ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

NOVEL DRUG DELIVERY SYSTEM FOR MANAGEMENT OF *S.AUREUS* INFECTIONS IN PATIENTS SUFFERING VENOUS STASIS ULCERS

S.Dugal*, A.Chaudhary

Dept.of Microbiology, Sophia College, University of Mumbai, Maharashtra, India.

**Corresponding author: suparnadugal@gmail.com*

ABSTRACT

Local disinfection of stasis ulcers using topical application of povidone iodine (PVP-I) has been reported to be a superior treatment regimen compared to the use of systemic antibiotics. However, contact burns and irritant dermatitis associated with sudden release of large quantities of PVP-I from bandages are a major drawback attributed to the use of this antiseptic. The current study investigates the use of niosomes as micro reservoirs aiding in the slow gradual release of this drug. *S.aureus*, one of the most common etiological agents associated with stasis ulcers, was chosen as the test organism. Niosomes prepared by thin film hydration technique were characterized using scanning electron microscope and nanoparticle analyzer. Zeta potential of the prepared niosomes was found to be -5.6mV. FTIR studies demonstrated no chemical alteration of the drug subsequent to its encapsulation. Polydispersity index of 0.541 revealed good homogeneity of the prepared formulation. Minimum inhibitory concentration of *S.aureus* by micro broth dilution technique was found to be 0.30% w/v of available iodine. The entrapment efficiency of PVP-I was found to be 80%. Further, an ex vivo goat skin model used to study the drug release profile revealed the bactericidal effect to extend over 35 hours, thus demonstrating the ability of niosomes to delay drug release.

Keywords: *S.aureus,* stasis ulcers, povidone iodine, niosomes, delayed release.

1. INTRODUCTION

Chronic stasis ulcers due to venous insufficiency are the most common type of skin ulcers. They mainly occur just above the ankle and usually affect geriatric populations. About 1:50 persons develop venous insufficiency at some stage. Venous stasis ulcers (VSU) are presented as areas of poorly healing wounds, generally of the medial malleolus. They may be exudative with local skin necrosis and irregular borders. Ulcerated stasis dermatitis is the end stage of venous insufficiency which occurs due to deep venous reflux and improper functioning of the valves within the veins. The elevated pressure developed within veins causes the capillaries to undergo changes such as fibrin cuffing. Weakening of the skin leads to ulceration and open wounds which predispose an individual to bacterial infections like cellulitis. Microbiota which have isolated and characterized from venous stasis ulcers include Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and E*scherichia coli* [1]. Healing of stasis ulcers is often poor and refractory to therapy, particularly when they are infected. Systemic antibiotic therapy is of little benefit in this setting because of poor penetration of antibiotics into the wounds. It has also been demonstrated that the relapse rate of superficial bacterial infections like impetigo and cellulitis, caused by *S.aureus* is significantly higher with the use of systemic antibiotics [2]. The mainstay of treatment of VSU therefore is the management of bioburden by use of topical antibiotics and antimicrobials impregnated in compression bandages. Commonly used antimicrobial agents include cadexomer iodine and povidone iodine [3].

PVP iodine is a broad spectrum biocidal agent which can react with enzymes of the respiratory chain and cell membrane proteins, damaging the microorganisms irreversibly [4]. Previous researchers have proven the effectiveness of topical povidone iodine compared to other topical antimicrobial agents as well as systemic antibiotics to prevent the progression of VSU infections [5]. However, though useful in the management of wounds, PVP iodine has also been reported to cause contact burns if excessive amount is used or released from absorbent bandages during application. Cases have been reported of skin irritation, delayed healing as well as blistering and patchy necrosis due to application of PVP-I on skin injuries [6]. Additionally, since venous ulcers are prone to being complicated by allergic contact dermatitis, care must be taken while employing PVP-I as a therapeutic measure. Faced with the prospect of increased sensitivity and complications associated with the current mode of application of PVP-I, a drug delivery system employing a novel approach needs to be considered.

Niosomes or non ionic surfactant vesicles have been recently explored by several investigators for the gradual and sustained release for various compounds including terbinafine hydrochloride, ofloxacin and miconazole. [7-9]. Niosomes being osmotically active, biodegradable and non immunogenic are emerging as superior alternatives to liposomes, which are chemically less stable. These vesicles, with the desired drug encapsulated within them, are known to act as microreservoirs and provide a depot effect. Previously we have reported the synthesis and use of niosomes for the control of *Candida* infections [10]. In the current study we have (i) formulated and further characterized PVP-I encapsulating niosomes (ii) evaluated them for invitro activity against *S.aureus*, selected as model organism representing Gram-positive pathogenic VSU colonization and (iii) studied the topical delivery profile of this medicament by employing an ex vivo goat skin model system. We have tested the hypothesis that niosomes would be able to delay the liberation of povidone –iodine thereby preventing adverse skin reactions associated with immediate drug release. We believe that such PVP-I niosomal preparations would not only be effective in controlling *S.aureus* infections and improve the quality of healing in patients suffering from venous stasis ulcers but also be useful in the general management of infections due to other chronic ulcers and wounds.

2. MATERIAL AND METHODS

2.1. Strains, Media and Chemicals

S.aureus culture obtained from a local hospital was grown at 37°C in Nutrient broth. Cells were harvested, washed in sterile phosphate buffer saline and standardized to an O.D. of 0.6 at 530nm. (ErmaInc. colorimeter). Betadine (povidone iodine) was obtained from Win Medicare Pvt. Ltd. New Delhi, Sorbitane monooleate (Span 80) was purchased from SD Fine Chemical Limited and cholesterol procured from Merck India Chemicals. Dialysis membrane was purchased from Himedia. All reagents used were of analytical grade.

2.2. Preparation of niosomes

Niosomes were synthesized by the thin film hydration technique using the surfactant, sorbitane monooleate and cholesterol in the ratio of 2:1. The two were dissolved in diethyl ether and the solvent evaporated under reduced pressure using rotary flash evaporator (Superfit™ India) at 150 rpm for 5- 10 min. Hydration was carried out with aqueous phase containing 75mg of povidone –iodine in 10ml of distilled water. It was vortexed and heated at 60-70°C for 1 hour. The resulting vesicles were cooled in an ice bath and then sonicated for 5 min.

2.3. Determination of minimum inhibitory concentration of povidone iodine against S.aureus

Using stock solution of 7.5% w/v povidone iodine (0.75%w/v available iodine), various dilutions were prepared. 0.1 ml of the 24 hour old *S.aureus* culture was added to all the tubes. Positive and negative controls were maintained. Tubes were incubated at 37°C for 24 hour and the lowest concentration that did not show growth corresponded to the minimum inhibitory concentration (MIC).

2.4. Determination of minimum bactericidal concentration of povidone iodine against S.aureus

0.1ml of culture from the tubes indicating minimum inhibitory concentration and all higher concentrations beyond it were surface spread on nutrient agar and incubated at 37°C for 24hrs. The minimum bactericidal concentration (MBC) endpoint was the minimum concentration of PVP-I at which at least 99.9% of the initial inoculum was eradicated and at which only one or no colonies could be seen on the agar.

2.5. Characterization of niosomes

Size of prepared niosomal preparation (with and without encapsulating PVP-I) was determined by nanoparticle analyzer based on laser scattering spectroscopy (Horiba Partica SZ100Z). Samples were checked at 25°C and average particle size calculated. Zeta potential analysis to determine particle surface charge, polydispersity index and viscosity were also done using the same nanoparticle analyzer. Inorder to check for any alteration in the chemical structure of the drug subsequent to entrapment the IR spectra of pure PVP-I solution and the niosomes entrapping PVP-I was recorded using Fourier transform infrared spectrophotometer.

2.6. Determination of drug entrapment and efficiency

Using a stock solution of 10µg of potassium iodide, various dilutions were prepared ranging from 0µg- 3.5µg. To obtain the calibration graph, a series of tubes were prepared containing 3 ml of the sample dilution along with 2 ml of 0.04% iodate, 1 ml of 22% sodium chloride and 1 ml of 6 M sulphuric acid. The contents were mixed well and kept aside for 10 min. One milliliter of 0.003% methyl red was added before diluting to 10 ml with distilled water. The absorbance of the solution was measured at 520 nm against water. The plot of absorbance *versus* concentration of iodine was a straight line with a negative slope.

Entrapment efficiency was determined by the dialysis method. 3ml niosomal dispersion was placed inside a dialysis bag and suspended in a beaker containing 50ml diffusion medium (distilled water).This was constantly stirred at room temperature for 4 hours after which the pure niosomal solution within the dialysis bag was removed. The amount of drug in the formulation was determined after lysing the niosomes using 50% n-propanol at 37°C for 30 minutes. The sample was filtered through Whatman Filter Paper no.1. After suitably diluting, the available iodine in the sample was assayed.

Absorbance values were plotted on the calibration curve and the concentration of the drug entrapped was calculated. Also the drug entrapment efficiency was calculated.

$$\text{Efficiency of entrapment} = \frac{\text{Conc. of drug entrapped} \quad \text{x } 100}{\text{Initial concentration of drug}}$$

2.7. Evaluation of the topical delivery of PVP-I encapsulated within Niosomes using ex vivo goat skin model.

To evaluate the gradual release of PVP-I entrapped within niosomes, 0.1 ml of *S.aureus* culture (adjusted to 0.01 OD) was added to several small pieces of goat abdominal skin (1cm x 1cm) and allowed to adhere for 24 hours. These pieces were then washed with phosphate buffer (pH7.4) and treated with niosomes for different time intervals. Appropriate controls were maintained. Viable count of *S.aureus* cells was determined by suspending and vortexing the mucosal pieces in sterile saline, followed by plating on sterile salt mannitol agar. Plates were incubated at 37°C for 24 hours and colonies counted.
All bacteriological experiments were done in triplicate and mean values have been reported.

3. RESULTS AND DISCUSSION

Chronic stasis leg ulcers are defined as open lesions between the knee and the ankle that remain unhealed for at least four weeks and occur in the presence of venous disease. General care of the skin surrounding an ulcer using a topical antiseptic like povidone iodine is essential to maintain skin integrity and minimize the risk of further ulceration. PVP-I is a stable chemical complex of polyvinylpyrrolidone and elemental iodine. The prolonged, non selective, anti-microbial action of PVP-I is unparalleled for surface microbicidal activity and is particularly effective in treating mixed infections. Its effectiveness has been clinically proven for all types of topical applications in both human and veterinary medicine.

However, previously researchers have reported cases of chemical burns induced by povidone iodine in patients undergoing surgeries and arteriographies [11-13]. A similar potential risk of contact burns exists in VSU patients who are predisposed to increased sensitivity due to damaged skin. Given the clinical significance of this condition, the current study explores a novel delivery system for the controlled release of this medicament to treat venous stasis wounds infected by *S.aureus.*

In order to determine the concentration of PVP-I to be encapsulated within niosomes the MIC of PVP-I was determined. It was found to be 0.30% w/v available iodine. The ratio of MIC/MBC was calculated to be less than or equal to 4 which indicated PVP-I as potent bactericidal agent against *S. aureus.* Niosomes prepared by thin film hydration technique were observed as discrete spherical vesicles using scanning electron microscopy (Fig. 1). Niosomes encapsulating PVP-I were comparatively larger than unloaded vesicles. The size of the niosomes with entrapped PVP-I ranged from 233.6 nm to 465.6 nm with an average size of 338.4 nm. The mean size of the smaller unloaded blank vesicles was found to be 220.8 nm by the light scattering method. The entrapment of the drug in niosomes results in an increase in vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayer thereby increasing vesicle size.

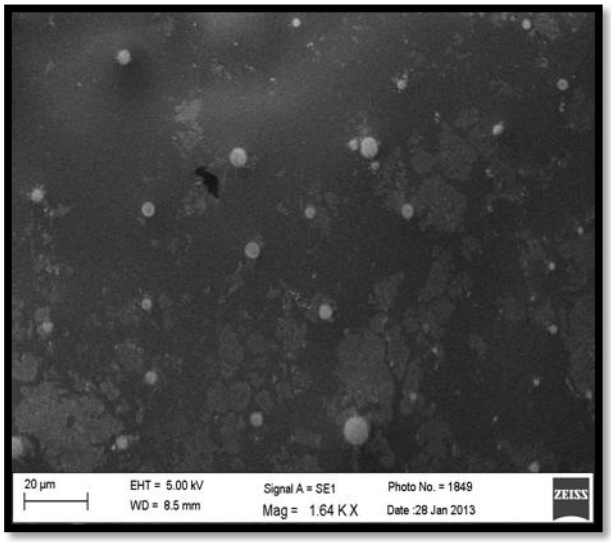
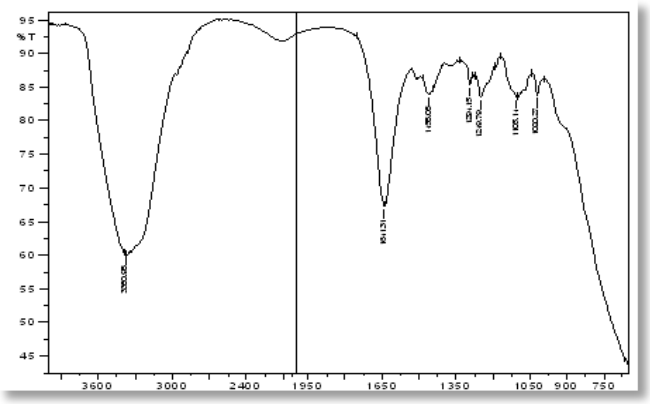


Fig 1: Scanning electron micrograph of the niosomes entrapping PVP-I

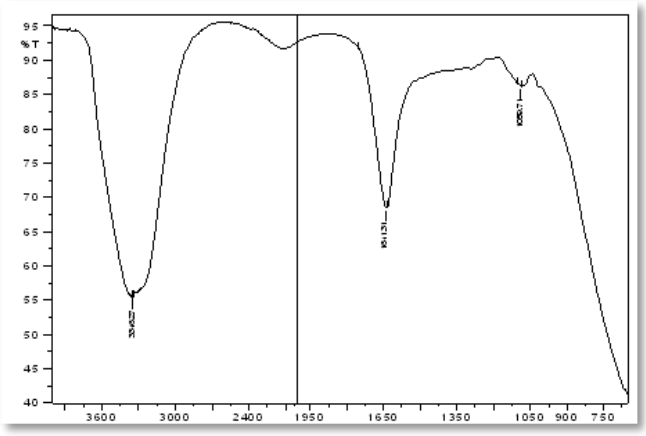
The zeta potential of the prepared niosomal suspension was found to be -5.6mV. Analysis of zeta potential is important since it indicates the surface charge of particles, their tendency to agglomerate as well as interact with microbial cells. Ideally, the optimum value of zeta potential should lie between +25 and - 25 mV [14]. Outside this range, the niosomes may not remain stable for a long period of time and this could adversely affect parameters such as entrapment efficiency and sustained release. A higher zeta potential indicates higher kinetic energy and tends to move particles towards agglomeration [14, 15]. Since *S. aureus* carry a surface charge of approximately −25.07mV [16] it is possible that since the microbial cells possessed a comparatively greater negative charge, it led to an increase in affinity and interaction with the particles, resulting in killing of the cells. The polydispersity index of 0.541 and viscosity of 0.896 mPa.s indicated good homogeneity of the formulation.

FTIR spectra of pure PVP-I as well as subsequent to its encapsulation is shown in Figure 2 A and B. The similarity in peaks indicates there was no incompatibility between the drug and the surfactants used for formulation. Pure PVP-I and PVP-I

after entrapment in niosomes revealed no appreciable change in the position of absorption bands and consequently demonstrated the absence of any chemical alterations in the drug. General minor changes in the obtained spectra were due to additional bands contributed by the surfactant.



(A)



(B)

Fig 2: FTIR spectra of (A) pure PVP-I (B) PVP-I entrapped within niosomes

The concentration of the drug added to prepare niosomal carriers by the thin film hydration technique was 6000 microgram/ml. Dialysis method performed showed that 4800 microgram/ml of the drug was entrapped by the vesicles. Hence the protocol selected for the formulation resulted in a high drug entrapment efficiency of 80% and suggests the ability of vesicles to prolong drug release. Previously A. Abdul Hasan Sathali *et al*, 2010 have demonstrated that as entrapment efficiency increases (with increase in surfactant concentration) more time is taken for maximum drug release i.e. the drug release is prolonged [7].

Topical delivery of PVP-I containing niosomes was evaluated using ex vivo goat skin model is [Figure 3].

Fig 3: Images of Fresh abdominal skin of goat (A) Before shaving (B) After shaving (C) Cut into 1 cm x 1cm pieces.

Release of the aqueous content of the niosomes occurred due to contact with bacterial cells which increased the permeability of the niosomal membrane. Viable count of the culture on the excised skin pieces after exposure to the formulation was determined at various intervals of time [Fig. 4].

The graph below shows that there was a gradual decrease in the number of bacterial cells since the niosomes acted as a local depot allowing the slow release of the antiseptic in a controlled manner. The release of PVP- I was prolonged for over 35 hours after which complete killing of the cells was achieved. At the end of 35 hours, the goat skin appeared intact, with no signs of blistering or any other adverse reaction.

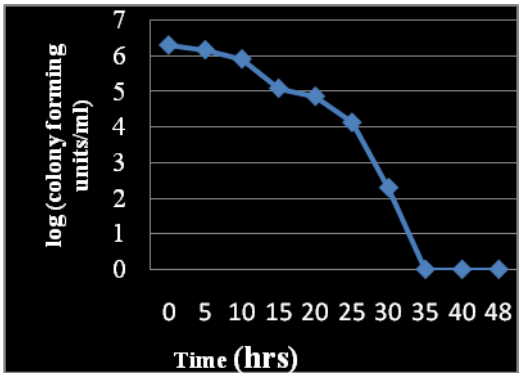


Fig 4: Ex vivo study of encapsulated PVP-I against S.aureus

In conclusion, the results of the current study demonstrated that local treatment with povidone-iodine niosomal carriers exhibited good efficacy and delayed the release of the medicament. This gradual release pattern of PVP-I could help prevent contact burns and other adverse reactions associated with the immediate delivery of large quantities of the antiseptic at the site of application. Further invivo studies would help to indicate any keratotic reactivity and the quality of wound healing.

4. ACKNOWLEDGEMENTS

The authors are grateful to the Biotechnology Department, Khalsa College Mumbai, for providing the facilities to carry rotary flash evaporation and Advanced Scientific Equipment Pvt. Ltd Mumbai, for providing facilities to carry out particle size and zeta potential analysis. The authors are also thankful to UICT Textile Dept. for the FTIR facilities.

5. REFERENCES

1. Ly de FF, Fabiana CP, Fernando de FF. *Brazil Jornal Vascular Brasileiro*, Sept. 2007; 6 (3).
2. Daróczy J. *Dermatology,* 2006; **212**:82-87.
3. Holloway GA, Johansen KH, Barnes RW, Pierce GE. *Western J Med,* 1989; **151(1)**: 35-8.
4. Schenck HV, Simak P, Haedicke E. *J.Pharm. Sci,* 1979; **68:**1505-1509.
5. O'Meara S, Al-Kurdi D, *et al*. *Cochrane Database Syst Rev*. 2010 Jan 20; (1):CD003557. doi: 10.1002/14651858
6. Murthy MB, Krishnamurthy B. *Indian J Pharmacol*, 2009 August; **41(4)**: 199-200.
7. Sathali AAH, Rajalakshmi G. *Int.J. PharmTech Res*, 2010; **2(3)**:2081-2089.
8. Gupta N, Shrivastava V. *Int J Phar Life Sci*. 2010; **1(7)**: 413-418.
9. Mohamed Firthouse PU, Mohamed Halith S, *et al*. *Int.J. Pharmtech res*. 2011; **3(2)**:1019-1022.
10. Dugal S, Chaudhary A. *Int. J. Pharm Pharm Sc*. 2013;**5(3)**
11. Demir E, O'Dey DM, Pallua N. *J Burn Care Res*. 2006; **27**:895-900.
12. Liu FC, Liou JT *et al.*, *Acta Anaesthesiol Sin*, 2003; **41:**93-6.
13. Nahlieli O, Baruchin AM *et al. Burns*. 2001; **27**:185-8.
14. Sinko PJ. *Martin's Physical Pharmacy and Pharmaceutical Sciences*. Edn **5**, New York: Lippincott Williams & Wilkins, 2006; pp 503- 509.
15. Tamizharasi S, Dubey A *et al. J. Young Pharm*. 2009; **3:**205-209.
16. Lee-Anne BR, James PO *et al. Journal of Medical Microbiology,* 2011, **60**:968-976.