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pH Dependent Encapsulation of Doxorubicin in PLGA

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ABSTRACT

PLGA nanoparticles loaded with doxorubicin hydrochloride (DOX.HCl) were prepared using Biodegradable poly (D, L – lactide-co-glycolide) – 75:25, by o/w and w/o/w emulsification solvent evaporation method using PVA (Mol. Wt. 9000) as surfactant. The encapsulation efficiency of the drug in w/o/w emulsification solvent evaporation method was found to be greatly affected by pH when the experiments were carried out at two pH i.e. 7 and 8. The maximum encapsulation efficiency was found to be 79% . Nanoparticles were morphologically characterized using SEM and particle size analyzer. The average size of the particles was 200nm. Doxorubicin attached PLGA was confirmed by FTIR analysis. In vitro drug release analysis was done at pH 7 and pH 7.4.It was found that drug release was faster at pH 7.

Keywords: PLGA, Doxorubicin, Drug-encapsulation, PVA, Drug-release

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INTRODUCTION

There are efforts to harness the potential of medicinal nanotechnology to improve the health of human beings and especially to fight the second largest disease of the world i.e. cancer. Doxorubicin hydrochloride is a widely used drug for treatment of a number of different types of cancers. Since FDA approval in the 1970s, this drug has been successfully used to treat a range of cancers including various leukemia, breast cancer, ovarian cancer, various lymphomas, etc. [1, 2]. Despite its potent activity, Doxorubicin- HCl has a number of disadvantages including short plasma circulation half-life, long elimination half-life, and nonspecific cell-cytotoxicity. These shortcomings can lead to dose-limiting side effects including cardiomyopathy and myelosuppression [3,4]. Encapsulating Doxorubicin- HCl in drug carriers like polymeric nanoparticles [5,6,7], liposomes [3, 8], microspheres [9, 10] and hydrogel [4]); is expected to inhibit these disadvantages [11, 12] e.g. Use of depot formulations such as microspheres and hydrogels can reduce toxic side effects while providing extended drug release period that may last up to months. Use of nanoparticulate carriers such as polymeric micelles, polymeric nanoparticles and liposomes to deliver doxorubicin-HCl can confer additional advantages such as prolonged circulation half-life and increased accumulation in tumors due to EPR (enhanced permeation and retention) effect, where the leaky vasculature and poor lymphatic drainage around tumors improve drug retention.

Drug delivery polymers that are biodegradable macromolecules have a variety of uses in drug delivery systems e.g. as scaffolds in tissue engineering, in hydrogel, for PEGylation, for polymer-drug conjugation, polymer therapeutics,

dendrimers, particles, and many other systems. Another advantage of polymers is that the linkage can be designed to control where and when the drug is released and its profile.

Drug delivery polymers can be synthetic (polyethylene glycol, poly(lactic-co-glycolic acid, and polyanhydride) or natural (collagen and hyaluronic acid) in origin. Most have a high molecular weight and are polydisperse. Some have irregular repeat units and many are branched. All of these things can hinder the best efforts to implement characterization studies.

Doxil a liposome based doxorubicin hydrochloride drug has already been FDA approved and used for treatment of cancer. Doxorubicin is highly hydrophilic hence its encapsulation into PLGA nanoparticles is a very challenging job. To improve doxorubicin-HCl loading into and release from polymeric nanoparticles, the strategies that are utilized includes chemical conjugation of doxorubicin to hydrophobic polymers [5,7] and chemical conversion of doxorubicin-HCl into its free base (hydrophobic) form through a chemical reaction with triethylamine (TEA) [11,13,10]

In this paper an effort to conjugate doxorubicin-HCL with PLGA is presented; because PLGA is biocompatible (non toxic, non thrombogeneic and low immunogenicity) and biodegradable. PLGA undergoes acid catalyzed hydrolysis at specific pH that is –COOH groups released on hydrolysis leads to further cleavage of the remaining polymer producing metabolites like lactic acid and glycolic acid which can be used as precursors in various metabolic pathways. Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water. Glycolic acid is either excreted unchanged in the kidney or it enters the tricarboxylic acid cycle and is eventually eliminated as carbon dioxide and water.

PLGA used in the present work was in the ratio of 75:25, because the time of degradation of PLGA depends on its monomeric ratio. PLGA 75:25 takes longer time for degradation than PLGA 50:50 as lactic acid is more hydrophobic as compared to glycolic acid thus lactic acid takes more time to degrade and sustain release of drug is possible.

MATERIALS AND METHODS

PLGA (75:25) was provided as gift by Purasorb. Doxorubicin hydrochloride was generously gifted by Dr. Ranjan Srivastava of RPG Life Sciences Limited. PVA (Mol. Wt. 9000) was purchased from Sigma Aldrich (Bangalore, India). All other reagents used were of analytical grade.

Doxorubicin loaded PLGA nanoparticles were prepared by single emulsion and double emulsion solvent evaporation method. In o/w emulsion solvent evaporation method, 50mg of PLGA was dissolved in acetone: dichloromethane mixture (0.5:2) and doxorubicin hydrochloride dissolved in methanol was added to it. This oil phase was homogenized with 15ml aqueous PVA solution. The experiments were carried out at pH 7. In w/o/w emulsion solvent evaporation method, 50mg PLGA was dissolved in acetone: dichloromethane mixture (0.5: 2). Doxorubicin hydrochloride was dissolved in water and added to the oil phase with homogenization. This mixture was then homogenized with 15ml of aqueous PVA at 15000rpm. The experiments were carried out at pH 7 and 8.

Assessment of Particle Size and Size Distribution- was done by a Dynamic Light Scattering (DLS) method using Zetasizer Nano ZS (Malven Instruments, UK).

Surface morphology of PLGA- Berberine nanoparticles -was studied using SEM. Particles were mounted on a metal hub using carbon adhesive tape and coated with a mixture of gold and palladium in an argon atmosphere.

Drug Content and Drug Entrapment Efficiency - The doxorubicin content of nanoparticles was determined by a spectrophotometer (Perkin- Erlenmeyer). The fluorescence intensity of free doxorubicin in centrifugate was measured at 480 nm. The concentration of drug was calculated from a standard curve, prepared by measuring the fluorescence intensity of known concentration of free doxorubicin. The encapsulation of drug was also confirmed by FTIR analysis. The drug entrapment efficiency was calculated as follows:

$$\text{Entrapment Efficiency} = \frac{\text{Wt. of the drug incorporated}}{\text{Wt. of the drug initially taken}} \times 100$$

FT- IR analysis: To confirm the loading of drug on PLGA Nanoparticles FT-IR spectral analysis was carried out.

In vitro release study- The *in vitro* release studies of doxorubicin loaded on to PLGA NPs were carried out in PBS at pH 7.4 and pH 7. The NPs were dispersed in 1ml of PBS in a dialysis bag and it was suspended in 50ml of PBS and placed in incubator at 37^{o}C. At regular intervals the absorbance of samples was checked with UV spectrophotometer at 480nm for the content of free drug.

RESULTS AND DISCUSSION

The PLGA nanoparticles encapsulating doxorubicin hydrochloride were prepared by o/w and w/o/w emulsification solvent evaporation method using a binary mixture of organic solvents like ACE, DCM and PVA as stabilizer. During the process organic solvent diffuses to the external phase leading to the formation of NPs. The size of NPs and encapsulation efficiency of the drug was found to be considerably affected by the method used, concentration of drug (table -1) and the pH (table - 2).

The poor encapsulation efficiency and larger particle size of the NPs can be observed in the single emulsion method which can be attributed to high water solubility of doxorubicin resulting in rapid leakage of the drug from NPs and swelling of particles. In single emulsion method the encapsulation efficiency of the drug increased with increase in concentration of the drug and maximum encapsulation efficiency was found to be 34%. Further increase in concentration of drug reduced the encapsulation efficiency.

When the encapsulation of drug was tried by w/o/w method at pH 7 no encapsulation was observed. However there was drastic improvement in the encapsulation efficiency of the drug when the double emulsion method was tried at pH8. The maximum encapsulation efficiency was 79% which could be due to the reduced solubility of the drug at alkaline pH.

Table 1: Effect of drug concentration and method used, on encapsulation efficiency at pH 7

Sample No.	Method	**d**rug concn	Encapsulation Efficiency	Particle Size (nm)
1	SE	531µg	-	420-700
2	SE	796µg	19%	790-1120
3	SE	1062µg	34%	393-775
4	DE	700µg	-	275

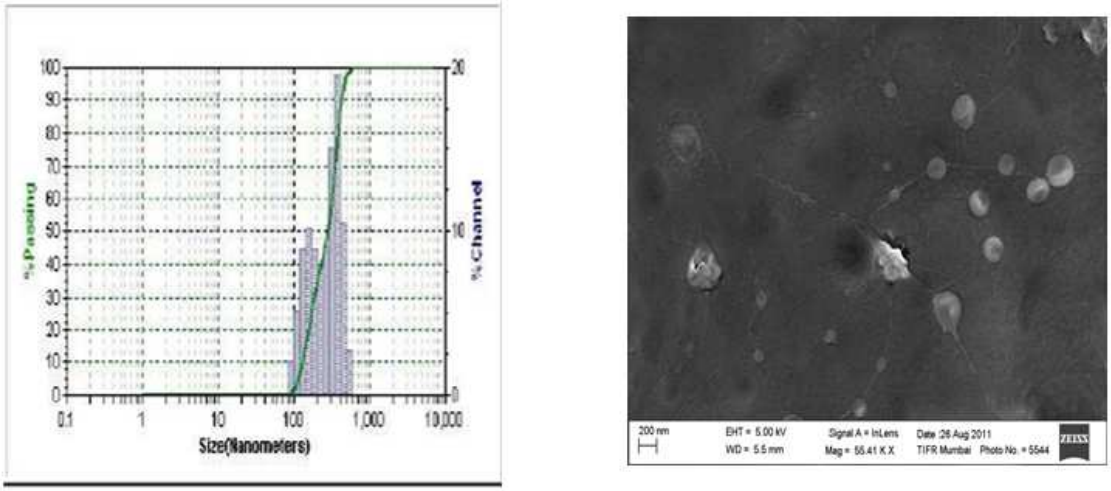


Figure 1: Doxorubicin loaded PLGA (Left) SEM image and
(Right) particle size of doxorubicin loaded PLGA nanoparticles prepared by DE method at pH8.

The *Particle size analysis* indicated that in all the samples prepared by double emulsion method at pH 8, the average size of the NPs was 275nm; whereas SEM micrographs indicated that the doxorubicin-HCl loaded PLGA NP size was in the range of 100 – 200 nm. With the increasing concentration of the drug the number of particles with larger size increased resulting in bimodal peak in particle size analysis graph. The encapsulation efficiency of the drug increased from 65% to79% with increase in concentration of the drug from700 µg to 1633 µg. Whereas the encapsulation efficiency decreased to 62% with further increase in concentration.

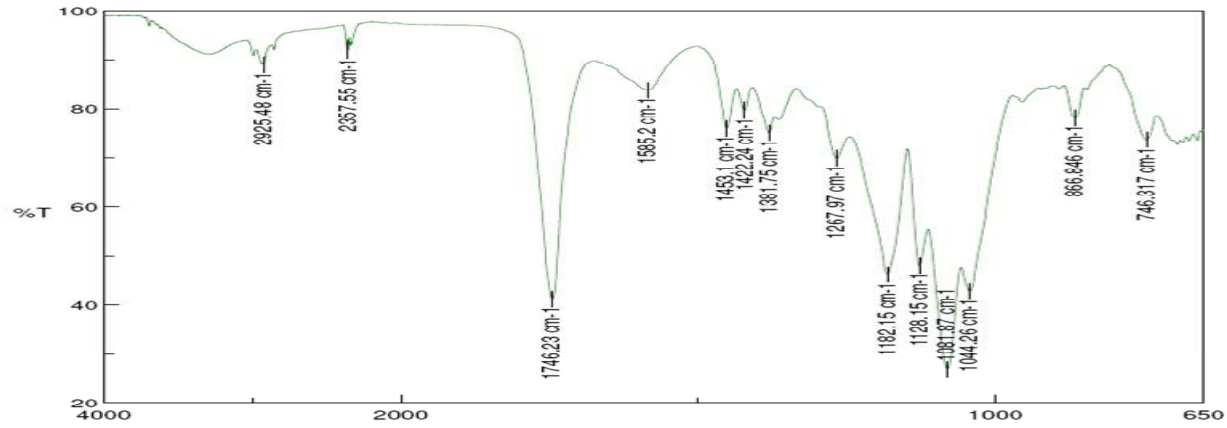
TABLE 2: Encasulation Efficiency AT pH 8

Sample No.	Method	Drug concn.	Encapsulation Efficiency
1	DE	700 µg	65 %
2	DE	1167 µg	68 %
3	DE	1633 µg	79 %
4	DE	1866 µg	62 %

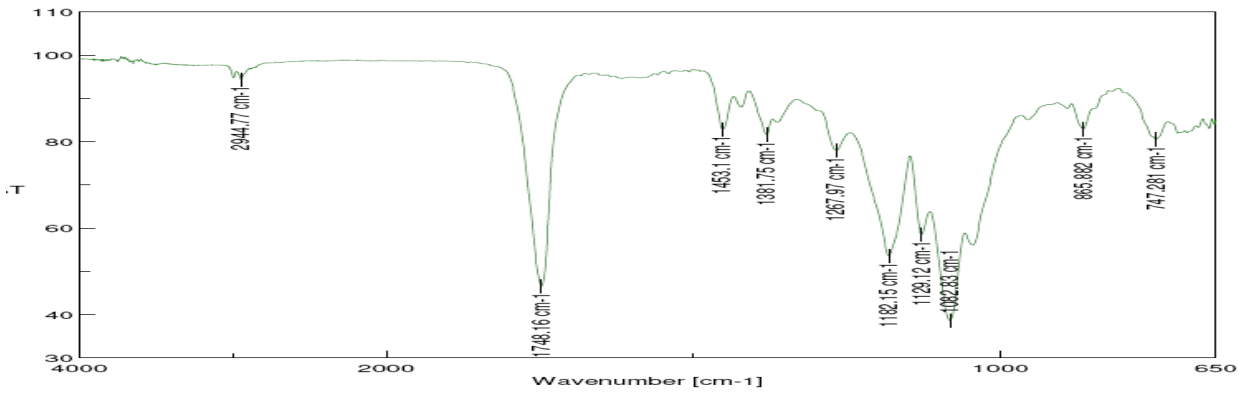
FTIR analysis: Figure - 2 exhibits the FT- IR spectra of doxorubicin-HCl +PLGA nanoparticles, PLGA (75:25) and doxorubicin-HCl

A broad strong characteristic peak at 3316 cm^{-1} corresponds to N-H and C-H stretching for the pure doxorubicin (Figure 2C) and the strong characteristic peak at 1746 cm^{-1} corresponds to C=O stretching (figure2B). The FT-IR spectra of doxorubicin loaded PLGA nanoparticles shows a peak at 2974- 3200 cm^{-1} due to N-H and C-H stretching of doxorubicin and PLGA (Figure 2A). Also shifting of the signal for N-H and C-H indicates chemical interaction between doxorubicin and PLGA.

A)



B)



C)

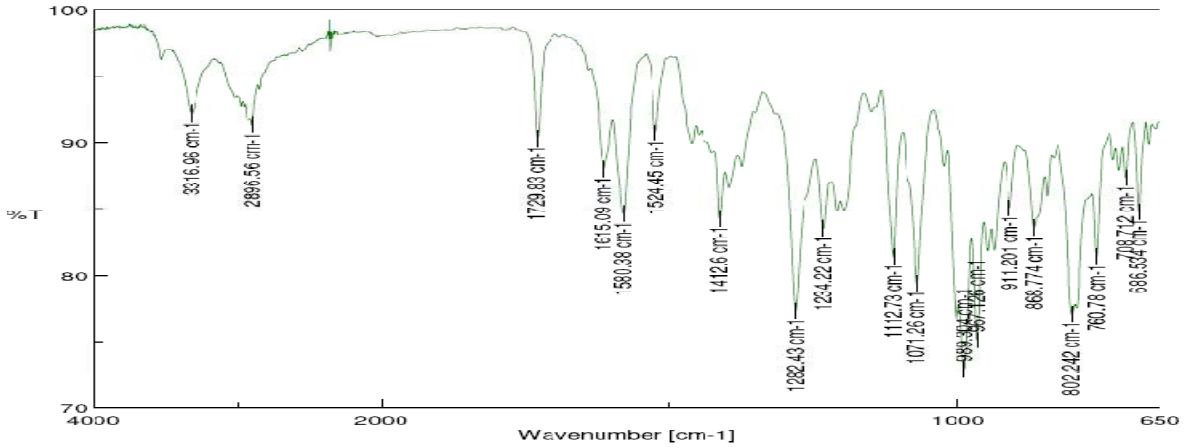


Figure2: FTIR spectra of (A) Doxorubicin-HCl loaded PLGA (B) PLGA (75:25) and (C) Doxorubicin-HCl

In vitro release study: In vitro release study indicates that release of drug from nanoparticles was faster at pH 7 than at pH 7.4. Almost 55% drug was released in first 6 hours at pH7; whereas only 25% drug was released at pH 7.4 during the same time period and thereafter a sustained release was observed up to 24 hours. This may be due to poor chemical interaction between drug and polymer at neutral pH.

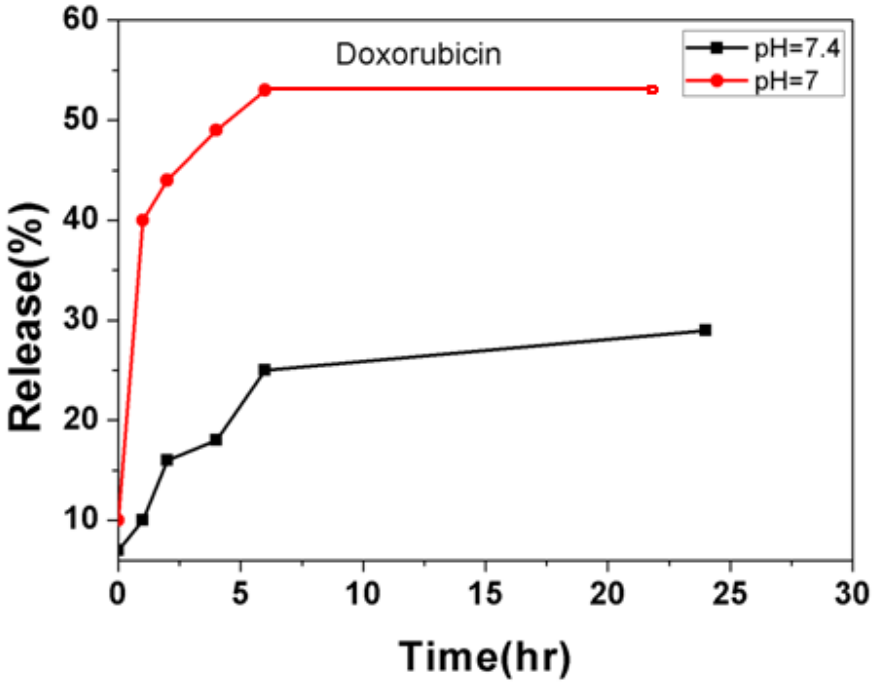


Figure3: In Vitro release of Doxorubicin encapsulated in PLGA (75:25) at pH 7.0 and 7.4

CONCLUSION

The doxorubicin loaded PLGA nanoparticles were successfully prepared. The NPs of different size and encapsulation efficiency can be prepared depending on the method used, drug concentration and pH. The study proved that encapsulation efficiency of doxorubicin in double emulsion method is effectively enhanced by selecting proper pH. Doxorubicin loaded PLGA nanoparticles showed a sustained drug release.

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