

FORMULATION AND IN VITRO EVALUATION OF NIOSOMAL POVIDONE –IODINE CARRIERS AGAINST *Candida albicans*

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ABSTRACT

Objective: Use of povidone-iodine (PVP-I) as a microbicidal agent is complicated by contact burns and other adverse reactions attributed to its sudden concentrated release from absorbent skin bandages. The current investigation examines the potential of niosomes or non-ionic surfactant vesicles to aid in the slow sustained release of the topical antiseptic in order to overcome this major drawback.

Method: *Candida albicans*, a fungus commonly associated with cutaneous infections was selected as the test organism since PVP-I has broad spectrum properties and been reported to be an effective antimycotic agent. After evaluating the antimicrobial effect of unencapsulated PVP-I on *Candida* cells, niosomes were developed by the thin film hydration technique, with a stoichiometric proportion of antiseptic: cholesterol: sorbitane monooleate of 1:1:2. The formulated vesicles were characterized for their particle size, zeta potential and drug entrapment efficiency. Further, in vitro agar cup tests and studies on the sustained release profile of the entrapped medicament were performed.

Results: The minimum inhibitory concentration of PVP-I for *Candida* was found to be 0.45% w/v available iodine and complete killing of cells was achieved at this concentration within 180 seconds. The average size of loaded vesicles was recorded to be 338.4 nm, with a drug entrapment efficiency of 80%. Subsequent to its encapsulation, PVP-I showed good permeability, retention of the fungicidal activity and the slow sustained effect lasted for almost 35 hours.

Conclusion: This is the first report of a study that has developed and characterized the in vitro efficacy and sustained drug delivery of PVP-I using niosomal carriers. The results demonstrate that niosomes can act as microreservoirs prolonging the release of PVP-I. They could thus help to prevent contact burns associated with the immediate release of the antiseptic when used for topical application.

Keywords: Povidone-iodine, *Candida albicans*, Niosomes, Encapsulation, Controlled release.

INTRODUCTION

Use of iodine as an antiseptic has long gone into disrepute as a result of its short lasting action and irritant properties. It has been replaced by iodophores such as povidone iodine, which has been advocated as a non-irritant, non-toxic compound for surgical scrubbing and disinfection of endoscopes [1]. However, it has now become increasingly evident that even though povidone iodine is classified as a non-irritant antiseptic, it is not completely devoid of corrosive action. It has been noted that the immediate release of PVP-I from absorbent bandages on application to wounds can result in contact burns [2]. Cases have been reported of skin irritation, delayed wound healing as well as blistering and patchy necrosis of the skin resembling chemical burns, in some exceptionally severe cases [3]. Hence a delivery system allowing slow release of this antiseptic needs to be explored.

Previously researchers have experimented with the encapsulation of PVP-I within liposomes in order to improve its efficacy, tolerability and bioavailability [4]. However, due to several problems such as expense, requirement for special storage and handling conditions, variability in the purity of natural phospholipids and instability of liposomes, commercial liposomal preparations of PVP-I have currently not yet been made available.

Niosomes or non ionic surfactant vesicles, though structurally similar to liposomes offer several advantages over them. They are composed of a bilayer of non-ionic surface active agent stabilized using cholesterol. They are osmotically active, stable, biodegradable, biocompatible, and non-immunogenic. In addition they can act as a depot, offering slow and controlled release of the entrapped drug. The structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as amphiphilic drug moieties and recently they have been explored for the delivery of a variety of compounds and drugs like aceclofenac[5], erythromycin[6] and nystatin[7].

Povidone-iodine is known for its antimycotic properties against *Candida albicans*, the causative agent of cutaneous candidiasis[8] and wound infections [9]. In addition, PVP-I also exhibits anti-

adherence property [10] and is used in gynaecology for vaginitis associated with *Candida* and trichomonas. Povidone-iodine which has been proposed for a long time as a topical agent for skin and mucosa disinfection is still chosen as a control in comparative studies concerning new antifungal topical formulations[11, 12].

The current investigation was designed to formulate PVP-I encapsulating niosomes which would allow slow permeation of the disinfectant while retaining its fungicidal activity. Such niosomal carriers could as serve as microreservoirs aiding in the controlled release of povidone –iodine and help reduce the irritant profile of this compound in the future.

MATERIALS AND METHODS

Candida albicans culture obtained from a local hospital was grown at 37° C in Saboraud's broth. Cells were harvested, washed in sterile phosphate buffer saline and standardized to an O.D. of 0.6 at 530nm. (Ermalnc. 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.

Determination of minimum inhibitory concentration of povidone iodine against *C. albicans*

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 48 hour old *C. albicans* culture was added to all the tubes. Positive and negative controls were maintained. Tubes were incubated at 37°C for 48 hour and the lowest concentration that did not show growth corresponded to the minimum inhibitory concentration (MIC).

Time kill experiment using unencapsulated disinfectant

Tube containing 0.45 % w/v povidone iodine (0.45 % w/v available iodine – the determined MIC value) was inoculated with *C. albicans*. Aliquots was removed at various time intervals and streaked on

Saboraud's agar. The time required for complete killing of the cells, indicated by the absence of growth, was noted.

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.

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 was also done using the same nanoparticle analyzer.

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.

$$\text{Efficiency of entrapment} = \frac{\text{Concentration of drug entrapped} \times 100}{\text{Initial concentration of drug}}$$

Agar cup diffusion assay

In vitro anticandidal activity of the unencapsulated PVP-I and the niosomal preparation of the antiseptic, was demonstrated by the agar cup-plate method [13]. Wells containing unloaded niosomes served as controls. Sizes of zones of inhibition were measured after incubation.

Evaluation of sustained antimicrobial activity of encapsulated povidone iodine

Niosomal vesicles containing PVP-I were incubated with the test culture in Saboraud's broth. Aliquots were withdrawn at various intervals of time and serially diluted 10-fold. Surface spread technique was performed using Saboraud's agar. The number of colony-forming units was then calculated from appropriate dilutions.

All microbiological experiments were done in triplicate and repeated three times. Mean values have been reported.

RESULTS AND DISCUSSION

The corrosive nature of iodine has resulted in its use as an antiseptic to be replaced by substances known as iodophores that contain an iodine molecule linked to a large molecular-weight organic compound. Povidone iodine is one such iodophore (Figure 1) that has poor irritant

properties despite preserved antiseptic efficacy. This stable chemical complex of polyvinylpyrrolidone and elemental iodine has been reported to be an effective disinfectant, active not only against bacteria and spores but also against fungi and viruses [14].

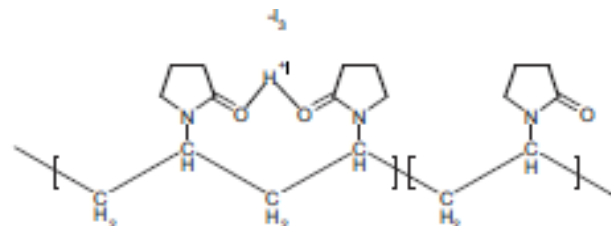


Fig. 1: It shows the chemical structure of 2-Pyrrolidone, 1-ethenylhomopolymer compound with iodine

Release of free iodine from the iodophore contributes to its biocidal activity. The liberated iodine is believed to penetrate the outer wall of microbes, and kill the cells by disrupting protein structure and nucleic acid synthesis [15].

On application, PVP-I forms a film that protects open wounds and is used topically in both human and veterinary medicine. Albeit uncommon occurrence, irritant contact dermatitis can be an unfortunate adverse reaction complicating its use as an antiseptic. Researchers have reported cases of chemical burns induced by povidone iodine in patients undergoing surgeries and arteriographies [16, 17, 18]. Despite this, no commercial product is currently available to aid in the sustained slow release of PVP-I from an absorbent material. Hence, in the current investigation niosome preparations encapsulating PVP-I were developed and studied.

Efficacy of the chemical antiseptic was determined in the absence of organic matter. The MIC of PVP-I by the broth micro dilution method for *C. albicans* was found to be 0.45% w/v available iodine. Time kill experiments demonstrated free povidone iodine to cause complete killing of cells within 180 seconds of coming in contact with them. A previous study by La Rocca et al, 1983 has similarly reported PVP-I to possess good antimycotic activity and achieve 100% killing of cells within a contact time of 10- 120 seconds [19]. 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 average zeta potential of the vesicles was recorded to be -5.6Mv which shows good stability in formulation. 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. Cationicity is undoubtedly important for the initial electrostatic attraction of antimicrobial particles to negatively charged membranes of microorganisms and a mutual electroaffinity likely confers selective antimicrobial targeting relative to host tissues. It has been proposed that a chemiosmotic potential may act in an electrophoretic manner to concentrate positively charged peptides on microbial surfaces [20]. *Candida albicans* have been reported to carry a surface charge of -12 to -20 mV. It is possible that as the fungal cells possessed a comparatively higher negative charge, it led to affinity with the drug loaded niosomes, and subsequent killing of the cells. 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 (Figure 2). 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. The impact of surfactant and cholesterol in the entrapment efficiency and release rate have been previously been shown to be significant [21]. A. Abdul Hasan Sathali et al, 2010 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 [22]. Similarly as the entrapment efficiency decreases (for the formulations with increased cholesterol ratio) less time is required for maximum drug release.

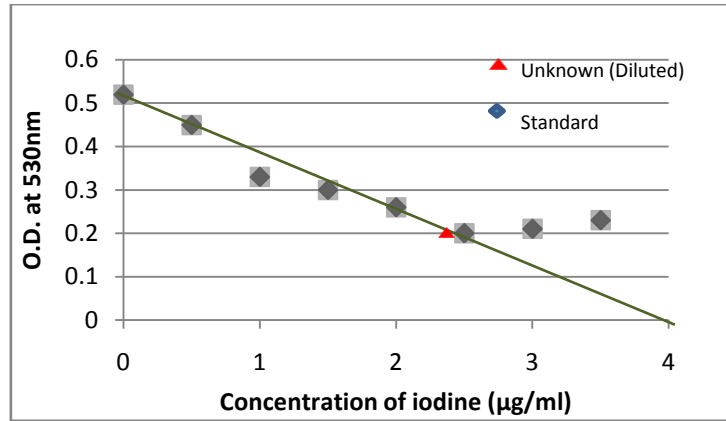


Fig 2: It shows the determination of PVP-I entrapment efficiency

The in vitro agar cup test performed to check for permeation and retention of fungicidal activity of PVP-I after encapsulation, demonstrated satisfactory permeability and antimicrobial efficacy (Table 1). As expected, the average zone sizes of inhibition around free PVP-I was significantly larger than those exhibited around the encapsulated medicament. This indicates that non-ionic surfactant vesicles were able to retain the PVP-I encapsulated within them, while allowing low concentrations to diffuse out slowly.

Table 1: It shows the comparative study of in vitro anticandidal activity of PVP-I before and after encapsulation

Well containing	Size of zone of inhibition (mm)
PVP-I without encapsulation	25
PVP-I encapsulated within niosome	14
Control (blank) niosome	No inhibition

Sustained release of the antiseptic was investigated by exposing the culture to entrapped PVP-I over a period of 48 hours. Viable count of cells was determined at every 5 h interval. Figure 3 indicates the pattern of decrease in the number of viable *Candida* cells with time.

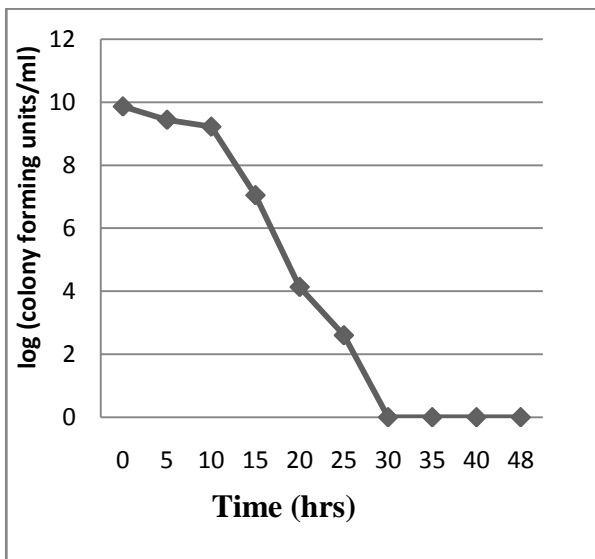


Fig 3: It shows the invitro study of sustained activity of encapsulated PVP-I

This graph is suggestive of the dynamics involved in the release of PVP-I from the encapsulating vesicles. The slow early decrease (up to 10 hrs) in the viable count of cells shows an initial retardation in the drug release from the vesicles. Cholesterol in the microcapsule composition has been reported to reduce the permeability of

niosomes, while improving the retention of the solute [23]. The faster decrease in the viable count observed thereafter, could be attributed to a gradual enhancement of the drug release rate due to the mechanical loosening and leakage of the vesicles. The prolonged antimicrobial effect of povidone iodine lasted for almost 30 hours. After this, there was complete disruption of all niosomes, leading to emptying of their contents, thereby killing all remaining fungal cells. These results thus indicate that non-ionic surfactant vesicles can act as microreservoirs prolonging the release of the active ingredient PVP-I.

CONCLUSION

The combination of povidone-iodine and niosomes unites the exceptional microbicidal activity of this antiseptic with the slow sustained release provided by niosomes. Niosomes could play an important role for improving the delivery of PVP-I and in preventing adverse reactions associated with the sudden immediate release of this medicament.

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