Formulation and Evaluation of a Nanoparticulate System for the Treatment of Lung Cancer

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ABSTRACT

Purpose: Over the past decade, there has been an increasing interest in using nanotechnology for lung cancer therapy.Recently, Chitosan based nanoparticles have received much attention by the researchers owing to its biodegradability, biocompability and the ability to deliver a wide range of drugs. Method: The aim of the present study was to formulate and evaluate chitosan nanoparticles containing Doxorubicin HCl. The chitosan nanoparticles were prepared by w/o emulsion method. Result: The drug loading capacity of the nanoparticles varied from 49.78 to 53.31 which depend on the concentration of drug in each formulation. The mean particle size of the selected batch was 129.9 nm with a polydipersity index 0.230 and zeta potential was found to be 43.9 mV. The study on *in vitro* release of all drug loaded batches in pH 7.4 phosphate buffer exerted a bi-phasic release pattern with and initial burst effect followed by a sustained release. The release kinetics studies showed that the release was Zero order diffusion controlled.Conclusion: Based on the observations, it can be concluded that the formulated nanopartilculate delivery system of Doxorubicin HCl exhibiting sustained release properties for a period of 24 hour, this may reduce concentration of drug to be administered along with frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability, and increase the effectiveness of the drug.

KEYWORDS: Lung cancer; Doxorubicin HCl; Chitosan; Zeta-potential; SEM; DSC

INTRODUCTION

Cancer is a term used for diseases in which abnormal cell divide without control and are able to invade other tissue and it not just one disease it's a combination of many diseases[1].Among them lung cancer continues to be the leading cause of death in both men and women in the world[2].Pulmonary diseases are treated by maintaining high and prolonged drug concentration in lungs either administered by pulmonary route or systemic route[3].

Systemic drug delivery of an anticancer drugs have narrow safety range spectrum because limited amount of drug reaches to the target tumor site[4]. Targeted drug delivery of anticancer drug on tumor site in lungs improves the therapeutic effect because it decreases the systemic exposure of drug[5]. Lungs provide large surface area ($100m^2$) and high vascularization which rapidly distribute the molecules. Lungs exhibit relatively low local metabolic activity and unlike the oral route of drug administration, pulmonary inhalation is not subject to first-pass metabolism[6].

In last decades, colloidal drug delivery system such as nanoparticles has received great attention[7]. It's defined as particulate dispersions or solid particles with a size in the range of 10-1000nm[8]. Because nanoparticles have small size these particles offer an alternative delivery system for cancer therapy that have the potential to control the release rate of drug, improve thebiodistribution, drug pharmacokinetics and reduce drug toxicity[9].

Doxorubicin Hydrochloride is one of the oldest drug uses for the treatment of lung cancer. It is a red-orange hygroscopic, crystalline powder- soluble inisotonic sodium chloride solution and water. There are two proposed mechanism by which doxorubicin acts in the cancer cell (i) Free radicals are generated which damage to cellular membranes (ii) DNA get intercalate and it cause disruption of topoisomerase-II- mediated DNA repair[10,11].

METHODOLOGY

Preparation of naoparticles

Step-I: The required quantity of paraffin oil was measured and maintained at temperature of 30˚C for 15-20 mins, required quantity span 40 was weighed and dissolve under magnetic stirrer.

Step-II: Chitosan solution in 1% v/v glacial acetic acid was prepared.

Step-III: The solution of chitosan was added drop-by-drop to the oil solution under Ultra Turrax at 10000 rpm for 10 min.

Step-IV: The doxorubicin hydrochloride was accurately weighed and dissolved in chitosan solution.

Step-V: A 0.5%w/v solution of STPP was prepared and 0.5ml of the same was added drop wise to the above formed emulsion which was further stirred under the Ultra Turrax at 1000 rpm for 10 mins.

Step-VI: The collected nanoparticle was centrifuged at 10000 rpm for 30 mins to remove the liquid supernatant, then the nanoparticle was washed with hexane (3 times) by which it was separated from the oil phase and dried.

Evolution of Nanoparticles

Drug content

The nanoparticles equivalent to 100 mg of the drug taken dissolved in 100 ml of

phosophate buffer pH 3.6 and subsequently diluted to get 100 µg/ml solution. From that 10 ml was taken by pipette and diluted with 100 ml of distilled water to get solution having

concentration of 100 µg/ml . From working standard solution 0.1, 0.2, 0.3 up to 1ml aliquots has been taken on a 10 ml volumetric flask, 2 ml of 0.05% w/v bromophenol blue and 3 ml of phosphate buffer were added. The solution was shaken for 2mins and 5ml of chloroform was added and then it was again being shaken for 2-3 mins and was even kept aside for the formation of coloured compound , finally the absorption was checked at 480nm.

Free drug content

Nanoparticle equivalent to 100 mg of the drug was taken and washed with water, to remove the free drug, the filtered was diluted with phosphate buffer pH 3.6 suitably and estimated calorimetrically by this above mention method.

Particle size analysis

The particles size was determined by dynamic light scattering, using Malvern system with vertically polarised light supplied by argon-ion laser (Cyonics) operated at 40 mW. Experiments were performed at a temperature of 25.0±0.1˚C at a measuring angle of

90˚ to the incident beam.

Surface charge analysis

The zeta-potential of the nanoparticles was determined by laser Doppler anemometry using a Malvern Zetasizer. Measurements were performed at 25.0±0.1˚C. The nanoparticles were dispersed in clear disposable zeta cell and measured.

Surface morphology

Scanning electron microscopy was performed to characterize the surface morphology of the formed nanoparticles and this was done using a JSM 6100 JEOL Scanning Electron microscope at 20 kV. Prior to examination, samples were gold-coated to render them electrically conductive and examined under the microscope.

***Invitro* diffusion study**

Cellulose dialysis bag (Cutt off 12000 Hi media) soaked overnight in PBS.

The wet sac was gently open and wash copiously with PBS then it was filled with PBS and examined for leaks. The sac was then emptied and 1 ml of nanoparticle formulation in PBS to be investigated was accurately transferred into the sac, which thus became the donor compartment. The sac was once again examined for any leaks and then was suspended in glass beaker containing 20 ml of PBS, which acted as receptor compartment. The content of the beaker was stirred using Teflon coated bar magnet and the beaker was closed with aluminum foil to prevent any evaporative losses during the experimental work.

At predetermined interval of time 1 ml aliquots were withdrawn from the receptor compartment and subjected to analysis. Fresh buffer was used to replenish the receptor compartment. Analysis was carried out immediately after withdrawal. All experiments were repeated thrice and the average values were taken.

Stability studies

Stability of a drug has been defined as the stability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf life.

All the formulations were packed in tightly closed amber-coloured bottlesand wrapped with aluminium foil and keep at 30º C ± 2ºC and (65±5%RH) for 30 days, 30 days in stability chamber at 2-8°C temperature in refrigerator and evaluated for their drug entrapment efficiency and drug content.

RESULT & DISCUSSION

Melting Point Determination

The melting point was found to be 204.5˚C

Solubility Determination

Soluble in water and in isotonic sodium chloride solution, slightly soluble in methanol.Practically insoluble in chloroform, in either and in other organic solvents.

Compatibility Study

The FT-IR spectrum of doxorubicin hydrochloride, chitosan and dox+ chitosan was carried Out. After 1 month the samples were visually observed. The drug was found compatible with All the excipients used in the formulation & with each other. These samples were also evaluatedfor the presence of impurity by FTIR method. No impurity was detected either in Initial samples or the samples kept at room temperature for 60 day.

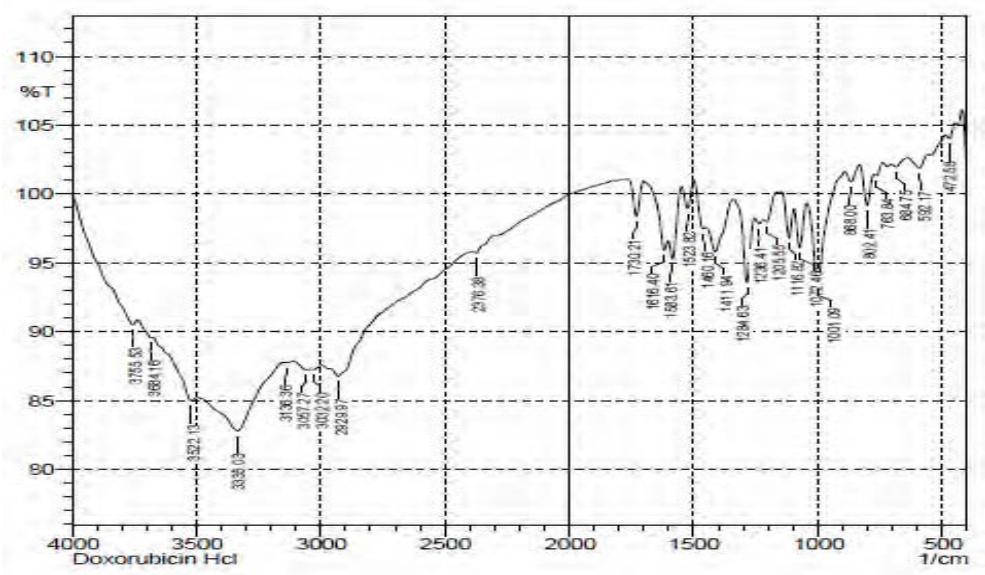

Figure no I: FT-IR spectra of Doxorubicin Hydrochloride

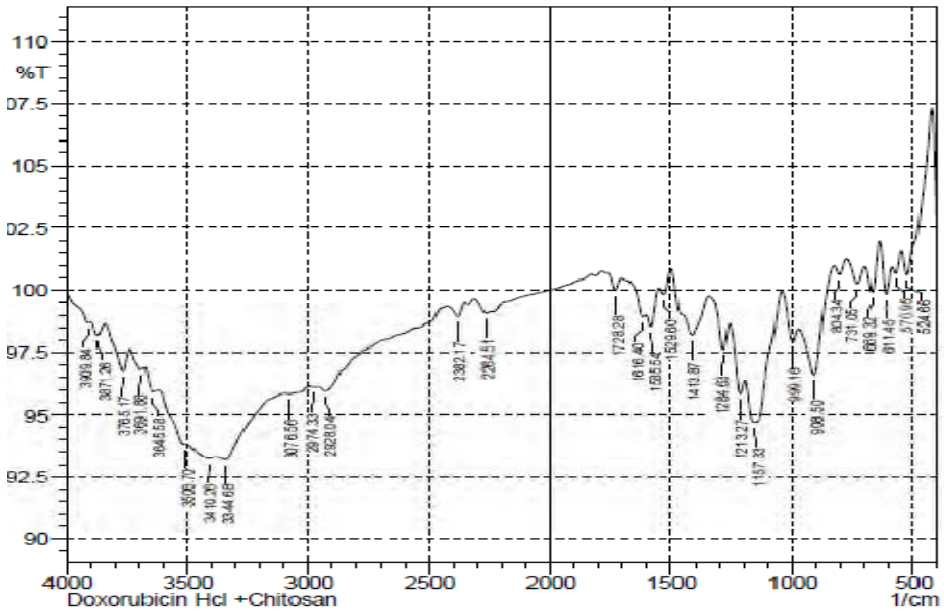

Figure no II: FT-IR spectra of Dox+chitosan

Existence of principle peaks revealed no considerable changes in the FT-IR peaks of Doxorubicin hydrochloride when mixed with excipients compared to pure doxorubicin Hydrochloride.

Differential scanning calorimetry

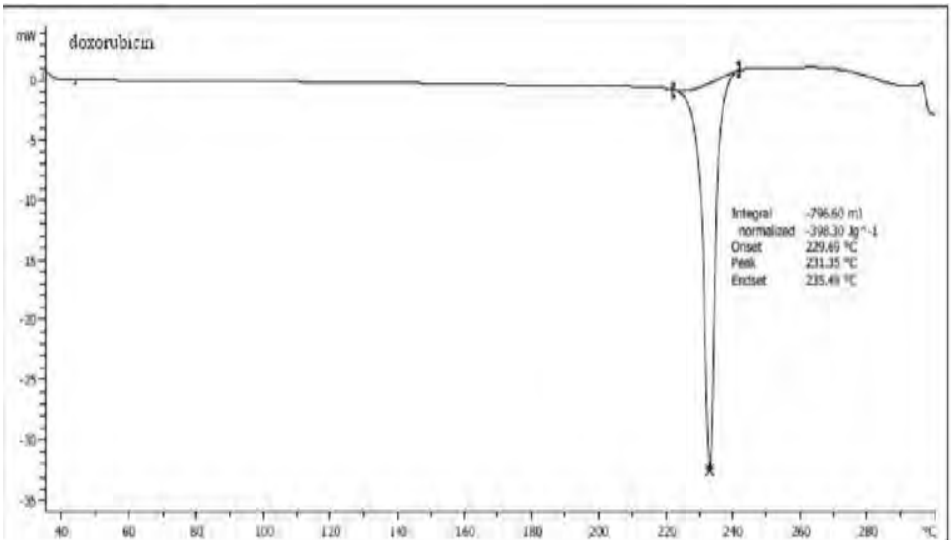

Figure no III: DSC of Doxorubicin Hydrochloride

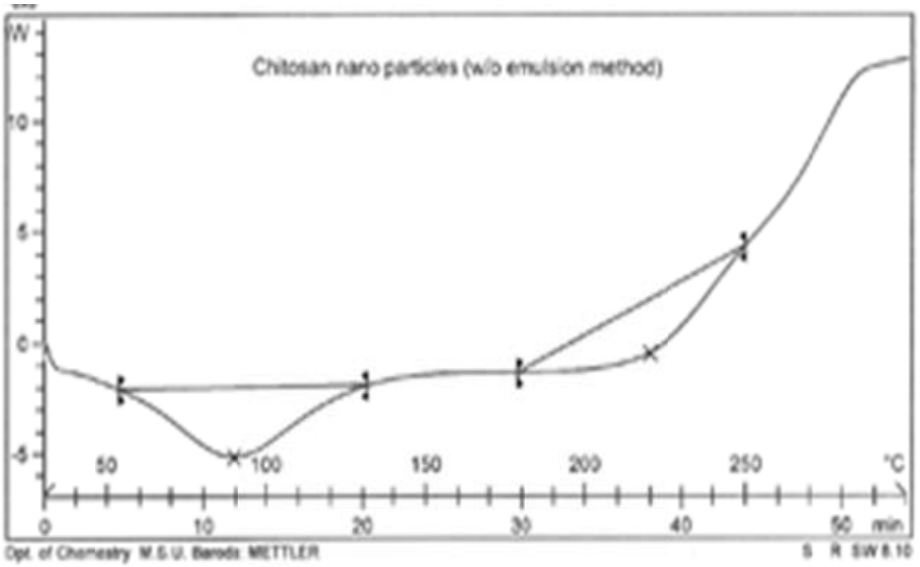

Figure no IV: DSC of dox+chitosan

Presence of Doxorubicin endotherm in nanoparticle thermogram suggests that the doxorubicin nanoparticles were completely entrapped in the polymer matrix.

Formulation table:

Table-I: Formulation table

Batch No.	Quantity of oil	Conc. of span – 20 (%)	Conc. of Chitosan (%)	Conc. of TPP (%)	Conc. of Drug(%)
B1	25 ml	1	2.0	1.0	2.0
B2	25 ml	**2**	2.0	1.0	3.0
B3	25 ml	3	1.0	1.0	2.0
B4	**25 ml**	**5**	**1.0**	**0.5**	**1.0**
B5	25 ml	5	1.0	0.25	3.0

Drug content& % Drug entrapment

Table-II: Drug content & % Drug entrapment

SL NO	Formulation	Drug content	%Entrapment
1	B1	71.8	49.78%
2	B2	81.93	50. 76%
3	B3	73.24	53.31%
4	**B4**	**87.56**	**53.52%**
5	B4	67.15	52.31

Particle size analysis

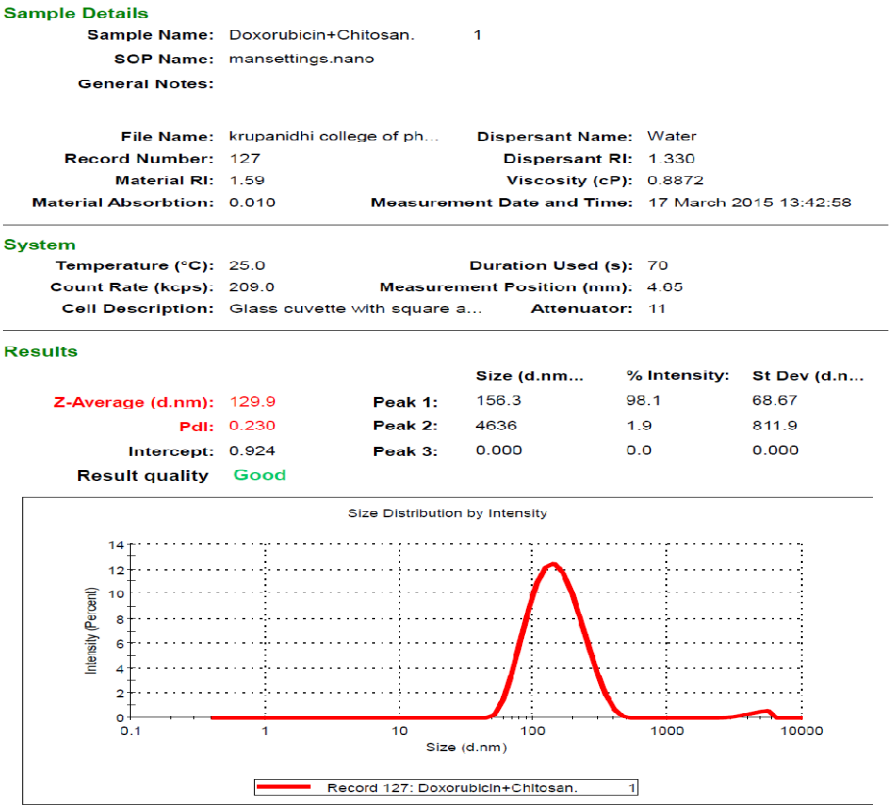

Figure-V: Particle size of nanoparticles

The particle size analysis of Nanoparticles prepared by W/O emulsion method (B4) was found to be d (nm) = 129.9 to 135.4 nm with a polydispersity index 0.230-0.2448. Nanoparticles prepared by W/O emulsion method gives small particle size and narrow size distribution because high shear stress was applied during the steps of preparation.

Surface charge analysis

Sample Details

Sample Name:	Doxorubicin+Chitosan. 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	krupanidhi college of pharm...	**Dispersant Name:**	Water
Record Number:	133	**Dispersant RI:**	1.330
Date and Time:	17 March 2015 13:57:31	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	26
Count Rate (kcps):	107.0	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	7

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	43.9	**Peak 1:**	43.9	100.0	13.6
Zeta Deviation (mV):	13.6	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	1.94	**Peak 3:**	0.00	0.0	0.00
Result quality	See result quality report				

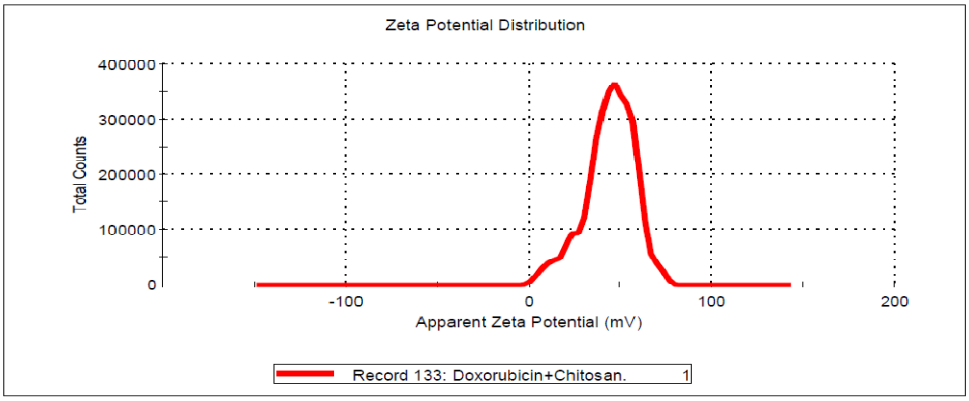

Figure-VI: Surface charge analysis

The zeta potential is a measure of the charge of the particles, as such the larger the absolute value of the zeta-potential the larger the amount of charge of the surface. In a sense, the zeta-potential represents an index for particle stability.

Scanning electron microscopy:

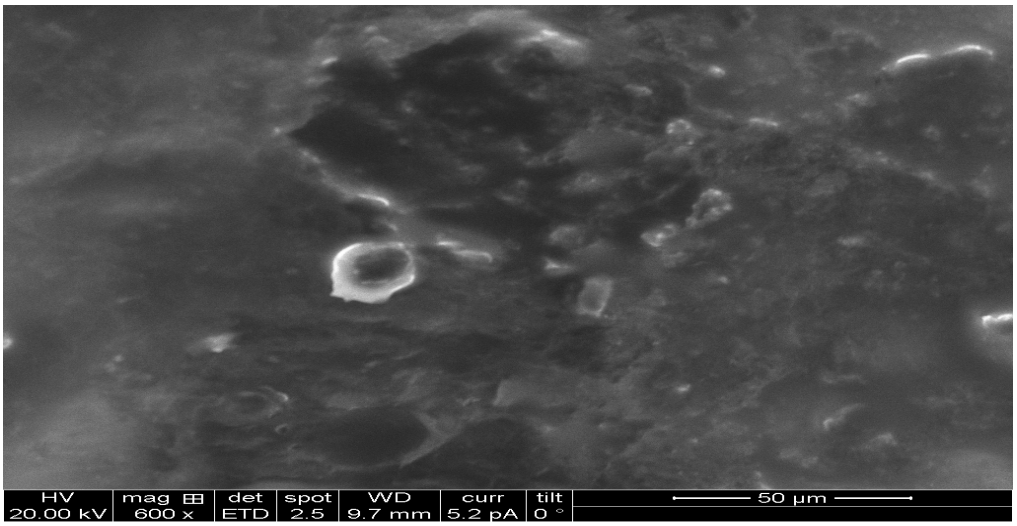

Figure- VII: SEM of formulation B4

The surface morphology indicated uneven surface which may be due to the high shear which is used in formulation.

Graphical presentation of % cumulative drug release

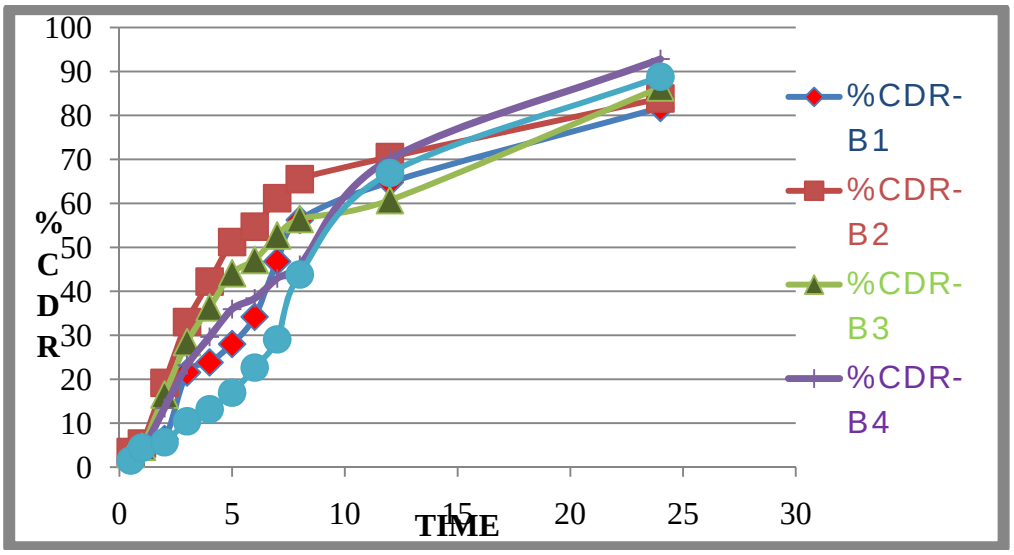

Figure-VIII: % cumulative drug release

The *in vitro* drug release of drug Doxorubicin HCl from the various nanoparticles formulations was carried out by using dialysis method in 7.4 pH phosphate buffer for 24 h. The cumulative percentage release of Doxorubicin HCl from the prepared nanoparticles varied from 81.73102% to 92.80369% which depended upon the drug concentration in the prepared formulations. The release profile graphically represented, where Formulation B4 showed the best release profile.

Regression coefficient value of different kinetic model for best formulation

Best formulation	Zero order	First order	Korsmeyer-Peppas	Higuchi
Regression coefficient (r^2)	0.9837	0.9465	0.9666	0.8704

Table-III: Regression coefficient value of different kinetic models

Zero order and Korsmeyer-peppas models revealed that the drug release was zero order diffusion controlled as indicated with higher r^2 values.

Stability studies

Formulation	30±2&60±5% RH for 30 days		2-8˚C for 30 days	
Best nanoparticle formulation (B4)	Drug content	Entrapment efficiency	Drug content	Entrapment efficiency
	86.56	53.01	87.56	53.52

Table-IV: Stability studies

The results of the stability study showed that there was no significant change in these parameters when stored at 2-8°C,while there is a decreased in drug content and entrapment efficiency when stored at 30±2°C (RH 60±5%) when compared to the initial results. Thus, it can be concluded that 2-8°C and ambient temperature & humidity are the most suitable for storage of prepared nanoparticles of doxorubicin hydrochloride.

CONCLUSION

In this present study, an attempt was made to develop a nanoparticulate delivery system for water soluble drug Doxorubicin HCl. The thesis describes the development, characterization and evaluation of Nanoparticulate drug delivery systems as carriers for anticancer agents for effective delivery to lung cancer. Chitosan nanoparticles of drug Doxorubicin HCl was prepared by W/O emulsion crosslinking method. This method was able to produce desired size and shape uniform nanoparticles.All the formulation showed good drug entrapment

and drug loading capacity. Among the different batches, formulation B4 was selected as the ideal formulation, after considering its drug entrapment and *in vitro* drug release.Particle size showed that the formed particles were in nano size and possesses a positive surface charge.All the formulation was able to sustain the drug release for a period of 24h. Release kinetics showed that the Doxorubicin HCl release from the nanoparticles was zero order diffusion controlled. The n value of Korsmeyer-Peppas equation indicated the release mechanism was Fickian.

Based on the observations, it can be concluded that the formulated nanopartilculate delivery system of Doxorubicin HCl widely accepted and physiologically safe polymer was capable of exhibiting sustained release properties for a period of 24 h. this may reduce concentration of drug to be administered along with frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability, and increase the effectiveness of the drug.

REFERENCE

[1] Yang W, Peters JI, Williams RO. Inhaled nanoparticles- a current review., 2008; 356: 239-47.
[2] Sukumar UK, Bhushan B, Dubey P, Matai I , Sachdev A, Packirisamy G. Emerging applications of nanoparticles for lung cancer diagnosis and therapy. inl-journal., 2013; 3: 5-7.
[3] Komaki, R, Cox, JD: Moss Radiation Oncology: Rationale, Technique. Results, Mosby-Year Book, St. Louis (1994).
[4] Behera D. Epidemiology of lung cancer-Global and Indian Perspective. JIACM., 2012; 13(2): 131-7.
[5] Ferlay J, Shin HR, Bray F et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer.,2010; 15(127):2893-917.
[6] Behera D, Balamugesh T. Lung cancer in India. Indian J Chest Dis Allied Sci.,2004; 46:269-81.
[7] Spiro GS and Silvestri AG. One Hundred years of Lung Cancer. American journal of Respiratory and critical care medicine., 2005; 172: 523-29.
[8] Li P, Dai Y, Zhang J, Wang A, and Wei Q. Chitosan-alginate nanoparticles as a novel drug delivery system for nefedipine. Int J Biomed Sci., 2008; 4(3):221-4.
[9] Dong X, Mattingly CA, Tseng M, Adams, Mumper RJ. Development of new lipid-based Paclitaxel nanoparticles using sequential simplex optimization. Eur J Pharm Biopharm., 2009; 72: 9-17.
[10] Xu Wang, Yiqing Wang, Zhuo (Georgia) Chen, Dong M. Shin. Advances of cancer therapy by Nanotechnology. Cancer Research and Treatment.,2009; 41(1): 1-11.
[11] Chetan C, Anajwala, Girish K. VijayendraSwamy S. M. Currents trends of nanotechnology for cancer therapy. Int J of PharmaSci and Nanotechnology.,2010; 3(3): 223-5.