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ENHANCEMENT OF ANTIBACTERIAL ACTIVITY OF TETRACYCLINE HYDROCHLORIDE BY NIOSOMAL ENCAPSULATION

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ABSTRACT:

Topical use of antibiotics is currently a widely accepted effective and safe treatment for topical infections. One potential strategy for improving topical administration of drugs is to encapsulate the drug in a colloidal carrier. Nonionic surfactant vesicles (niosomes) are promising drug carriers since they have potential applications in the delivery of hydrophobic or amphiphilic drugs. In this study niosomes encapsulated tetracycline hydrochloride (TC-HCl) were prepared by using lipid film hydration technique. The effect of processing variables such as surfactant structure, drug concentration, lipid concentration and charge inducing agents e.g. stearyl amine (SA) and dicetyl phosphate (DCP) on the percentage drug entrapped (PDE) and in-vitro release of TC-HCl has been determined. The results showed that niosomes prepared from span 60 and 40 gave the highest PDE, (34.6, 30.29% respectively) followed by span 20 and span 80 (15.6, 14.6% respectively) using 300 µmole total lipid. Increasing the total lipid concentration from 150-500 µmole for span 60 resulted in an increase in PDE from 33-38.7%. Changing drug concentration from 25 to 100 mg produced non significant effect on PDE. Moreover, concerning the charge inducing agent, incorporation of DCP and SA showed an increase in PDE to 67.14 and 46% respectively. Niosomes mean size showed a dependence on the HLB of span used. The lower the HLB the smaller the niosomal mean size. In-vitro release studies indicated that niosomal vesicles could retain a considerable amount of TC-HCl over prolonged period of time (24 hrs) for span 60 : chol (1:1) niosomes. The in-vitro antimicrobial activities of niosomes-encapsulated TC-HCl in comparison with that of free TC-HCl against clinical isolates isolated from skin infection showed that, TC-HCl encapsulated in either cationic or anionic niosomes was more active than TC-HCl encapsulated in neutral niosomes. The minimum inhibitory concentrations (MICs) for niosomes-encapsulated TC-HCl were significantly lower than those of the corresponding free TC-HCl. Niosomes-encapsulated TC-HCl showed greater antimicrobial activities than the free form. In conclusion, these drug formulations may have potential in the treatment of topical bacterial infections.

INTRODUCTION

To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task[1]. In the formulation of topical dosage forms, more attention has been devoted to new structures, which can ensure either adequate localization of drug within the skin to enhance the local effect or can increase the penetration through the stratum corneum. For these purposes various systems such as niosomes and liposomes have been investigated by several groups[2]. Drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs.

Niosomes or non-ionic surfactant vesicles are now widely studied as an alternative to liposomes and produce sustained release of drug topically. An increasing number of non-ionic surfactants have been found to form vesicles capable of entrapping hydrophobic and hydrophilic solutes. These non-ionic surfactant vesicles are regarded either as inexpensive alternatives, of non-biological origin, to liposomes, or perhaps in-vivo as a carrier system to carry drug molecules like liposomes[3-5].

Niosomes are supposed to give desirable interactions with human skin when applied in topical preparation by improving especially the horny layer characteristics, both by reducing transepidemal water loss and increasing smoothness via replenishing lost skin lipid[6].

The niosomal methotrexate (0.25%) in a chitosan gel used as a topical agent significantly resolved psoriatic lesions and was shown to be quite effective in treating patients without any side effects[7].

Niosomes forming non-ionic surfactants are chemically stable, available by synthesis in pure form at lower cost compared to liposomes forming phospholipids. Among various non-ionic surfactants used to formulate niosomes, spans are most suitable for commercial exploitation since they are safe and widely used as emulsifiers in food and pharmaceutical industry[8].

TC-HCl is a broad spectrum antibiotic produced by streptomyces aureofaciens and indicated for use against many bacterial infections. It is commonly used to treat acne. Topical use of antibiotics is currently a widely accepted effective and safe treatment for acne. Several articles revealed that topical application of antibiotic such as tetracycline showed clinical effectiveness for mild to moderate inflammatory acne[9,10].

Staphylococcus aureus and coagulase-negative staphylococci (*Staphylococcous epidermidis*) are commonly found in acne vulgaris and ciprofloxacin, penicillin, erythromycin, tetracycline, clindamycin, fusidic acid and gentamicin had potent activity against these organisms[11,12].

Several diseases and lesions such as lacrimal canaliculitis, caries and periodontal disease, inflammation following use of intrauterine pessaries, various types of abscess, septicaemia, and acne vulgaris have been attributed to the direct or indirect pathogenic effects of fermentative actinomycetes, propionibacteria, or eubacteria[13].

The present investigation was under taken (a) to encapsulate TC-HCl in niosomes, study the effect of different processing variables on the entrapment efficiency and to asses the release properties of entrapped drug in-vitro. (b) To evaluate the in-vitro antimicrobial activity of niosomes entrapped TC-HCl compared with the free drug against clinical isolates isolated from human skin infection.

EXPERIMENTAL

Materials:

Tetracycline hydrochloride, Sorbitan monolaurate (span 20), Sorbitan monopalmitate (span 40), Sorbitan monostearate (span 60), Sorbitan monooleate (span 80), cholesterol (chol), Stearyalamine (SA) and dicetylphosphate (DCP) were purchased from Sigma Chemical Co., St-Lowis, Mo, USA. All chemical were of analytical grade and used without further purification. Methanol and Chloroform were obtained from El-Nasr Company for pharmaceutical chemicals, Cairo, Egypt. Semi-permeable Cellophane Membrane 30/32 was purchased from Fisch Scientific Co., London, England.

Methods:

Preparation of non ionic surfactant vesicles (NSV):

Niosomes were prepared by using the lipid film hydration technique[2]. Non ionic surfactants and cholesterol with or without charged lipid were weighed as indicated in Table I and dissolved in chloroform/methanol system (2:1) in a 100 ml round bottom flask. The solvent mixture was evaporated in a rotary evaporator under a vacuum at a temperature of 25 ± 2°C and the flask was rotated at 100 rpm until a smooth, dry lipid film was obtained. The film was hydrated with 3 ml of TC-HCl aqueous solution for 45 min at 60°C with gentle shaking. The niosomal suspension was further hydrated at 2-8°C for 24 h. Niosome pellets were then separated by centrifugation at 14000 rpm for 30 min.

Entrapment efficiency:

The entrapment efficiency of niosomes was determined by centrifugation of the refrigerated niosomes (2-8°C) suspension at 14000 rpm for 30 min. The prepared niosomes were analyzed for percent drug entrapment by spectrophotometric method (λ_{max} 355 nm) after separation of free drug according to the following equation:

$PDE = D_t - D_f / D_t$

Where D_t = total drug amount

D_f = concentration of free drug

The effect of variables on the entrapment efficiency and in-vitro release rate were investigated. The type of surfactant as span 20, span 40, span 60 and span 80, the ratio of lipids, concentration of drug and presence of charged lipid. The volume of the aqueous phase was kept constant unless other wise indicated.

Drug release studies:

The in-vitro release of TC-HCl from niosomal vesicles was determined by a simple dialysis method[14]. One ml of the vesicles suspension after separation of free drug was placed into glass tube to which a cellophane membrane was attached to one side, the tube was suspended in 250 ml beaker containing 100 ml distilled water. The beaker was maintained at 37±2°C and stirred at 100 rpm in a water bath shaker. Three milliliter samples were withdrawn at specified interval and replaced with an equal volume of fresh distilled water at the same temperature to keep the volume constant during the experiment. The samples were analyzed spectrophotometricly at λmax 355 nm against distilled water as a blank. Each experiment were carried out in triplicate and the mean values were taken.

Optical microscopy:

Photo micrographs of the prepared vesicles were taken with an optical microscope (Zeiss, Me 63 C, West Germany). The mean diameter for 100 vesicles was determined, Each experiment were carried out in triplicate and the mean values were taken.

Sizing of the vesicles:

Vesicles were mounted on a glass slide and viewed under a phase contrast microscope (MISR-FATRMO 2001, EGYPT) with a magnification of 400x to determine the size , using a stage micrometer.

Microbiological study:

Determination of Tetracycline-HCl concentration encapsulated into the niosomes.

The amount of TC-HCl encapsulated into the niosomes was determined by a microbiological assay as previously described[17]. Briefly, 100 µl from an overnight culture of *Staph. aureus* grown in Müller-Hinton broth were transferred aseptically on to 3 ml saline to obtain turbidity visually comparable to 0.5 McFarland of about 10^6 CFU/ ml.

100 µl of standardized broth cultures of test bacteria were accurately spread on to the surface of Müller–Hinton agar plates using glass spreader. The surfaces of the seeded media were allowed to dry at room temperature for 30 min. Wells (cups) of 7 mm diameter were aseptically made in the seed Müller–Hinton agar using sterile cork borers[18]. Accurately measured 100 µl of each of the standard free TC-HCl solutions (50, 25, 12.5, 6.25 and 3.125 µg/ml) or niosomal antibiotic were added into the respective wells in duplicates.

The niosomes were first lysed by adding 20 µl of 1% Triton X-100 (v/v, with PBS) to 150 µl of niosomal dispersion and then incubated for 15 min at 50°C. to release their content. This level of Triton X-100 was sufficient to release all encapsulated tetracycline without any effect on the performance of the assay and this is the only experiment where a detergent was used. The plates were incubated for 24 h at 37°C and the zones of inhibition around the known concentrations of TC-HCl were used to generate a standard curve. The unknown niosomal antibiotic concentrations were determined by linear regression analysis.

Bacterial strains and growth conditions:

The four clinical samples of bacterial isolates used in this study were isolated from skin swabs. The isolates were morphologically, microbiologically and biochemically characterized as *Staphylococcous aureus, Staphylococcous epidermidis, Corynebactrea jeikeium and Actinomyces spp* according to Cruickshank et al and Buchanan et al.[15,16]. Bacteria were routinely cultured in nutrient broth and on nutrient agar plates. The isolates used in this study were maintained and characterized in Microbiology department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Determination of minimum inhibitory concentration (MIC) of TC-HCl:

The MIC is defined as the minimum inhibitory concentration of antibiotic sufficient to prevent bacterial growth in-vitro. The MICs of free or niosome-encapsulated tetracycline against tested organisms were determined using the cup plate agar diffusion method[19,20]. Briefly, serial dilutions of free or niosomes-encapsulated tetracycline (50, 25, 12.5 and 6.25 µg/ml) were prepared. Wells (cups) of 7 mm diameter were made in the seeded Müller–Hinton agar using sterile cork borers as previously mentioned. 100 µl of each of the free or niosomes-encapsulated tetracycline dilutions were transferred into the respective wells in duplicates. The plates were allowed to stand for about 30 min at room temperature to allow for proper diffusion of the noisomes to take place. The free tetracycline and plain niosomes (without tetracycline) were used as positive and negative controls, respectively. The inverted plates were then incubated at 37°C for 24 h after which diameters of zones of inhibition were measured and recorded. The minimum inhibitory concentration (MIC) was determined by linear regression analysis. Two separate experiments were performed to confirm the results.

Statistical analysis

Statistical significance between niosome-encapsulated TC-HCl and the free TC-HCl was tested with Student *t* test for unpaired values. Statistical significance was defined as a P value of <0.05.

RESULTS AND DISCUSSION

The lipid film hydration technique was selected to prepare niosomes containing TC-HCl as reported by Azmin *et al.*[5] with slight modification. Experiments were designed to incorporate TC-HCl into niosomes using lipid film hydration technique by changing the HLB (Hydrophilic Lipophilic Balance) using different Spans, keeping cholesterol concentration the same (Table 1).

Table 1: Composition of TC-HCl niosomal batches

Surfactant	Lipid composition in molar ratio (µmol) Surf : Chol : SA : DCP	Surf : Chol : SA : DCP Weighed quantity (mg)	Drug conc. (mg)	HLB
Span 20	150:150	5.19:5.8:0: 0	50	8.6
Span 40	150:150	6:5.8:0:0	50	6.7
Span 60	150:150	6.4:5.8:0:0	50	4.6
Span 80	150:150	6.43:5.8:0: 0	50	4.3
Span 60	148.5:148.5:0: 2.97	6.4:5.87:0:0.016	50	4.6
Span 60	148.5:148.5:2.97:0	6.4:5.74:0.053:0	50	4.6
Span 60	75:75	3.23:2.9:0:0	50	4.6
Span 60	250:250	10.7: 9.67: 0: 0	50	4.6
Span 60	150:150	6.4:5.8: 0:0	25	4.6
Span 60	150:150	6.4:5.8:0:0	100	4.6

The prepared niosomes were analyzed for percent drug entrapment (PDE) and results are recorded in table 2. The highest entrapment (34.7% ±0.2) was observed with span 60, this may be attributed to the surfactant structure. It is known that span 20, span 40, span 60 and span 80 have the same head group and different alkyl chain. For these nonionic surfactants only span 80 has unsaturated alkyl chain. The introduction of such double bond made the chain bend and adjacent molecules cannot be tight when they form the membrane of niosomes. Span 40 and span 60 are solid at room temperature and have highest phase transition temperature (Tc)[21]. The surfactant having the highest Tc produces the highest entrapment efficiency, so the results her investigated the influence of Tc on the entrapment efficiency. Hao *et al.*[22] reported that the length of alkyl chain of non-ionic surfactant is an important factor for permeability of the membrane. Accordingly, span 60 was the selected surfactant in further experiments. As for vesicle size, mean size of niosomes showed a regular increase with increasing HLB from Span 80 (HLB 4.3) to Span 20 (HLB 8.6), Table 2.

Niosomal vesicles prepared from Span 20 had the greatest vesicle size and vesicles prepared from Span 80 had the lowest vesicle size, table2. This effect is related to the inverse relationship between hydrophobicity of the non-ionic surfactant and vesicular diameter of niosomes[23,24].

Table 2: Effect of surfactant structure on percentage drug entrapped of TC-HCl.

Span	Structure	HLB	*PDE±**SE	Particle size (µm)
20	Sorbitan monolaurate	8.6	14.6±0.01	6.86
40	Sorbitan monopalmitate	6.7	30.29±0.09	5.21
60	Sorbitan monostearate	4.6	34.66±0.02	4.47
80	Sorbitan mono-oleate	4.3	15.8±0.013	3.12

* Percent drug entrapped
** Standard error

Effect of total lipid concentration on the entrapment efficiency:

The effect of total lipid concentration on the entrapment efficiency of TC-HCl in span 60: cholesterol (1:1) niosomal vesicles was examined by changing the total lipid concentration while keeping the drug concentration constant (50 mg/ml). The results are shown in table 3, for span 60 and cholesterol in the molar ratio 1:1, the PDE increased from 33±0.031 to 38.7±0.1 as the total lipid concentration was increased from 150 µmol to 500 µmol. A similar increase in the PDE by increasing the lipid concentration was observed by Helmi[24]. However, the amount entrapped of TC-HCl mg per mmol lipid decreased as the total lipid concentration increased. This means that the amount of lipid taking part in the encapsulation decreased as the lipid concentration increased.

Table 3: Effect of total lipid concentration on the percentage drug entrapped of TC-HCl in span 60: cholesterol (1:1) niosomes.

Total lipid conc. (µmol)	*PDE±**SE	mg : mmol lipid
150	33±0.031	72.0±.03
300	34.66±0.02	35.33±0.012
500	38.7±0.1	28.58±0.025

* Percent drug entrapped
** Standard error

Effect of drug concentration on the entrapment efficiency:

The effect of drug concentration on entrapment efficiency of TC-HCl in the niosomes was examined by changing the amount of drug incorporated while keeping total lipid concentration constant (300 µmol), the results are shown in table 4. For span 60 : cholesterol (1:1), the PDE scarcely increased as the drug concentration in the aqueous phase was increased. This means that the total aqueous volume has nearly constant value. As the percentage of drug encapsulation depends upon the amount of aqueous phase enveloped in the niosomal vesicles during preparation, this result suggested that the enveloped aqueous phase was the same irrespective of drug concentration[25]. However, the amount entrapped of TC-HCl mg per mmol lipid increased as the drug concentration increased from 25 to 100 mg/ml. This means that the total aqueous volume has a nearly constant value. As the percent drug encapsulated depends upon the amount of the aqueous phase enveloped in the niosomal vesicles during preparation.

Table 4: Effect of drug concentrations on the percentage drug entrapped of TC-HCl in span 60: cholesterol (1:1) niosomal formulations.

Drug concentrations (mg/mL)	*PDE±**SE	mg : mmol lipid
25	31.2±.03	32.92±.02
50	34.66±0.02	35.33±0.012
100	36.3±.02	77±0. 1

* Percent drug entrapped
** Standard error

Effect of charge inducing agents on the entrapment efficiency:

The effect of charge on the entrapment efficiency of TC-HCl in niosomes was examined by incorporation of charge-inducing agents as stearyl amine (SA) for induction of positive charge or dicetyl phosphate (DCP) for induction of negative charge, the results are shown in table 5. Negatively charged niosomes showed the highest entrapment efficiency followed by positively charged nisomes and neutral niosomes, it is likely that increased entrapment of TC-HCl is due to the formation of lipophilic ion pair between TC-HCl and DCP, which partitions into the lipid bilayers. The increased efficiency of drug entrapment in the presence of charges may be attributed to the presence of charged interface, so there is an electrostatic repulsion between adjacent bilayers causing an increase in the distance between the bilayers. This leads to a rise in the volume of the internal aqueous compartment of the vesicles. The presence of charges also prevents aggregation of niosomal vesicles and increases the stability of niosomal dispersion[26,27].

Table 5: Effect of charged lipid on the percentage drug entrapped of TC-HCl in span 60: cholesterol (1:1) niosomal formulations.

Lipid composition	*PDE±**SE
Span60 : Chol	34.66±0.02
Span60 : Chol :DCP	67.14±0.08
Span60 : Chol : SA	46 ±0.05

* Percent drug entrapped
** Standard error

In-vitro release studies:

From the data of in-vitro release of TC-HCl (figure. 1), the rate of TC-HCl release through a dialysis membrane, for all the prepared formulation, was slower than that obtained from TC-HCl solution. It was noticed that there is an efficiency of the niosomal preparations in slowing down the rate of release of the drug compared with the free drug solution. While the release from the solution was about 92% with in 6 hrs, the release from the vesicles was 44.9, 34, 26.8 and 61.1 from spans 20, 40, 60 and 80 niosomal formulations respectively after 6 hrs.

Effect of surfactant structure on the in-vitro release of TC-HCl from niosomal vsicles:

Figure 1 shows the effect of surfactant structure on the in-vitro release of TC-HCl from the prepared niosomes. The rate of release of TC-HCl from vesicles was the lowest from span 40 and span 60 and highest from the other types of spans. This finding may be attributed to the higher phase transition temperature (Tc) for span 40 and span 60 (22). So, span 40 and span 60 form vesicles with less permeable, less leaky and rigid bilayers than other spans. Moreover, span 80 possess an unsaturated alkyl chain which makes more permeable bilayer membrane[28].

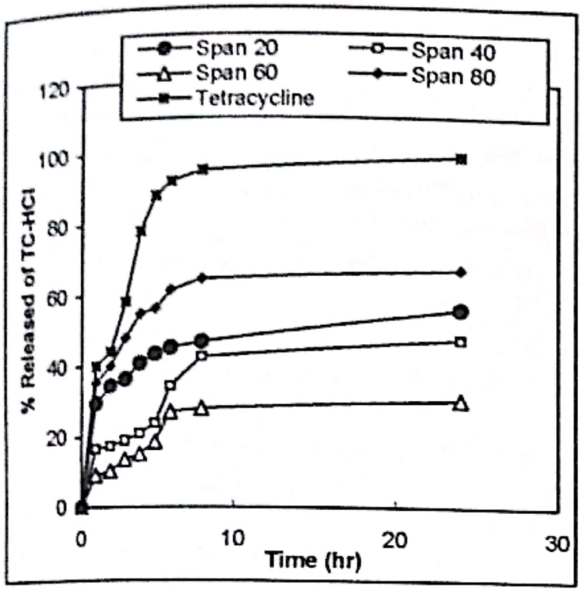

Figure 1: Effect of span type on in-vitro release of tetracycline hydrochloride from niosomal formulations.

Effect of charge inducing agents on the in-vitro release of the TC-HCl from niosomal vesicles:

Figure 2 shows the effect of charge inducing agents as DCP and SA on the in-vitro release of TC-HCl from prepared niosomes. The neutral niosomes showed the highest release rate of TC-HCl followed by charged niosomes. This is ascribed to that charged lipids may serve to tighten the molecular packing of the vesicles bilayers resulting in decreased rate of drug release from charged niosomes[29]. The negatively charged niosomes showed the lowest release rate, this may be due to the dipole attraction force induced between the positively charged TC-HCl and the negatively charged DCP.

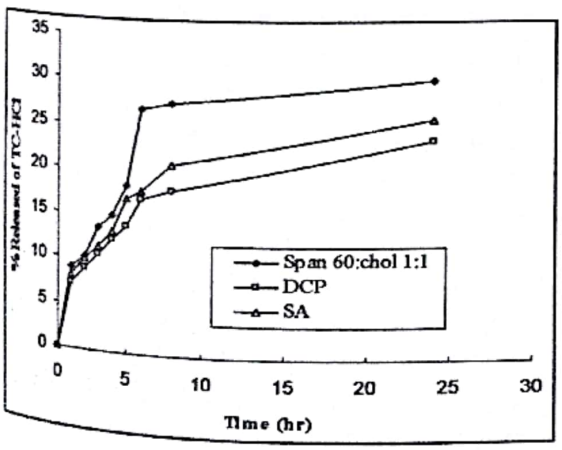

Figure 2: Effect of charged lipid on in-vitro release of tetracycline hydrochloride from niosomal formulations.

Optical microscopy:

The photo micrograph of niosomes vesicles are shown in figure 3. The vesicles are spherical in shape with some multivesicular niosomes. The size distribution of vesicles tends to be fairly wide.

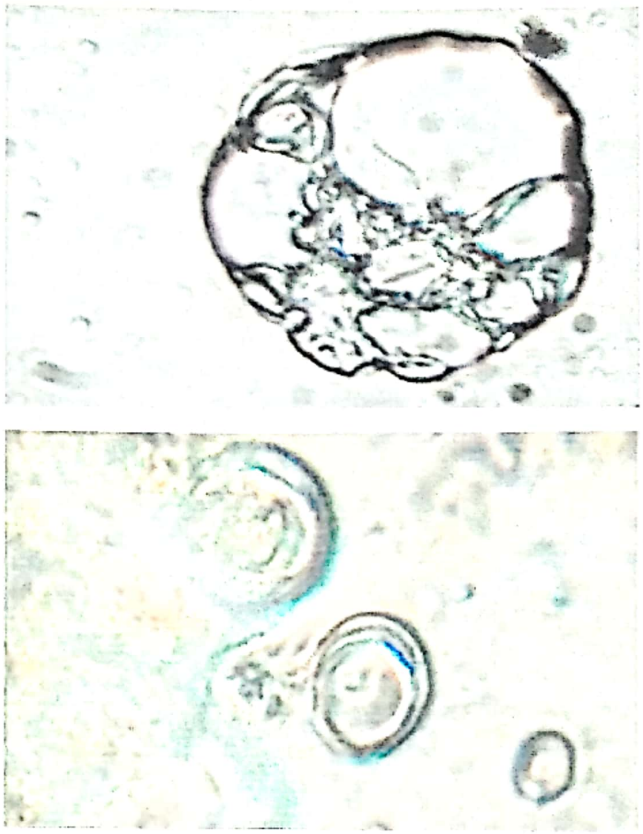

Figure 3: Optical micrographs of niosomes vesicles.

Microbiologically assay of tetracycline concentration encapsulated into the niosomes.

The amount of tetracycline encapsulated into the niosomes was assayed microbiologically against *Staph. aureus* after noisomal lysis with Triton-X-100. From the standard curve of free tetracycline, the % amount of tetracycline encapsulated in neutral, cationic and anionic niosomes was 37.2±1.4, 46.4±0.24 and 64.6±3.4 %, respectively. The results are comparable with that obtained by centrifugation method.

Antimicrobial activity of free and niosomal encapsulated tetracycline:

Antimicrobial activities of free and niosomal encapsulated tetracycline against four clinical isolates of microorganisms isolated from skin are shown in table 6. The MIC values for niosomal entrapped tetracycline were significantly lower than those of the corresponding free tetracycline for all the tested isolates. We found that, tetracycline encapsulated in cationic and anionic niosomes was more active than tetracycline encapsulated in neutral niosomes for all tested organisms.

A highly significant difference in MICs was observed for *Staph. aureus*, *Staph. epidermidis*, *Corynebacteria* and Actinomyces which exhibited a MICs of 8.1, 7.5, 5.7 and 5.5 µg/ml for free tetracycline, respectively, versus 2 and 1.9, 2.6 and 2.3 µg/ml for tetracycline encapsulated in cationic niosome, respectively, and 2.3, 1.7, 2.7 and 2.5 µg/ml for tetracycline encapsulated in anionic niosomes, respectively.

We also observed that, TC-HCl encapsulated in cationic and anionic niosomes was more active against all tested organisms, whereas the MICs were reduced to 1/4 fold reduction than MICs of free tetracycline. On the other hand, there was a significant difference between the MICs of tetracycline-encapsulated in neutral niosome than MIC of free tetracycline for all tested organisms.

Empty niosomes (free from antibiotic) containing PBS have no antibacterial activity. Likewise, the combination of empty niosomes with free drug had no additive effect on the antibacterial activity of tetracycline[26].

Table 6: In vitro activities of free and niosomal encapsulated tetracycline against four clinical isolates.

Tetracycline formulations	MIC (µg/ml) Mean of MIC ± SEM (P value)			
	Staph aureus	*Staph epidermidis*	*Coryne-bacteria*	*Actino-myces spp*
Free tetracycline	8.1±0.8	7.5±1.3	5.7±0.5	6.0±0.2
Neutral niosomes	5.2±0.2 (0.036)	2.5 ± 0.6 (0.029)	3.6±0.2 (0.019)	3.4±0.3 (0.026)
Cationic niosomes	2.0±0.3 (0.002)	1.9±0.5 (0.019)	2.6±0.4 (0.003)	2.3±0.3 (0.004)
Anionic niosomes	2.3±0.4 (0.003)	1.7±0.1 (0.015)	2.7±0.6 (0.008)	2.5±0.1 (0.005)

MIC: minimum inhibitory concentration
SEM: standard error of mean
Statistical significance between niosomal encapsulated TC-HCl and the free tetracycline was tested with Student *t* test for unpaired values. Statistical significance was defined as a *P* value of < 0.05.

CONCLUSION

Sorbitan esters of different HLB values in the presence of cholesterol and charged lipids have been successfully used for the preparation of niosome vesicles. The percentage drug entrapped, particle size and drug release rate depended on the structure of span and the charge inducing agents. Tetracycline encapsulation in niosome vesicles has potential enhancement of the antimicrobial activity and consequently the treatment of topical bacterial infections. Further experiment studies in-vivo are needed in order to ascertain the enhancement of topical treatment by tetracycline encapsulation in niosomes.

REFERENCES

1. Majeti, N.V., Ravi, K. and Niraj, K., **Drug Dev. Ind. Pharm.**, 27, 1 (2001)
2. Singh, R. and Vyas, S.P., **Indian J. Pharm Sci.**, 58(1), 9 (1996)
3. Namdeo, A. and Jain, N.K., **Indian J. Pharm. Sci.**, 58(2), 46 (1996)
4. Shahiwala, A. and Misra, A., **J Pharm. Sci.**, 5, 220 (2002)
5. Azmin M.N, Florence A.T, Handjani-Vila R.M, Stuart J.F, Vanlerberghe G and Whittaker J.S., **J Pharm Pharmacol.**, 37, 237 (1985)
6. Junginger, H.E., Hofland, H.E.J. and Bouwstra, J.A., **Cosmet. Toil.**, 106, 45 (1991).
7. Lakshmi,P. K.; Gayathri Devi, S.; Bhaskaran, S. and Meenakshi, MBBS., **The Int. Journal of Dermatology.**, 3 (1), (2005).
8. Yoshioka, T.; Sternberg, B. and Florence, A., **Int. J. Pharm.**, 105, 1 (1994)
9. Toyoda, M and Morohashi, M., **Dermatology**, 196 (1), 130 (1998)
10. Norris, J.F.; Hughes, B.R.; Basey, A.J.; and Cunliffe, W.J., **Clin. Exp. Dermatol.**, 16(1), 31 (1991)
11. Vogt, K.; Hermann, J.; Blume, U.; Gollnick, H.; Hahn, H.; Haustein, U.F. and Orfanos, C.E., **European Journal of Clinical Microbiology & Infectious Diseases.**, 11 (10), 943 (1992).
12. Miller,Y.W.; Eady, E.A; Lacey, R.W.; Cove, J.H.; Joanes, D. N. and Cunliffenb W. J., **Journal of Antimicrobial Chemotherapy.**, 38, 829 (1996)
13. Schaal, K. P.; Schofield, G. M. and Pulverer, G., **Infection.**, 8, S122-S130 (1980)
14. Sammour, O.A. **Egypt. J. Biomed. Sci.**, 7, 121 (2001)
15. Cruickshank, R.; Duguid, J.P; Marmion B.P. and Swain R.H.A, 'Medical Microbiology'. 12th ed. Vol. II, The practice of medical microbiology. Churchill Livingstone, Edinburg, London and New York, P. 170 (1975)
16. Buchanan, R. E.; Gibbons N. E., and Co-Editors, Williams and Wilkins Co. 'Bergey's Manual of Systemic Bacteriology'. 9th Edition, Baltimore, USA. P. 141 (1980)
17. Omri A; Suntres ZE and Shek PN. **Biochem Pharmacol.**, 64, 1407 (2002)
18. Azoro, C., **World J. Biotechnol.**, 3, 347 (2002)
19. Yongabi, K.A., Studies on the potential uses of medicinal plants and macrofungi (lower plants) in water and waste water purification FMENV/ZERI Research centre, Abubakar Tafawa Balewa University Bauchi Nigeria, P.1 (2003)
20. Ebi, G.C. and Ofoefule, S.I., **Phytother. Res.**, 11, 47 (1997).
21. Yoshioka, T.; Sternberg, B. and Floernce, A., **Int. J. Pharm.**, 105, 1 (1994).
22. Hoa,Y.; Zhao,F.; Li, N.; Yang, Y. and Li, K., **Int. J. Pharm.**, 244, 73 (2002).
23. Yoshioka, T.; Sternberg, B; Moody M. and Florence, A., **J. Pharm. Pharmacol.**, 44, 1044 (1992).
24. Helmi M.A. Controlled release formulations of certain anti-inflammatory drugs. MS. Thesis, Zagazig university, Egypt (2005).

Zagazig J. Pharm. Sci., December 2005
Vol. 14, No. 2, pp. 11-17

25. Sammour, O.A.; **Zag. J. Pharm. Sci.**, 1(1-2), 34 (1992).
26. Kulkarni,S.B.; betageri, G.v. and Singh, M., **J. Microencap.**, 12(3) 229 (1995).
27. Gianasi, E.; Cociancich, F.; Uchegbu, I.F.; Florence, A.T. and Duncan, R., **Int. J. Pharm.**, 148, 139 (1997).
28. De Gier, J.; Mandersloot, J. and Van Deeren, L., **Biochim. Biophys. Acta**, 150, 666 (1998).
29. Omaima, N.E. and Ahmed, H.H., **Int. J. Pharm.**, 158, 121 (1997).
30. Omri, A.; Ravaoarinoro, M.; and Poisson, M., **J Antimicrob. Chemother.**, 36, 631 (1995).

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تحسين النشاط المضاد للبكتريا لعقار التتراسيكلين باستخدام حويصلات منشطات السطوح الغير متأينة (النيوزومات) كحاملات للعقار.

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اشتملت هذه الدراسة على تصميم العديد من منشطات السطوح الغير متأينة والحاملة لعقار التتراسيكلين للاستخدام الموضعي واستخدمت طريقة التبخير بانعكاس الوسط في تحضير هذه النيوزومات وتم دراسة عدة متغيرات على معدل احتواء العقار وانطلاقة من هذه النيوزومات هي : نوع منشطات السطوح الغير متأينة مثل السوربيتان استر ٢٠، ٤٠، ٦٠، ٨٠ وتركيز الدواء وكذلك تركيز الدهون وتأثير استخدام مواد ذات شحنات كهربية مختلفة مثل ثنائي أسيتيل الفوسفات وستيريل أمين.

وأوضحت النتائج أن النيوزومات المحضرة من سبان ٤٠ و٦٠ لها أعلى قدرة على احتواء العقار ويتبع ذلك سبان٢٠ ثم سبان ٨٠ ، وعند زيادة التركيز الكلي للمادة الدهنية من ١٥٠ إلى ٥٠٠ ميكرومول أدى ذلك إلى زيادة نسبة احتواء العقار من ٣٣% إلى ٣٨,٣% وبتغير تركيز العقار من ٢٥ إلى ١٠٠ مليجرام زادت نسبة الاحتواء من ٣٢,٩٢ إلى ٧٧ مليجرام لكل مللي مول من المادة الدهنية، ولقد لوحظ أن استخدام الشحنات الكهربية يتبعها زيادة في معدل الاحتواء للعقار بنسبة ٤٦% للستيريل أمين و ٦٧,١٤% لثنائي أسيتيل الفوسفات أما بالنسبة لمعدل انطلاق العقار فقد تبين أنة قد تم انطلاق حوالي ١٨ % و٣٦% من الحويصلات المخضرة من سبان ٦٠ و ٤٠ في خلال ٦ ساعات مقارنة ب ٤٥ % و ٥٨ % من سبان ٢٠ و٨٠.

وبدراسة حجم الحويصلات المحضرة تبين أن متوسط حجم الحويصلات يعتمد على نسبة الميزان المائي الزيتي لمنشطات السطوح الغير متأينة.

وأوضحت الدراسة المعملية على البكتريا المستخلصة من الجلد المصاب بالعدوى البكتيرية أن تأثير التتراسيكلين المتحوصل في النيوزومات له تأثير مضاد للبكتريا أكبر من تأثير العقار الحر.