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Preparation and Evaluation of Povidone Iodine based Microsponge for Wound Healing Activity in Rats

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Abstract

Microsponges are polymeric delivery systems composed of the porous microparticle. They remain small sponge-like spherical particles with a great porous surface. Moreover, they may improve stability, inferior side effects and modify drug release beneficially. Povidone-iodine is a complex of the potent bactericidal agent iodine and the carrier molecule povidone. This study aimed to prepare and evaluate ethyl cellulose microsponges containing povidone-iodine in the various ratio of polymer and a fixed ratio of drug, solvent and combination of surfactants by quasi- emulsion solvent diffusion method. The microsponges were characterized by % of available iodine content, % production yield, % entrapment efficacy, average particle diameter, angle of repose, FTIR, DSC and SEM studies followed by wound healing activity.

Keywords: Povidone Iodine, Microsponges, Sodium Cholate, Wound Healing Activity, Probe Sonicator

INTRODUCTION

Povidone-Iodine was recommended to the pharmaceutical market as an antiseptic agent and is as active as iodine and its effectiveness has been clinically recognized for all sorts of topical applications in human medicine. Topical application of Povidone-Iodine (PVP-I) useful and effective in the treatment of burns and infected skin ulcers were control of bacterial growth or upholding of low bacterial count along with protection of developing epithelium is essential [1].

PVP-I can be used on mucous membranes without the risk of burns and it is not only antiseptic but also appears to augment wound healing [2]. It has been revealed to be effective, rapid-acting and a safe wound healing disinfectant [3]. The problems occurring due to growing resistance against other antibiotics and antiseptics and the presence of cross-resistance between them should turn into the use of Povidone-Iodine.

Microsponge is porous spherical microparticles having a particle size range of 5-300 µm with a capability to between wide range of active ingredients and are used as a carrier for topical drug delivery. These microparticles act like microscopic sponges, storing the active drug until its release is triggered by application to the skin surface. The release of the drug into the skin can be initiated by various triggers like rubbing, concentration gradient, higher skin temperature, application of pressure, etc. Conventional formulations of topical drugs are intended to work on the outer layers of the skin. Typically, such products release their active ingredients upon application, producing a highly concentrated layer of active ingredient that is rapidly absorbed. The Microsponge system can prevent excessive accumulation of ingredients within the epidermis and the dermis. Thus, it can significantly reduce the irritation of effective drugs without reducing their efficacy. Further, these porous microspheres with active ingredients can be incorporated into formulations such as gels, creams, lotions, and powders [4]

Recent formulations offering a great improvement in wound healing. Thus, current delivery (microsponge) might enhance the wound healing process. They often provide a less irritation and delay release of drug substance compared to powder of PVP-I.

Keeping the above facts in mind it was thought worthwhile to develop topical dosage forms, namely microsponges powder of Povidone-Iodine and compare its characteristics with available marketed preparation. This study aims to formulate microsponges containing povidone-iodine, and then evaluate the % of available iodine content, % production yield, % entrapment efficacy, average particle diameter, angle of repose, FTIR, DSC and SEM studies followed by wound healing activity compared with the market product (powder) and povidone-iodine microsponges.

MATERIAL

The ingredients required for the present work were procured from various supplies. Povidone-iodine was received as a gift sample from a pharmaceutical company, Malaysia and ethyl cellulose and tween 80 were purchased from RM marketing, Essex, UK. Sodium cholate procured from Bio Basic Canada Inc, Canada and all the other ingredients used were of analytical grade and were used as procured.

METHODS

Preparation of povidone-iodine Microsponges

Povidone-iodine microsponges were prepared by a quasi-emulsion solvent diffusion method [5-7] as shown in table 1 and figure 1.

Table 1: Composition of povidone-iodine microsponges

Formulation Code	PVP iodine (mg)	Ethyl cellulose (mg)	Dichloromethane (ml)	Surfactant ratio (1:3) Sodium cholate : Tween 80
RI	500	100	10	0.15 : 0.45
R2	500	150	10	0.15 : 0.45
R3	500	200	10	0.15 : 0.45
R4	500	300	10	0.15 : 0.45

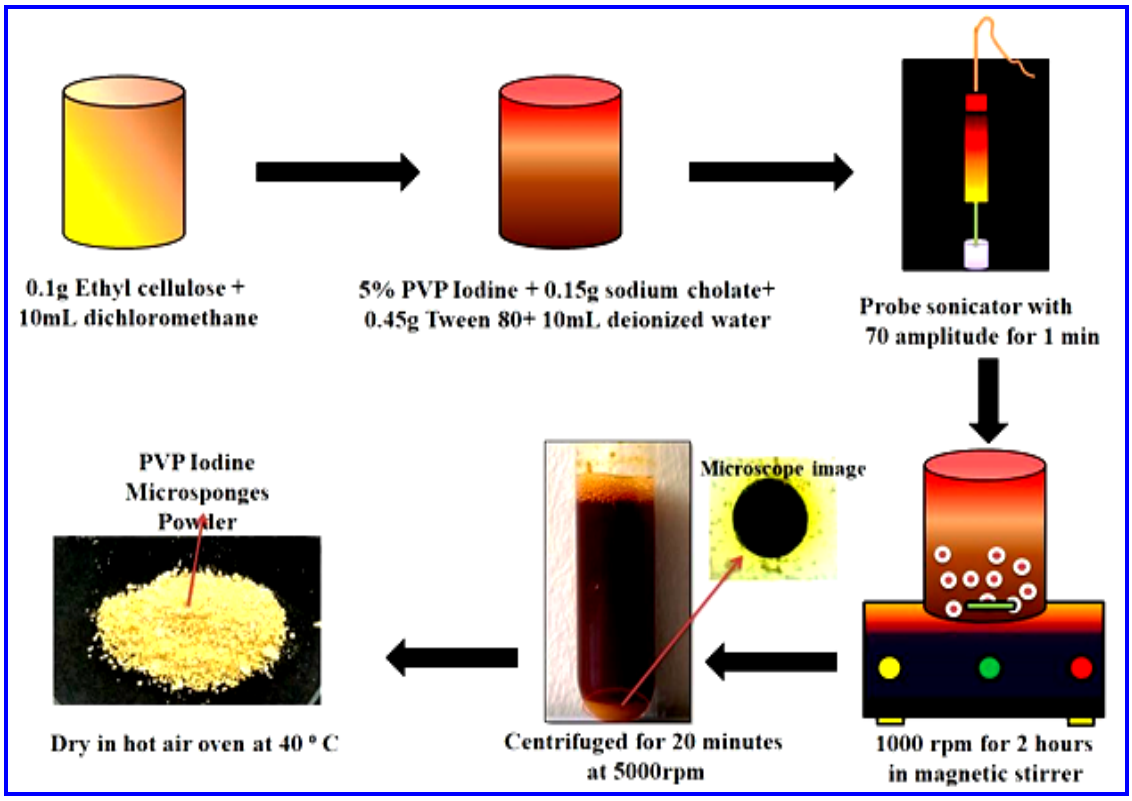

Figure 1: Preparation of povidone-iodine microsponges

IN-VITRO EVALUATION

Particle size analysis [8]

Particle size analysis of prepared microsponges was carried out using optical microscopy (Microscope with Olympus 70 G Camera scope 9-DN-117M).

Available iodine

Transfer 1.0 gm of povidone-iodine microsponges into a round bottom stoppered iodine flask containing 150 ml of water and stir for 1 hour. Add 0.1 ml of diluted acetic acid and titrate against 0.01M sodium thiosulphate using starch solution as an indicator towards the end [9].

1ml of 0.1M sodium thiosulphate is equivalent to 12.69 mg of available iodine.

$$\frac{\text{Titre volume} \times \text{Molarity factor of sodium thiosulphate} \times \text{equivalent factor}}{\text{Weight taken in (gm) sample}} \times 100 = \%\ \text{gm of available iodine}$$

Production yield

The production yield of microsponges was determined by formula mentioned as follows 10]

$$\frac{\textit{Practicle mass of microsponge}}{\textit{Production yield}} = \textit{Theoretical mass}\ (\textit{Polymer} + \textit{Drug}) \times 100$$

Entrapment efficiency

The percentage of entrapment efficiency of the prepared microsponges was determined by measuring the concentration of free iodine in the dispersion medium. The amount of free iodine was detected in the filtrate and the amount of incorporated iodine was determined as a result of the initial iodine minus the free iodine by titration method.

Angle of repose [11]

The angle of repose is a property associated with inter-particulate friction, density, surface area and shape of the particles. The angle of repose was calculated by the fixed height cone method. Accurately-weighed povidone-iodine microsponges powder (10 g) were taken in a funnel and formulations were allowed to fall on a plain sheet of paper. The distance between the tip of the funnel and the peak of

the conical heap was fixed at 3 mm. The angle of repose was determined by measuring the height of the cone of powder and the radius of the base. All measurements were done in triplicate and mean and the standard deviation was calculated for each povidone-iodine microsponge formulation.

Infrared spectroscopy

Using FT-IR spectrophotometer (FT-IR, Shimadzu 8400 S, Japan) implying KBr pellet method, spectra of povidone-iodine, ethylcellulose and physical mixtures of povidone-iodine and ethyl cellulose were recorded in the wavelength range of 4000– 400 cm^{-1}.

Differential scanning calorimetry

The samples were characterized by physical appearance and chemical interaction by DSC and FTIR. Thermal analysis was performed using NETZSCH STA 449F3 thermal analyzer. The samples were scanned between 30°C to 350°C under nitrogen at the heating rate of 20°C.

Scanning electron microscopy (SEM)

For assessing the morphology of prepared povidone-iodine microsponges were examined under a scanning electron microscope (Phenom ProX) operating at 5 kV.

EXPERIMENTAL PROCEDURE

For the experiment, the animals received general anesthesia by inhalation of diethyl ether. Then the hair on the back of the dorsal region was removed by shaving. The excision wound of a diameter of 4 cm full-thickness piece of skin was removed for creating a cutaneous wound in the dorsum back of the rats. The wound was left untreated with any wound healing or antimicrobial agents and undressed to the open environment for the first 2 days post- wounding. This model was used to monitor the rate of wound closure. The animals were randomly divided into 3 groups; control group, reference group povidone-iodine powder (5% w/v), and treatment group povidone-iodine microsponges powder (5% w/v). Each rat in each group was housed separately and provided with free access to a standard laboratory diet and water following the completion of the surgical procedure. Daily monitoring of animals and the wound were done.

Experimental design

Each group was consists of 5 Wistar rats.

Group A: control group animals were applied topical iodine-povidone powder once daily for 20 days.

Group B: reference group animals were left to spontaneous healing.

Group C: treatment group animals povidone-iodine microsponges (5 % w/v) powder were applied once daily for 20 days.

Measurement of healing

The progressive changes in wound closure area were monitored planimetrically in mm^2 by tracing the wound boundaries on a clean transparent polyethylene paper on every alternate day without producing any damage to the injured site. The area of the wound was calculated by counting the number of boxes on a graph paper and expressed as the percentage (%) reduction of original wound size. The epithelization period of the wound was expressed as the number of days taken for complete epithelization. The calculations of the percentage reduction of the original wound size were calculated.

Statistical analysis

All the results were expressed as mean ± S.D. for five animals in each group. Statistical analysis was made using a one-way analysis of variance (ANOVA) followed by Dunnett's t-test for posthoc analysis. *P* - values < 0.05 were considered to be statistically significant.

RESULT AND DISCUSSION

Physical appearance

Povidone-iodine microsponge particles with fairly golden yellow colour were obtained by quasi-emulsion solvent diffusion method; with good particle size, shape and flow property as shown in figure 2 A, B and figure 3.

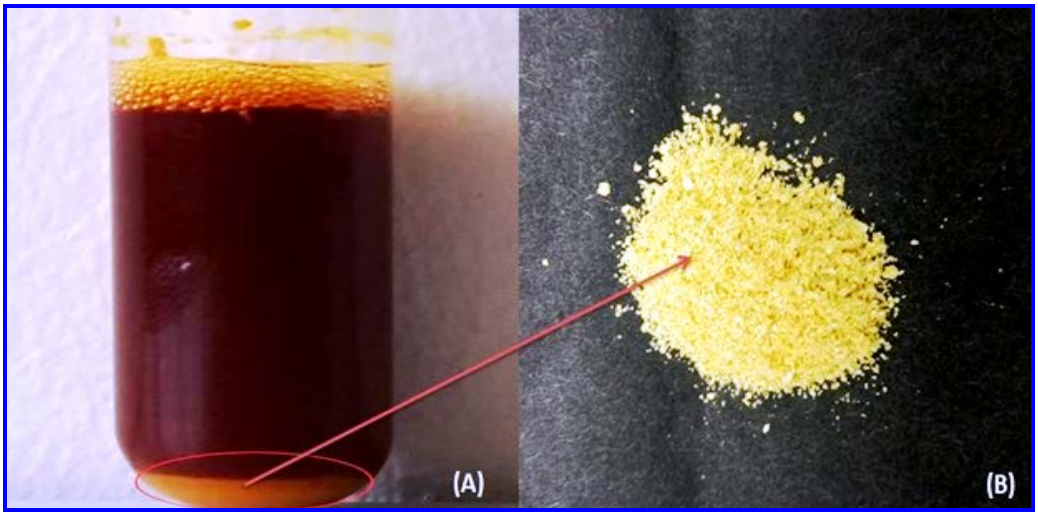

Figure 2: (A) Appearance of microponges containing solution. (B) Appearance of microponges powder

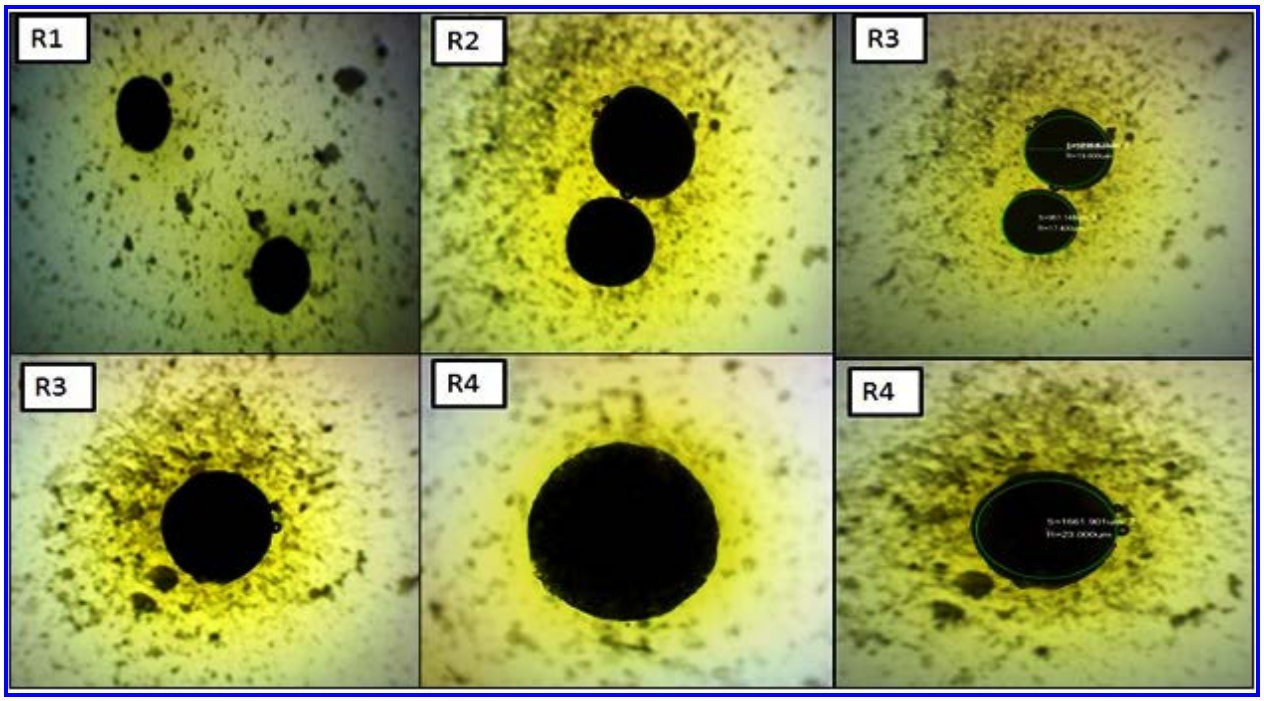

Figure 3: Optical microscopy view of various batches of povidone iodine microsponges (40X)

Table 2: Available iodine content, production yield, entrapment efficacy, average particle diameter and angle of repose

Formulation Code	Available Iodine Content (%)	Production Yield (%)	Entrapment Efficacy (%)	Average Particle Diameter (µm)	Angle of Repose (Degrees)
RI	58.4 ± 2.45	78.4 ± 1.01	55.2 ± 1.2	6.60 ± 0.09	10.95 ± 0.39
R2	59.7 ± 1.91	82.6 ± 2.83	57.6 ± 2.2	8.40 ± 1.26	12.87 ± 0.42
R3	62.4 ± 2.65	85.2 ± 2.57	59.8 ± 1.9	17.60 ± 0.86	17.87 ± 0.21
R4	65.3 ± 1.73	87.8 ± 1.69	61.5 ± 2.4	23.80 ± 1.15	25.93 ± 0.51

Particle size analysis

Particle size analysis of povidone-iodine microsponges was found to be in the range of 6.60 – 23.80 µm as shown in table 2 and figure 4. The visual inspection of all formulation using the optical microscope for particle size revealed that the average particle diameter has increased with an increase in the concentration of ethyl cellulose. It was because polymer available at a higher concentration was in more amount thereby increasing the viscosity of external phase and droplet size which leads to the greater size of povidone-iodine microsponges.

Scanning electron microscopy

Formulated povidone-iodine microsponges were exposed to scanning electron microscopy (SEM) analysis for measuring their morphology. The SEM images of povidone-iodine microsponges are shown in Figure 5a. SEM photomicrographs reflected that microsponges formed were extremely porous, mostly spherical. By the diffusion of solvent (DCM) from the surface of microsponges, pores were generated and the pore size was in the range of 1.18µm to 5.58 µm Figure 5b. Furthermore, it was showing that the typical internal structure contained spherical cavity enfolding a stiff shell constructed of drug and polymer.

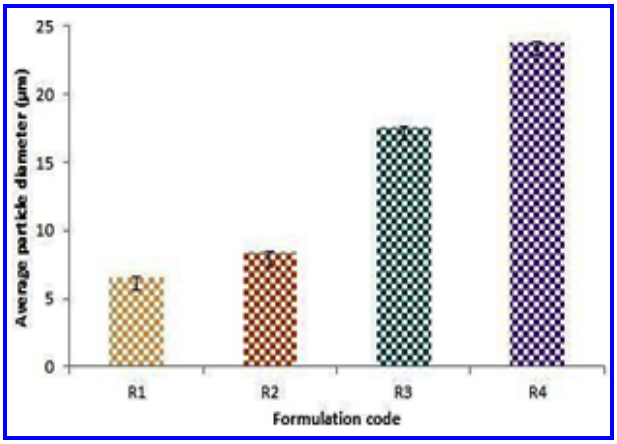

Figure 4: shows average particle diameter of povidone-iodine microsponges

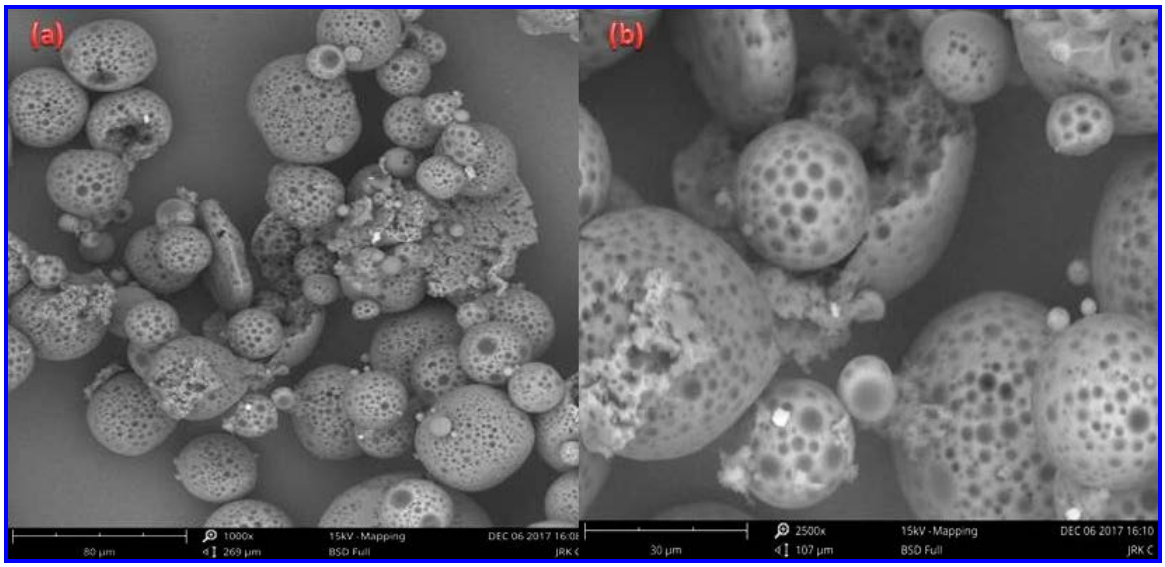

Figure 5: Scanning electron microscopy image of povidone-iodine microsponges

Available iodine

Available iodine content results reflected that a higher concentration of ethyl cellulose led to increasing iodine loadings. Available iodine content ranges of all the formulation were 58.4% to 65.3% as shown in figure 6. Due to the hydrophilic nature of PVP-I in external phase shows more affinity towards the aqueous medium of surfactant mixture during the formulation shows moderate loading capacity.

Production yield

The production yield of all formulation of microsponges ranged from 78.4% to 87.8% as shown in table 2. Polymer concentrations were found to affect the production yield slightly. In the case of ethylcellulose 100 mg (R1), production yield was low, i.e. 78.4% while for ethylcellulose 300 mg (R4), it was 87.8% as shown in figure 7.

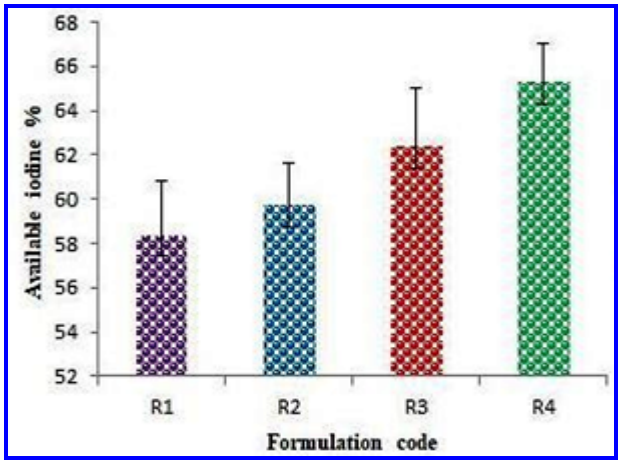

Figure 6: Shows % of Available iodine of povidone-iodine microsponges

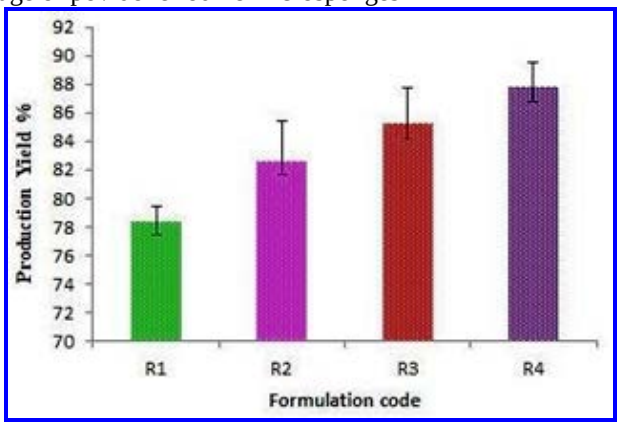

Figure 7: Shows % of Production yield of povidone-iodine microsponges

Entrapment efficiency

A high concentration of polymer caused an increase in dispersed phase viscosity. On the diffusion of solvents from the inner phase, almost all of the dispersed phase was converted to solid microsponges and separated particles appeared. The highest entrapment efficiencies of these formulations could be attributed to the availability of maximum polymer amount to each PVP-I unit in contrast to the rest of the formulations. The entrapment efficiencies were moderate in the range of 55.2–61.5% as given in Table 2 and figure 8.

The angle of repose

The angle of repose obtained also indicated that formulations R1, R2, R3, and R4 had good flow since their angle of repose fell within the range: 10 – 25 while formulations R1, R2, R3 had an excellent flow (≥25º) due to lesser in particle size and formulation R4 had a good flow (≤ 25º) due to slightly higher in particle size (Figure 9a).

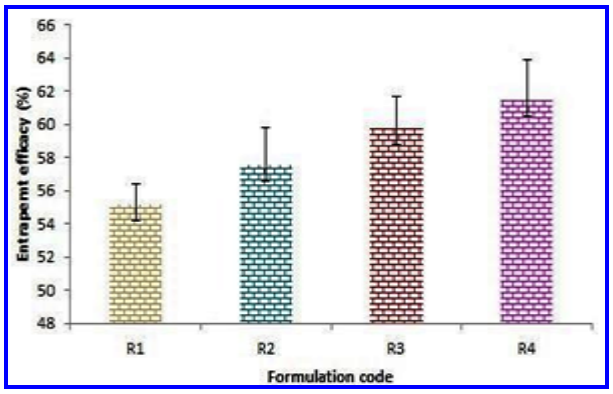

Figure 8: Shows % entrapment efficiencies of povidone-iodine microsponges

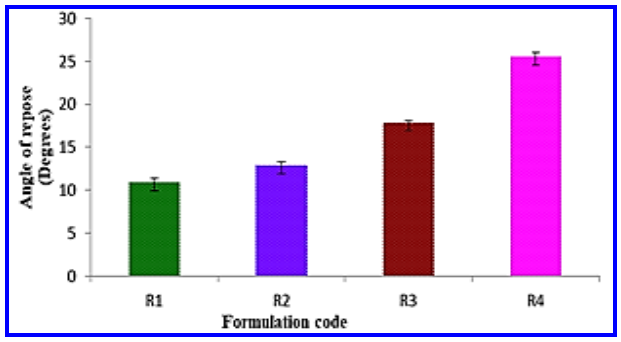

Figure 9a: Shows angle of repose of povidone-iodine microsponges powder

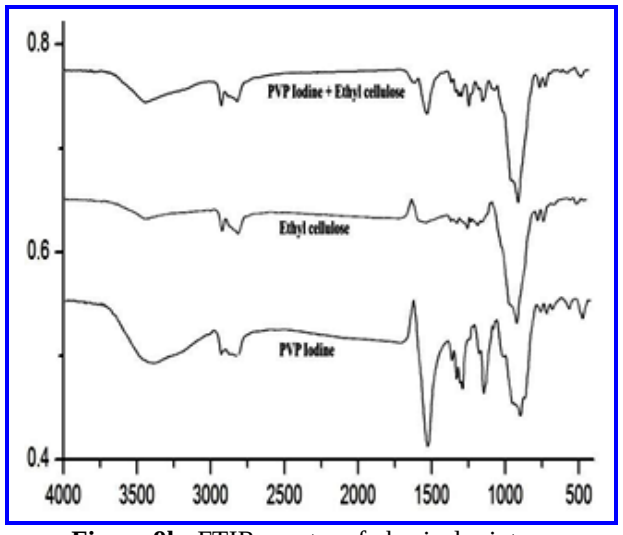

Figure 9b: FTIR spectra of physical mixture

FTIR spectra

Povidone-iodine and ethyl cellulose combination shows no disturbance in the functional group, Therefore a polymerized active constituent has no change of effect after formulation (Figure 9b).

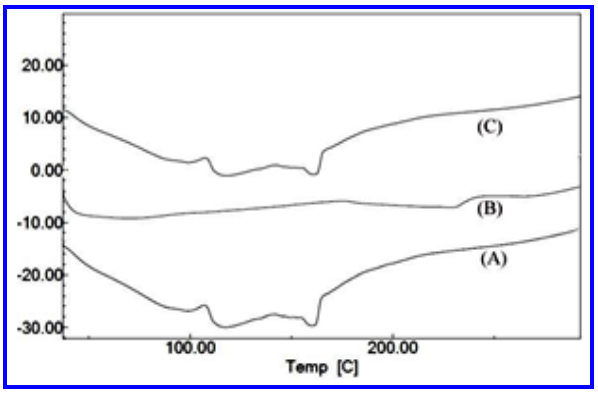

Figure 9c: DSC spectra of physical mixture

DSC spectra

DSC studies of povidone-iodine showed a long and sharp characteristic endothermic peak at 160.90 ˚C. The DSC thermograms of physical mixtures of povidone-iodine and ethyl cellulose showed peaks at 160.90 °C. These findings indicate that there was no interaction occurs between povidone-iodine and ethyl cellulose combination (Figure 9c). Therefore, povidone-iodine and ethylcellulose can be used as excipients in the formulation of microsponges as shown in Figure 1.

Table 3: Percentage of wound closure rate of different groups on excision model in rats

Post-wound healing days	Percentage of wound closure rate (%)		
	Control group	Reference group povidone-iodine powder (5% w/v)	Treatment group povidone iodine microsponges powder (5% w/v)
2	6.83	8.64	9.35
4	18.03	35.07	29.92
6	32.57	46.78	51.45
8	57.89	66.53	58.578
10	72.43	78.84	73.67
12	77.95	85.32	80.33
14	82.21	86.52	83.31
16	70.53	88.82	89.52
18	86.17	90.86	90.58
20	87.62	92.46	93.53

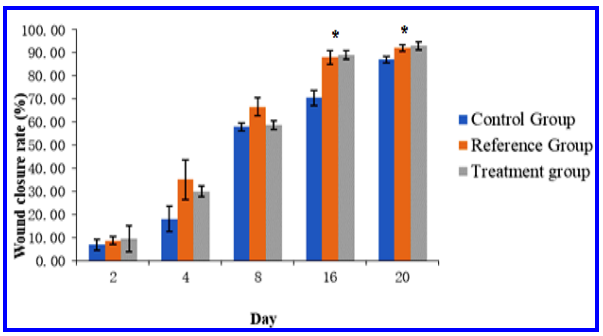

Figure 10a: Percentage wound closure in the control, reference and treatment groups at different time intervals. Results are presented as mean ±SD (n=5). * indicates a significant difference at $p < 0.05$ compared with the control group.

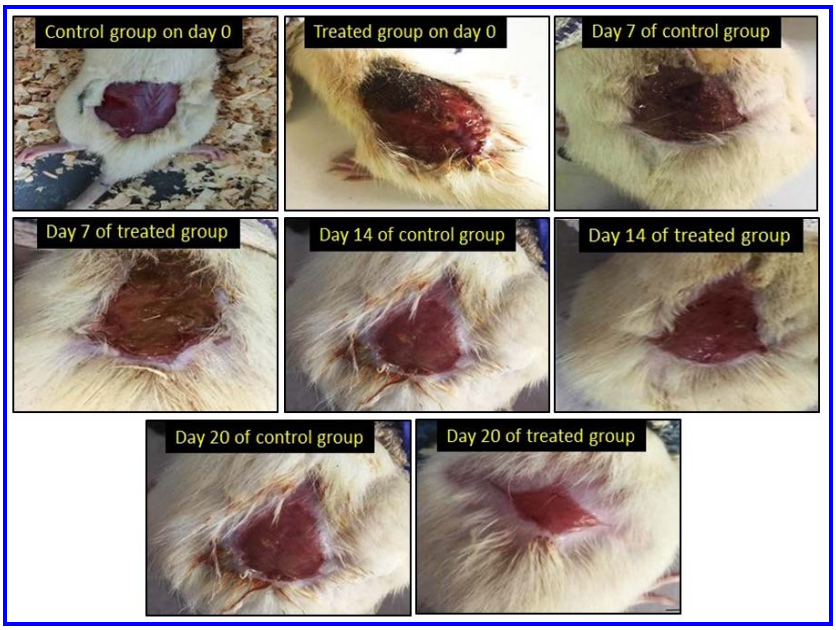

Figure 10b: Wound healing activity of marketed povidone-iodine powder and povidone-iodine microsponges.

Wound healing activity

The application of marketed povidone-iodine powder and povidone-iodine microsponges on excision wound model in rats compared to control group and day 0 data self-control group. The significant effect was observed on day 20. The percentage wound closure rate was indicated in table 3 and figure 10 a. The photograph excision wound of rat was illustrated in figure 10b.

CONCLUSION

PVP iodine-based microsponge system was developed effectively using a combination of surfactants like sodium cholate and tween 80 for highly effective topical delivery up to an extended period to reduce application frequency, hypersensitive reactions allied to the conventional marketed formulation. The executed technique was found to be simple, reproducible and rapid; which led to the formation of highly porous, spherical microsponges with excellent flow property. Varied polymer concentration reflected a remarkable effect on Available iodine content, production yield, entrapment efficacy, average particle diameter and angle of repose. The PVP iodine-based microsponge topical delivery system developed and investigated in the present research approach was seems to be promising with respect to the eradication of wound healing.

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