

Rifampicin Niosome: Preparations, Characterizations and Antibacterial Activity Against *Staphylococcus aureus* and *Staphylococcus epidermidis* Isolated from Acne

Kohinur Begum¹, Ashfia Fatima Khan², Humayera Kabir Hana², Jakaria Sheak² and Reza-ul Jalil³

¹Department of Pharmacy, ASA University Bangladesh, ASA Tower, Shyamoli, Dhaka-1207, Bangladesh

²Department of Pharmacy, Primeasia University, Banani, Dhaka-1213, Bangladesh

³Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Received: May 11, 2015; Accepted: June 09, 2015; Published (web): June 15, 2015

ABSTRACT: Niosomes, a vesicular formulation, has been explored extensively for topical application to enhance skin penetration as well as to improve skin retention of drugs. In this study, three different rifampicin (1% w/w) niosomal formulations were prepared. Span 60, propylene glycol, dimethyl sulfoxide (DMSO), methanol and distilled water were used in the formulations. Different rifampicin niosomal formulations containing 12% (RN-F1), 16% (RN-F2) and 20% (RN-F3) of Span 60 were prepared by injection method. Rifampicin content of each formulation was determined by UV spectrophotometer at 475 nm. Niosome particle size was measured by laser scattering method using Mastersizer 2000. Volume average diameter, d_{50} of different formulations were found 8.488 nm (RN-F1), 12.533 nm (RN-F2) and 12.375 nm (RN-F3). Niosome preparations were also characterized by entrapment efficiency and *in vitro* drug release and pH stability test. Results of entrapment efficiency were found to be 55.11%, 57.66% and 60.17% for RN-F1, RN-F2 and RN-F3, respectively. Drug release pattern of RN-F2 and RN-F3 formulations showed sustained release at controlled rate. All formulations were more stable at pH 5.8 to 7.0. Antibacterial effect of rifampicin niosomes was evaluated against *S. epidermidis* and *S. aureus* isolated from acne by antibiotic sensitivity and time kill study. The results of time kill study revealed that up to 96% of bacteria were killed within 4 hours. In this experiment, rifampicin niosomes were successfully prepared for the intention of targeting antibacterial effect in acne.

Key words: Rifampicin, niosomes, antibacterial effect, acne, *S. epidermidis*, *S. aureus*.

INTRODUCTION

Niosomes are promising vehicle for drug delivery. It is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Niosomes have better stability and can prolong the circulation of the entrapped drug because of the presence of non-ionic surfactant with the lipid. This allows better targeting of drugs to tumour, liver and brain. One of the major advances in vesicle found that some modified vesicles possess properties that allowed them to successfully deliver drugs into the

deeper layers of skin. Transdermal delivery is important because it is a non-invasive procedure for drug delivery. Furthermore, problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided.¹ Niosomes have been investigated for drug delivery through the most common routes of administration, such as intramuscular, intravenous, subcutaneous, ocular, oral and transdermal.²⁻⁵ Certain factors such as amount of drug and type of surfactant affect vesicle size, entrapment efficiency and release characteristics. Rifampicin is a broad spectrum antibiotic. It is active against *Mycobacterium tuberculosis* and *M. leprae*. Rifampicin is slightly

Correspondence to: Kohinur Begum
Cell: +8801713-080285
E-mail: kohinur025@yahoo.com

soluble in water and fairly soluble in other solvents.⁶ In the assay against bacterial flora of acne lesions, *Propionibacterium acnes* and *Staphylococcus epidermidis* were found to be sensitive to the antibiotic used in the treatment of acne. The highest antibacterial effect against both of these species was demonstrated by rifampicin and tetracycline.⁷ However, only few studies have been reported as a targeted delivery of rifampicin by niosomal drug delivery system. In this study, an attempt has been taken to prepare formulation of rifampicin niosomes and evaluate its antibacterial activity against the bacteria isolated from acne.

MATERIALS AND METHODS

Materials. Chemicals used as rifampicin (Cipla, India), Span 60, propylene glycol, dimethyl sulfoxide, propanol, methanol (Merck, Germany), Mueller Hinton agar (Himedia). Instruments used such as Mastersizer 2000 (Malvern, UK), Ultrasonic bath (Sonoswiss, Switzerland), Ultraviolet Spectrophotometer (Shimadzu, Japan), Centrifuge machine (Boeco, Germany).

Bacterial strains. Bacterial strains were previously isolated from acne patients.⁸ *S. epidermidis* and *S. aureus* were used in this study.

Calibration curve of rifampicin. 10 mg rifampicin was taken in a 100 ml volumetric flask and methanol was added up to the mark to obtain a concentration of 100 mg/l. Dilutions were made to obtain solutions of 5 different concentrations (10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l and 50 mg/l). The absorbance of each solution was measured at 475 nm using ultraviolet spectrophotometer. A calibration curve was plotted using the absorbance data against drug concentration and the curve was used to estimate the drug content of the niosomal formulations (RN-F1, RN-F2 and RN-F3).

Formulation and preparation of rifampicin niosomes. Three rifampicin niosome products were formulated and designated as RN-F1, RN-F2 and RN-F3. Ingredients and amounts are given in table 1. Rifampicin niosomes were prepared according to the injection method with some modifications.^{4,9} Briefly, 0.5 g rifampicin was taken in a glass vial (20 ml). 2 g

of DMSO was added slowly and the mixture was heated at 70°C using an ultrasonic bath at ultrasonic wave (45Hz). 36.5 g water was taken in 100 ml bottle and placed inside the ultrasonic bath at 70 °C. 6 g Span 60 was weighed in 50 ml beaker and 5 g propylene glycol was added and sonicated at 70 °C in the ultrasonic bath to make a clear solution. The solution of rifampicin in DMSO was added to the mixture of Span 60 and propylene glycol and sonicated for 15 minutes. Then the mixture (rifampicin, DMSO, Span 60 and propylene glycol) was injected into the water by disposable syringe using 14G needle. The force applied during the injection produced unilamellar vesicles. The preparation was vigorously shaken and sonicated further for 5 minutes and then bottle was placed in ice cold water to cool.

Particle size analysis. Laser scattering method was used to measure particle size of niosome formulations. Mastersizer 2000 (Malvern UK) was used for particle size determination and the procedure was carried out according to the instrumental instruction. Approximately 20 mg of the niosome formulations were taken in sample chamber containing water for injection, sonicated for 5 minutes and count was made when sufficient obscuration was achieved.

Determination of entrapment efficiency. The amount of drug entrapped was analyzed by standard entrapment efficiency method. Niosomes containing drugs were separated from untrapped drug by centrifugation. The niosomal dispersion was centrifuged at 4500 rpm for 30 minutes and supernatant was collected for further studies. The entrapped drug (1 ml) was disrupted using 3 ml 50% propanol for 5 minutes which was analyzed spectrophotometrically for drug concentration at 475 nm to calculate the concentration of entrapped drug against 50% propanol as blank. The % of entrapped drug was calculated by applying the following equation.¹⁰

% of entrapment = $A_e \times 100/A_i$ where A_e is the amount of entrapped drug and A_i is the initial amount of drug in lipid phase.

In vitro drug release. The study was performed on the release pattern of the drug from the niosomal formulations using dialysis method. The entrapped drug containing supernatant was pipetted into a dialysis bag (Visking, DTVO3500) and placed in 100 ml of phosphate buffer (pH 7.4) and kept under constant agitation using magnetic stirrer at temperature of 37° C. Each periodical time sample was withdrawn and same volume of buffer was replaced. Then samples were assayed by spectrophotometer at 475 nm using medium as blank. The release was compared with pure drug solution.¹

pH stability test. 1 mg entrapped niosomes in 5 ml of phosphate buffer (pH 5.8, 7.00, 8.00) were kept in a screw cap test tube for 2 hours at 25°C and the sample was withdrawn. The supernatant was analyzed spectrophotometrically at 475 nm after centrifugation at 4500 rpm for 30 minutes.¹⁰

Antibiotic sensitivity test. Mueller Hinton agar plates were used for antibiotic sensitivity test. *S. epidermidis* and *S. aureus* were used for testing antibiotic sensitivity by Kirby-Bauer method.¹¹ A sterile swab was used to spread the microorganism uniformly on the surface of the medium. Cork borer was used to make holes in the medium. 50 µg/25 µl of rifampicin and formulations (RN-F1, RN-F2 and RN-F3) were added in different holes in the medium. The plates were incubated at 37°C for 24 hours. Diameter of zone of inhibition around each niosome formulation was recorded in millimeters. Average diameter of zone of inhibition in duplicate test was taken to determine its sensitivity.

Time kill study. Time-kill study was performed using a method based on the European Standard quantitative suspension test.¹² An initial inoculum of *S. epidermidis* was prepared using a single colony in 10 ml nutrient broth. The actively growing culture was diluted in nutrient broth and verified by a total viable count. 1 ml of the initial inoculum was added to 9 ml of the solution of the sample of rifampicin niosome. The final concentration of the sample was 1mg/ml. The screw cap test tube containing the suspension for each sample was shaken at 37 °C and samples (100 µl) were taken at 1, 2, 3 and 4 h intervals and plated on nutrient agar. The total viable

count was determined after overnight incubation at 37 °C.

RESULTS AND DISCUSSION

Calibration curve of rifampicin. The calibration curve of rifampicin was prepared using five different concentrations (10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l and 50 mg/l) and absorbances of each solution was measured at 475 nm using ultraviolet spectrophotometer. Standard curve is shown in Figure 1.

Drug content in formulations. The drug content of each formulation was calculated by using standard curve (Figure 1) as shown in Table 2. The drug content of all formulations showed satisfactory result.

Table 1. Formulation of rifampicin niosomes.

	RN-F1	RN-F2	RN-F3
Ingredients	Weight in g	Weight in g	Weight in g
Rifampicin	0.5	0.5	0.5
DMSO	2	2	2
Propylene glycol	5	5	5
Span 60	6	8	10
Water	36.5	34.5	32.5
Total	50	50	50

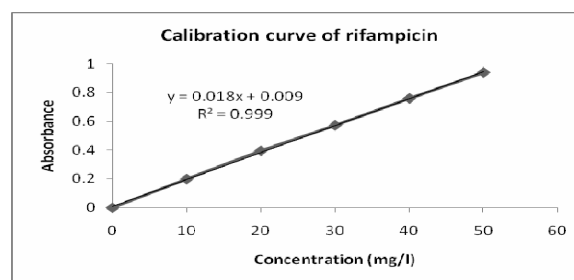


Figure 1. Calibration curve for determination of rifampicin content of the formulations.

Determination of particle size. Niosome particle size was measured by light scattering method using Mastersizer 2000. From this study d_{50} of different formulations were found to be 8.488 nm (RN-F1), 12.533 nm (RN-F2) and 12.375 nm (RN-F3). The particle size of three formulations was found to be different due to variation in the composition of the formulation as shown in table 3 and figure 2.

Determination of entrapment efficiency. The entrapment showed around 55% to 60% for all

formulations with the highest value of 60% in RN-F3 in figure 3. Entrapment efficiency results depend on several factors, including the ratio of surfactant, the structure of the surfactant and the method of

preparation. In this study, entrapment of drug was increased with increasing of amount span 60 was added.

Table 2. Content of rifampicin in formulations.

Formulation	Concentration (mg/l)	Drug content % w/w	Mean drug content % w/w
RN-F1	20.11	1.005	0.9875
	19.4	0.97	
RN-F2	17.94	0.897	0.8735
	16.94	0.85	
RN-F3	17.89	0.897	0.9015
	18.11	0.906	

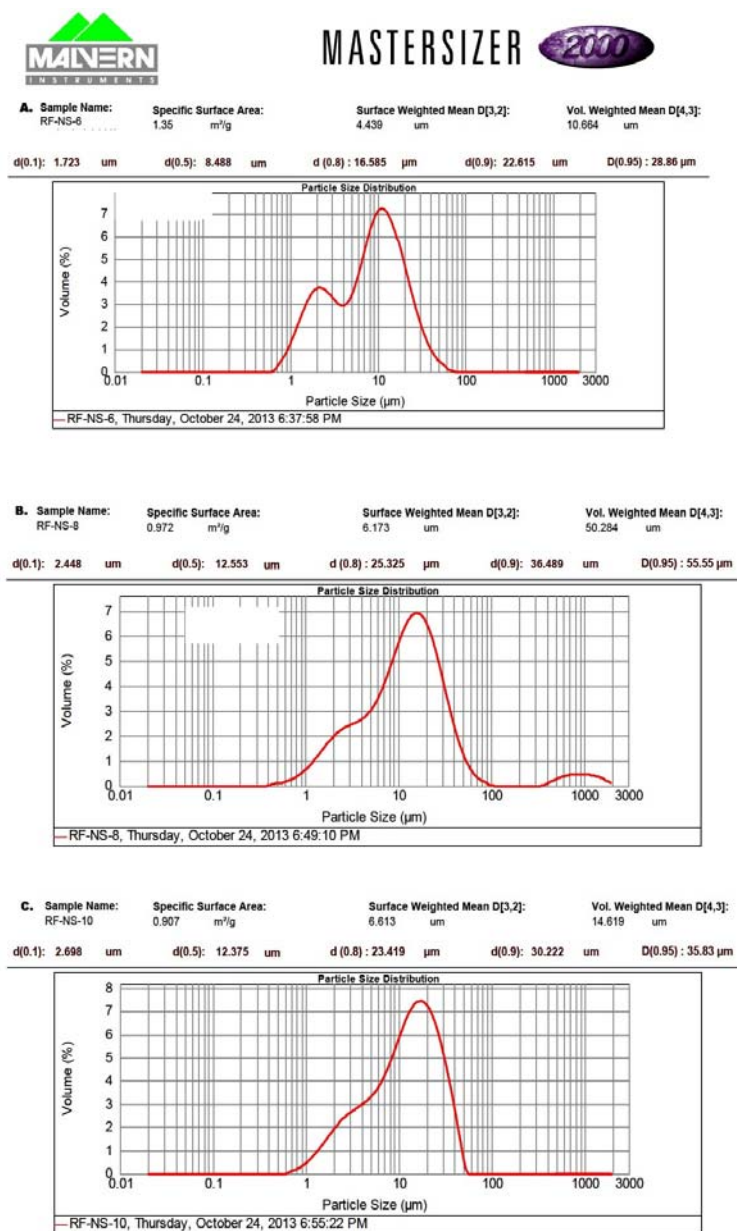


Figure 2. Graphical presentation of particle size of rifampicin niosomes.

Table 3. Particle size at different fraction levels of rifampicin niosome formulations.

Rifampicin niosome	d (90) μm	d (50) μm	d (10) μm
RN-F1	22.615	8.488	1.723
RN-F2	36.489	12.533	2.448
RN-F3	30.222	12.375	2.689

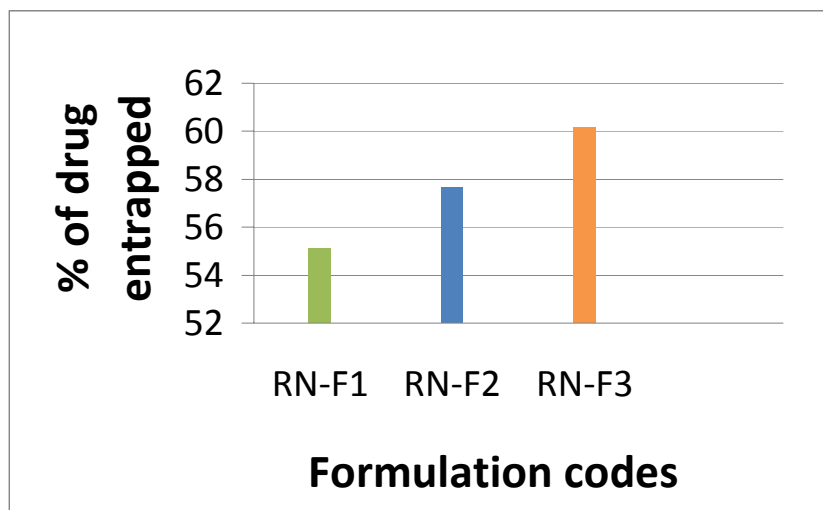
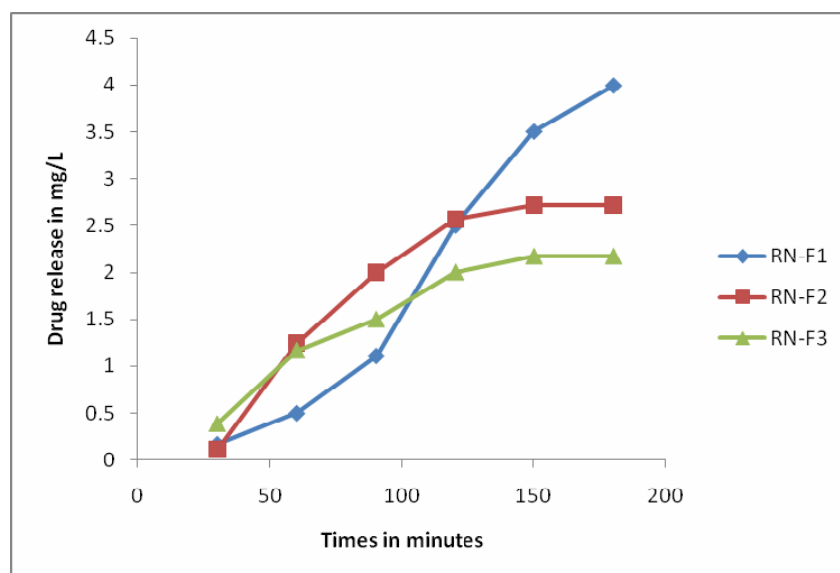


Figure 3. Entrapment efficiency of rifampicin niosomes.

Figure 4. *In vitro* drug release of rifampicin niosomes.

***In vitro* drug release.** The membrane permeability at different time intervals was considered to check the *in vitro* release of drug. The absorbances were compared with standard values to know the drug release. On estimating the drug release, the amount released seems to be higher gradually after few minutes until it reached end of

drug release. An equilibrium was established when all drugs were released from dialysis bag. Drug release pattern is shown in Figure 4.

pH stability test. Niosomal formulations had a reservoir effect for drug at skin pH leading to drug entrapment in niosomal composition and in skin layers showing a retardation effect. Results of pH

stability study showed that maximum concentration of drug was retained at weakly acidic to neutral pH (Figure 5). So formulations were suitable for targeting the drug at the site of action on skin. As a topical preparation, the drugs will easily penetrate the skin and reach the target site.

Antibacterial activity of rifampicin niosome.

S. aureus and *S. epidermidis* strains were found to be sensitive to rifampicin niosomal formulations as shown in figure 6.

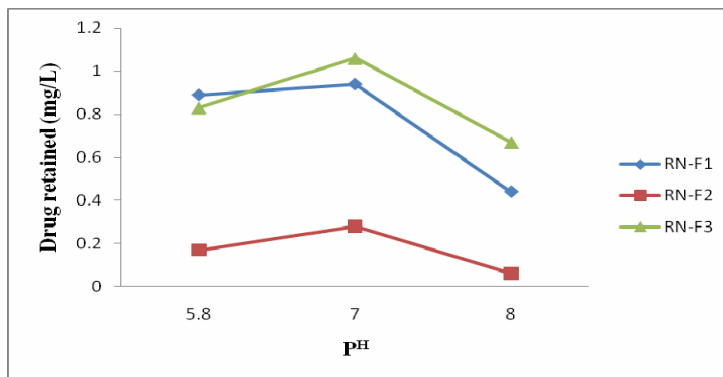


Figure 5. pH stability of rifampicin niosomes.

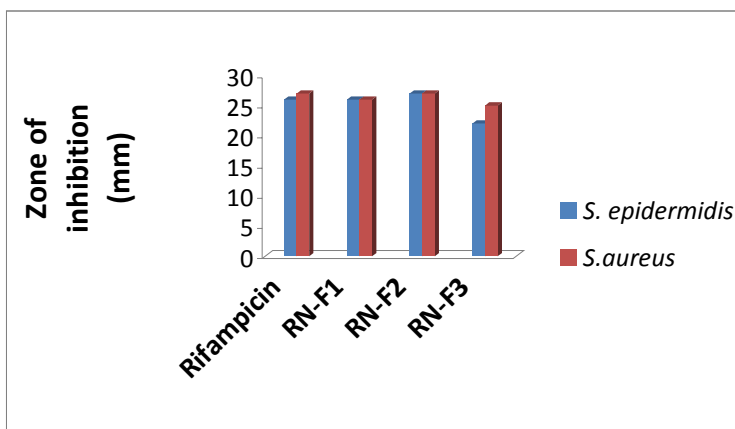


Figure 6. Antibacterial activity of rifampicin and rifampicin niosomes.

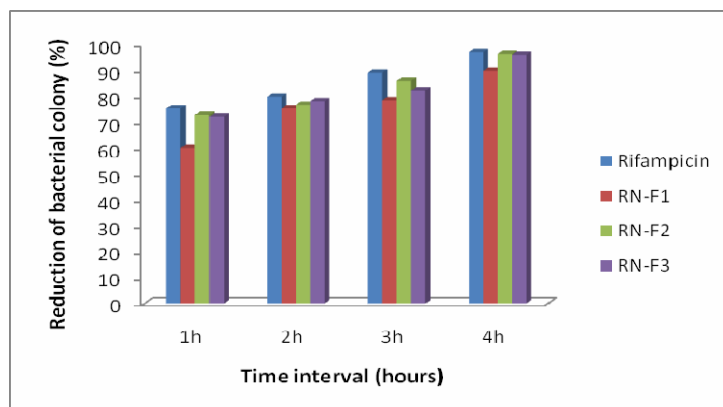


Figure 7. Results of kill time of rifampicin and rifampicin niosomes against *S. epidermidis*.

Time kill study. The results of the time kill study are given in figure 7. After three hours 78% to 80% bacterial cells were reduced by different niosomal formulations. 89% to 96% of bacteria were killed by the different formulations within 4 hours.

CONCLUSION

The main objective of this study was to design suitable niosome encapsulated drug delivery system, to study the *in vitro* behavior of the prepared system and to investigate the niosome encapsulated drug for its activity. The formulations were showing satisfactory particle size, entrapment efficiency, *in vitro* drug release and pH stability. This study showed that niosomal formulation can provide consistent and prolonged release of rifampicin from different niosomal formulations. It will lead to sustained action of the entrapped drug with reduced the side effects associated with frequent administration of the drug and potentiate the therapeutic effects of the drug. Results of *in vitro* antibacterial effect studies also are encouraging. However, extensive research should be carried out to establish its human applications in the future.

REFERENCES

- Albery, W.J. and Hadgraft, J. 1979. Percutaneous absorption: theoretical description. *J. Pharm. Pharmacol.* **31**, 129-139.
- Biju, S.S., Talegaonkar, S., Misra, P.R. and Khar, R.K. 2006. Vesicular systems: an overview *Indian J. Pharm. Sci.* **68**, 141-153.
- Azmin, M.N., Florence, A.T., Handjani-Vila, R.M., Stuart, J.F., Vanlerberghe, G. and Whittaker, J.S. 1985. The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. *J. Pharm. Pharmacol.* **37**, 237-242.
- Baillie, A.J., Florence, A.T., Hume, L.R., Muirhead, G.T. and Rogerson, A. 1985. The preparation and properties of niosomes non-ionic surfactant vesicles. *J. Pharm. Pharmacol.* **37**, 863-868.
- Hu, C. and Rhodes, D.G. 1999. Proniosomes: a novel drug carrier preparation. *Int. J. Pharm.* **185**, 23-35.
- Martindale, The Complete Drug Reference (2009). Ph Press, RPS Publishers, USA, 36th edition, pp. 3037-3044.
- Załuga, E., Giedrys-Kalemba, S. and Hałasa, J. 1996. Bacterial flora of acne lesions in diagnostic material of the Microbiology and Immunology Department, Pomeranian Medical Academy (PAM) in Szczecin. *Med. Dosw. Mikrobiol.* **48**, 55-59.
- Khan, A.F., Hana, H.K., Sheak, J. and Begum, K. Antibiotic sensitivity of *Staphylococcus aureus* and *Shaphylococcus epidermidis* isolated from acne patients. *Bangladesh Pharm. J.* (in press)
- Vyas, S.P. and Khar, R.K. 2002. Targeted and Controlled Drug Delivery: Novel Carrier Systems. CBS Pub. New Delhi, India.
- Iyyanuchamy, S.K. and Blessy, J. R. 2013. Entrapment of antibiotics in biosystem by niosomal drug delivery method. *J. Drug Deliv. Therapeutics* **3**, 6-8.
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **36**, 493-496.
- European Committee for Standardization 1997. Chemical disinfectants and antiseptics. Basic Bactericidal Activity: Test Methods and Requirements. European Standard EN 1040. Brussels: European Committee for Standardization.