RJC

RASĀYAN *J. Chem.*
Vol. 9 | No. 3 |300 - 308 | July - September | 2016
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SILICA COATED LIPOSOMES FOR DRUG DELIVERY TOWARDS BREAST CANCER CELLS

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ABSTRACT

The main aim of the work was to synthesize a drug delivery vehicle such as silica coated liposomes, to incorporate epirubicin-hydrochloride and to find its anti-cancer activity against MCF-7 cell lines. Multilamellar liposomes (phosphatidylcholines) were prepared using thin film hydration using egg yolk. Epirubicin-Hydrochloride, an anti-cancer drug was encapsulated within the fluidic phosphatidylcholine multilamellar lipid vesicles by thin film hydration. Silica was prepared from rice husk ash and the layer of silica was formed above lipid bilayer by acid catalysis method. The variations for the transmittance were noted by FTIR analysis for the coated and uncoated samples. The entrapment efficiency was calculated by UV-Vis Absorbance of the coated and uncoated drug encapsulated liposomes. The coating of silica was confirmed using TEM and Particle Size Analyzer. Silica coating enhances the stability of drug-loaded delivery vehicles. In vitro study reveals that the uncoated drug loaded liposomes showed greater growth inhibition than silica coated formulations. Silica coated liposomes are best suited for in-vivo therapeutic formulations and targeting cancer cells due to their better bio-stability.

Keywords: Multilamellar Liposomes, Anti-cancer, Drug delivery, Epirubicin-Hydrochloride, thin film hydration.

INTRODUCTION

Drug delivery systems, offering controlled delivery of biologically active agents, are rapidly gaining importance in pharmaceutical research and development. To achieve controlled drug delivery, sophisticated systems containing different carriers have been developed. The use of anticancer delivery systems would result in enhanced concentrations of the anticancer agent at the site of infection, due to targeting of drug to the infected tissues, increased intracellular drug concentrations and reduced toxicities of potentially toxic drugs resulting from the targeting of anticancerous drugs to the infectious organism[1]. Liposomes are possible carriers for controlled drug delivery and targeting by the intravenous route. Liposomes are microscopic vesicles consisting of multiple concentric lipid bilayers formed when lipid films are dispersed in an aqueous medium. As with most drug carriers, liposomes have been used extensively in an attempt to improve the selective delivery and the therapeutic index of antimicrobial agents and anticancer drugs. Liposomes, artificial phospholipids membranes, are usually produced from naturally occurring, biodegradable, and nontoxic lipids such as lecithin, cholesterol, and phosphatidylcholine[2].

The use of anticancer drug loaded liposomes may result in increased anticancer drug concentrations at the site of infection (passive or active targeting), increased intracellular anticancer concentrations (enhanced liposome-cell interaction), and reduced toxicities of potentially toxic anticancer agents (biodistribution, away from host cell). It is established that silica coating is chemically inert, biocompatible, hydrophilic and inexpensive. Here, we have prepared liposome having layers of silica to enhance the stability of the formulation. This formulation has improved encapsulation efficiency of macromolecule within the liposome by inhibiting leakage of epirubicin drug due to presence of an outer silica coat[3].

EXPERIMENTAL

Materials and Methods

Fresh Eggs were purchased from the shop to extract lecithin. Rice husk was bought and made into ashes. The drug used for the anticancer study was epirubicin-hydrochloride which is mainly used against Breast cancer cell lines (MCF-7).

Synthesis of Multilamellar liposomes

40g yellow yolks from hen's eggs were separated. To this, 100ml chloroform: methanol mixture in the ratio 2:1 were added. The entire conical flask was then covered with black paper. The conical flask containing the mixtures was shaken vigorously and then agitated for 3 hours at 20^0C at 250rpm. The agitated mixture was then allowed to separate out 60ml of the clear yellow solution that appeared below the creamy layer. The separated clear solution was then concentrated under reduced pressure for 2 days in a vacuum oven. To this mixture, ice cold acetone was added for the lipids to precipitate. The whole mixture was then dried in an oven and chloroform was then added to make the stock solution. About 1ml of the stock solution was taken in a round bottom flask[4]. To this, warm water (that was heated to about 55 to 60^0C) was added. The whole mixture was then stirred. Milky white suspensions of liposome were observed.

Synthesis of Epirubicin encapsulated liposomes

About 5ml of the milky white suspension were taken and heated to the lipids melting temperature (85^0C). 5µl of the drug (Epirubicin Hydrochloride) were added to the heated solution and stirred continuously[5]. Continuous freezing and thawing were done.

Synthesis of silica

100g of rice husk was treated with 50ml of dilute HCl and EDTA at 60-80^0C for 1 hour. It was then washed with double distilled water several times to remove all the acid and was allowed to dry at 110^0Cfor 12 hours in an oven. The dried husk was then burned at 650-700^0C for 4-6 hours to get rice husk ash. 50g of NaOH and 10 times double distilled water was then added to the ash and heated at 100^0C under constant stirring for 4 hours with a pH 9-10. It was then filtered to get a clear and colorless solution of sodium silicate. Dilute HCl was then added to the sodium silicate solution under constant stirring till a precipitate was formed. Distilled water was then added and continued stirring and then dilute HCl was added until a turbid solution with pH 2 was obtained. The turbid solution was then filtrated and separated to get silica gel (UFACS). Silica gel was then dried to obtain the required silica[6].

Synthesis of Silica coated Epirubicin encapsulated Liposomes

0.05g of the prepared silica was taken and was added to the above drug loaded liposomes with continuous stirring and freezing thawing process[7].

Characterization

UV-Visible spectra of silica coated, uncoated multilamellar liposomes, and uncoated drug encapsulated multilamellar liposomes were taken by PC Based Double Beam spectrophotometer 2202, Systronics. The range of measurement is taken from 200-1100cm^{-1}. The entrapment efficiency is calculated from the supernatant solution by determining its absorbance value and can be calculated using the following equation[8] :

$$\text{Entrapment Efficiency}(\%) = \frac{UV\ Absorbance\ of\ Drug - UV\ Absorbance\ of\ drug\ in\ supernatant}{UV\ Absorbance\ of\ Drug}$$

Infrared spectra of silica coated, uncoated multilamellar liposomes, and uncoated drug encapsulated multilamellar liposomes were taken by Fourier Transform Spectrometer (FT-IR), Bruker Optics, Alpha-T, Germany. The range of measurement was taken in the region 4000-500 cm^{-1}.The size of silica coated,

uncoated multilamellar liposomes and uncoated drug encapsulated multilamellar liposomes were visualized by TEM *(*Hitachi H-7650 Tokyo, Japan) operating at 80 kV.The mean particle size of silica coated, uncoated multilamellar liposomes and uncoated drug encapsulated multilamellar liposomes were estimated using Particle Size Analyzer, DC 12000, CPS Instruments USA. The anti-cancer activity in coated and drug encapsulated Multilamellar liposomes were performed using 2 types of cell lines, i.e. in fibroblast cell lines (L929) and breast cancer lines (MCF-7).

RESULTS AND DISCUSSION

UV-VIS Spectroscopy

The comparison of UV- Vis spectra for Liposome, drug encapsulated liposomes and silica coated drug encapsulated liposomes is shown in Fig.-1. The UV-Vis wavelength of the drug was found to be at 484nm and its absorbance at 2.909 as shown in Fig.-2**.** Multilamellar liposomes showed a peak at 383.6nm and its absorbance 2.83. The UV- Vis spectra for Epirubicin Hydrochloride (Drug) are shown in Fig.-2. The entrapment efficiency of the coated and uncoated liposomes was determined after 1h and 24 h[9]. The UV-Vis spectra of drug encapsulated multilamellar liposomes after 1h and 24hrs.

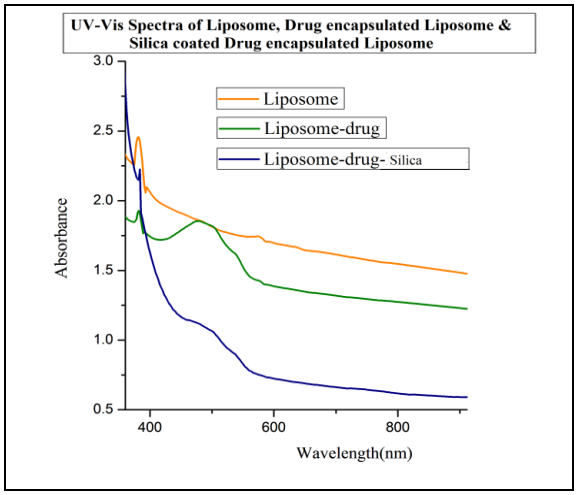

Fig.-1: UV- Vis spectra for Liposome, drug encapsulated liposomes and silica coated drug encapsulated liposomes

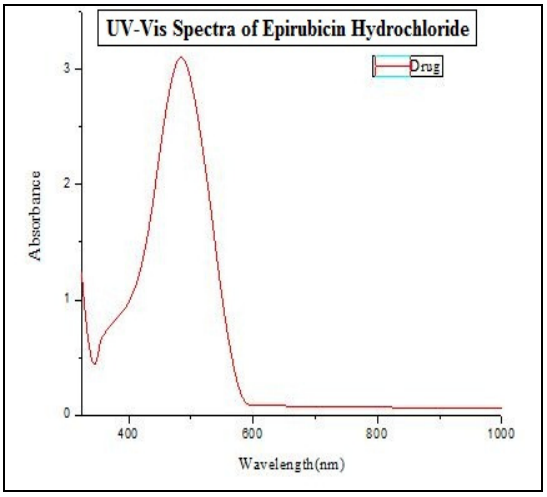

Fig.-2: UV- Vis spectra for Epirubicin Hydrochloride(Drug)

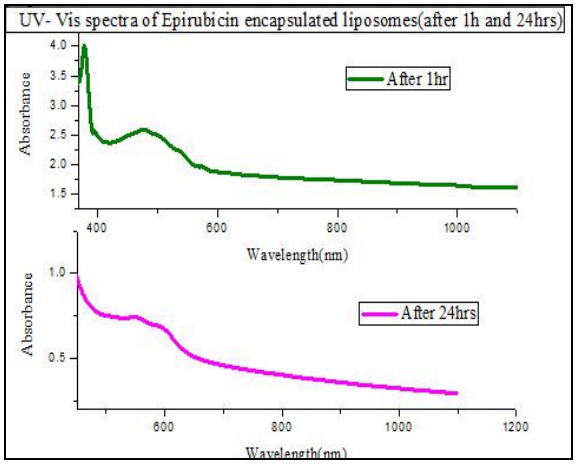

Fig.-3: UV-Vis spectra of drug encapsulated multilamellar liposomes after 1h and 24hrs.

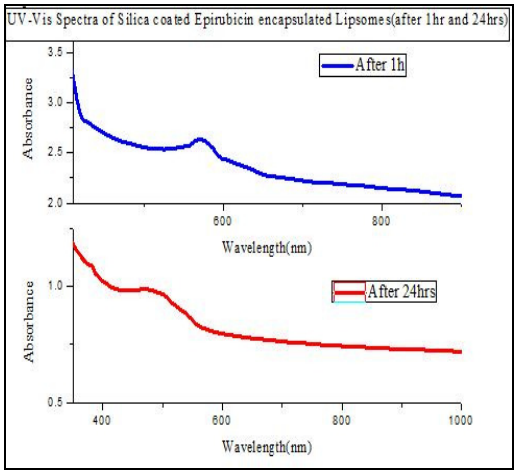

Fig.-4: UV- Vis spectra for silica coated Epirubicin encapsulated liposomes (after 1h and 24hrs)

The comparison of UV- Vis spectra for Epirubicin encapsulated liposomes (after 1h and 24hrs) is shown in Fig.-3. The UV-Vis spectra of silica coated drug encapsulated multilamellar liposomes after 1h and 24hrs is shown in Fig. 4. The entrapment efficiency of various samples calculated after 1h and 24hrs is shown in Table-1. From the results obtained, the drug encapsulated multilamellar liposomes showed higher entrapment efficiency of 76% (after 24h) than the silica coated drug encapsulated multilamellar liposomes (68% after 24h).

Table-1: Drug Loading Efficiency

Name of the sample	Entrapment Efficiency (after 1hr) (in %)	Entrapment Efficiency (after 24hr) (in %)
Epirubicin encapsulated Liposomes	16.45	76
Silica coated Epirubicin encapsulated liposomes	15.06	68

FTIR Spectroscopy

FTIR of silica coated and uncoated multilamellar liposomes were taken by Fourier Transform Spectrometer (FT-IR), Bruker Optics, Alpha-T, Germany. The FTIR of liposomes, drug encapsulated liposomes and silica coated Liposome are shown in Figures- 5 to 7.

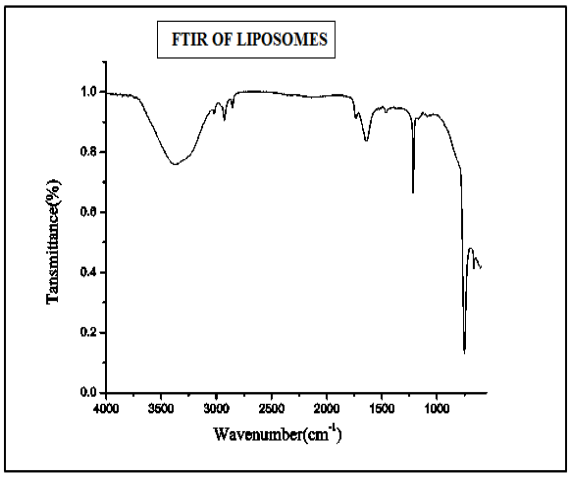

Fig.-5: FTIR spectra of Multilamellar liposomes.

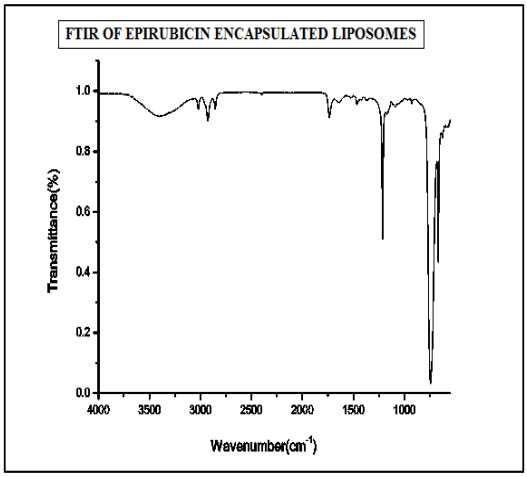

Fig.-6: FTIR spectra of Epirubicin encapsulated liposomes.

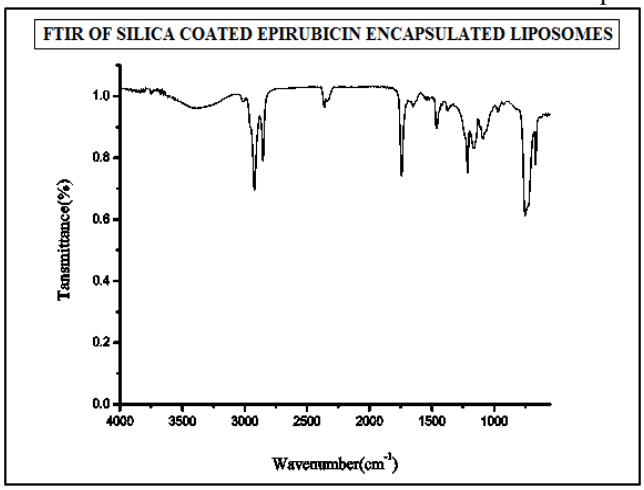

Fig.-7: FTIR spectra of Silica coated Epirubicin encapsulated liposomes.

The FTIR spectra of multilamellar liposomes is shown in Fig.-5 and it showed a curved band at 3366 cm-1 and were assigned to a region containing –OH intermolecularly and intramolecularly H bonds. The sharp peak at 2865 cm^{-1} near 2930- 2915 cm^{-1} showed the presence of $–CH_2$ asymmetric bonds ie; due to the –CH. Absorptions due to methyl groups occur in this region The sharp peak at 1736 cm^{-1} showed strong bands due to C=O stretching in this region The sharp peak at 1214 cm^{-1} was assigned to $[PO_2^-]$ bonds ranging from 1225-1195 cm^{-1}. This peak shows the phosphatidyl groups present in the sample[10]. The peak at 749 cm^{-1} was due to the $–(CH_2)_n$ bonds stretching in the region 755-735 cm^{-1}. Finger print region is found at 670 cm^{-1}.

The FTIR spectra of drug encapsulated liposome is shown in Fig.-6. The FTIR spectra of drug encapsulated multilamellar liposomes showed a curved band at 3402 cm^{-1} and were assigned to a region containing –OH intermolecularly and intramolecularly H bonds. The peak at 2928 cm^{-1} is due to the –CH stretching and is due to the presence of alkanes. The sharp peak at 2854 cm^{-1} near 2930- 2915 cm^{-1} showed the presence of $–CH_2$ asymmetric bonds ie; due to the –CH. Absorptions due to methyl groups occur in this region The sharp peak at 1737 cm^{-1} showed strong bands due to C=O stretching in this region The sharp peak at1214 cm^{-1} was assigned to $(CH_2)_3$-C bonds ranging from 1225-1195 cm^{-1}. This peak shows the phosphatidyl groups present in the sample. The peak at 742 cm^{-1} was due to the $–(CH_2)_n$ bonds stretching in the region 755-735 cm^{-1}.

The FTIR spectra of silica coated Epirubicin encapsulated multilamellar liposomes are shown in Fig.-7. This graph showed a curved band at 3402 cm^{-1} and were assigned to a region containing –OH intermolecularly and intramolecularly H bonds. The peak at 2921 cm^{-1} is due to the –CH stretching and is due to the presence of alkanes. The sharp peak near 2930- 2915 cm^{-1} showed the presence of $–CH_2$ asymmetric bonds ie; due to the –CH. Absorptions due to methyl groups occur in this region The sharp peak at 1745 cm^{-1} showed strong bands due to C=O stretching in this region The sharp peak at 1214 cm^{-1} was assigned to $(CH_2)_3$-C bonds ranging from 1225-1195 cm^{-1}. This peak shows the phosphatidyl groups present in the sample. The peak at 751 cm^{-1} was due to the $–(CH2)_n$ bonds stretching in the region 755-735 cm^{-1}. Silica coating present in the formulation was evident from Si-O[11] stretching at 1100 cm^{-1}, Si-OH at 980 cm^{-1} and the Si-O-Si bending at 770 cm^{-1}.

TEM

The TEM images of liposomes, drug encapsulated liposomes and silica coated liposomes are represented in Fig.-8 and Fig.- 9.

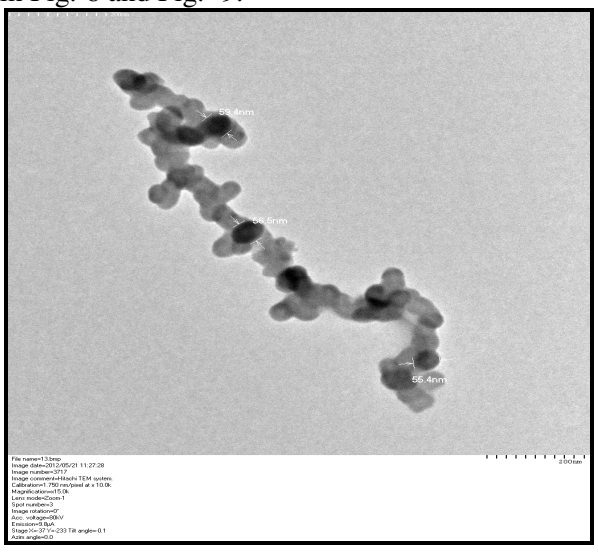

Fig. 8: TEM image of Multilamellar Liposomes

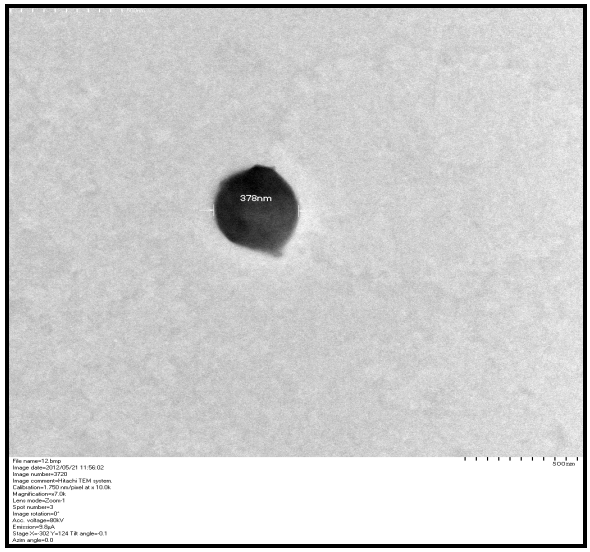

Fig. 9: TEM image of Silica coated Epirubicin encapsulated multilamellar Liposomes

It is evident from the above figures that the coated and uncoated liposomes are spherical in shape. The mean particle size obtained from TEM photomicrographs of multilamellar liposomes was in the range of 55-80 nm. This value was correlated well with the results from particle size analysis. The silica coated drug loaded liposomes showed particles of size ranged 300-500 nm. The above figures clearly demonstrate the morphological differences occurred between the uncoated liposomes and the liposomes that are sterically stabilized by silica[12].

Particle Size Analysis

The particle size of liposomes and silica coated drug encapsulated liposomes were measured in dilute aqueous suspensions. The size of the particle determines where the light is scattered. The particle size distribution of liposomes and silica coated drug encapsulated liposomes are shown in Fig.-10 and Fig.-11.

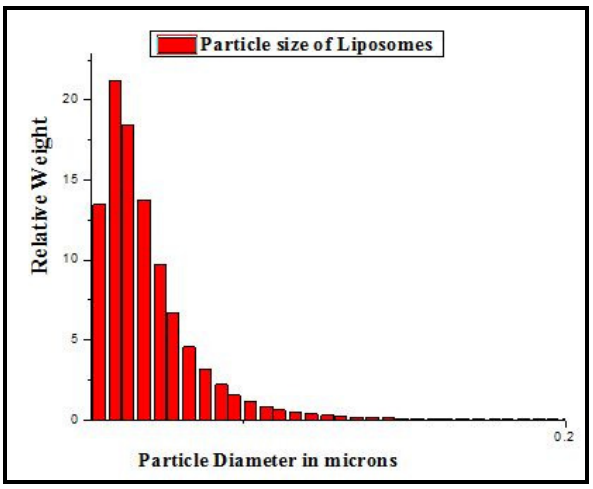

Fig.-10: Particle size analysis for Multilamellar liposomes

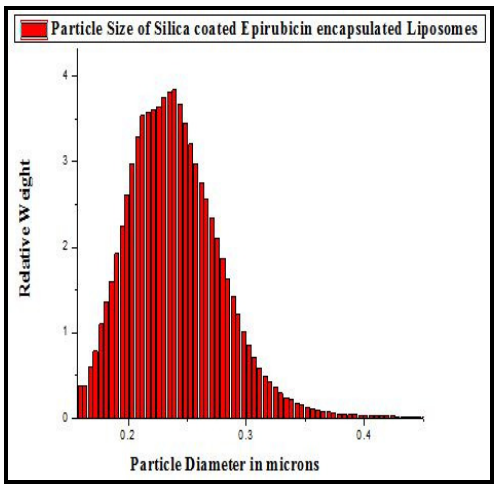

Fig.-11: Particle size analysis for silica coated Epirubicin encapsulated Liposomes.

Multilamellar liposomes showed a mean size of 76.2nm while the silica coated drug encapsulated liposomes showed a considerable variation in its particle size ie, 297.8nm. This showed an increase in its size due to the silica coating. This result was correlated with the TEM images of silica coated multilamellar liposomes[13].

MTT assay for apoptosis evaluation

MTT assay was done against fibroblast cells lines (L929) and breast cancer cell lines (MCF-7). MTT assay result revealed higher cell death in breast cancer cell lines (MCF-7) than fibroblast cell lines when the drug and the silica coated and uncoated drug loaded liposomes were supplied at different concentration. The below figures shows the in vitro results of the drug encapsulated silica coated and uncoated liposomes adduct against 2 cell lines. Cytotoxicity increases with increasing adduct concentration more dramatically for Breast cancer (MCF-7) tumor cells[14] than for fibroblast cells (L929). At a concentration of 10µl, 80.7% of MCF-7 was killed by the drug encapsulated liposome while the silica coated drug encapsulated liposomes showed cell death of 75.2%.

Figure-12 denotes the control used in fibroblast cells lines (L929). Figures- 13 to 15 show various concentrations (1µl, 5µl and 10µl) of silica coated Epirubicin encapsulated liposomes in fibroblast cells lines (L929). fig. 16 shows the control used in breast cancer cell lines. Figures-17 to 19 show various concentrations (1µl, 5µl and 10µl) of silica coated Epirubicin encapsulated liposomes in breast cancer cell line (MCF-7)**.** From the figures, the silica coated Epirubicin encapsulated liposomes has higher cell death in MCF-7 cell lines than L929 cell lines.

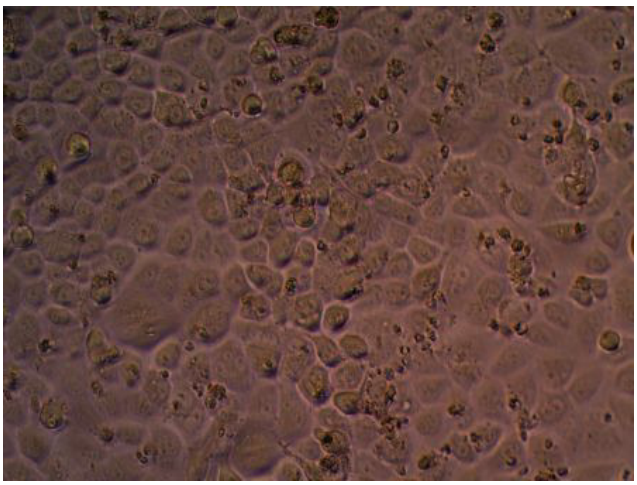

Fig.-12: Control used in L929 cell lines

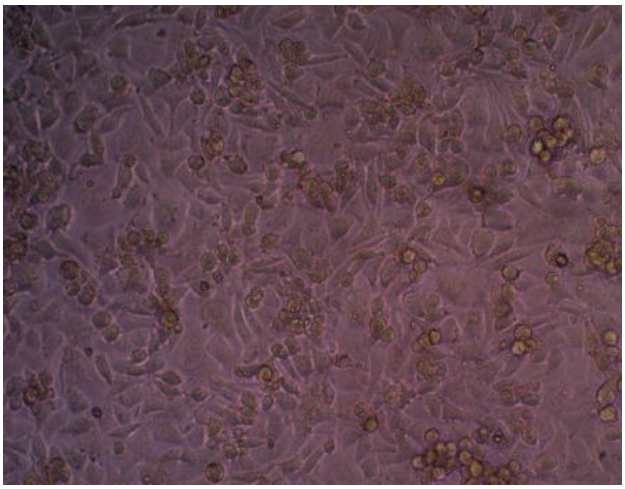

Fig.-13: 1µl of Silica coated Epirubicin encapsulated liposomes in fibroblast cells lines (L929)

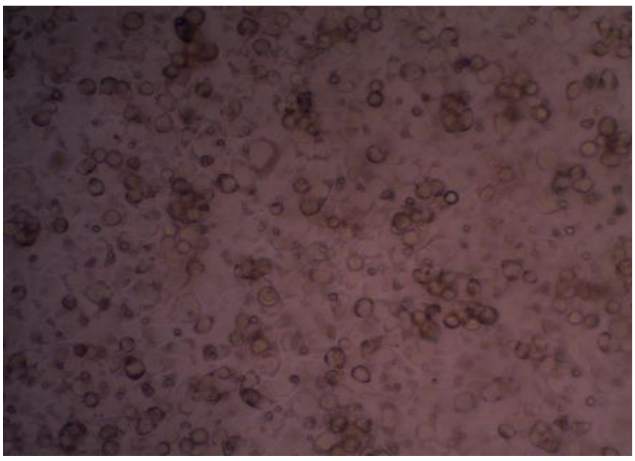

Fig.-14: 5 µl of Silica coated Epirubicin encapsulated liposomes in fibroblast cells lines (L929)

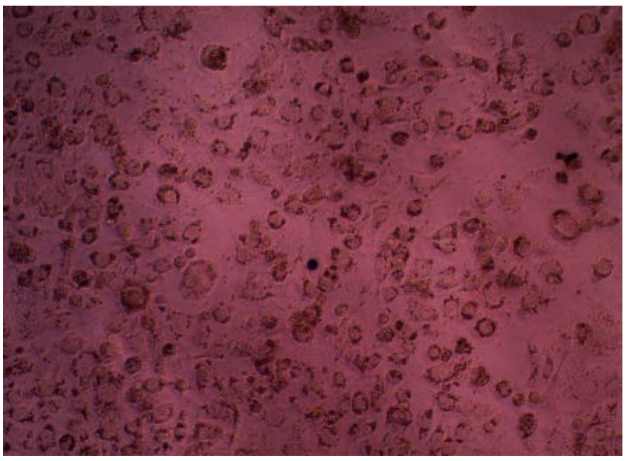

Fig.-15: 10µl of Silica coated Epirubicin encapsulated liposomes in fibroblast cells lines (L929)

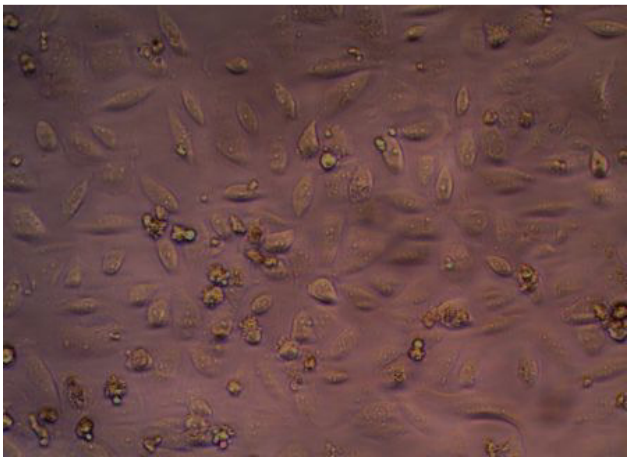

Fig.-16: Control used in MCF-7 cells

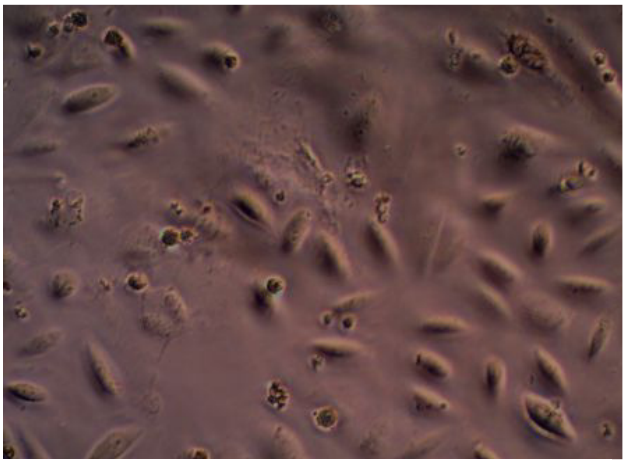

Fig.-17: 1 µl of Silica coated Epirubicin encapsulated liposomes in breast cancer cell lines (MCF-7)

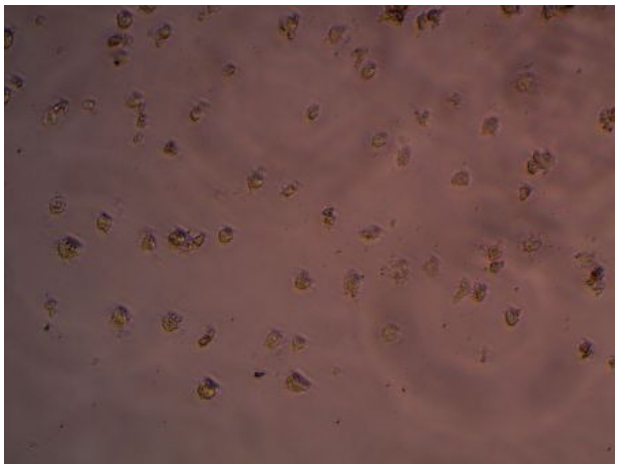

Fig.-18: 5µl of Silica coated Epirubicin encapsulated liposomes in breast cancer cell lines (MCF-7)

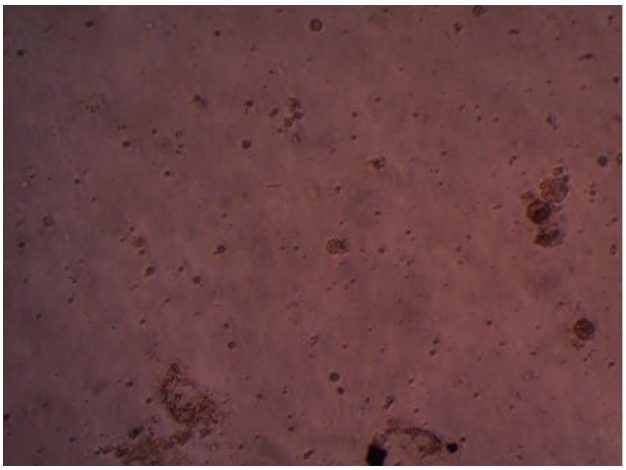

Fig.-19: 10µl of Silica coated Epirubicin encapsulated liposomes in breast cancer cell lines (MCF-7)

In-Vitro analysis against fibroblast cells lines (L929)

In-vitro analysis were done for (epirubicin) drug, epirubicin loaded liposomes (DL)[15] and silica coated epirubicin loaded liposomes (SDL) against normal cell lines (L929) which is shown in Fig.-20.

In-Vitro analysis against breast cancer cell lines

As shown in Fig.-21, in breast cancer cells the incorporation of Epirubicin in liposomes enhanced the cytotoxic effect of the drug as compared to free drug and silica coated epirubicin encapsulated liposomes. Cytotoxicity results showed higher cell death for Epirubicin encapsulated liposomes than silica coated epirubicin encapsulated liposomes. The reason for higher growth inhibition relies on the advantage of liposomes to penetrate through the cell membranes whereas silica coated liposomes, due to its stability[16], the penetration will be less.

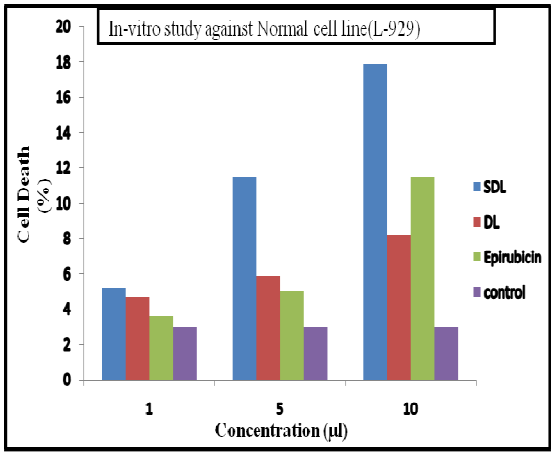

Fig.-20: In-vitro study against fibroblast cells lines (L929)

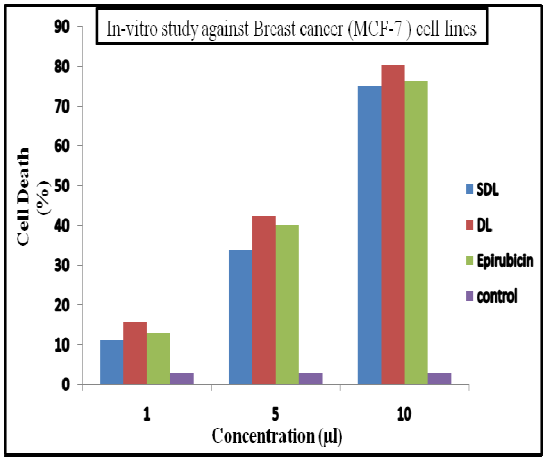

Fig.-21: In-vitro analysis against Breast cancer cell lines

CONCLUSION

Multilamellar liposomes were successfully prepared using thin film hydration method. Silica was successfully prepared from rice husk ash. Epirubicin- hydrochloride drug was successfully encapsulated inside the liposomes and was coated with silica which was synthesized using acid catalysis method. This was characterized using UV-Vis Spectroscopy, FTIR, Particle Size Analyzer and TEM. The drug entrapment efficiency after 1hr and 24hrs was calculated using the absorbance of the UV-Visible spectra.

In case of Epirubicin encapsulated liposomes, the efficiency was calculated to be 16.45% in 1hr and after 24 hrs the efficiency was found to be 76%. 15.064% entrapment efficiency was calculated after 1hr and that at 68% after 24hrs for silica coated Epirubicin encapsulated liposomes. The results showed higher entrapment efficiency in Epirubicin encapsulated Liposomes than silica coated Epirubicin encapsulated liposomes after 24hrs than 1 hr. FTIR results confirmed a silica coating over the Epirubicin encapsulated multilamellar liposomes and the variations in transmittance for uncoated and coated liposomes. In comparison with the spectra of the coated and uncoated drug encapsulated liposomes, strong bands were found at 3300-3500 cm^{-1} which was due to OH-groups linked to the H-bonds. The bands at 2852 cm^{-1} by the liposomes were shifted to 2924 cm^{-1} for the drug encapsulated liposomes and to 2865 cm^{-1} for the silica coated liposomes and this band is due to the symmetric stretching of $[(CH_3)_4]$. The average particle size estimated for the multilamellar liposomes was approximately of 50-200nm and that for silica coated multilamellar liposomes obtained was in the range of 300-500nm. This was confirmed using TEM and particle size analysis. In-vitro cytoxicity studies were done against cell lines ie; Fibroblast cells lines (L929) and Breast Cancer (MCF-7). From the results obtained, the Epirubicin encapsulated liposomes showed higher growth inhibition than the silica coated epirubicin encapsulated liposomes. Breast cancer cell lines showed the highest percentage of cell death for the concentrations (10:50:100) ie; (15.7:42.3:80.5) for uncoated epirubicin encapsulated liposomes than the silica coated epirubicin encapsulated liposomes (11.1:33.9:75.2). The complete work proved the anti-cancer activity for the Epirubicin encapsulated liposomes were higher in breast cancer cell lines than the silica coated Epirubicin encapsulated liposomes and the drug itself. The present formulation can also be used as a drug delivery vehicle for pharmaceutical industries. Anti-cancer activities for in-vivo studies are also possible.

ACKNOWLEDGEMENTS

The author Princy Alexander is very thankful to the Chancellor, Noorul Islam University, Kumaracoil for their valuable guidance, constant encouragement and providing basic facilities to carry out the present research work.

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[RJC-1423/2016]