (2016), 5379424. doi: 10.1155/2016/5379424

