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DESIGN, PREPARATION AND VALIDATION OF AMINOACID LOADED NATURAL TRANSDERMAL FILMS FOR SKIN NOURISHMENT ACTIVITY

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ABSTRACT

Nature has been instrumental as a source for therapeutics. Marine organisms comprise approximately a half of total biodiversity thus offering a vast source to discover useful therapeutics. Transdermal drug delivery systems (TDDS), also known as "medicated adhesive patches," are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. The evidence of percutaneous drug absorption may be found through measurable blood levels of the drug, detectable excretion of the drug and its metabolites in the urine and through the clinical response of the patient to the administered drug therapy. Supporting skin repair reducing the effects of oxidative stress slows the ageing process of the skin. Essential amino acids are the most important for skin cells. Amino acid permeation studies show may help the skin nourishing activity defend itself against oxidative stress and protect it from sun damage and it has shown encouraging preliminary skin health benefits.

Key words: Transdermal drug delivery system, Medicated adhesive patches Skin permeation, Amino acid permeation studies.

INTRODUCTION

A transdermal patch or skin patch is a medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and into the blood stream. Often, this promotes healing to an injured area of the body. The lipophilic stratum corneum is a major barrier to the penetration of hydrophilic compounds into and through the skin [1]. Natural polymers are useful as biomaterials and these are biodegradable polymers. These are used in regenerative medicine, implantable materials, controlled release carriers or scaffolds for tissue engineering. These polymer are used as drug delivery carriers, they are degraded into biologically accepted compounds, often through the process of hydrolysis, which leave the incorporated medications behind [2]. Biological dressings like fibrin glue gelatin sheets chitosan films are popular for quicker wound healing [3, 4]. The major advantages of natural polymers are good cytocompatibility, biodegradable and do not require any surgery for removal of polymers [5]. Gelatin is also a natural polymer derived from collagen of animal skin and bones. It is translucent, colorless, brittle and tasteless [6]. It is biodegradable in nature Chitosan, gelatin combination shown many advantages when used in other preparations like sponges, scaffolds etc. This combination also showed good compatibility in XRD and FTIR studies [7].

The common ingredients which are used for the preparation of TDDS are as follows.

Drug: Drug solution is in direct contact with release liner. Ex: Nicotine, Methotrexate and Estrogen.

Liners: Protects the patch during storage. The liner is removed prior to use. Ex: polyester film.

Adhesive: Serves to adhere the components of the patch together along with adhering the patch to the skin. Ex: Acrylates, Polyisobutylene, Silicones.

Membrane: Controls the Release of the drug from the reservoir and multi-layer patches, Ex: Terpenes, Terpenoids, Pyrrolidones. Solvents like alcohol, Ethanol, Methanol. Surfactants like Sodium Laurylsulfate, Pluronic F127, Pluronic F68.

Backing layer: Protect patch from the outer environment. Ex: Cellulose derivatives, polyvinyl alcohol, Polypropylene Silicon rubber [8].

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MATERIALS AND METHODS

Essential aminoacid was obtained as sample from Integrated Marketing Co., Hyderabad. Chitosan was extracted from crab shells. Gelatin was procured from Qualigens fine chemicals, Mumbai, India. Sorbital was procured from Central drug House, Mumbai.

Drying and size reduction of crab shell

First, remove all the crab's tissues except the exoskeleton of it, then wash the crab's exoskeleton with distill water for several times. After that, grind the crab's exoskeleton as small as possible and place the crab's exoskeleton at room temperature and desiccate it under the sun light [9].

Demineralization

To prepare chitosan, 10 grams of dried crab shell wastes proceeded with the demineralization process by adding 1.5N HCl at room temperature for 1hour. The spent acid was discarded and the shells were repeatedly washed with distilled water until the pH is neutral.

Deproteionization

The demineralized shells were then deprotinized 0.5% NaOH AT 100^0 C for 30 minutes. This method helped to weaken the protein tertiary structure of the shells. Protein solution was removed and washed thoroughly with distilled water and the pH was checked removal of remaining protein from the shells for that 3%NaOH was added to the sample 100^0 C for 30 minutes. After draining the residual proteins along with the effluents, the sample once again washed and the pH was observed till it was approximately near to neutral. This step also helped in decolourization of the shells. Hence the chitin slurry was obtained [10].

Isolation of chitosan from chitin

The Chitosan was prepared by deacetylation of chitin by treating with 42% aqueous NaOH at 95°C for 1.5 hour .After deacetylation the alkali was drained off and washed thoroughly with distilled water until the pH is less than 7.5 and then dried at ambient temperature (30 ± 2°C).

Transdermal film formation

Preparation of chitosan films

The chitosan films were prepared by casting chitosan (which is collected from crab shell) dissolved in 0.5M Acetic acid (2%) solution in the plastic tray and air dried at room temperature.

Preparation of gelatin films

The gelatin films were prepared by casting gelatin (2%, 3%, 4%, 5%) solution which is dissolved in hot water maintained 60^0C in the plastic tray and air dried at room temperature.

Preparation of composite films

The films were prepared by casting chitosan (2%), gelatin (2%) solution separately and different proportions (2:2, 2:3, 2:4 and 2:5) of both chitosan and gelatin (composite films) as shown in table1 with 0.2%. Sorbital as plasticizer after vacuum filtration for removal of entrapped air bubbles on the plastic tray and air dried at room temperature. Based on the results of physical parameters of the films, the ratio of 2:3 was selected for preparation of the drug loaded films.

Preparation of aminoacid loaded chitosan-gelatin film

Stock solutions of essential amino acid were prepared in the concentrations of 1, 2, 3,4%w/v. Drug solution was added to 1:5 ratio of chitosan and gelatin polymer solution such that 2.5,5,7.5,10 of essential amino acid was present in 0.19sq.cm area of the film respectively. As shown in table 2.

Film formation-Glass Substrate Method

The polymeric solutions are kept aside for swelling then required quantity of plasticizer and drug solution are added and stirred for 10min. Further, it is set-aside for sometimes to exclude any entrapped air and is then poured in a clean and dry petriplate. The rate of solvent evaporation is controlled by inverting a glass funnel over the petriplate. After overnight, the dried films are taken out and stored in desiccators [11].

Evaluation of Transdermal films

Weight uniformity

The prepared patches are to be dried at 60°C for 4hrs before testing. A specified area of patch is to be cut in different parts of the patch and weigh in digital balance. The average weight and standard deviation values are to be calculate from the individual weights.

Percentage of Moisture content

The prepared films are to be weighed individually and to be kept in a desiccator containing fused calcium chloride at room temperature for 24 hrs. After 24 hrs the films are to be reweighed and determine the percentage moisture content from the below mentioned formula.

Percentage moisture content =

[Initial weight - Final weight/ Final weight] ×100.

Percentage Moisture uptake

The weighed films are to be kept in a desiccator at room temperature for 24 hrs containing saturated solution of potassium chloride in order to maintain 84% RH. After 24 hrs the films are to be reweighed and determine the percentage moisture uptake from the below mentioned formula.

Percentage moisture uptake =

[Final weight- Initial weight/ initial weight] ×100

Thickness of the patch

Thickness of film influence the time required to absorb the polymer into the body. To determine the uniformity in thickness of film and change in thickness film after drug loading, it was measured for each film using screw gauge at three different sites of the film and the mean was calculated.

Folding endurance

The folding endurance was determined to determine flexibility of film. The flexibility of the film is needed to handle the film easily and for comfortable, secured application of film on the wound. It was determined by repeatedly folding one film at same place till it breaks or folded up to 200 times manually. The number of times of film could be folded at the same place without breaking give the value of folding endurance.

Tensile strength

Tensile strength measures the ability of film to withstand rupture, mechanical pressures or the force required to break the film. Tensile strength of the film was determined by using the Instron tensile testing machine at SDDC section in CLRI. It was expressed in MPa units. Tensile strength was done for all composite films.

***In vitro* drug release studies**

A specified area of the patch is to be dissolved in a suitable solvent in specific volume. Then the solution is to be filtered through a filter medium and analyse the drug content with the suitable method (UV or HPLC technique). Each value represents average of three samples.

***In vitro* skin permeation studies**

An *In vitro* permeation study can be carried out by using diffusion cell. The epidermal layer of goat skin hair from the skin region is to be removed carefully by using a electric clipper the dermal side of the skin was thoroughly cleaned with distilled water to remove any adhering tissues or blood vessels, equilibrated for an hour in dissolution medium or phosphate buffer pH 7.4 .The temperature of the cell was maintained at 32 ± 0.5°C using a thermostatically controlled heater. The isolated epidermal layer of goat skin piece is to be mounted between the compartments of the diffusion cell, with the epidermis facing upward into the donor compartment. Sample volume of definite volume is to be removed from the receptor compartment at regular intervals, and an equal volume of fresh medium is to be replaced. Samples are to be filtered through filtering medium and can be analyzed by spectrophotometrically. Flux can be determined directly as the slope of the curve between the steady-state values of the amount of drug permeated vs. time in hours.

Stability studies

Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines by storing the TDDS samples at 40 ± 0.5°C and 75 ± 5% RH for 3 months. The samples were withdrawn at 0,30,60,90 days and analysed suitably for the drug content.

Statistical Analysis

The results are expressed as mean ± S.D. Statistical analysis was performed by paired t-test, one-way and two-way analysis of variance (ANOVA) tests for multiple comparisons. Statistical significance was set accordingly at p (0.05) level.

RESULTS AND DISCUSSION

All the films were prepared by solvent casting technique and were evaluated by weight variation, moisture content, moisture uptake. Table 7 shows the tensile strength parameters of composite films and selected drug loaded film. As gelatin proportion is increased, parameters like maximum extension, elongation at break (%) and tensile strength were increased. As per paired t-test of statistical analysis there was no significant difference ($p<0.05$) in tensile strength of blank and drug loaded films. It indicated that the tensile strength of film was not changed significantly after loading the drug into film. In composite films, with increase in gelatin concentration, thickness also increased significantly.

There is slightly difference in thickness of aminoacid loaded films (AF8) when compared to blank composite films (F4) which indicated that the loading of drug did not influence the thickness of films as shown and the folding endurance values of all prepared films. With increase in concentration of gelatin, there was significant increase in folding endurance of composite films. There was significant difference ($p<0.05$) in folding endurance between optimized blank composite film F4 and the drug loaded films may be due to alteration of flexibility of films by drugs. AF8 shown maximum folding endurance among aminoacid loaded films, which indicated that it may have maximum flexibility.It was found that, there was significant difference in capacity to the flexibility between aminoacid loaded chitosan gelatin films. It indicated that the aminoacid concentration is in decreasing the flexibility of the film will be decreased. Among aminoacid loaded films, AF8 i.e., 5 milli molar./0.19 sq.cms. Loaded film, shown maximum flexibility.

Table 9.shows the cumulative percentage drug release of selected chitosan gelatin film F4 and aminoacid loaded chitosan gelatin films. The percentage of drug release from these films were determined by *In vitro* diffusion studies to find the time taken by film to release the complete aminoacid loaded chitosan gelatin film AF8 for skin nourishing activity. 99% of aminoacid was released within 1 hour (60 minutes) indicated that the film is not interfering in aminoacid release on skin. The *Invitro* skin permeation of aminoacid loaded films was more than the blank chitosan and composite films. It was confirmed that the more skin permeation activity of the aminoacid loaded films than blank films. It confirmed that increased skin permeation activity of the aminoacid loaded chitosan gelatin films than blank films, so aminoacid loaded chitosan gelatin films may show skin nourishing activity than blank films.

CONCLUSION

Based on above findings it can be concluded that the composite film of aminoacid loaded chitosan gelatin films is successful in skin permeation for skin nourishing activity with improved skin repaired properties than chitosan gelatin film F4 film.

Table 1. FTIR absorption ranges of chitosan

Functional group	*Characteristic Absorption(s)*(cm^{-1})	Chitosan from crab	Standard chitosan
Amine N-H Stretch	3500 - 3300	3428	3420.06
Alkyl C-H Stretch	2950 - 2850	2905	2923.73
Alkenyl C=C Stretch	1680 - 1620	1659	1643.59
Aromatic C=C Bending	1700 - 1500	1558	1556.16

Table 2. Percentage of Weight variation

S.NO	Name of the films	Initial weight (mg)			Final weight			% of weight variation
1.	F1	25.6	25.5	25.6	22.4	22.6	22.5	11.97
2.	F2	28.8	28.6	28.7	25.2	25.4	25.3	11.84
3.	F3	26.8	26.7	26.8	23.7	23.9	23.6	11.32
4.	F4	24.3	24.2	24.4	21.4	21.6	24.3	7.69
5.	F5	29.1	29.3	29.3	25.2	25.4	25.1	13.68
6.	F6	30.2	30.4	30.4	26.7	26.9	26.8	11.63

Table 3. Percentage of Moisture content

S.NO	Name of the films	Initial weight (mg)			Final weight (mg)			% of moisture content
1.	F1	25.6	25.5	25.6	24.4	24.2	24.3	4.95
2.	F2	24.3	24.2	24.4	23.4	23.6	23.1	3.84
3.	F3	26.8	26.7	26.8	25.7	25.8	24.6	5.20
4.	F4	28.8	28.6	28.7	27.9	27.5	27.4	11.49
5.	F5	29.1	29.3	29.3	28.7	28.2	28.0	3.19
6.	F6	30.2	30.4	30.4	29.2	29.3	29.3	3.51

Table 4. Percentage of Moisture uptake

S.NO	Name of the films	Initial weight(mg)			Final weight (mg)			% of moisture content
1.	F1	25.6	25.6	25.8	29.8	29.8	29.4	15.88
2.	F2	24.3	24.3	24.6	28.6	29	29.2	18.57
3.	F3	26.8	26.9	26.7	29.2	29.2	30.4	9.95
4.	F4	28.9	28.8	28.9	30.4	30.1	30.5	5.08
5.	F5	29.6	29.1	29.8	32.2	32.4	32.6	9.83
6.	F6	30.5	30.2	30.7	34.5	36.4	34.5	15.31

Table 5. Thickness of Chitosan – Gelatin film

Name of the films	Thickness (mm)			Mean ±SD
	Trail1	Trail2	Trail3	
F1	0.03	0.05	0.04	0.040±0.010
F2	0.05	0.04	0.04	0.043±0.006
F3	0.05	0.05	0.04	0.047±0.005
F4	0.06	0.05	0.06	0.057±0.006
F5	0.05	0.06	0.06	0.055±0.005
F6	0.05	0.06	0.06	0.055±0.005

Table 6. Folding endurance of Chitosan – Gelatin film

Name of the films	Folding endurance			Mean ±SD
	Trail1	Trail2	Trail3	
F1	159	154	164	159±5.00
F2	185	182	178	181.6±3.51
F3	108	107	105	106.6±1.52
F4	248	254	252	251.3±3.05
F5	264	262	260	262.0±2.0
F6	267	265	268	266.0±1.5

Table 7. Tensile Strength of Chitosan – Gelatin film

Name of the films	F1	F2	F3	F4	F5	F6
Maximum Load (N)	3.25	4.28	5.23	5.62	5.69	5.72
Extension at Maximum Load (mm)	17.00	16.23	25.32	46.74	50.63	51.69
Elongation At Break (%)	3.5	3.7	3.5	5.1	5.4	5.2
Tensile Strength (MPa)	22.25	39.25	43.21	65.34	69.23	72.59

Table 9. *In vitro* drug release studies of amino acid loaded Chitosan Gelatin film

Time interval (minutes)	5 mmol Amino acid loaded Chitosan Gelatin film AF7			Mean ±SD
	Trail1	Trail2	Trail3	
0	0	0	0	0±0
10	0.49	0.49	0.48	0.487±0.006
20	0.54	0.52	0.53	0.530±0.01
30	0.68	0.69	0.67	0.680±0.01
40	0.74	0.74	0.73	0.737±0.06
50	0.74	0.74	0.74	0.740±0.00
60	0.74	0.74	0.74	0.740±0.00

Figure 1. Transdermal films

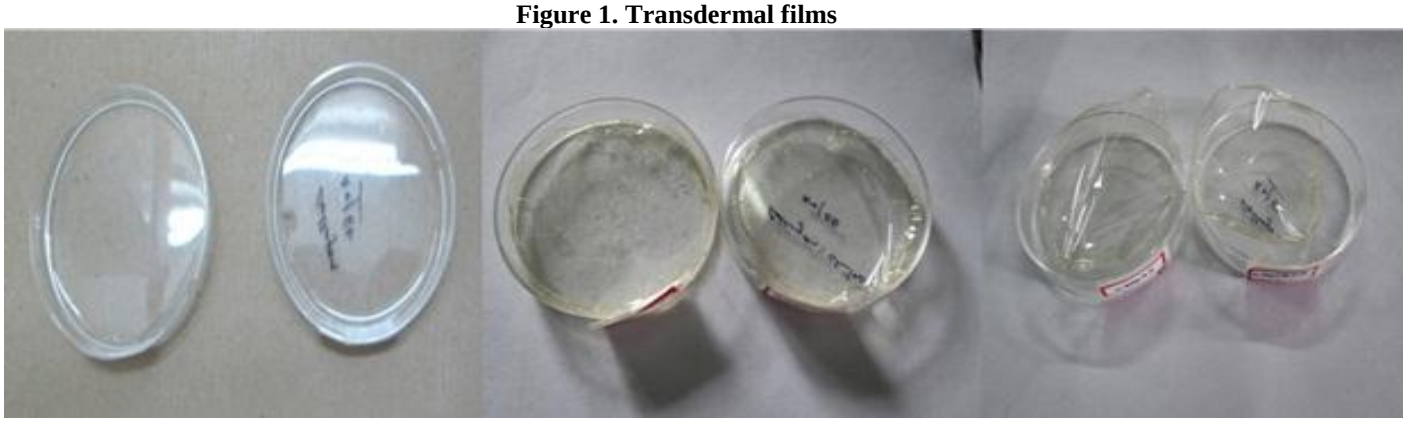
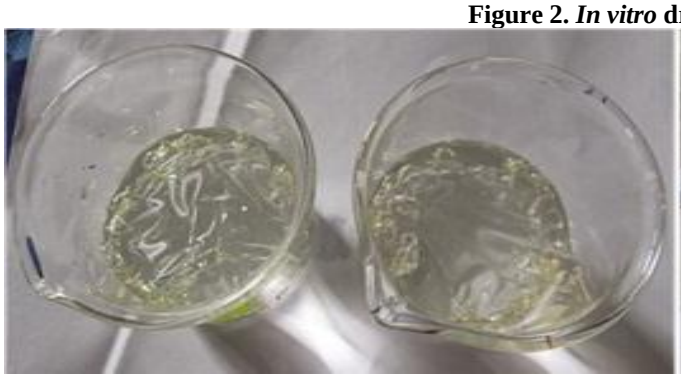


Figure 2. *In vitro* drug release studies



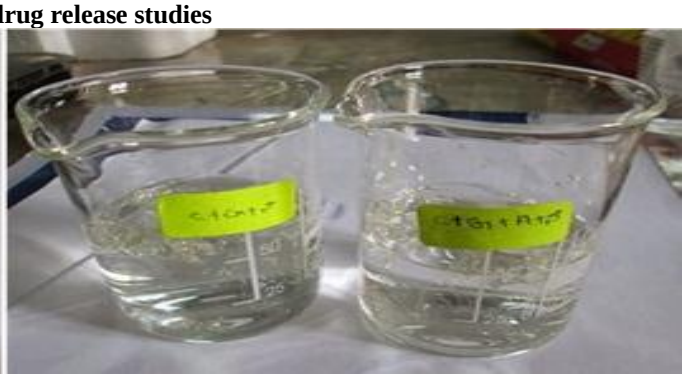
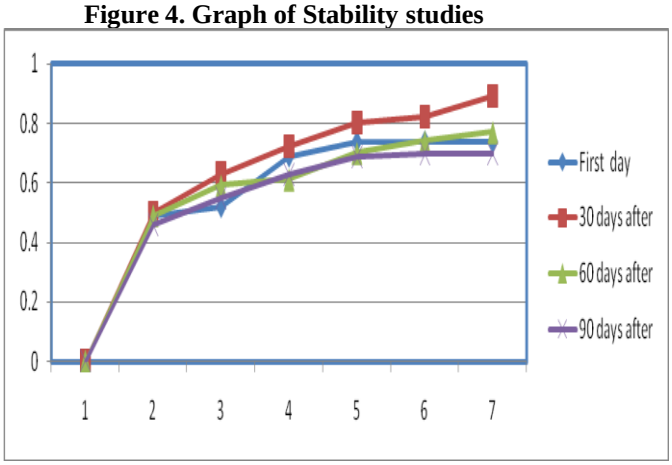


Figure 3.*In vitro* skin permeation studies

Figure 4. Graph of Stability studies



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