Advances in Bioresearch
Adv. Biores., Vol 11 (5) September 2020: 47-55

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.11.5.4755

Advances in Bioresearch

ORIGINAL ARTICLE

Formulation and Evaluation for Samudraphen Transdermal Drug Delivery System

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ABSTRACT
Transdermal drug delivery is the externally applicable medicine available in the form of patch. It can release dose of drug in systemic circulation topically, it facilitates a balanced blood level profile, resulting to reduced systemic side effects. In the present investigation we have prepared transdermal patch drug delivery system for wound healing activity by using Samudraphen Drug (Cuttle fish bone) as an active drug. The patch is prepared by using different ingredient like Carbopol 940, Polyvinyl alcohol, Ethanol and Methyl paraben. The evaluation of prepared patch is carried out by using Film thickness, Folding endurance, Weight variation and Percentage moisture content. The Full Factorial Design study also carried out.
Key words: *TTDS, Samudraphen, Characterization, Animal study of patch*

Received 11.04.2020 Revised 20.05.2020 Accepted 27.07.2020

How to cite this article:
Krishna S. Yadav, Ajit S. Kulkarni. Formulation and Evaluation for Samudraphen Transdermal Drug Delivery System.. Adv. Biores., Vol 11 (5) September 2020: 47-55

INTRODUCTION

During previous few years, there was an increasing the importance in the improvement of newer drug delivery system (DDS). These developed new DDS are beneficial in term of improved patient compliance, overall therapeutic effect with significant level. When these systems are designed to overcome the problems with conventional methods of drug delivery i.e. the drugs which can degrade in GI tract or degradation before reaching to the area of action could be effectively delivered with pulsatile as gastro resistant drug delivery. One of the novel drug deliveries is transdermal drug delivery system. [3,7]

Transdermal drug delivery suggests controlled release of drug dose to the patient, it facilitates a balanced blood level profile, resulting to reduced systemic side effects and, sometimes, improved efficacy over other dosage forms. The topical product or DDS has been used for eras for the cure of local skin disorders. The use of the skin as a route for systemic drug release of many drugs has been proved.[8,12]

By transdermal route the administration of drugs offers the advantages of being comparatively painless. Delivery of drug through the skin for systemic effect, called transdermal delivery was first used in 1981, when Ciba-Geigy marketed transdermV (present day marketed as transderm scope) to prevent the nausea and vomiting relations with motion sickness.[13,20]

In the present investigation we have prepared transdermal patch drug delivery system for wound healing activity by using Samudraphen Drug (Cuttle fish bone) as an active drug. The patch is prepared by using different ingredient like Carbopol 940, Polyvinyl alcohol, Ethanol and Methyl paraben. The evaluation of prepared patch is carried out by using Film thickness, Folding endurance, Weight variation and Percentage moisture content. The Full Factorial Design study also carried out. [14]

1) To explore Samudraphen drug for its therapeutic activity in form of transdermal drug delivery system.
2) To formulate Samudraphen transdermal drug delivery system.
3) To evaluate prepared Samudraphen containing transdermal drug delivery system.

MATERIAL AND METHODS

Materials

The materials required for the present work were procured from diverse sources. The material required are Samudraphen drug, Carbopol 940, Polyvinyl alcohol, Ethanol and Methyl paraben and purified water.[21]

Method:

Procedure for preparation of TDDS PATCH is as follows:

Polyvinyl alcohol was dissolved in distilled water and prepared polymeric aqueous solution using continued stirring with magnetic stirrer at 400rpm for 1 H. Carbopol 940 was dissolved in ethanol in second beaker. These two beakers were kept aside for removal of the air bubbles. Both phases were mixed with each other with continuous stirring. Drug solution was prepared by using solvent like 95% ethanol. Then these solutions were added in above polymeric solution as per sequence with continue stirring then finally added methyl paraben. After adding all components mixture was stirred for 2hrs. This solution was poured in petriplates and air dried for an hour. We have prepared nine formulations i.e. F1 to F9. [4,9]

Formulation table for Samudraphen patch:

Ingredient	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Samudraphen (gm)	2	2	2	2	2	2	2	2	2
Polyvinyl alcohol(gm)	2	2.2	2.4	2	2.2	2.4	2	2.2	2.4
Carbopol 940 (gm)	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3
Ethanol (ml)	15	15	15	15	15	15	15	15	15
Water (ml)	80	80	80	80	80	80	80	80	80

Characterization of Samudraphen transdermal patch:[9,14,15]

Film thickness: Thickness of film was calculated at five different randomly selected spots using micrometer screw gauge. Thickness of film is directly related to the accuracy of dose in the film.

Weight variation: Weight uniformity has done by randomly selected patches about 10 in number. A specified area of patch had cut in 3'3''different parts and weighted on digital balance. Average weight of transdermal patches was calculated and its standard deviation.

Folding endurance: Folding endurance of patches has done by repeatedly folding a small strip of film (2x2cm) at the same place till it breakdowns. The number of time the film possibly will be folded at the similar place with no breaking is the folding endurance value.

Percentage moisture content: Test was performed by taking individually weight of prepared films and will be kept in desiccators containing CaCl2 at room temperature for 24 hr. weight of film was taken after a stated interval until they show a constant weight. The percent moisture content was measured using following formula.

$$\textbf{Percentage moisture content} = \frac{\text{FINAL WIGHT} - \text{INITIAL WIGHT}}{\text{FINAL WIGHT}} \times 100$$

***In-vivo* wound healing activity in animal model**:[10,23]

The *In-vivo* wound healing activity is carried out on wistar rats for 15 days.

Experimental animals: Wister rats (150-180g) were selected for present study. The animals as maintained at weal-ventilated temperature controlled animal chamber for 7 day earlier to the experimental period.

Procedure: The animals were divided into 3 groups of four rats each as follow:

Group-I (control) were treated with simple ointment base.

Group-II rats were treated reference standard Dicloplast patch.

Group- III rats be treated with transdermal patch containing Samudraphen (test formulation) on the dayof experiment the all rats were anesthetized. A full thickness of the removed wound with circular area of 2×2 mm^2 (width 1.5 cm and depth 0.2 cm) was made on the shaved back (dorsal thoracic region) of the rats. The wounding day was considered as day 0. The wound were cured with topical claim. The wound contraction was calculated by a tracing paper on the wounded margin and calculated as percentage decline in wound area. The wound were observed and the area of wound size was measured on 3, 6, 9, 12, 15 and 18th of post wounding day. The percentage of wound cessation was calculated using the following equation: % wound closure = Wound Area on Day '0' – Wound Area on Day 'n' ×100 Wound Area on Day '0' Where, n = number of days.

RESULTS AND DISCUSSION

Collection of material:


Fig. No.1 Samudraphen (Cuttlebone) Fig. No. 2 Samudraphen powder (Source – Google Images)

Authentication of material:

The authentication of Samudraphen was done from Mankarnika Aushadalya, Zoology department Pune.

Characterization of drug:

Identification:

Samudraphen (cuttlefish bone) is 1 to 3 inches in width and 5 to 10 inches in length. The skeleton is an oblong, elliptical or oval substance, of whitish colour. Samudraphen is flat, broad and oval in shape. The shell is entirely dead and composed of calcareous rather than horny matter.

Fig. No.3 Samudraphen

Microscopic view:

A part of transverse section of bulk part of the drug was viewed under binocular microscope and it was seen that crystalline form is like multisteriod structured on evenly distributed pillars.

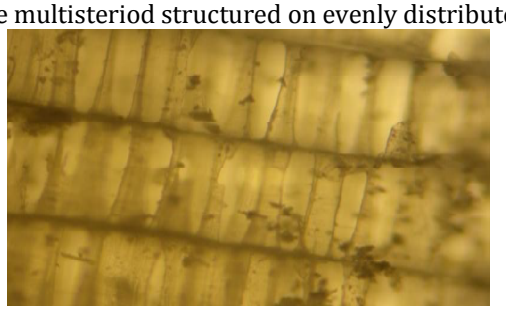


Fig. No.4 Microscopic view of T.S. of part of Cuttlebone

Loss on drying / Moisture content:

It was found that weight of the dried drug was 4.9gm. Thus loss of weight was calculated i.e.0.1gm. which represent 2.04%.Moisture content of drug had shown very less loss in drying 0.1 gm as the drugs available in market are already dried.

Total ash value:

Ash value was found to be 87.64% this value was high as compared to other plants and animals origin because, of its high mineral content.

Fig. No. 5 Crucible containing ash

Acid insoluble ash value:

In this process it was found that in a sample of 5 gm drug, average of total ash was 4.373 gm and acid insoluble ash was found to be 9.46% which means ash was very soluble in acids.

Fig. No.6 Crucible containing acid soluble ash

Water soluble ash value:

Total ash was 4.373 gm and total insoluble ash was found to be 4.359gm. Thus water soluble ash was 0.014gm which was 0.32% of drug taken.

Fig. No.7 Crucible containing water soluble ash

Evaluation parameters of transdermal drug delivery system. (Patch)

Physical Evaluation of patch:

The prepared patch formulations of Samudraphen was inspected visually for their colour, Texture and appearance. All prepared formulations was whitish in colour with smooth texture.

FILM THICKNESS:

Table 1: Film thickness results of transdermal patch

BATCH NO.	THICKNESS (µm)
F1	0.45± 0.05
F2	0.45±0.05
F3	0.42±0.04
F4	0.42±0.40
F5	0.45±0.04
F6	0.47± 0.04
F7	0.42±0.05
F8	0.45±0.05
F9	0.40±0.40

The film thickness ranged from 0.40 to 0.47um. Film thickness is a function of polymer concentration so, in the formulation of polymer concentration dependent film thickness was observed. The observation film thickness was sufficient to release the drug.

WEIGHT VARIATION:

Table 2: Weight variation results of transdermal patch

BATCH NO.	WEIGHT(mg)
F1	0.491±0.09
F2	0.533±0.01
F3	0.488±0.09
F4	0.544±0.01
F5	0.541±0.02
F6	0.537±0.01
F7	0.492±0.09
F8	0.541±0.02
F9	0.581±0.07

The weight of films varied from 0.488 to 0.581 mg. this variation was because of change in polymer concentration and other excipients.

FOLDING ENDURANCE:

Table 3: Folding Endurance results of transdermal patch

BATCH NO	FOLDING ENDURANCE
F1	304.5 ±6.42
F2	311.7±2.86
F3	332.2±8.95
F4	322.7±11.2
F5	282.2±6.17
F6	315.2±17.07
F7	286±26.97
F8	315.5±8.64
F9	276.5±40.95

Folding endurance is a function of polymer concentration i.e., carbopol 940 and polyvinyl alcohol. It ranged from 276.5 to 332.2. Less folding endurance was observed with high concentration of polyvinyl alcohol and carbopol 940. Highest folding endurance was observed with high polyvinyl alcohol concentration and lous carbopol 940 concentration. F1 to F3, keeping carbopol 940 concentration fixed lous level, (0.01mg) and chasing polyvinyl alcohol concentration from 2, 2.2 and 2.4mg respectively, it was observed that, there was increase in folding endurance from F1 to F3. So, it indicated that polyvinyl alcohol was having significant effect on folding endurance.

PERCENTAGE MOISTURE CONTENT:

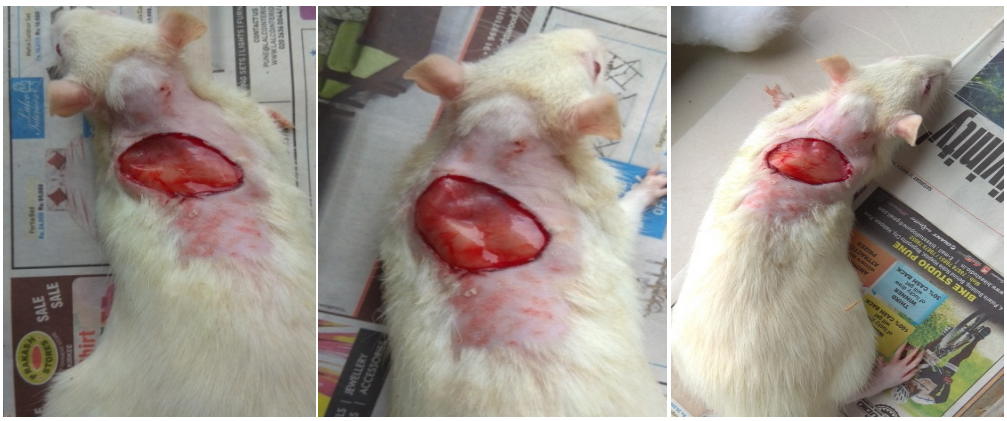
Table 4: Percentage moisture content results of transdermal patch

BATCH NO.	(%) MOISTURE CONTENT
F1	1.66±1.15
F2	1.69±0.170
F3	1.84±0.136
F4	1.69±0.025
F5	1.68±0.064
F6	1.73±0.010
F7	1.63±0.085
F8	1.60±0.083
F9	1.74±0.062

Percentage moisture content is a function of polymer concentration i.e., carbopol 940 and polyvinyl alcohol. It ranged from 1.74 to 1.84 %. Less percentage moisture content was observed with high concentration of carbopol 940 and polyvinyl alcohol. Highest percentage moisture content was observed with high carbopol 940 concentration and lous polyvinyl alcohol concentration. It was observed that there was an increase in percentage moisture content from F1 to F3. So, it indicated that carbopol 940 was having significant effect on percentage moisture content.

***In-vivo* wound healing activity in animal model**:**[2,10,23,19]**

Day '0':



Control **Standard** **Test (Formulation)**

Day '15':

Control **Standard** **Test (Formulation)**

Figure 8 Effect of transdermal Film on wound size at different day's interval

Table 5 Effect of transdermal patch on excision wound at different days interval.

Groups	Wound size area in mm²(mean± SEM)					
	0 day	3rd day	6th day	9th day	12th day	15th day
Control	7.0225±0.037 (0)	7.0025±0.036 (0.28%)	6.9025±0.014 (1.70%)	6.546±0.15 (6.78%)	6.235±0.10 (11.21%)	4.46±0.027 (40.76%)
Standard	7.0575±0.029 (0)	6.7225±0.078 5 (4.74%)	3.1425±0.057*** (55.47%)	2.275±0.010*** (67.76%)	1.337±0.13*** (81.04%)	0.847±0.06*** (87.99%)
Test	7.0475±0.012 (0)	6.32±0.079* (10.30%)	4.2875±0.146*** (39.16%)	3.412±0.05*** (51.58%)	1.7175±0.14*** (75.62%)	0.73±0.04*** (89.46%)

N=6, values are experiment as mean ± SEM. one way ANOVA followed by Dunnet t- test , t- value, denotes significance at a; P<0.05, b; P<0.01% as correlate with control.

1. The excision wounds created as per method described animals were shaved on back dorsal portion using alovera (veet) and were using anesthetized. An impression was made on shaved back dorsal region and zone of the wound to be produced was marked.
2. A full thickness excision wound with a spherical area of 2mm².was produced along the marking using toothed forceps, a surgical scissors.
3. The formulation test drug samudraphen patch (2gm) and standard drug dicloplast patch were applied once from the same day (0day) after3 day of the operation, until the complete healing.
4. In this model, wound contraction and epithelization period were evaluated wound contraction was measured as percent contraction each 3 day after wound creation.
5. The entire test patches used in excision wound model revealed significant wound healing effect from 3rd day to 15th day, as compared to control and standard groups.
6. The significant decrease in excision wound zone was observed in Test group on 3rdday (P<0.005).
7. The significant decrease in excision wound zone was observed in Standard group on 6th, 9th, 12th and 15th day (P<0.001).
8. The significant decrease in excision wound zone was observed in Test group on 6th, 9th, 12th and 15th day (P<0.001).[23]

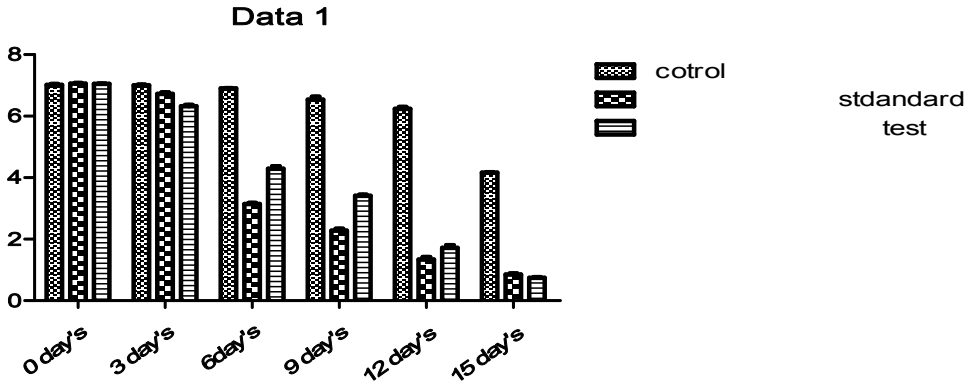


Fig .9:Effect of transdermal patch on excision wound at different day's interval.

Table no. 6: The wound healing activity for percentage wound closure.

Sr. No.	'n' day	Control	standard	Test
1	0 day	0	0	0
2	3 day	0.28%	4.74%	10.30%
3	6 day	1.70%	55.47%	39.16%
4	9 day	6.78%	67.76%	51.58%
5	12 day	11.21%	81.04%	75.62%
6	15 day	40.76%	87.99%	89.49%

From the above graph, it was observed that, percentage of wound closure of test showed that, as days passed it showed better wound closure from day 6 to day 15 than standard one.

Histopathological studies of wounded skin:[1,10,23,]

The characteristics observed during histopathological examination were the proliferation of fibroblasts, blood vessels collagen fibre and tissue remodeling etc. This histopathological observation also provided additional evidence for the experimental wound healing activity. The details of histopathological data are given in figure no.10, 11, 12.

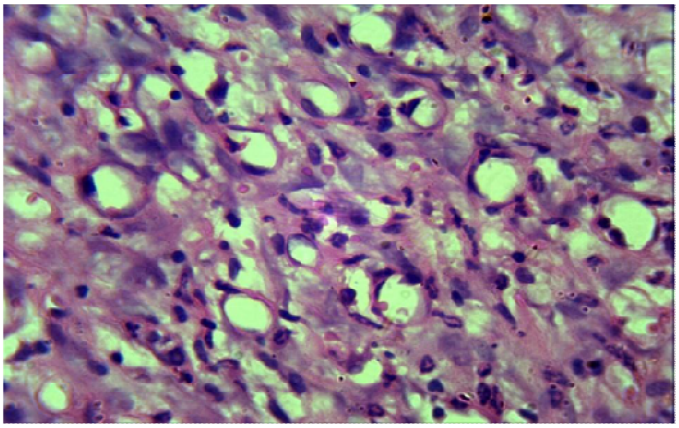


Fig. 10. Histopathological image of control group [1]

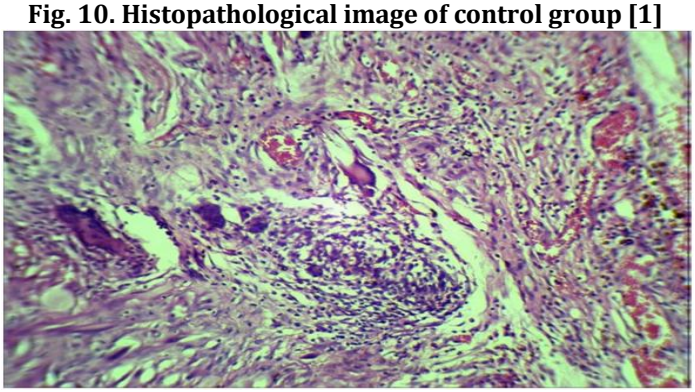


Fig.11: Histopathological image of Standard group [10]

Histopathology characteristics of standard group (Dicloplast patch) showed poor fibroblast cells, increased fibroblast cells, blood vessels in excision wound, blood vessels and collagen fibers in excision wound.

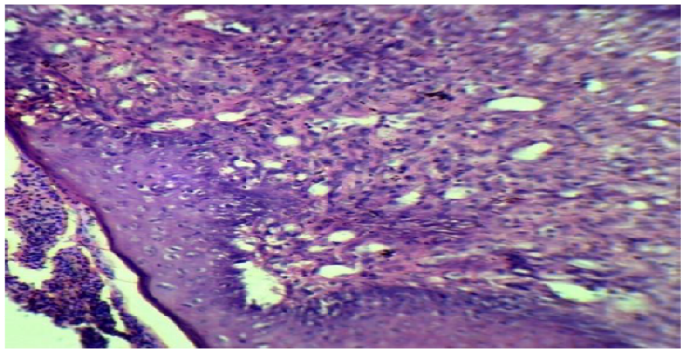


Fig. 12: Histopathological image of Test group [23]

Histopathology characteristics of test group (Samudraphen patch) showed increased fibroblast cells, blood vessels and collagen fibers in excision wound.

CONCLUSION

This research work was undertaken with an objective to explore marine origin drug 'Samudraphen' for the wound healing activity by modifying ayurvedic medicine system into modern pharmaceutical concept such as transdermal drug delivery system i.e.(patch). Formulation was having optimum evaluation parameters wound healing activity proved traditional clams.

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