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Formulation and characterization of Ciprofloxacin encapsulated liposomes: In vitro antimicrobial activity against multi drug resistant *Salmonella typhi*

Formulación y caracterización de liposomas encapsulados de ciprofloxacina: actividad antimicrobiana in vitro frente a *Salmonella typhi* multirresistente

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Abstract

Since decades antibiotics have been used to combat diversified bacterial infections. They have also been used for a variety of other medicinal applications too. They have been a blessing to human beings in the battle against microbes, saving millions of lives. Globally, the infections caused by multidrug-resistant (MDR) bacteria are on the rise as they are gaining resistance towards antibiotics. *Salmonella typhi* is one such pathogen responsible for typhoid fever. It is on its way to become resistant towards antibiotics. Thus, there is a need to combat this infectious pathogen. Different nanocarriers have been used for this purpose and liposomes are well-established systems due to their high biocompatibility, bioavailability and possibility to vehiculate drugs. They are designed to carry drug safely to the action site. Currently liposomal formulations were designed to encapsulate ciprofloxacin and to analyze its in vitro efficacy against MDR *Salmonella typhi*. Formulations were prepared with Bx-DSPE-PEG$_{(2000)}$ (0.1%), 100mg lipids: Phosphatidylcholine (PC) and Cholesterol (CH) at ratio of 5:3 which resulted in 93.95% encapsulation efficiency. Ciprofloxacin encapsulated liposomes reflected a faster release than usual with stability gradually reduced from 93.95% to 71.9% over the period of 9 months, with 0.7 Polydispersity index, 95.27nm particle size and -20.58mV zeta potential. Minimum Bactericidal concentration (MBC) and minimum inhibitory concentration was at lower drug concentrations i.e., 15 μg/ml, and 10 μg/ml, respectively. Conclusively, the prepared liposomes proved an effective in vitro drug delivery method against MDR *Salmonella typhi*.

Keywords: *Salmonella typhi*, Nanocarriers, Liposomes, Ciprofloxacin, Multidrug-resistant (MDR).

Resumen

Desde hace décadas se han utilizado antibióticos para combatir diversas infecciones bacterianas. También se han utilizado para una variedad de otras aplicaciones medicinales. Han sido una bendición para los seres humanos en la batalla contra los microbios, salvando millones de vidas. A nivel mundial, las infecciones causadas por bacterias multirresistentes (MDR) están aumentando a medida que adquieren resistencia a los antibióticos. *Salmonella typhi* es uno de esos patógenos responsables de la fiebre tifoidea. Está en camino de volverse resistente a los antibióticos. Por lo tanto, existe la necesidad de combatir este patógeno infeccioso. Se han utilizado diferentes nanotransportadores para este propósito y los liposomas son sistemas bien establecidos debido a su alta biocompatibilidad, biodisponibilidad y posibilidad de vehicular fármacos. Están diseñados para llevar el fármaco de forma segura al sitio de acción. Actualmente se diseñaron formulaciones liposomales para encapsular ciprofloxacino y analizar su eficacia in vitro contra MDR *Salmonella typhi*. Las formulaciones se prepararon con Bx-DSPE-PEG$_{(2000)}$ (0,1%), 100 mg de lípidos: Fosfatidilcolina (PC) y Colesterol (CH) en una proporción de 5:3, lo que resultó en una eficiencia de encapsulación del 93,95 %. Los liposomas encapsulados con ciprofloxacina reflejaron una liberación más rápida de lo habitual con una estabilidad reducida gradualmente del 93,95 % al 71,9 % durante el período de 9 meses, con un índice de polidispersión de 0,7, un tamaño de partícula de 95,27 nm y un potencial zeta de -20,58 mV. La concentración bactericida mínima (MBC) y la concentración inhibitoria mínima estaban en concentraciones de fármaco más bajas, es decir, 15 μg/ml y 10 μg/ml, respectivamente. En conclusión, los liposomas preparados demostraron ser un método eficaz de administración de fármacos in vitro contra MDR *Salmonella typhi*.

Palabras clave: *Salmonella typhi*, Nanoportadores, Liposomas, Ciprofloxacino, Multirresistente (MDR).

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1 Introduction

Gastroenteritis is a diarrheal disease, characterized by an increase in the frequency of bowel movement with or without fever, stomach pain and vomiting (Barrett and Fhogartaigh. 2017). It is commonly known as "Typhoid fever" caused by a Gram-negative bacteria *Salmonella typhi* (Gómez-Montaño *et al*., 2021; Malik-Kale *et al*., 2011); a causative agent of *Salmonella typhi* (Azhar *et al*., 2019). The typhoid fever is responsible for morbidity and mortality in humans (Masuet-Aumatell *et al*., 2021). However, on the global scale the burden of typhoid fever through human history is not cleared and determined so far. As the antibiotics used against it are no longer effective in curing it resulting in thousands of deaths. The symptoms include prolonged fever and abdominal discomfort which can become serious and can cause complications too, like intestinal perforation (Pitzer *et al*., 2019). Once the bacteria; *Salmonella typhi* enters into the body of its host, it starts multiplying causing the disease. Therefore, there is a need to overcome this infection by using antibiotics, through an antimicrobial therapy (Muñoz-Correa *et al*., 2020; Gibani *et al*., 2019). Reportedly, the typhoid fever has an estimated global incidence of 11-21 million cases annually, with 120 000-160 000 deaths approximately (Cao *et al*., 2021). Antibiotics are the only means to cure infectious diseases (Yayehrad *et al*., 2022). *Salmonella typhi* strain has become resistant to all antibiotics used for its treatment so far (Azhar *et al*., 2019) which requires utmost attention in order overcome the disease.

Multiple drugs with known susceptibility towards pathogens, are now becoming ineffective due to mutations in bacterial genome which is attributed to continuous antibiotic exposure (Gashe *et al*., 2018). In current study, we have selected ciprofloxacin as our model drug, due to its characteristic of being commonly used and still effective for the treatment with low toxicity, low bacterial resistance and broad-spectrum antimicrobial activity (Xu *et al*., 2019 ; Al-Joufi *et al*., 2022). At present typhoid is being treated with fluoroquinolones i.e. ciprofloxacin and cefixime (Gonzalez-Escobedo *et al*., 2011), however the pace with which pathogen is acquiring resistance towards antibiotics is making the health professionals to opt for new remedies to cure the pathogenic infections (Gómez-Montaño *et al*., 2020; Dahiya *et al*., 2019). *Salmonella typhi* has also shown resistant towards ciprofloxacin (Hughes *et al*., 2021), in concern to which the current study focuses on the use of ciprofloxacin encapsulated liposomes to not only to reduce drug load on the body but also its effectiveness at this concentration. Researchers all over the world are looking for alternative ways of treating bacterial infections. Right quantity and immediate delivery are important factors to eradicate the pathogen from developing resistance. Drug targeting through drug delivery system (DDS) is a new promising tool currently being used to solve various problems related to drug delivery. This approach comprises of designing a system, capable of releasing the drug in a controlled manner by enhancing the therapeutic efficiency, reducing the side effects and drug leakage into rest of the body (Sur *et al*., 2019).

Among all the presently available nano-carriers, liposomes have gained attention as most favorable drug delivery system due to their non-toxic nature. The encapsulation of hydrophilic and hydrophobic drugs can be achieved by liposomal nanocarriers as they have a great potential to respond (Meng *et al*., 2020). Different antibiotics are encapsulated in liposomes to enhance their site specificity. Ciprofloxacin is one of the antibiotic, which when encapsulated in liposomes, helps in achieving site specificity by treating infections caused by bacterial pathogens and is used to treat various bacterial infections (Ferreira *et al*., 2021).

Conclusively, it can be stated that this study has successfully constructed unilamellar ciprofloxacin encapsulated liposomes modified by Bx-DSPE-$PEG_{(2000)}$. This is the first study that shows the effectiveness of nano-carriers against antibiotic resistant *Salmonella typhi*.

2 Materials and methods

2.1 Materials

All the materials i.e. dialysis bags (12,000-14,000 MW cut off), Phosphatidylcholine, Cholesterol, Bx-DSPE-$PEG_{(2000)}$, Ammonium sulphate, Ciprofloxacin, phosphate buffer, saline used in the study were purchased from Sigma-Merck.

2.2 Methods

2.2.1 Ciprofloxacin encapsulated liposomes

Liposomes were prepared using Bx-DSPE-$PEG_{(2000)}$ (0.1%), 100mg lipids i.e. Phosphatidylcholine (PC)

and Cholesterol (CH) at the ratio of 5:3, dissolved in 1ml organic solvent. Excess organic solvent was evaporated (60 ºC and 120 rpm). A thin dry lipid film was formed inside the flask which was hydrated with 4ml, 250 mM ammonium sulfate (pH 5.5) buffer in rotary. After that, the liposomal suspension was sonicated for about 10 min at 25 ºC at room temperature for the reduction of the vesicle size and to avoid aggregation.

The lipid vesicles were filtered using a 0.1 μm (100 nm) pore sized polycarbonate membrane filters to obtain small unilamellar vesicles (SUVs). A trans-membrane ion gradient of ammonium sulphate was generated by removing the untapped ammonium sulphate through ultracentrifugation at 150,000×g (equivalent to 29,600 rpm) at 4 °C for 90 min. The pellet containing the ammonium sulphate encapsulated liposomes were washed and suspended in Phosphate buffer saline (PBS) (7.4). Finally ciprofloxacin 20 mg/ml (CIP, Sigma Aldrich) prepared in PBS (pH 6.8) was added to the liposomes mixture for the formation of ciprofloxacin encapsulated liposomes by incubating the solution at 60 °C for 60 min (Ghosh *et al*., 2019).

2.2.2 *Separation of free drug*

After the ciprofloxacin encapsulation, free drug was removed by ultracentrifugation at 10,000 g for 30 min at 4 °C. The pellet containing the drug encapsulated liposomes was washed and suspended in 1ml PBS (pH 7.4) whereas the supernatant (containing free drug) was used to calculate the encapsulation efficiency of the liposomes (Corrêa *et al*., 2019).

2.2.3 *Encapsulation efficiency*

Encapsulated efficiency (EE) of liposomes was determined by following Liu *et al*. (2015). According to which drug concentration was measured in the supernatant that was previously separated. The encapsulation efficiency of drug was expressed as the percent of drug encapsulated and calculated (Naveed and Waheed. 2014). The encapsulation efficiency was calculated as follows:

$$EE\% = \frac{[\text{Total drug}] - [\text{Free drug}]}{[\text{Total drug}]} \times 100\% \qquad (1)$$

2.2.4 *Stability of liposomal formulations*

Stability of liposomal formulations was determined to evaluate the rate of drug leakage from liposomes. After the removal of the non-encapsulated drug, liposome dispersions were maintained at 4 °C for 9 months. After every 30 days interval, encapsulation efficiency was determined using UV-Visible spectrophotometer at 277 nm (Jain and Shastri. 2011).

2.2.5 *In vitro release assay*

Drug release from liposomes was assessed using dialysis membrane (DM) method. Dialysis bags (12,000-14,000 MW cut off; Sigma-Aldrich) were soaked prior to use in distilled water at room temperature for 12 h for the sake of removal of the preservative. Each dialysis bag contained 100 μl free drug, 100 μl non-encapsulated liposomes, and 100 μl drug encapsulated liposomes. For the drug release, each dialysis bag was separately immersed in a conical flask containing 20 ml of PBS (pH 7.4) and stirred through magnetic stirrer at 100 rpm at 37 °C. Throughout the experiment, 1 ml of the aliquot in different time intervals was removed from the receptor chamber and replaced with an equivalent volume of fresh PBS. The release run was continued at assigned time periods (1 h, 3 h, 5 h and 24 h). The absorbance of sample was taken at 277nm and drug release was calculated (Weng *et al*., 2020).

2.2.6 *Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)*

MIC of both ciprofloxacin and ciprofloxacin encapsulated liposomes was determined by agar dilution method. For this purpose the antibiotic concentration ranging from 5-40 μg/ml was used and the bacterial inoculum was adjusted to 0.5 McFarland standard. MIC was determined as the lowest concentration of the antibiotic completely inhibiting the microbial growth (Kashef *et al*., 2020).

MBC was determined by time kill technique; best studied assay for the determination of bactericidal effect. Overnight cultures of *Salmonella typhi* adjusted to 0.5, McFarland standard were taken and proceeded further. MBC was observed at 0 h, 2 h ,4 h ,6 h ,8 h and 24 h with drug concentration ranging from 5-40 μg/ml. MBC was established as the lowest concentration of antibiotic, able to promote a 99.9% reduction of the initial bacterial inoculum (Alhariri *et al*., 2017).

2.2.7 *Characterization of liposomes and ciprofloxacin encapsulated liposomes*

The particle size, zeta potential and polydispersity index (PDI) of the liposomes (control and ciprofloxacin encapsulated liposomes) were measured by zeta sizer (Malvern) (Popovska. 2014). Morphological information of liposomes and ciprofloxacin encapsulated liposomes was measured through atomic force microscopy (AFM) (Haeri *et al*., 2014).

2.2.8 *Statistical analysis*

One-way ANOVA was used to find out the significance ($p \leq 0.05$) difference between means of different treatments. SPSS program version 16 was used along with application of Duncan multiple range test. Each experiment was repeated thrice.

3 Results and discussion

3.1 *Encapsulation efficiency*

EE of ciprofloxacin encapsulated liposomes was determined using the dialysis technique. Initially, ratios (5:1, 5:2, 5:3, 5:4, 5:5, 5:6, 5:7) of phosphatidylcholine (PC) and cholesterol (CH) were used to determine the encapsulation efficiency, where PC was kept constant while CH was gradually increased to check its effect on the encapsulation efficiency (Fig. 1a). Conclusively, the molar ratio 5:3 was considered optimum for the current study as it yielded high (93.95%) encapsulation efficiency of ciprofloxacin encapsulated liposomes. The encapsulation efficiency obtained in the current study was higher than the efficiency reported earlier where it was reported 85.2% (Wang *et al*., 2018). Similarly in another study 71-79% of the encapsulation efficiency was achieved (Khatib *et al*., 2019). Whereas more than 90% encapsulation efficiency of the ciprofloxacin encapsulated liposomes was also reported in another study (Ghosh *et al*., 2019). According to Hosny (2010), the encapsulation efficiency of ciprofloxacin encapsulated liposomes increased to 73% with an increase in CH content (Hosny. 2010). Therefore, it can be concluded that the CH content has profound effect on the encapsulation efficiency of liposomes. The increase in CH content results in an increased encapsulation efficiency due to the rigidity of the lipid bilayer. It helps in achieving increased stability, reduced permeability leading to the maximum drug delivery with maximum encapsulation efficiency (Zhang *et al*., 2014).

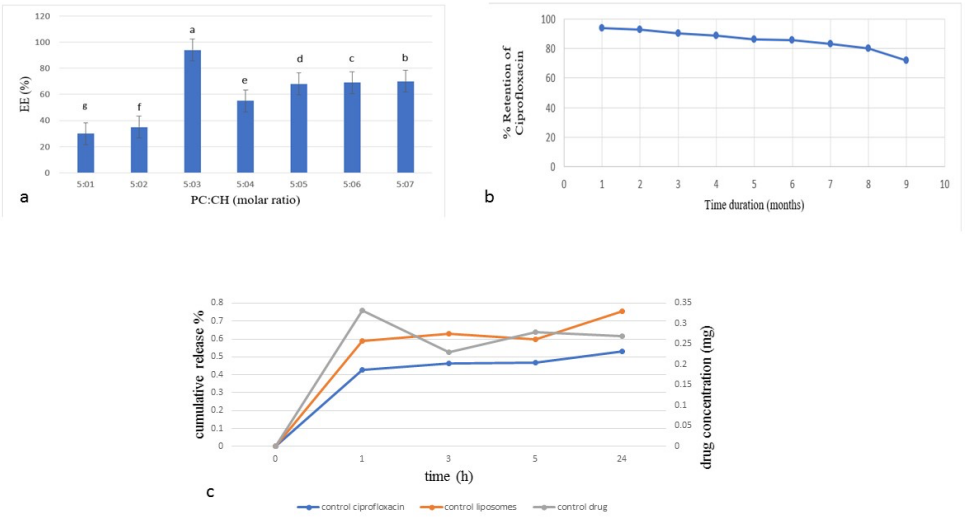

Figure 1: Ciprofloxacin encapsulated liposomes profiling a) Encapsulation Efficiency of Ciprofloxacin encapsulated liposomes with different cholesterol concentration b) Stability of Ciprofloxacin encapsulated liposomes stored at 4 ºC for period of nine months c) Release profile of control ciprofloxacin, control liposomes and Ciprofloxacin encapsulated liposomes.

Another reason for increase encapsulation efficiency could be attributed towards using the pH gradient method for drug encapsulation as also stated in another study where ~95% encapsulation efficiency was achieved with active loading of ciprofloxacin by a transmembrane pH gradient method (Munaweera *et al*., 2018).

3.2 Stability of liposomal formulations

The stability was observed for ciprofloxacin encapsulated liposomal formulations over a period of 9 months. Initially the ciprofloxacin encapsulated liposomes showed high stability with 94% encapsulation efficiency. However the stability was reduced to 71.9 % (Fig. 1b) over the period of 9 months. Whereas in one of the study 80% encapsulation efficiency was observed over the same period. The stability retained for a longer period when stored in refrigerator i.e. 4°C, and as also reported by another study it was reported that stability can reduce if the formulations are not stored properly and slow reduction was observed in stability over a longer period (Pignatello *et al*., 2018).

In another study by Jain and Shastri (2011), the liposomal formulation showed decrease stability when CH content was raised considerably that might resulted in increased efflux of the encapsulated drug, and consequently resulting in the delayed retention of drug. It was stated that liposomes remain much stable under refrigerator storage as all the features of the lipid membrane were retained (Jain and Shastri. 2011). Thus, it can be concluded that if the CH content is raised higher, it leads to decrease in encapsulation efficiency which ultimately results in reduced stability. This phenomenon is also coined in many of related studies which explains that the increase in CH content above a certain limit profoundly decreases the stability and encapsulation efficiency of the liposomal formulations (Munaweera *et al*., 2018; Weers. 2019).

Therefore under refrigerated conditions nano-carriers can be stored for longer period of time with not so much effect on the stability.

3.3 In vitro release assay

In the current study, In vitro release assay of the ciprofloxacin encapsulated liposomes initially showed slow drug release, which increased with the passage of time. Whereas ciprofloxacin (control) showed fast release initially which slowed down with the passage of time (Fig. 1c).

In one of the study it was reported that the encapsulation of ciprofloxacin within liposomes extended the drug release time and the release rate of drug from liposomes depends upon the liposome surface burst effect (Cipolla *et al*., 2016). Same findings were reported from other studies (Panwar *et al*., 2010 ; Torge *et al*., 2017 ; Munaweera *et al*., 2018). The efficacy of slow and prolonged release of drugs is several-fold. First, it reduces the number and frequency of doses required that minimizes patient noncompliance and eliminates the night time administration of drugs. Secondly, when a fast-release drug is taken, there is a rapid surge of the drug throughout the body. As metabolism of the drug proceeds, the concentration of the drug diminishes. A slow-release drug would eliminate these peaks and valleys of fast-release drugs, which place a strain on cells. Because a constant lower concentration of the drug is being released, it reduces the possibility of toxic levels of drugs. It also reduces gastrointestinal side effects (Silverman, 2004).

3.4 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

3.4.1 MIC and MBC for ciprofloxacin encapsulated liposomes

The MIC for ciprofloxacin was determined through agar dilution method and MBC through the time kill assay following the Clinical and Laboratory Standards Institute (CLSI) guidelines. The MIC value for ciprofloxacin was 15 μg/ml during 4 h of incubation, whereas MBC was 20 μg/ml where growth rate reduced from 10^9 to 10^2 log i.e., 99.9% reduction as compared to growth obtained on 2 h of incubation. The other tested concentrations i.e. 25-40 μg/ml showed reduction in the growth rate from 6 h of incubation (Fig. 3a). In few of the studies the break point MIC values of ciprofloxacin in case of *Salmonellae Typhi* infection was <0.125 μg/mL as susceptible, 0.125 μg/mL - 1 μg/mL as reduced susceptibility, and ≥ 1 μg/mL as resistant (Ingle *et al*., 2019; Lee *et al*., 2021). Some studies have reported MIC and MBC of ciprofloxacin ranging from 5.68 μg/ml to 44.45 μg/ml while others reported range from 1-0.5?μg/ml against resistant Salmonella isolates during 24 h of incubation (Girish *et al*., 2013; Khanal *et al*., 2017). In one of the study 0.016μg/ml MIC against *Salmonella typhi* strain, declared sensitive towards the antibiotic

(Jin *et al*., 2019). In another study the MIC value for ciprofloxacin ranged from 1.2 - 9.5 μg/mL, while the MBC ranged from 78.1 - 312.5 μg/mL for 24 h of incubation, respectively (Seanego and Ndip. 2012). In the current study bacterial growth varied with the increase and decrease in ciprofloxacin concentration as also observed in other studies (Girish *et al*., 2013; Chang *et al*., 2021).

Thus, it can be concluded that in the current study MIC (15 μg/ml) for ciprofloxacin was effective in reducing growth of bacterial up to 99.9% as early as 4 h of incubation.

3.4.2 MIC and MBC for ciprofloxacin encapsulated liposomes

MIC and MBC was also determined for ciprofloxacin encapsulated liposomes with different concentrations of entrapped drug (5-40 μg/ml). The MIC value obtained in the current study for ciprofloxacin encapsulated liposomes was 10 μg/ml. For MBC different drug concentrations (5-40 μg/ml) were studied at 0 h, 2 h, 4 h, 6 h, 8 h and 24 h, where at 5 μg/ml countless growth was observed at 24 h and at 10 μg/ml, 4 log reduction within 4 h of incubation was observed. At 15 μg/ml drug concentration the growth rate reduced from countless to nil within 2 h, no growth was observed from the 4 h of incubation and this pattern continued to be the same till 24 h of incubation. Thus, it was considered optimum MBC. Whereas at high drug concentrations i.e., 20, 30, 35 and 40 μg/ml, bacterial growth was not inhibited. Conclusively, liposomes carrying low drug concentration were more effective in controlling the bacterial growth in less time span as compared to ciprofloxacin alone. This condition might be due to the low drug encapsulation as also reported in another study (Cipolla *et al*., 2014).

In the current study the minimum concentration of the ciprofloxacin encapsulated liposomes that killed the bacteria within 2 h of incubation after its release from liposomes (MBC) was 15μg/ml, while the minimum concentration (MIC) that inhibited the growth within the 4 h of incubation was 10 μg/ml (Fig. 3b). Thus, it can be concluded that the ciprofloxacin encapsulated liposomes were efficient in killing the microbe much earlier than ciprofloxacin alone and is not reported earlier at this concentration and therefore drug encapsulated liposomes showed an effective drug delivery system.

3.5 Characterization of liposomes and ciprofloxacin encapsulated liposomes

In the current study liposomes had 0.4 polydispersity index, 102.5nm particle size and -39.2mV zeta potential. Whereas for ciprofloxacin encapsulated liposomes the polydispersity index was 0.7, particle size was 95.27nm and zeta potential -20.58mV (Fig. 2a and b). The size of encapsulated liposomal formulations after dilutions was decreased in the current study. This was also observed in another study where liposomal formulations upon diluted with PBS resulted in significant decrease in size (Lujan *et al*., 2019). Apart from dilutions another factor that influenced the reduction in size was the addition of Bx-DSPE-PEG$_{(2000)}$ in the liposomal formulations that resulted in small sized unilamellar vesicles. Biotin in current study played an important role not only in reducing the size of liposomes but also increasing the PDI of liposomes as also explained in another study (Salem *et al*., 2015).

In the current study reduction in the zeta potential of ciprofloxacin encapsulated liposomes was observed as compared to liposomes. This phenomenon is coined up in one of the previous work according to which the reduction in zeta potential is due to the distribution of a drug in liposomes and on the physico-chemical properties of the drug at certain pH (Kashef *et al*., 2020). Slight reduction of negative surface charge due to encapsulation of Ciprofloxacin was also reported in other studies (Torge *et al*., 2017 ; Munaweera *et al*., 2018 ; Kashef *et al*., 2020). Therefore the particle size was reduced in the current study, after the encapsulation of drug, that played a significant role in maintaining the long-term stability of liposomes with slow reduction in the encapsulation efficiency and same findings were reported in another study (Shu *et al*., 2019).

Surface morphology of liposomes and ciprofloxacin encapsulated liposomes was measured through atomic force microscopy (AFM) (Fig. 2c,d). In the current study AFM was done in tapping mode for the observation of surface modifications of liposomes and ciprofloxacin encapsulated liposomes just like done in previous studies, resultantly the liposomes were observed as small globular structures in our current study same structure was also observed in studies conducted years back (Ruozi *et al*., 2011 ; Robson *et al*., 2018).

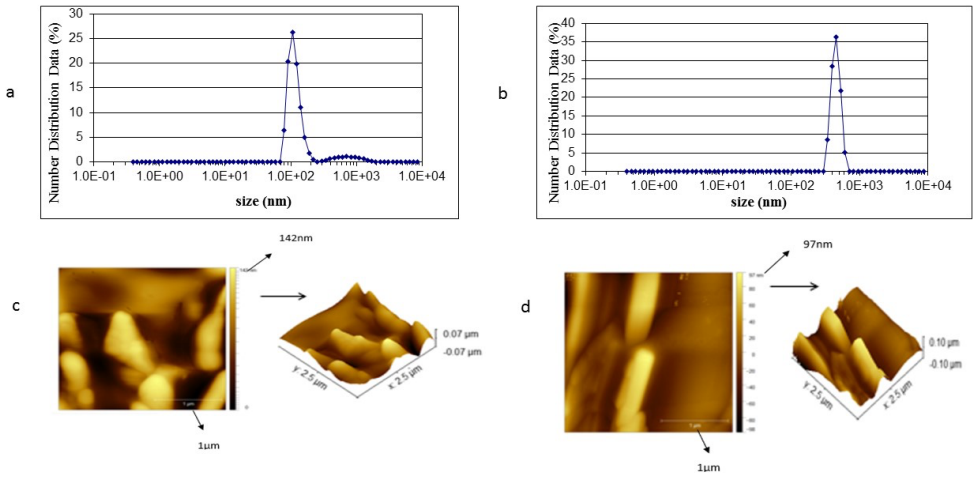

Figure 2: Characterization of drug loaded liposomes a) zeta size analysis and peak representing the size of control liposomes b) zeta size analysis and peak representing the size of drug loaded liposomes. c) Control (blank) liposomes visualized by AFM d) Ciprofloxacin encapsulated liposomes visualized by AFM.

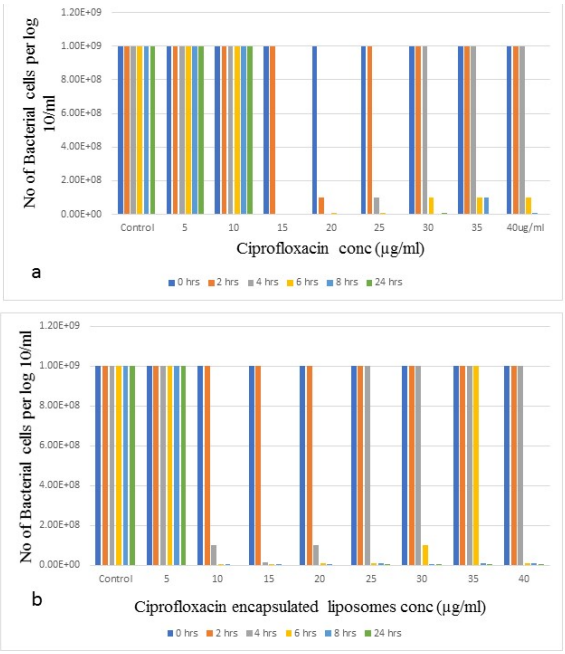

Figure 3: Minimum inhibitory concentration (MIC) of *Salmonella typhi* at different time intervals a) Ciprofloxacin b) Ciprofloxacin encapsulated liposomes.

Tapping mode was used for the imaging of liposomal formulations in the current study because of their delicate nature. Same mode was applied for their imaging back in year 2020 (Khanal *et al.*, 2020). Resultantly AFM results showed light and dark areas in the current study indicating a smooth surface texture for both liposomes and ciprofloxacin encapsulated liposomes. The dark area depicted the collapsed structure, characterized by a flattened lipid layer, while the bright contour suggested the hydration of lipids. Similar explanation of dark and light area were explained in the previous studies (Ruozi *et al.*, 2011 ; Topal *et al.*, 2018). In one of the previous study the flattening of liposome was observed which is thought to be considered because of their high surface density preventing the tip from direct contact with the liposomal surface (Das *et al.*, 2019). Moreover, AFM was being used in current study to examine the lipid bilayers and to observe the significant differences of liposomes and ciprofloxacin encapsulated liposomes. It was also used for the size confirmation of the formulations, which was almost same as obtained through zeta sizer. Same phenomena has been used in one of the previously conducted study where AFM technique was used for size confirmation, and bilayers profiling of lipids (Benne *et al.*, 2020).

Conclusions

In the current study ciprofloxacin encapsulated liposomes that were prepared with Bx-DSPE-$PEG_{(2000)}$, lipids, Phosphatidylcholine (PC) and Cholesterol (CH) at ratio of 5:3, resulted in 93.95% encapsulation efficiency, with stability that gradually reduced from 93.95% to 71.9% over the period of 9 months. These liposomes had 0.7 polydispersity

index, 95.27nm particle size and -20.58mV zeta potential. Minimum bactericidal concentration and minimum inhibitory concentration was at lower drug concentrations i.e., 15 μg/ml, and 10 μg/ml, respectively. The ciprofloxacin encapsulated nano-carriers produced, proved an effective drug delivery system having reduce MIC and MBC value with respect to ciprofloxacin used alone. Further in detail in vivo studies can be conducted to confirm their efficiency as substitute for conventional drug.

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