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Polymeric Sustained Biodegradable Patch Formulation for Wound Healing

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***Abstract*—**It is the patient compliance and stability in combination with controlled drug delivery and biocompatibility that forms the core feature in present research and development of sustained biodegradable patch formulation intended for wound healing. The aim was to impart sustained degradation, sterile formulation, significant folding endurance, elasticity, biodegradability, bio-acceptability and strength. The optimized formulation comprised of polymers including Hydroxypropyl methyl cellulose, Ethylcellulose, and Gelatin, and Citric Acid PEG Citric acid (CPEGC) triblock dendrimers and active Curcumin. Polymeric mixture dissolved in geometric order in suitable medium through continuous stirring under ambient conditions. With continued stirring Curcumin was added with aid of DCM and Methanol in optimized ratio to get homogenous dispersion. The dispersion was sonicated with optimum frequency and for given time and later casted to form a patch form. All steps were carried out under strict aseptic conditions. The formulations obtained in the acceptable working range were decided based on thickness, uniformity of drug content, smooth texture and flexibility and brittleness. The patch kept on stability using butter paper in sterile pack displayed folding endurance in range of 20 to 23 times without any evidence of crack in an optimized formulation at room temperature (RT) (24 ± 2°C). The patch displayed acceptable parameters after stability study conducted in refrigerated conditions (8±0.2°C) and at RT (24 ± 2°C) up to 90 days. Further, no significant changes were observed in critical parameters such as elasticity, biodegradability, drug release and drug content during stability study conducted at RT 24±2°C for 45 and 90 days. The drug content was in range 95 to 102%, moisture content didn't exceeded 19.2% and patch passed the content uniformity test. Percentage cumulative drug release was found to be 80% in 12h and matched the biodegradation rate as drug release with correlation factor R^2>0.9. The biodegradable patch based formulation developed shows promising results in terms of stability and release profiles.

***Keywords*—**Sustained biodegradation, wound healing, polymeric patch, stability.

I. Introduction

WOUNDS on skin heal naturally regenerating dermal and epidermal tissue. Although wound healing occurs naturally, however few complications like sepsis, disruption of tissue and skin layer, maggot's formation, extension of infection to adjacent and interior organs occur in majority of cases. Skin grafting and biological dressings are utilized to prevent extensive loss and damage to the tissue [1]-[3]. Repair of injured tissues undergoes continuously, which include inflammation, proliferation, and migration of different cell types [4], [5]. The process includes sequence of inflammation and repair in which epithelial endothelial inflammatory cells, platelets and fibroblasts briefly come together outside their normal domains, interact to restore structure of their usual discipline and having done to initiate their functions [6], [7]. Today healthcare system includes dermal patch based technology as one of the well-known and widely utilized approach for delivering bioactives through the skin avoiding needles for systemic actions.

The patch proposed for the wound healing might include an ultra-concentrated formula infused into a small discreet dermal patch which adheres to the skin surface. This technology uses advanced technology based on physiological to introduce the formulation as a controlled delivery unit that can extend drug's presence during the wound healing phases. It has proven to be fastest, easiest, safest and most economical way to help wound to heal [6]-[8]. Nowadays, more focus is paid to pollution causing non-biodegradable synthetic polymers that can be replaced by biodegradable substances such as polysaccharide, lipid, protein and other composite films [6]-[9]. The use of biodegradable polymers and actives of natural origin would be quite fruitful in developing wound healing patches not only with respect to environment but also to patient compliance physiologically, economically and psychologically. The present study is proposed to develop a biodegradable patch of Curcuminoid for wound healing that can counter issues pertaining to variability of natural active and the frequency of physician's visit ensuring complete wound care during skin's healing process.

II. Materials and Methods

A. Materials

Curcumin was procured from Vision India Technology; Hydroxymethylpropyl cellulose and Ethyl Cellulose was obtained from SD Fine chemicals; Polyvinylpyrrilidone and Polyethyleneglycol were obtained from Sigma-Aldrich. Other solvents and materials were of analytical grade.

B. Methods

Statistical Analysis: All methods were performed in triplicate and outcomes were prepared using one way ANOVA in order to mean comparison of more than two groups using SPSS software. Results were reported as meaningful for $P<0.05$.

Synthesis of G2 CPEGC dendrimer: According to [10] divergent method G1 and G2 dendrimers were prepared. CPEG dendrimers are synthesized in two steps including

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esterification and combination with citric acid. The monomer units of ester-liked fragment were citric acid and diacyl halide poly (ethylene glycol) was used as the dendrimer core. Diacid poly ethylene glycol was chlorinated using thionyl chloride ($SOCl_2$) and reacted with citric acid as the monomer to generate the G1. Then, the G1 was coupled to citric acid to produce the G2 with the aid of dicyclohexylcarbodiimide (DCC) in the pyridinemedium. We obtained Compound G1 through the DCC–PEG–DCC reaction with citric acid. Then, the G2 was produced by coupling the G1 to citric acid. The reaction should be functionalized in dry condition. Then we used dimethyl sulfoxide (DMSO) as resolvent that reacted with Calcium chloride in portion of 10:1 was prepared to get dried condition.

Calibration curve: Reverse phase HPLC system (LC-2010 CHT; Shimadzu, Japan) comprising of Phenomenex C18 column (250 mm x 4.6 mm) was used for analysis of CUR. Mobile phase was a isocratic mixture of acetonitrile: HPLC water (57:43 %v/v), at pH 3.3 maintained using citric acid. Elution was carried out at a flow rate of 1.0 mL/min at room temperature (37^0C), with the UV-Vis detection wavelength of 425 nm. Method was validated using various parameters such as accuracy, precision, linearity, limit of detection and limit of quantification [11].

Formulation of Biodegradable Wound Healing Patches

The formulation methodology is followed with significant modifications in research published as dissertation [12]. The present studies involved the utilization of dendrimeric polymer base for designing biodegradable novel drug delivery system. Prior to the preparation of biodegradable wound healing patch, preliminary study trial batches were formulated without drug and checked for their integrity. Gelatin processed at 42ºC to attain molten state with ethylene glycol. Then CPEGC triblock dendrimer was dissolved separately with acetic acid in aqueous phase. Thereafter the content was transferred to ethylene glycol and gelatine mixture and sonicated in bath sonicator at 42ºC continuously for 15 minutes. The mixture was poured in petridish and kept at room temperature to cool down gradually. The placebo patch was tested for various *in vitro* parameters at RT and 4°C for 7, 15 and 30 days and evaluated for physical description (color and texture), folding endurance, percentage moisture content and rate of biodegradation at skin's pH. In further, trials mixture of molten Gelatin and ethylene glycol with specified quantity (0.6-1.5%wt) of CPEGC triblock dendrimer dissolved in purified water together. Thereafter drug dispersed in purified water was incorporated in the mixture with continuous stirring. Then Hydroxy propyl methyl cellulose (HPMC K4M) and ethyl cellulose in specified quantity were dispersed in above mixture with aid of dichloromethane and methanol in molar equal ratio.

Evaluation of Biodegradable Patches

1. Physical Appearance: Formulated patches were evaluated for their physical appearance, uniformity, which on a large part determines patient acceptability of the patch and also therapeutic efficacy.
2. Thickness Uniformity: The thickness of each film was measured by using screw gauze. The thickness was measured at six different places on each film and the average thickness of the film was taken as the thickness of the film.
3. Weight Variation: Weight variation was studied by individually weighing 10 randomly selected patches and average weight was calculated. The individual weight should not deviate significantly from the average weight.
4. Folding Endurance: Folding endurance of the patches was determined by repeatedly folding one patch at the same place till it broke or folded up to 300 times manually, which is considered satisfactory to reveal good patch properties.
5. Drug Content: The films were tested for the content. A film of size 2x2 cm^2 was cut and placed in a volumetric flask. Ten ml of methanol was added and the contents were stirred in a shaker bath for 24 h to dissolve the film. Subsequent dilutions were made with phosphate buffer (pH 7.4). The absorbance of the solution was measured against the corresponding blank solution using UV-VIS spectrophotometer.
6. Percentage of Moisture Content: The patches were weighed individually and kept in a desiccator containing activated silicate room temperature for 24 hrs. Individual patches were weighed repeatedly until they showed a constant weight. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight.
7. Percentage of Moisture Uptake: A weighed film kept in a desiccator at room temperature for 24hrs, which contains saturated solution of potassium chloride in order to maintain 84% RH. After 24 h, the films were reweighed to determine the percentage moisture uptake.
8. Uniformity of the Dosage Unit Test: An accurately weighed portion of the patch is to be cut into small pieces and transferred to a specific volume using a volumetric flask, dissolved in a suitable solvent and sonicate for complete extraction of the drug from the patch and made up to the mark with the same. The resulting solution was allowed to settle for about 1 h and the supernatant was suitably diluted to give the desired concentration with the suitable solvent. The solution was filtered using a 0.2µm membrane, filtered and analysed by a suitable analytical technique UV or HPLC and the drug content per piece was to be calculated
9. Swelling Studies: Weight and area increase due to swelling were measured: Weight increase due to Swelling: The drug-loaded patch of size 1 x 1 cm2 was weighed on a pre-weighed cover slip. It was kept in a petridish and 50 ml of phosphate buffer (pH 7.4) solution was added. After every five min, the cover slip was removed, wiped with tissue paper, and weighed up to 30 min. The difference in the weights gives the weight increase due to absorption of water and swelling of patch.

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10. *In vitro* Drug Release Studies: The basket assembly method employed for assessment of the release of the drug from the prepared patches. Dry films of known thickness are to be cut into a definite shape, weighed and fixed over on a basket. The basket was then placed in 250ml of the dissolution medium or phosphate buffer (pH 7.40 and the apparatus was equilibrated to 32±0.5ºC. The paddle was then set and operated at a speed of 50rpm. Samples (3ml aliquots) can be drawn at appropriate time intervals up to 24hrs and analysed by a UV spectrophotometer or HPLC.
11. Stability Studies: Stability studies are to be conducted according to the ICH guidelines by storing the patches at 25± 0.5ºC/60±3% RH and 5-8ºC and for 6 months. The samples were withdrawn at prespecified time points as well and analysed suitably for the physical appearance and drug content.

III. Results and Discussion

In the present study, the formulation trials of wound healing patches were conducted in order to develop a product with acceptable design criteria that included physical appearance, folding endurance, content, drug's controlled release, moisture content, stability, and controlled biodegradation. The optimum formulation was selected based on aligning of *in vitro* parameters within acceptable range.

Physical Appearance of patch formulations were evaluated for different parameters to show its strength, efficacy, patient compliance and its importance. Physically it is observed as an orange yellowish coloured patch with smooth texture.

No change in consistency and film formation was observed in formulation F1; it was in the suspended form. As there was no thickening agent and plasticizer was also in fewer amounts in F1, thus changes were done in F2 formulation. F2 too showed no film formation, however slight improved consistency was observed possibly due to low content of polymers including gelatin and dendrimer. However, viscosity was increased probably due to addition of dendrimer and gelatin, and less elasticity might be due to low amount of ethylene glycol. Hence, further modifications were planned in F3 formulation to address issues related to viscosity and film formation. Further, to reach to a better conclusion, addition of drug was also subsequently planned which would occupy medium space and contribute in viscosity building as it is insoluble in aqueous phase. In F3D, film formation was observed with no adhesive property. It is possible that as there was lack of water soluble polymeric content that can also act as bulking agent, patch displayed less adhesive property.

The thickness of the wound healing patches was found to be in between 0.17±0.1 to 0.21±0.2. The thickness of all the patches did not varied significantly around optimized formulations. Test was performed and repeated number of times till the patch started showing breaks. The folding endurance of patches was found to be in between 23±1.2 to 34±4.5. In case of F4 formulation the patch was formed with ambient adhesive property, however, texture was not very smooth. As there was no improvement in folding endurance of patch due to addition of excess polymeric base, further modifications were done in formulation F6. Formulation F6 patch displayed no brittleness, quite good elasticity, and folding endurance compared to earlier batches. Film formation was rapid, uniform and texture of the film was found to be improved compared to earlier trials. Although, adhesion is not an important parameter in wound healing patch, in contrast to conventional dermal patches, however, significantly small adhesion is acceptable, which F6 formulation displayed upon physical examination. As changes were done in quantity of plasticizer to improve the property of patch in F6, some parameters were taken as critical in further formulations to get the best results.

The drug content of the formulations was observed to be within 75%±0.2% and 88%±0.5% range. Formulation trials F6D to F8D displayed above 90%, which was considered as an acceptable figure.

The percentage moisture content of formulated batches was found to be in between 8.7±0.3% and 21.9±0.8%. Thus controlled humidity conditions are necessary for such formulation during manufacturing and storage. Therefore the moisture uptake nature by the different formulation techniques follows the order: F3D > F4D > F6D> F7D> F8D. Also taking view from other formulation the amount of moisture is optimum with regard to overcoming brittleness and controlling the sustained degradation rate of patch. As represented in the *in vitro* release data the highest dissolution rate and drug release was shown by F8 followed by, F7 and F6 formulation as release was higher in F8 due to ratio of Drug: HPMC: EG and CPEGC : Drug. The highest dissolution rate in Phosphate Buffer (pH 6.8) and drug release was shown by F8 and F4 formulation as there was early burst observed in it and ethylene glycol was providing medium in matrix of patch.

The degradation rate of formulations were observed to be consistent with release rate and sustained too. It was observed during development trials the difference in performance among F6, F7 and F8 formulation batches is minimized and acceptance criteria after optimization**.** The pattern of degradation is erodible and due to consistency in the patches as also observed due to uniformity of drug's distribution is attributable to use of blend of water soluble cellulosic and degradable dendritic polymers. This consistency in degradability is also reflected in *in vitro* release pattern. The mechanism of degradation and presentation of drug involves two steps; namely degradation of patch and release of part of drug in solubilized or finely dispersed state due to its close association with CPEGC dendrimers. The release patter of the drug is also dependent upon the rate of degradation of the patch on the skin surface and its dissociation from the CPEGC dendrimers. Thus it can be stated that while macro-polymers including HPMC and Ethyl Cellulose take care of degradation of patch, while dendrimers create a microenvironment suitable for presentation of drug in finely dispersed and soluble form. The comparison among drug release and biodegradation rate was done simultaneously which shows that release of drug increases with time as the patch degrades, due to the erosion

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mechanism; drug erodes from the patch and the weight of final patch also decrease. The optimum drug release was shown by F6 formulation thus found to display higher acceptability to be considered as a wound healing patch that can release around 75% of drug after 8 h and patch formulation's biodegradation was up to 84% (after 8 h). All optimization trial formulations displayed stability under tested condition for up to 6 month. The drug's presentation as solubilized form due to incorporation of CPEGC dendrimers play critical role in release control and uniform application of drug on the skin surface.

TABLE I
FORMULATION TRIALS

INGREDIENTS (in mg or solvent in ml)	F1	F2	F3D	F4D
Curcumin	NT	NT	10	10
CPEGC Dendrimer	NT	0.5	0.5	1.0
Gelatin	NT	25	25	25
HPMC K4M	3	5	7	3
Ethyl cellulose	5	5	5	5
PVP	NT	20	NT	NT
PEG	NT	NT	NT	NT
Ethylene Glycol	0.3	0.3	0.3	0.7
Acetic acid	0.2	0.2	0.2	0.2
Dichloromethane	2.5	2.5	2.5	2.5
Methanol	2.5	2.5	2.5	2.5

TABLE II
OPTIMIZATION TRIAL FORMULATION

INGREDIENTS (in mg or solvent in ml)	F6	F7	F8
Curcumin	10	10	10
CPEGC Dendrimer	0.25	0.5	1.25
Gelatin	25	25	25
Hydroxypropyl Methyl cellulose K4M	5	7	3
Ethyl cellulose	5	5	5
PVP	NT	NT	NT
PEG	NT	NT	NT
Ethylene Glycol	0.3	0.3	0.7
Acetic acid	0.2	0.2	0.2
Dichloromethane	2.5	2.5	2.5
Methanol	2.5	2.5	2.5

PEG: polyethyleneglycol; PVP: polyvinylpyrrolidone

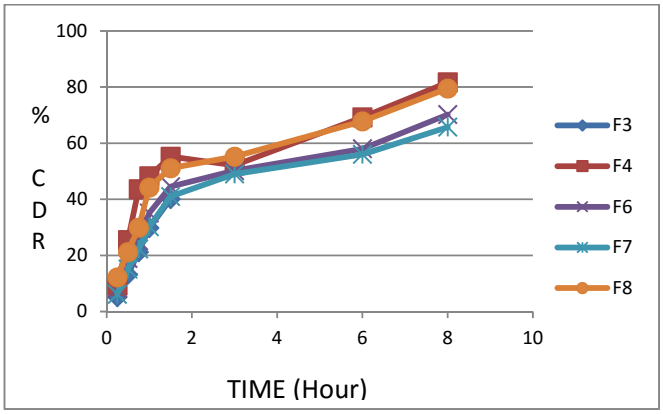


Fig. 1 *In vitro* drug release profile of formulations; % CDR: Percentage cumulative drug release

IV. CONCLUSION

The optimized formulations displayed improved folding endurance, degradability and *in vitro* release pattern that presents it as the acceptable choice as wound healing patch.

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