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RESEARCH ARTICLE

Niosomal encapsulation of the antitubercular drug, pyrazinamide

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Abstract

It is estimated that more than one-third of the world population is infected with *Mycobacterium tuberculosis*. Pyrazinamide (PZA) plays a unique role in shortening therapy because it kills a population of semilatent tubercle bacilli residing in an acidic environment. Niosomes are vesicles made up of non-ionic surfactant and exhibit behavior similar to liposomes *in vivo*. Preparation of PZA niosomes took place using different molar ratios of Span 60 and Span 85, with cholesterol (CH) i.e. Span: CH (1:1) and (4:2). Dicetyl phosphate and stearyl amine were used in preparation of negative and positively charged niosomes, respectively. Free PZA was separated by cooling centrifugation and estimated spectrophotometrically at 268.4 nm. Niosomes were characterized by electron microscopy and differential scanning calorimetry. The highest percentage PZA entrapped was obtained using Span 60 and the molar ratio (4:2:1) negatively charged niosomes. This was followed by the neutral PZA neutral (4:2) Span 60 niosomes. Biological evaluation of selected PZA niosomal formulations took place on guinea pigs infected with *M. tuberculosis*. The present work is an attempt to target maximum concentration of PZA to the affected site (lungs) and to exclude undesirable side effects and decrease toxicity. Macrophage targeting and overcoming drug resistance is our final goal.

Keywords: Tuberculosis, pyrazinamide, niosomes, biological evaluation, characterization

Introduction

Human tuberculosis (TB) is a contagious-infectious disease mainly caused by *Mycobacterium tuberculosis*, an aerobic pathogenic bacterium that establishes its infection usually in the lungs[1]. It is estimated that more than one-third of the world population is infected with *M. tuberculosis*. In 2005, there were an estimated 8.8 million new active TB cases and 1.6 million people died of TB[2]. TB is a disease that thrives in conditions of poverty, malnutrition and limited access to healthcare. Developing countries bear the brunt of global TB burden[3].

Pyrazinamide (PZA), an important frontline TB drug, plays a key role in shortening TB therapy from 9 to 12 months to the current 6 months[4,5]. The ability of PZA to shorten the TB therapy is related to its activity against a population of non growing, persisted tubercle bacilli residing in an acid pH environment that are not killed by other TB drugs[4–6].

Non-ionic surfactant vesicles (niosomes) result from the self assembly of hydrated surfactant monomers[7]. They are similar in physical structure and form to the more widely studied phospholipid vesicles (liposomes), consisting of single or multiple surfactant bilayers (lamellae) enclosing an aqueous core[8]. *In vivo*, niosomes have showed similar activity to liposomes in improving the therapeutic performance of drugs[9] and their distribution in the body follows the pattern of other colloidal drug delivery systems[10]. Niosomes are advantageous to liposomes as they are less expensive, more stable.

The encapsulation of PZA in niosomes would promote the drug's efficacy, reduce the administered drug dose and decrease the toxic side effects via reducing the

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drug dose and dosing frequency, thus improving patient compliance for better management of TB. This study was approved by an investigational review board of animal care and use committee.

Materials and methods

Chemicals

PZA (El-Nasr Pharm. Chem. Co., Egypt), Span 60, >98% (Merck, Germany), Span 85, >98% (Fluka, Germany), cholesterol (CH), minimum 99% (Sigma Chemical Co., St. Louis, MO), dihexadecyl phosphate (dicetyl phosphate, DCP), >98% (Fluka, Germany) and octadecylamine (Stearylamine, SA), minimum 97% (Sigma Chemical Co.). All other chemicals were of analytical grade.

Biological materials

Guinea pigs, of either sex, were used in the biological experiments. *M. tuberculosis*, strain H37Rv was supplied by Veterinary Serum and Vaccine Research Institute, Cairo, Egypt. The biological indicator used in sterilization experiment, *Bacillus pumilus* E601, was supplied by the control department of the Statens Serum Institute, Copenhagen, Denmark.

Preparation of PZA niosomes

PZA niosomes were prepared by applying the Vortex Dispersion Method[11]. Preparation took place using two main non-ionic surfactants, namely Span 60 and Span 85. Two different molar ratios were used in the preparation of PZA niosomes using Span along with CH, i.e. Span: CH (1:1) and Span: CH (4:2). Charge inducing agents DCP and SA, were incorporated in order to impart negative and positive surface charge, respectively employing the molar ratios Span: CH: charge inducing agent (1:1:0.1) and Span: CH: Charge inducing agent (4:2:1). A list of the compositions of all niosomal formulations prepared is included (Table 1).

Free PZA was separated from entrapped drug by cooling centrifugation at around 5200*g* and –4°C and estimated by spectrophotometric measurement at 268.4 nm[12]. Entrapped PZA was determined by difference from the amount added at the start of the experiment[13–16].

Niosomal characterization

Characterization of the prepared niosomes was conducted by employing transmission electron microscopy, where the vesicles were stained using 2% potassium phosphotungstate[17]. Also DSC was performed for the lyophilized pellets of the niosomal formulations. A scan rate of 5°C/min was adopted. The individual constituents used in niosomal formulations were also investigated.

In vitro release of PZA niosomes

Three molar ratios of PZA niosomes were selected for this study namely, Span 60: CH: DCP (1:1:0.1) and Span 60: CH: DCP (4:2:1) along with Span 60: CH (4:2). The diluted niosomal pellets were shaken at 37°C and a rate of 150 strokes/min. Samples were drawn at specified time intervals (3, 6, 24, 48, and 96 h) and the amount of PZA released was estimated, spectrophotometrically, for each time interval.

Biological Evaluation of PZA Niosomes

This experiment has been approved by the institutional review board of the division of pharmaceutical and drug research, NRC, Cairo, Egypt. Guinea pigs were infected by a 0.1 ml suspension (equivalent to a load of about 10^6 bacilli) of *M. tuberculosis*, strain H37Rv. Three weeks after infection, the guinea pigs were divided into six groups, each of five animals. The first group was given PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) by a dose equivalent to 25 mg/kg PZA[18,19] twice weekly. The second group received PZA niosomes of the molar ratio Span 60: CH (4:2), by a dose equivalent to 25 mg/kg PZA twice weekly. The third group was given free PZA, 25 mg/kg twice weekly. The fourth group was given free PZA 6 days per week. A fifth group was given drug-free niosomes twice weekly and the control group was given saline 6 days per week. The formulations were administered subcutaneously for 3 weeks. Seven days after the last treatment dose, guinea pigs were sacrificed, dissected, and the organs separated were used for measurement of three parameters selected for biological evaluation namely, evaluation of root specific lung weight (RSLW), bacterial counts of lung homogenates and histopathological examination.

Evaluation of RSLW

The evaluation of RSLW of the lungs of guinea pigs aims to evaluate the organomegaly for the lungs of guinea pigs of different groups undergoing this experiment. The value of this parameter comes from the fact that TB infection results in an increase in the organ weight because of the presence of mycobacteria and increases the

Table 1. Composition of pyrazinamide niosomal formulations.

Niosome composition	Molar ratio of components				
	Span 60	Span 85	CH	DCP	SA
Span 60: CH (1:1)	1	—	1	—	—
Span 60: CH: DCP (1:1:0.1)	1	—	1	0.1	—
Span 60: CH: SA (1:1:0.1)	1	—	1	—	0.1
Span 60: CH (4:2)	4	—	2	—	—
Span 60: CH: DCP (4:2:1)	4	—	2	1	—
Span 60: CH: SA (4:2:1)	4	—	2	—	1
Span 85: CH: DCP (1:1:0.1)	—	1	1	0.1	—
Span 85: CH: SA (1:1:0.1)	—	1	1	—	0.1
Span 85: CH: DCP (4:2:1)	—	4	2	1	—
Span 85: CH: SA (4:2:1)	—	4	2	—	1

concentrations of macrophages and lymphocytes which get activated because of the presence of tubercle bacilli.

The weight of the guinea pigs was determined before the animals were sacrificed. After dissection the lungs, of each guinea pig, were removed and weighed. The RSLW was calculated according to the equation[20]:

$$\text{RSLW} = \sqrt{\frac{lung\ weight\ (mg) \times 10}{body\ weight\ (g)}}$$

Bacterial counts of lung homogenates

The animals were dissected and the lungs of each animal were processed by homogenization by a method modified from that applied by Marks[21]. The lung homogenates were diluted with sterile saline and centrifuged at 2000 rpm for 20 min. The supernatant of the lung homogenates was cultured on a duplicate set of sterile Lowenstein–Jensen slants. The slants were incubated at 37°C for 8 weeks and examined weekly for visible growth. The number of live bacilli was counted as number of colony forming units (CFU)/g lung, 8 weeks after culture.

Histopathological studies

Histopathological examination was performed on lungs, livers, and spleens of animals obtained from the six groups of the experiment. The organs were stained by hematoxylin and eosin stains for histopathological examinations through the light microscope and examined for the type and severity of lesions.

Sterilization of PZA niosomes by γ-irradiation

This study aimed at investigating the optimum sterility conditions required to provide a sterile parentral PZA niosomal formulation which could be safely injected. The study was conducted utilizing Span 60: CH: DCP (4:2:1) and Span 60: CH (4:2), for each sterilizing dose of γ-irradiation. Niosomes were prepared under hygienic conditions by washing instruments with ethyl alcohol 70%. Niosomal pellets were stored in screw capped glass vials and irradiated using a Cobalt-60 source at ambient temperature by Canadian Gamma Cell. Two γ-irradiation doses were attempted, viz., 15 and 25 KGy where eight vials, four for each niosomal preparation, were exposed to each irradiation dose and eight vials were kept as control.

In the sterility testing, the medium employed was sterile Brewer's thioglycollate medium, the contents of each vial were inoculated into a sterile test tube of thioglycollate medium. This process took place in a laminar flow cabinet and in the vicinity of a Bunsen burner in order to achieve aseptic conditions. One test piece of the biological indicator *B. pumilus* E601, recognized as one of the challenge microorganisms used for the certification of commercial radiation sterilization cycles[22], was inoculated into a sterile test tube of thioglycollate medium at each dose of irradiation. Two sterile thioglycollate tubes were inoculated with 24 h broth culture of *Escherechia coli* and *Clostridium sporogens* as positive control for aerobic and anaerobic bacteria, respectively to test the growth promoting ability of the medium. One sterile test tube of sterile thioglycollate was left uninoculated acting as negative control i.e., to check the sterility of the medium. All tubes were incubated at 32°C for 14 days and were examined regularly to monitor any growth.

Statistical analysis

The data of RSLW and bacterial counts were analyzed by using unpaired student's *t*-test.

Results and discussion

PZA niosomal entrapment

The results of PZA entrapment in niosomes prepared using Span 60 are illustrated in (Table 2). The PZA entrapment in niosomes prepared using Span 85 are illustrated in (Table 3). PZA niosomes prepared using Span 60: CH: DCP (4:2:1) show the highest percentage of PZA entrapped, for formulations employing the molar ratio Span 60: CH: charge inducing agent (4:2:1), followed by Span 60: CH (4:2) and Span 60: CH: SA (4:2:1) PZA niosomes. In addition, Span 60: CH: SA (1:1:0.1) show the highest entrapment for formulations employing the molar ratio Span 60: CH: charge inducing agent (1:1:0.1) (Table 2). Similar results were obtained with niosomes prepared using Span 85 (Table 3). PZA niosomes prepared using Span 85 show a lower percentage of PZA entrapment compared to niosomes prepared using Span 60, of the same molar ratio and surface charge for all the formulations investigated (Table 3).

Charcterization of PZA niosomes

The electron microscope micrographs of selected PZA niosomal formulations show a clear view of the niosomes revealing the outline of the vesicles and the surfactant-lipid bilayers. The particle diameter of some of the vesicles is also recorded. Positively charged PZA niosomes

Table 2. Percentage of pyrazinamide entrapped in niosomal formulations prepared using Span 60.

Niosomal formulation	Molar ratio*	Surface charge†	Entrapment (%) ± SD
Span 60: CH (1:1)	1:1	Neutral	6.8 ± 1.9
Span 60: CH: DCP (1:1:0.1)	1:1:0.1	Negative	10.1 ± 2.2
Span 60: CH: SA (1:1:0.1)	1:1:0.1	Positive	10.5 ± 2.2
Span 60: CH (4:2)	4:2	Neutral	9.1 ± 1.4
Span 60: CH: DCP (4:2:1)	4:2:1	Negative	11.2 ± 2.9
Span 60: CH: SA (4:2:1)	4:2:1	Positive	7.6 ± 2.3

*Molar ratio of Span 60: CH: charge inducing agent.
†Surface charge is assumed according to the charge inducing agent added to the formulation.

show marked aggregations compared to negatively charged and neutral PZA niosomes (Figure 1). Some of the micrographs show multilamellar structure. Most of the niosomes prepared employing Span 60 as the main component show a particle diameter of <1 μm and having a particle size range 255–701 nm, while niosomes prepared employing Span 85 as the main component show higher particle diameter reaching up to 1.9 μm. Niosomes prepared with SA as a positive charge inducer show aggregations as previously reported[15] compared to niosomes prepared with DCP as a negative charge inducer and niosomes prepared with no charge inducing agent.

Table 3. Percentage of pyrazinamide entrapped in niosomal formulations prepared using Span 85.

Niosomal formulation	Molar ratio*	Surface charge†	Entrapment (%) ± SD
Span 85: CH: DCP (1:1:0.1)	1:1:0.1	Negative	5.5 ± 1.2
Span 85: CH: SA (1:1:0.1)	1:1:0.1	Positive	6.1 ± 1.3
Span 85: CH: DCP (4:2:1)	4:2:1	Negative	6.0 ± 1.0
Span 85: CH: SA (4:2:1)	4:2:1	Positive	6.0 ± 0.8

*Molar ratio of Span 85: CH: charge inducing agent.
†Surface charge is assumed according to the charge inducing agent added to the formulation.

Figure 2 reveals the thermodynamic behaviour of PZA niosomes individual components and PZA niosomes of different molar ratios and surface charges, as shown by their DSC thermograms. Characterization by differential scanning calorimetry shows that a decrease in the peak transition temperature (T_m) of the main constituents is generally observed in the DSC thermograms of plain niosomes of different molar ratios investigated. A second peak is generally observed which could represent a number of possible components viz., CH, SA, DCP, or their interactions. Incorporation of PZA generally leads to an increase in the (T_m), for the two peaks examined, as observed in the DSC thermograms of PZA niosomes compared to plain niosomes.

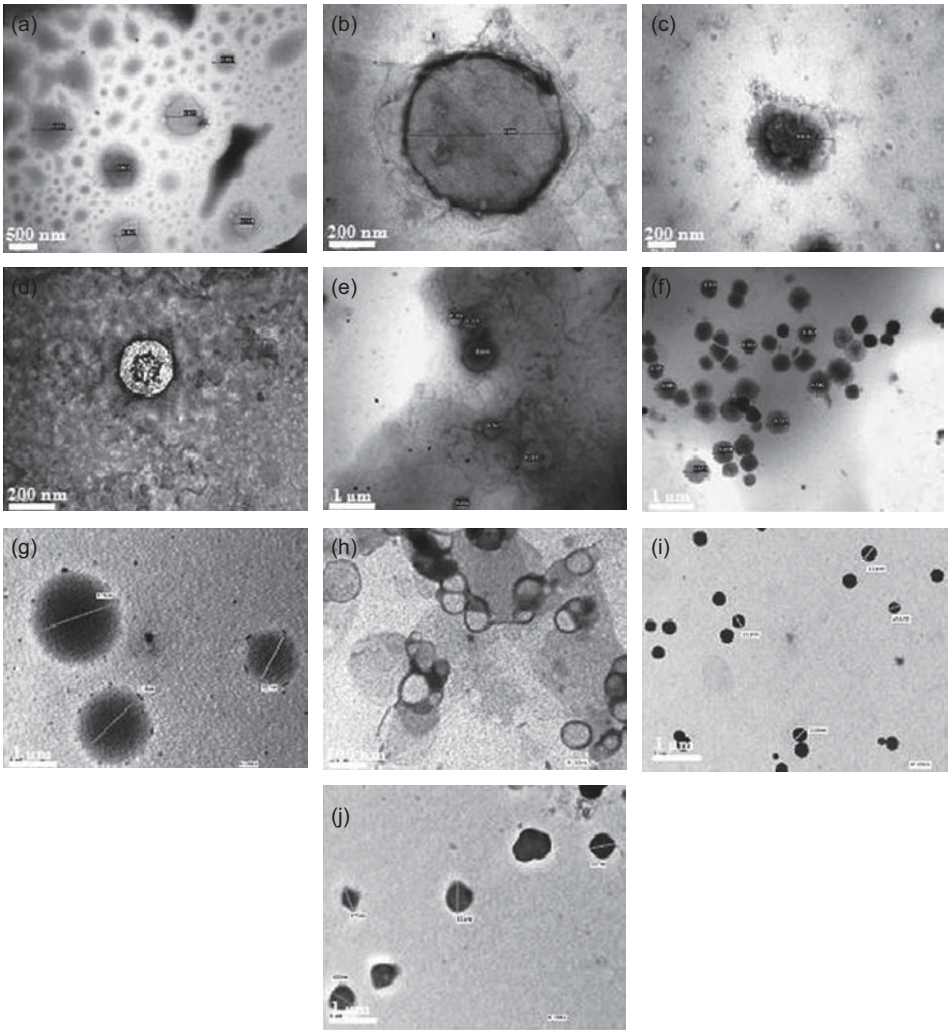

Figure 1. Electron microscope micrographs of pyrazinamide niosomes of different molar ratios. (A) Span 60: CH (1:1); (B) Span 60: CH: DCP (1:1:0.1); (C) Span 60: CH: SA (1:1:0.1); (D) Span 60: CH (4:2); (E) Span 60: CH: DCP (4:2:1); (F) Span 60: CH: SA (4:2:1); (G) Span 85: CH: DCP (1:1:0.1); (H) Span 85: CH: SA (1:1:0.1); (I) Span 85: CH: DCP (4:2:1); (J) Span 85: CH: SA (4:2:1).

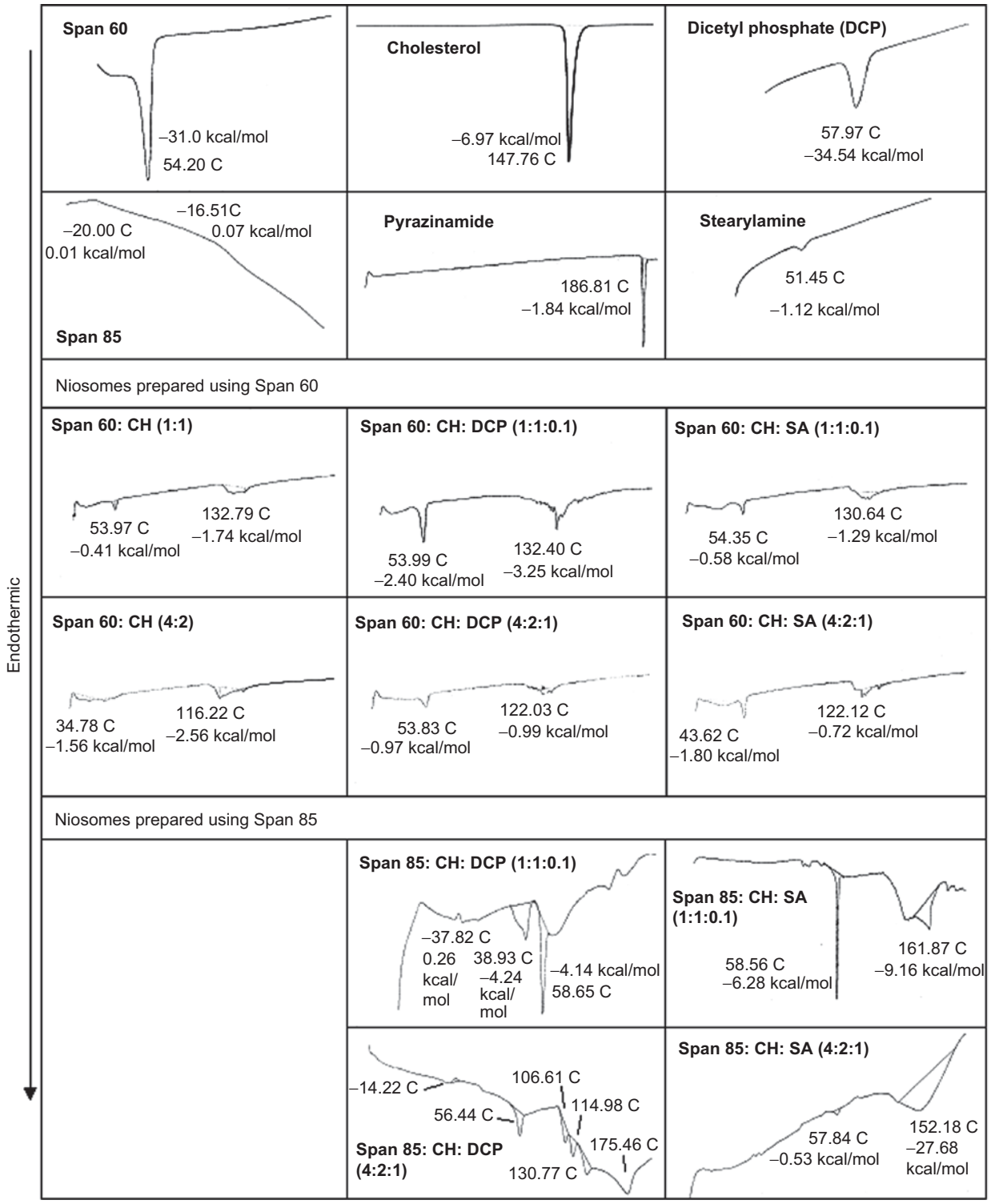

Figure 2. DSC thermograms of pyrazinamide niosomes and their individual components.

In vitro release of PZA niosomes

Figure 3 and Table 4 show the percentage of PZA retained in three selected niosomal formulations investigated at different periods of time. The formulations exhibiting the highest percentage of PZA entrapped were selected for this experiment, with exclusion of niosomes prepared using SA as a positive charge inducer due to reported *in vivo* aggregation and toxicity[15,23]. The release behavior is biphasic, with an initial burst release appearing clearly after 3 and 6 h. This is followed by a slower release of PZA from the niosomal vesicles, starting at 24 h. PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) exhibit lower drug retention compared to the PZA niosomes of the molar ratio Span 60: CH (4:2) (65.1% compared to 83.3% after 96 h). PZA niosomes of the molar ratio Span 60: CH: DCP (1:1:0.1) exhibit a higher percentage of drug retention

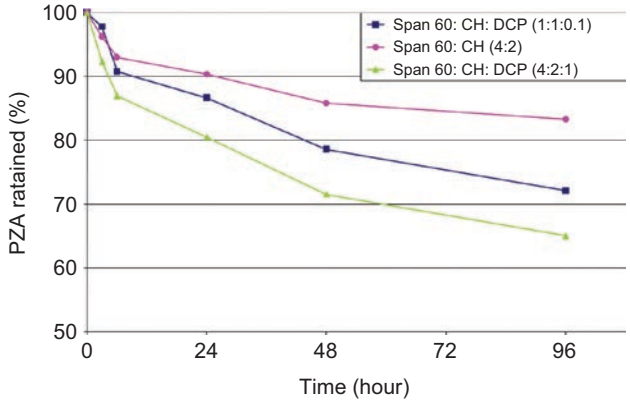

Figure 3. *In vitro* release of pyrazinamide niosomes.

Table 4. *In vitro* release of pyrazinamide niosomes.

	Mean PZA retained (%) ± SD		
Time (h)	Span 60: CH (4:2)	Span 60: CH: DCP (4:2:1)	Span 60: CH: DCP (1:1:0.1)
Zero	100 ± 0	100 ± 0	100 ± 0
3	96.2 ± 3.0	92.3 ± 1.6	97.8 ± 0.6
6	93.0 ± 3.8	87.0 ± 2.6	90.7 ± 2.7
24	90.3 ± 4.8	80.6 ± 3.5	86.7 ± 1.2
48	85.8 ± 5.6	71.6 ± 5.1	78.6 ± 0.7
96	83.3 ± 5.8	65.1 ± 5.1	72.1 ± 1.0

compared to PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) i.e. 72.1% compared to 65.1% after 96 h. The biphasic behaviour of niosomes could be explained by the first fast release of the drug molecules adsorbed on the niosomal surface, whereas drug molecules encapsulated in the inner bilayers of the niosomal vesicles take more time to be free. Charged molecules such as DCP are reported to help in preventing the aggregation of niosomes[24]; providing a larger free surface area on the surface of the niosomal vesicles, thus allowing release of larger amounts of entrapped drug viz. PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1). The higher retention of drug in niosomes of the molar ratio Span 60: CH: DCP (1:1:0.1), compared to (4:2:1) would be attributed to the greater amount of CH incorporated in the preparation. CH is known to abolish the gel to liquid phase transition of liposomal and niosomal systems resulting in less leakiness of the vesicles[25], thus an increase in the amount of CH leads to higher retention of entrapped drug.

Biological evaluation of PZA niosomes

Evaluation of RSLW

Figure 4 reveal the results of RSLW values for guinea pigs of different groups. Control group receiving drug-free niosomes showed the highest RSLW (21.3 ± 0.5), followed by guinea pigs receiving saline buffer (19.9 ± 0.2). The RSLW of lungs of guinea pigs of the treatment groups are arranged as follows: free PZA twice weekly (16.4 ± 1.1) > free PZA six times weekly (14.5 ± 0.9) > PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) twice weekly (10.9 ± 1.9) > PZA niosomes of the molar ratio Span 60: CH (4:2) twice weekly (10.5 ± 0.6).

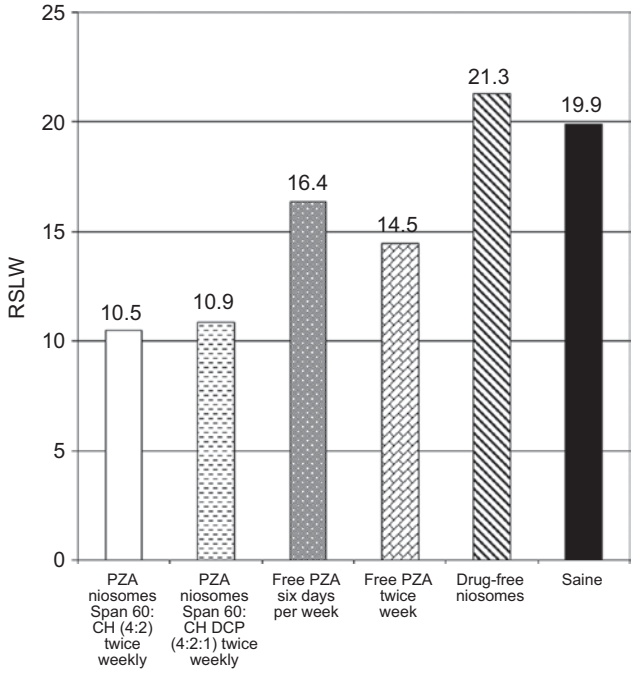

Figure 4. RSLW values for guinea pig lungs of different treatment groups.

The group receiving drug-free niosomes shows no significant difference ($P > 0.05$), while all other groups show a significant difference from the control group at ($P < 0.05$) as demonstrated (Table 5). Group receiving PZA niosomes of the molar ratio Span 60: CH (4:2) shows no significant difference from the group receiving PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) ($P > 0.1$), while a significant difference from the groups receiving free PZA twice weekly and 6 days weekly ($P < 0.005$) is observed. The group receiving PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) also shows a significant difference from the treatment group receiving free PZA twice weekly and free PZA 6 days weekly ($P < 0.05$) (Table 3). It should be noted that mean RSLW of lungs of healthy uninfected guinea pigs of the same age and average weight, included here for comparative purposes, is equal to 8.2 ± 0.5.

Treatment with both PZA niosomal formulations has led to a significantly lower RSLW values for lungs of infected guinea pigs compared to the same dose of free PZA, and also compared to free PZA given three times the dose given in niosomal formulations. TB infection results in an increase in the organ weight because of the presence of mycobacteria and increases the concentrations of macrophages and lymphocytes which get activated because of the presence of tubercle bacilli[26], thus a lower value of RSLW indicates less severity of infection and better efficacy in treatment of TB.

Bacterial counts of lung homogenates

The results of bacterial counts of lung homogenates of guinea pigs (Figure 5) reveal that the control group and group receiving drug-free niosomes exhibited the highest bacterial count among all groups investigated. (9.6 ± 0.2 and 9.8 ± 0.5 log CFU/g lung). Guinea pigs receiving

Table 5. Values of RSLW for lungs of guinea pigs receiving free and niosomal PZA.

Treatment group	Mean RSLW ± SD	*P* value*	*P* value†
PZA niosomes Span 60: CH (4:2) twice weekly	10.5 ± 0.6	—	—
PZA niosomes Span 60: CH: DCP (4:2:1) twice weekly	10.9 ± 1.9	0.736631 (NS)	—
Free PZA 6 days weekly	14.5 ± 0.9	0.002640 ($P < 0.005$)	0.037379 ($P < 0.05$)
Free PZA twice weekly	16.4 ± 1.1	0.004009 ($P < 0.005$)	0.035118 ($P < 0.05$)

*Level of significance with respect to PZA niosomes Span 60: CH (4:2).
†Level of significance with respect to PZA niosomes Span 60: CH: DCP (4:2:1).

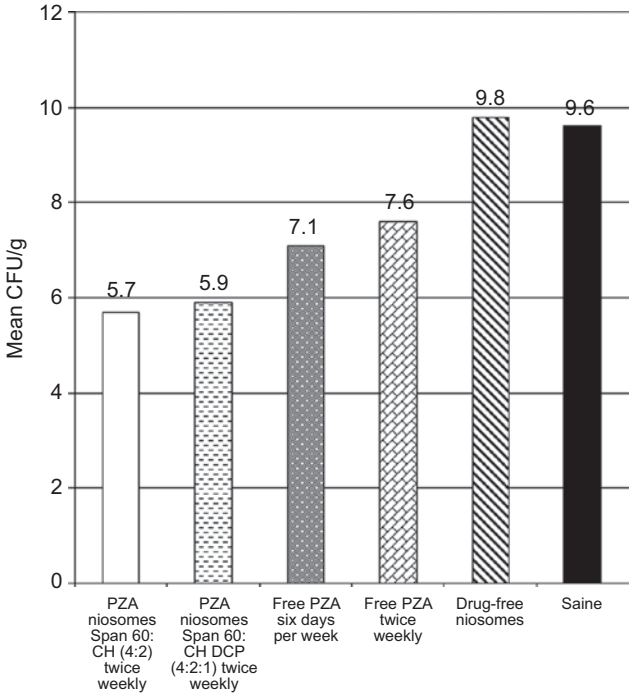

Figure 5. Bacterial counts for guinea pig lung homogenates of different treatment groups.

niosomal PZA twice weekly, for the two formulations investigated, exhibited lower bacterial counts compared to guinea pigs receiving free PZA twice weekly and 6 days per week (5.9 ± 0.3 and 5.7 ± 0.2, compared to 7.6 ± 0.3 and 7.1 ± 0.4 log CFU/g lung, respectively).

Table 4 reveals the statistical significance of the data which was tested employing student's *t*-test. The group receiving drug-free niosomes showed no significant difference from the control group ($P > 0.1$). All treatment groups exhibited a significant difference from the control group at $P < 0.05$ or less (Table 6). The bacterial count of guinea pigs receiving PZA niosomes of the molar ratio Span 60: CH (4:2) showed no significant difference from the treatment group receiving PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) ($P > 0.1$). Guinea

Table 6. Bacterial counts for guinea pig lung homogenates receiving different treatments.

Treatment group	Mean Log CFU/g lung ± SD	*P* value*	*P* value†
PZA niosomes Span 60: CH (4:2) twice weekly	5.7 ± 0.2	—	—
PZA niosomes Span 60: CH: DCP (4:2:1) twice weekly	5.9 ± 0.3	0.282158 (NS)	—
Free PZA 6 days weekly	7.1 ± 0.4	0.004109 ($P < 0.005$)	0.015056 ($P < 0.05$)
Free PZA twice weekly	7.6 ± 0.3	0.002491 ($P < 0.005$)	0.009942 ($P < 0.01$)

*Level of significance with respect to PZA niosomes Span 60: CH (4:2).
†Level of significance with respect to PZA niosomes Span 60: CH: DCP (4:2:1).

pigs receiving PZA niosomes of the molar ratio Span 60: CH (4:2), showed a significantly lower bacterial count compared to guinea pigs receiving free PZA twice weekly and free PZA six days per week ($P < 0.005$). The bacterial counts of the group of guinea pigs receiving negatively charged PZA niosomes, also showed a significant difference from the treatment group receiving free PZA twice weekly ($P < 0.01$) and free PZA six days weekly, ($P < 0.05$), (Table 6). Accordingly, it could be concluded that the two PZA niosomal formulations investigated have the advantage of reducing the bacterial counts in lungs of infected guinea pigs compared to the same dose of free PZA given twice weekly, and also compared to free PZA given at a triple dose (Figure 5).

Histopathological studies

The results of examination of the lungs of guinea pigs treated with PZA niosomes of the molar ratio Span 60: CH (4:2) show mild granulomatous reaction (+) (Figure 6A), liver shows also a mild reaction (+) (Figure 6B). Spleens of guinea pigs show moderate granulomatous reaction (++). Guinea pigs treated with PZA niosomes of the molar ratio Span 60: CH: DCP (4:2:1) show lungs exhibiting moderate granulomatous reaction (++) showing multiple number of circumscribed round granuloma formation (Figure 6C). Livers and spleens exhibit also a moderate granulomatous reaction (++). Lungs of guinea pigs receiving free PZA six times per week are exhibiting moderate granulomatous reaction (++) (Figure 6D). The liver shows severe granulomatous rection (+++).The spleen of guinea pig shows moderate reaction (++) with focal necrosis with circumscribed round granuloma formation. Guinea pigs treated with free PZA twice weekly show lungs having severe granulomatous reaction (+++) with focal circumscribed round cellular granuloma formation (Figure 6E). Liver and spleen show severe granulomatous reaction (+++). The group receiving drug-free niosomes shows severe reaction (+++) in the three organs investigated. The control group shows lungs having severe reaction (+++) with the cellular granuloma formation replacing the air alveoli, mainly epitheloid

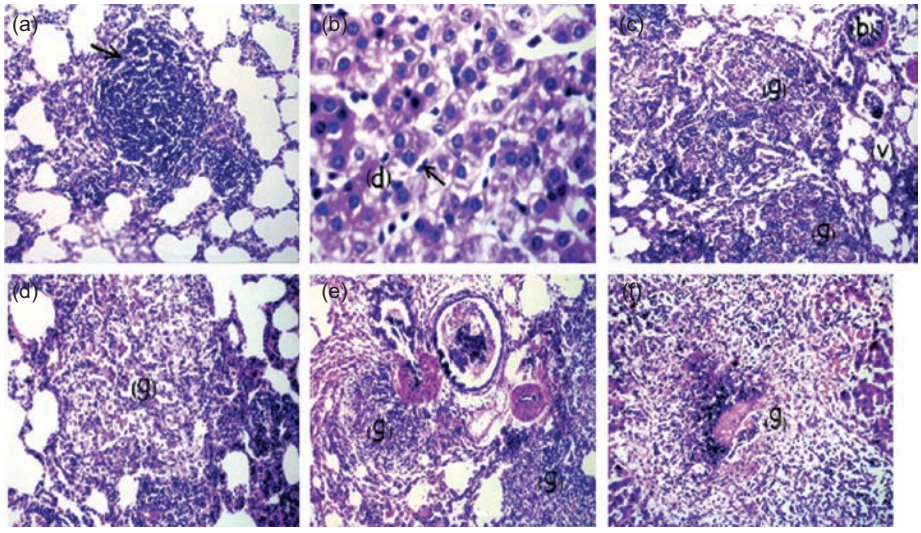

Figure 6. Histopathological examination showing: (A) lung of guinea pig treated with PZA niosomes [Span 60: CH (4:2)] showing focal aggregation of lymphoid cells (arrow) (H&E, ×64). (B) Liver of guinea pig treated with PZA niosomes [Span 60: CH (4:2)] showing Kupffer cells proliferation (arrow) in between the vacuolar degenerated hepatocytes (D) (H&E, ×160). (C) Lung of guinea pig treated with PZA niosomes [Span 60: CH: DCP (4:2:1)] showing multiple circumscribed round granuloma formation (G) replacing the lung alveoli (v) (b = bronchiole) (H&E, ×24). (D) Lung of guinea pig treated with free PZA 6 days weekly showing circumscribed granuloma formation (G) (H&E, ×40). (E) Lung of guinea pig treated with free PZA twice weekly showing focal circumscribed round granuloma formation replacing the air alveoli (G) (H&E, ×24). (F) Liver of guinea pig of the control group showing granuloma formation with central necrosis and calcification (G) (H&E, ×40).

Table 7. Sterility testing of pyrazinamide niosomes.

Radiation dose (KGy)	Pyrazinamide niosomes on thioglycolate		Biological indicator *Bacillus pumilus* E601 on thioglycollate	Control tests		
				Positive control		
	4:2 Neutral	4:2:1 Negative		*E. coli*	*Cl. sporogenes*	Negative control
\	+	+	+	+	+	–
15	+	+	+			
25	–	–	–			

(+), growth; (–), no growth; *E. coli, Escherechia coli* aerobic bacteria; *Cl. sporogenes, Clostridium sporogenes* anaerobic bacteria.

cells in nature, liver shows a very severe granulomatous reaction (++++) with granuloma formation observed all over the hepatic tissue and characterized by central calcification and necrosis surrounded by epitheloid cells, giant cells (Figure 6F). The spleen shows a severe reaction (+++).

Histopathological findings support the favourable use of PZA niosomes in treatment of *M. tuberculosis* compared to free PZA. Results are consistent with those of the RSLW.

Sterilization of PZA Niosomes

The data presented (Table 7) shows that there is no growth in negative control group, while growth took place in the two positive control groups. In tubes inoculated with the biological indicator, *B. pumilus* E601, growth is detected in the radiation doses of zero and 15 KGy while no growth is observed at the radiation dose of 25 KGy. Tubes inoculated with PZA niosomal suspensions show growth at the doses zero and 15 KGy while no growth is observed at 25 KGy in the two PZA niosomal formulations investigated.

The results obtained indicate that the irradiation dose of 25 KGy is the optimum sterilization dose for PZA niosomal formulations investigated when prepared under hygienic conditions. This study was performed for elucidating the optimum γ-radiation dose that could be used for sterilization of PZA niosomes expected to be used for future studies.

Conclusions

Clinical management of TB poses serious problems because the efficacy of chemotherapy has been reduced, reasons for failure of chemotherapy may be the difficulty in achieving adequately high drug concentrations at the infection site, inadequate penetration into macrophages and low stability levels in cells[26]. Carrier or delivery systems such as liposomes and microspheres have been developed for the sustained delivery of anti-TB drugs and have demonstrated better chemotherapeutic efficacy when investigated in animal models[16,27]. Niosomes containing rifampicin were prepared and characterized using Span 85 and CH in various molar fractions[28]. Liposomes and niosomes have been reported as drug delivery systems for encapsulating rifampicin, and have been evaluated *in vitro* and *in vivo*[29,30]. Niosomes entrapped rifampicin were evaluated for the amount of drug present in the lymphatics compared to free rifampicin[31].

The results obtained in this work pave the way for the use of PZA in niosomal formulations for the treatment of TB, with more efficacy and safety compared to its free form. Also there is a possibility of dose reduction which would aid in adherence to treatment and improve patient compliance.

Declaration of interest

The authors report no declarations of interest

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