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A research on wound healing activity of cuttlefish bone

Anuja Nanasaheb Nikam[1], Rohit Rangnath Kale[1], Ananda Bhimrao Waghmode[2]
[1] Poona College of Pharmacy, Pune, Maharashtra, India
[2] Assistant Professor, Mandesh Institute of Pharmaceutical Science and Research Center, Mhswad, Maharashtra, India

Abstract
During the past few years, there was an increasing the interest in the development of novel drug delivery system in these novel drug delivery system. These developed novel drug delivery systems 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 treat or degradation before reaching to the site of action could be effectively delivered with pulsatile as gastro resistant drug delivery. One of the novel drug deliveries is transdermal drug delivery system.
Wound healing is very complex process with many potential factors that can delay healing of wound. It may be caused by an act such as a gunshot, chemicals, cut, heat, cold pressure, fall or surgical intervention by infectious diseases. Samudraphen is drug from marine origin which is also referred as cuttle fish bone. This is an internal shell of Sepia Officinalis belonging to the Sepiodia. Cuttle bone contains Chitin, Chitosan, amino acids, and calcium carbonate. Which makes the successful development of formulation which could be used to hasten wound healing and induced cell migration and proliferation, and antibacterial activity. The description about the Samudraphen is available from Samhita period.

Keywords: samudraphen, novel drug delivery system, developed, transdermal drug delivery system, wound healing

Introduction
During the past few years, there was an increasing the interest in the development of novel drug delivery system in these novel drug delivery system. These developed novel drug delivery systems 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 treat or degradation before reaching to the site of action could be effectively delivered with pulsatile as gastro resistant drug delivery. One of the novel drug deliveries is transdermal drug delivery system.
Transdermal drug delivery offers controlled release of drug dose to the patient, it enables a steady blood level profile, resulting in reduced systemic side effects and, sometime, improved efficacy over other dosage forms. The topical product or drug delivery system has been used for centuries for the treatment of local skin disorders. The use of the skin as a route for systemic drug delivery of many drugs has been proved.
The administration of drugs by transdermal route offers the advantages of being relatively painless. Delivery of medication through the skin for systemic effect, called transdermal delivery was first used in 1981, when Ciba-Geigy marketed transderm V (present day marketed as transderm scope) to prevent the nausea and vomiting associates with motion sickness.

Wound
Wounds are inescapable events of life; they are physical injuries that results in an opening or breaking of the skin. Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. It is produced by physical, chemical, thermal, microbial, or immunological damage to the tissue. Every wound is unique and deserves individual care.

Classification of wounds
Wounds are classified as open wounds and closed wounds on the basis of underlying cause of wound creation and as acute and chronic wounds on the basis of physiology of wound healing.

Open wound
From an open wound blood escapes the body and bleeding is clearly visible. Open wound is again classified into following types according to the object that cause the wound.

- Incised wound: It is an injury with no tissue loss and minimal tissue damage. Bleeding in such case can be profuse, so immediate action should be taken.
- Abrasions or Superficial wounds: It is caused by sliding fall on to a rough surface. During abrasion the topmost layer of the skin i.e. epidermis is scraped off that exposes nerve ending resulting in a painful injury.
- Gunshot wound: They are caused by a bullet or similar driving into or through the body.
- Puncture wounds: They are caused by some object puncturing the skin, such as nail or needle. In this type of wound there are chances of infection because dirt can enter into the depth of wound.
- Penetration wounds: This type of wound caused by entering any sharp object like knife and coming out from the skin.

Closed wound
In closed wounds blood escapes the circulating system but remain in the body. It includes systems but remain in the body. It includes,

a. Bruises: Bruises are caused by a blunt force trauma that damaged tissue under the skin.

b. Hematomas or blood tumor: They are caused by damage to blood vessel that consequently causes blood to collect under the skin.

c. Crush injury: This type of injury caused when great or extreme amount of force is applied on the skin overlong period of time.

Wound can also be Acute or Chronic.

1. **Acute wounds:** In this type of wound the tissue injury that normally proceeds through an orderly and timely reparative process those results in sustained restoration of anatomic and functional integrity. It mainly occurs due to cuts or surgical incisions and completes the wound healing process within the expected time frame.

2. Chronic wounds

These wounds are that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation. This type of required more time to heal or recur frequently. Most common causes of chronic wounds are local infection, hypoxia, foreign bodies, trauma, and systemic problems such as diabetes mellitus, malnutrition, immunodeficiency or medications.

Factors Affecting Wound Healing. [15]

1. Infection at the wound site.
2. Insufficient oxygen supply and tissue perfusion to the wound area.
3. Drugs.
4. Improper diet.
5. Elderly age.
6. Diabetes and other disease Conditions.

Need of Present Investigation

Wound healing is very complex process with many potential factors that can delay healing of wound. It may be caused by an act such as a gunshot, chemicals, cut, heat, cold pressure, fall or surgical intervention by infectious diseases.

Samudraphen is drug from marine origin which is also referred as cuttle fish bone. This is an internal shell of Sepia Officinalis belonging to the Sepiodia. Cuttle bone contains Chitin, Chitosan, amino acids, and calcium carbonate. Which makes the successful development of formulation which could be used to hasten wound healing and induced cell migration and proliferation, and antibacterial activity. The description about the Samudraphen is available from Samhita period.

In Ayurveda like CharakSamhita, SusrutaSamhita, and AsthangaHridaya detail account on wound management is written. The shasthiupakrama (60 measures) discussed in Susruta Samhita are one of the most comprehensive wound management account and provide many insights to manage difficult, non-healing wounds.

Literature Review

Samudraphen

A. Austin *et al*. (1982): evaluated the effect of cuttlebone and its compositions &formulations. This is applied on treating wound healing. The compositions are finely divided Cephalopod skeleton i.e. cuttlebone in a compatible topical carrier. This shows improved effectiveness in promoting healing of the wounds. The ground cephalopod skeletal material may be dusted directly on the wound or may be applied a compatible carrier which may be a gas, liquid or solid. There are some following formulations by which it promotes the healing effect of wound which are those: Dressing to a wound, ointment or salve, paste, stable dispersion, smooth thick cream, etc. The products of the invention are applicable to abrasions, scratches, chapped skin, bed sores, cuts, burns & hemorrhoids.

B. Dasari Srilakshmi *et.al*. (2012): reviewed that, rasashastra is an integral part of Ayurveda that deals chiefly with mercury, metals, minerals and animal origin drug having therapeutic and all chemical importance. Use of mineral and metallic preparations for health care may be a unique feature of Rasashastra.

a. **C.** Siddiquee M.R.A. *et.al*. (2014): the author works out to update the knowledge on cuttlebone as a drug including its structure, identification, Pharmacological effects and medicinal uses. And the second part of the paper includes original research work on characterization of this drug on physiochemical laboratory parameters.

D. Dr. Pramod Kumar *et.al*. (2013): reviewed that Samudraphen is one of the Pranijiasudhavargadravya (animal origin drug) which posses immune medicinal value and used for eye and ear aliments since time immemorial. Since samhita period it is being used to treat various ailments and hence, occupied a special place in Ayurveda.

Drug Profile

Samudraphen (Cuttle bone)

Samudraphen is also called cuttlebone which belongs to marine origin and now - days- very important marine origin product due to its own numerous applications. Cuttlefish belongs to the family Sepiodia, Class Cephalopoda, Which forms parts of Phylum Mollusca. Sepioidea is characterized by a thick internal calcium- containing shell called the cuttlebone.

Physical property

a. Samudraphen (Cuttlefish bone) is white brownish in colour having bright white surface.
b. It is oblong in shape, tapering at the ends.
c. It is light and fragile.

- Origin, Collection and distribution:

1. Origin

Synonyms given for Samudraphena

The following all meaning indicates that the synonyms given for Samudraphena (Cuttle fish bone) are on the basis of its place origin.

According to the Rasa texts, Samudraphen (Cuttlefish bone) is originated as separated body part of specific variety of fish having 10 arms lengths, round body, delicate with ugly look. It is having fearful eyes and its back is difficult as that of turtle.

2. Collection and distribution

Cuttlefish having very less life span (1-3 years), it availability is never a concern. When the fish dies naturally the Samudraphen is collected near the coast so there is no

question for ethical objection and are easily available in the market for fewer coasts. Cuttlebone, earlier used in traditional medicines in India loose importance in 20th century but now lots of research work are being carried out to establish its pharmacological effects.

Excipient Profile
1. Water
Nonproprietary Names

BP: Purified Water

JP: Purified Water

PhEur: Aqua purificata

USP: Purified Water

Synonyms
Aqua, Hydrogen oxide

Chemical Name and CAS Registry Number
Water (7732 – 18 - 5)

Empirical Formula and Molecular weight
H2O, 18.02

Functional Category
Solvent

Application in pharmaceutical Formulation or Technology
Water is the most widely used excipient in pharmaceutical production operations. Specific grades of water are used for particular applications in concentration up to 100%; Table No. Purified water and water for injection are also used for cleaning operations during production of Pharmaceutical products.

Description
The term 'Water' is used to described potable water that is freshly drawn direct from drinking. Although potable and safe to drink, for most pharmaceutical applications, ion-exchange treatment, reverse osmosis or some other suitable process to produce 'Purified Water. Water is a clear, colorless, odorless and tasteless liquid.

Typical Properties
Boiling point: 100 C.
Critical pressure: 21.1 Mpa (218.3atm)
Critical temperature: 374.2 C.
Dielectric constant: D25=78.54.
Dipole moment: 1.76 in benzene at 25 C, 1.86 indioxane at 25 C
Ionization constant: 1.008 × 10-14 at 25 C.
Latent heat of fusion: 6 kJ/ mol. (1.436 kcal / mol)
Melting point: 0 C
Refractive index: nD20 = 1.3330.
Solubility: Miscible with most polar solvents.
Specific heat (liquid): 4.184 J/ 91 C (1.00 cal/ g / C) at 14 C.
Surface tension: 71.97 m N / m (71.97 dynes/ cm) at 25 C.
Vapor pressure: 3.17 Kpa (23.76 mmHg) at 25 C
Viscosity (dynamic): 0.89 m Pas (0.89cp) at 25 C.

Stability and storage Conditions
Water is chemically stable in all physical states (ice, liquid. and vapor) Water for specific purposes should be stored in appropriate containers.

Incompatibilities
In pharmaceutical formations, Water can react with drugs and other excipients that are susceptible to hydrolysis at ambient and elevated temperature. Water can react violently with alkali metals and rapidly with alkaline metals and their oxides such as calcium oxide and magnesium oxide.

Safety
Water is the base for many biological life forms and its safety in pharmaceutical formulations is unquestioned provided it meets standard of quality for portability and microbial content.

2. Carbopol 940
Nonproprietary Names
BP: Carbomers
PhEur: Carbomers
USP-NF: Carbomer

Synonyms
Acrypol, acritamer, acrylic acid polymer, carbomera, carbopol, carboxypolymethylene,
polyacrylic acid, carboxyvinyl polymer.

Chemical Name

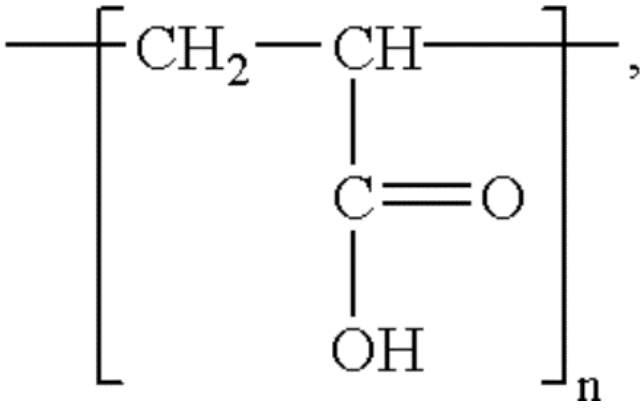

Fig 1: Structure of carbopol 940

Carbomers are synthetic high molecular weight polymers of acrylic acid that are cross linked with either allyl sucrose.

Functional Category
Bioadhesive material, controlled-release agent, emulsifying agent, emulsion stabilizer, rheology modifier, stabilizing agent, suspending agent and tablet binder.

Applications in pharmaceutical formulation
Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers.
Formulations include creams, gels, lotions and ointments for use in ophthalmic, rectal, topical and vaginal preparations.
In tablet formulations carbomers are used as controlled release agents.

Use Concentration (%)
- Emulsifying agent: 0.1–0.5.
- Gelling agent: 0.5–2.0.
- Suspending agent: 0.5–1.0.
- Tablet binder: 0.75–3.0.
- Controlled release agent: 5.0–30.0.

Typical Properties
- pH = 2.5–4.0 for a 0.2% w/v aqueous dispersion;
- Dissociation constant pKa = 6.0-0.5.
- Glass transition temperature 100–1050 C.
- Melting point Decomposition occurs within 30 minutes at 260 ^{0}C.

Solubility
Solubility swells able in water and glycerin and, after neutralization, in ethanol (95%). Carbomers do not dissolve but merely well to a remarkable extent, since they are three dimensionally cross linked micro gels.

Stability and storage Conditions
Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104 ^{0}C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Complete decomposition occurs with heating for 30 minutes at 2600C.

Safety
Carbomers are used extensively in nonparenteral products, particularly topical liquid and semisolid preparations. Grades polymerized in ethyl acetate may also be used in oral formulations. Acute oral toxicity studies in animals indicate that carbomer 934P has a low oral toxicity.

3. Polyvinyl Alcohol
Nonproprietary Names
PhEur: Poly (Vinyl Alcohol)
USP: Polyvinyl Alcohol

Synonyms
Airvol; Alcotex, Celvol, Elvanol, Gelvatol, Gohsenol, Lemol, Mowiol, Polyvinol, PVA.

Chemical Names
Ethenol, homopolymer

Functional Category
Coating agent, lubricant, stabilizing agent, viscosity-increasing agent.

Applications in Pharmaceutical Formulation
Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic Formulations. It is used as a stabilizing agent for emulsions (0.25–3.0% w/v). Polyvinyl alcohol is also used as a viscosity increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solutions for lubrication purposes. In sustained-release formulations for oral administration and in transdermal patches.

Typical Properties
- Melting point 288 ^{0}C.
- Refractive index 1.49–1.53.

Solubility
Soluble in water; slightly soluble in ethanol (95%); insoluble in organic solvents. Dissolution requires dispersion (wetting) of the solid in water at room temperature followed by heating the mixture to about 900C for approximately 5 minutes.

Viscosity
Grade Dynamic viscosity of 4% w/v aqueous solution at 208C
- High viscosity 40.0–65.0.
- Medium viscosity 21.0–33.0.
- Low viscosity 4.0–7.0.

Stability and Storage Conditions
Polyvinyl alcohol is stable when stored in a tightly sealed container in a cool, dry place. Aqueous solutions are stable in corrosion resistant sealed containers. It is stable on exposure to light.

Safeties
Polyvinyl alcohol is generally considered a nontoxic material. It is non-irritant to the skin and eyes at concentrations up to 10% and concentrations up to 7% are used in cosmetics.

4. Methyl Paraben [20]
Nonproprietary Names
BP: Methyl Hydroxybenzoate
JP: Methyl Parahydroxybenzoate
USP-NF: Methylparaben

Synonyms
4-hydroxybenzoic acid methyl ester; metagin; Methyl Chemosept;

Functional Category
Antimicrobial preservative.

Applications in Pharmaceutical Formulation
Methyl paraben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations.
The methyl paraben are effective over a wide pH range and have a broad spectrum of antimicrobial activity.
Although they are most effective against yeasts and molds.

Description
Methyl paraben occurs as colourless crystals or a white crystalline powder. It is odourless and has a slight burning taste. Methyl paraben exhibits antimicrobial activity of pH 4–8. Preservative efficacy decreases with increasing pH owing to the formation of the phenol ate anion. Methyl parabens are more active against yeasts and moulds than against bacteria. They are also more active against gram-positive bacteria than against gram-negative bacteria.

Properties
- Density 1.352 g/cm3
- Dissociation constant (pKa) 8.4 at 228 ^{0}C
- Melting point 125–128 ^{0}C

Stability and Storage Conditions
Aqueous solutions of methyl paraben at pH 3–6 may be sterilized by autoclaving at 1200C for 20 minutes, without decomposition. Methyl paraben always stored in well closed container in a cool, dry place.

Incompatibilities
The antimicrobial activity of methyl paraben and other paraben is considerably reduced in the presence of non-ionic surfactants, such as polylobate 80, as a result of micellization.

Safety
Methyl paraben and other parabens are widely used as antimicrobial preservatives in cosmetics and oral and topical pharmaceutical formulations.

Materials and Methods
Materials
The materials required for the present work were procured from diverse sources. Following drugs excipients and ayurvedic were used for the formulation and evaluation of transdermal drug delivery system preparation (patch).

Experimental Work
Selection of Drug
Samudraphen is a marine origin drug which is also referred as Cuttlefish bone. Samudraphen contains Chitin, Chitosan, Amino acid and Calcium carbonate.

Collection and authentication of plant martial:
The pure drug Samudraphen was collected from MankarnikaAushadalya, Pune. The material was authenticated from Zoology department, Yashwantrao Chavan Institute of Science, Satara. (Maharashtra)

Preformulation studies
Characterization of Samudraphen
1. 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 color. Samudraphen is flat, broad and oval in shape. The shell is entirely dead and composed of calcareous rather than horny matter.
After Identification of this drug on above description drug was characterized on parameters of microscopic view, loss on drying (moisture content), density, pH, successive extraction and ash values like total ash, water insoluble ash, acid insoluble ash, sulphated ash.

2. 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 multi steriod structured on evenly distributed pillars.

3. Loss on drying / Moisture content
The drug was powdered and a sample 5g was placed on a dry Petri dish. The Petri dish along with the drug was dried at 105 ^{0}C for 2 hrs. In oven and weighed. The drying was continued until two successive reading matched with each other.

Formulation design of transdermal drug delivery form containing Samudraphen.
Optimization of formula
For optimizing formula for transdermal patch the quantity of excipients were changed and the evaluation test for transdermal patch were carried out.

Full Factorial Design
A factorial design is used to evaluate effect of two or more factors simultaneously. The full Factorial design is a technique that allows identification of factor involved in a process and assesses their relative importance. A factor is simply a categorical variable with two or more values referred to as levels. Construction of factorial a design involves the selection of parameters and the choice of response. A study, in which there are 3 factors with 2 levels, is called 2/3 Factorial Design.
A 2/3 Full Factorial Design (FFD) was Constructed where the amount of Polyvinyl alcohol (X1), Carbopol 940 (X2) were selected as a factors. High and low levels of each factors were coded as + 1, - 1 respectively shown in the table the ranges of a factor must be chosen in order to adequately measure its effects on the response variables.

Table 1: Amount of variables in 2^3 factorial design batches

Coded value	Actual value	
	X_1	X_2
-1	2 gm	0.1gm
+1	2.4gm	0.3gm

Table 2: Formulation of 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

Preparation of Samudraphen containing transdermal drug delivery system (patch) []
Procedure for preparation of TDDS PATCH is as follows:
In one beaker polyvinyl alcohol dissolved in distilled water and was prepared polymeric aqueous solution using continued stirring with magnetic stirrer at 400rpm for 1 H. carbopol 940 dissolved in ethanol in second beaker. These two beakers kept aside for remove the air bubble. After remove the air bubble both mixtures mixed with continuous stirring. Drug solution was prepared by solvent like 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 stir for 2H. Then make a transdermal patch with help of petriplate.
We have prepared Nine formulations i.e. F1 to F9, F1 formulation was prepared without carbopol 940 and compared with other formulations. F2 to F9 formulations were prepared with variable concentration of polyvinyl alcohol i.e. 2.5-4% and constant concentration of carbopol 940.

Evaluation of patch: []
Patches were evaluated for their thickness, weight uniformity, folding endurance, percentage moisture content and wound healing activity by using suitable animal model.

1. Thickness

Table 3

Batch No.	Thickness (mm)
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

Weight Uniformity

Table 4

Batch No.	Weight (mg)
F1	0.491 ± 0.09
F2	0.533 ± 0.01
F3	0.489 ± 0.09
F4	0.545 ± 0.01
F5	0.542 ± 0.02
F6	0.537 ± 0.01
F7	0.493 ± 0.09
F8	0.542 ± 0.02
F9	0.482 ± 0.07

2. Percentage Moisture Content

Table 5

Batch No.	% moisture content
F1	1.66 ± 0.069
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

Determination of physical appearance:
The colour of various formulations was determined by visual inspection.

***In- Vivo* studies for wound healing activity by using suitable animal model:**
Wound healing activity: []
Animals required
Species/Common name –
Wistar rats male and female 200 to 300gm (n= 18)

Table no. 6. Data of animal requirements

Table 6

a.	Age	More than 2 month
b.	Gender	Either male or female

The rats were divided into three groups namely,

1. Group I: Control
2. Group II: (Standard)
3. Group III: transdermal patch containing Samudraphen (topical formulation)

Procedure
The rats were divided into three groups namely control, Standard, and transdermal patch containing Samudraphen (test formulation) on the day of experiment the whole rats were anesthetized. A full thickness of the excision 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 treated with topical application. The wound contraction were measured by a tracing paper on the wounded margin and calculated as percentage reduction in wound area. The wound were monitored and the area of wound size was measured on 3,6,9,12,15 and 18 th of post wounding day.
The percentage of wound closure was calculated using the following formula:

$$\% \text{ wound closure} = \frac{\text{Wound Area on Day '0'} - \text{Wound Area on Day 'n'}}{\text{Wound Area on Day '0'}}$$

Where n = number of days.

Histopathological studies of wounded skin: [ ]
At the 18th the experiment was terminated and tissue of wound area was removed from the surviving animals for histopathlogical examination. Sample tissue were fixed in 10 % formalin and were embedded in transdermal patch serial section (5µm thickness) of paraffin embedded tissues' were cut the tissues stained by haematoxylin & eosin (HE staining) and after that they were examine by electronic microscopy.

Result and Discussion
A. Collection of material:

Fig 2: Samudraphen (Cuttlebone)

B. Authentication of material:
The authentication of Samudraphen was done from Mankarnika Aushadalya, Pune.

Characterization of drug:
1. 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 color. Samudraphen is flat, broad and oval in shape.

The shell is entirely dead and composed of calcareous rather than horny matter.

Fig 3: Samudraphen

After Identification of this drug on above description drug was characterized on parameters of microscopic view, loss on drying (moisture content), density, pH, successive extraction and ash values like total ash, water insoluble ash, acid insoluble ash, sulphated ash.

2. 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.

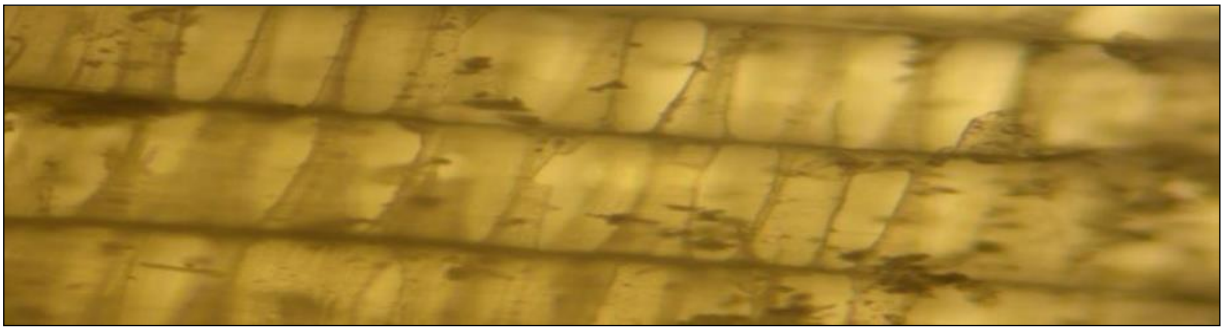

Fig 4: It shows microscopic view of T.S. of part of Cuttlebone

3. 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. 0.1 gm. Which represent 2.04%. Moisture content of drug had shown very minor loss in drying that was 0.1 gm as the drugs available in market are already dried.

4. 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.

5. 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.

6. 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.

Results of Preformulations studies

Table 7

Sr. no.	Test	Observation	Standard reading
1	Identification a) Size b) Shape	1 to 3 inches width, 5 to 10 inches length. Flat, broad oval shape.	to 3 inches width, 5 to 10 inches length. Flat, broad oval shape.
2	Microscopic view (Binocular microscope)	Crystalline form is likening multistoried structure on evenly distributed pillers.	Crystalline form is likening multistoried structure on evenly distributed pillers.
3	Loss on drying	4.9 gm, 0.1 gm, 2.04%	4.92 gm, 0.08 gm, 1.6%
4	Total ash value	87.64%	93.87%
5	Acid insoluble ash value	0.0473gm 9.46%	0.48gm 9.6%
6	Water soluble ash value		
7	Successive Extraction		
	a) Petroleum ether	0.02%	0.02%
	b) Chloroform	0.02%	0.02%
	c) Methanol	0.42%	0.54%
	d) Water	2.14%	2.36%

Evaluation parameters of transdermal drug delivery system. (Patch)

Physical Evaluation of patch

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

Film Thickness

Table 8: Film thickness results of transdermal patch

Batch NO.	Thickness (mm)
F1	0.82± 0.19
F2	0.84±0.19
F3	0.88±0.19
F4	0.88±0.19
F5	0.89±0.19

Thickness of transdermal films were determined by using micrometer screw gauge. The average thickness values were prepared in table no. 9.15. It was found that there is increased in thickness of transdermal patches from F3 to F5. The thickness range was about 0.82 to 0.89. F3 and F4 formulations showed optimum thickness due to flexibility and cohesive film forming nature of polymer which has used in transdermal patches. Uniform film thickness was not found in all formulations. Formulation F1 was having less thickness because of inpresence of carbopol 940. Formulations F3 and F4 shown same results. So it showed good film former. Concentration of polyvinyl alcohol was increased in all formulation it was affect on its thickness of tranderml patch. F5 formulation got good results and it slightly matches with F3 and F4 formulation.

The results of folding endurance of film were given in table no.9.16. The formulation F5 shown highest folding endurance as compared to other formulations. Formulation F1 and F2 were having less folding endurance as compared to F3 to F5. As the concentration of polyvinyl alcohol was increased flexibility of film was found to be increased to a certain extent. In F1 formulation concentration of polyvinyl alcohol was less than other formulations so folding endurance was less than other formulation it was about 320 only. Formulation F3, F4 and F5 got better results. F5 formulation got highest folding endurance because of increased concentration of polymer which increased the flexibility of formulation at certain extent. The folding endurance of all prepared films ranged between 320-396

The films of all formulation were tested for weight variation. Weight variation study of transdrmal patches were shown in table no. 9.17. In all formulations concentration of polyvinyl alcohol was different and carbopol 940 was same but in F1 formulation carbopol 940 was not added. The use of carbopol 940 was for moisture absorbing agent. So it affects on weight of all formulations. Weight of F1 formulation was less than other formulations which was about 140mg. weight of F2 formulation was greater than F1 formulation because of increase in polymer concentration. Formulation F3 and F4 got same results. Even though in concentration of polymer was increased in F3 and F4 formulations it didn't affected on weight of transdermal patches. Concentration of polymer increases viscosity was also increase so weight of F5 formulation was greater than other formulations.

The results of % moisture content are given in table no. 9.20. From table it was found that, increased concentration of carbopol 940 and polyvinyl alcohol there was increased in moisture content of transdermal films. The average ranges of % moisture content of all prepared films were between 3 to 7%. Less % of moisture content was found in formulation F1 and F2. In F1 it was because of absence of carbopol 940 in F1 formulation and in F2 it was because of concentration of polyvinyl alcohol was less than other formulations. Formulation F3 got optimum result. Formulation F4 and F5 got same results. Those were slightly similar to F3 formulation. All these occurred due to concentration of polymer only as cabopol940 because it was act as moisture absorbing polymer.

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