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Formulation and Evaluation of Curcumin-Honey Hydrogels UsingBox Behnken Design

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ABSTRACT

The main objective of this research work is to develop polymeric formulation to be applied in the treatment of skin wounds. Hydrogels are one of the most popular types of wound dressings. In addition to this, hydrogels have been found to promote fibroblast proliferation by reducing the fluid loss from the wound surface and protect the wound from external noxae necessary for rapid wound healing. Curcumin and honey have proven wound healing activities. It is envisaged to utilize the properties of both the herbal actives by formulating them in a suitable dosage form for the effective management of wounds. On the basis of DOE, four formulations were selected as optimizedformulation for preparation of hydrogel because the results of experimental values for composition of gels are more similar to the predicted values and also these are within limit. It was observed from the data as shown that collagen and Chitosan solution concentration affected the percentage release of drug from the dosage form. It was found that cumulative drug release from formulation was reduced insignificantly when Collagen and Chitosan increased. It was due to the fact that with chitosan at concentration of 0.25% and Collagen concentration 1.25% showed good porosity and swelling ratio that allowed the better absorption of buffer media through polymeric network and hence release was prolonged upto 24 hrs.

Key words: Hydrogels, DOE, Curcumin, Formulation, Evaluation

INTRODUCTION

The skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. The skin is continuous, with the mucous membranes lining the body's surface (Kanitakis, 2002). When the epithelia line integrity of the skin disrupts or when cellular and anatomical or functional continuity of living tissue breaks or lost, the condition is regarded as wound. Due regards to some world estimations about 6 million

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people suffer chronic wounds world over. Inflammatory mediators produced at the site of wound results into pain and swelling when the condition of unhealed wound continues for long. Wounds are invitation to many kind of infection and may lead to enhance the recovery of injured patients. Thus an appropriate method for healing of wounds is must in restoration of disrupted anatomical continuity and disturbed functional status of the skin which may result into overall health management (Kumar *et al.*, 2007;Roberts *et al.*, 1998).

Briefly, hydrogels are three-dimensional, hydrophilic, polymeric networks proficient in absorbing a great amount of water or biological fluids. Owing to their high water content, porosity and soft consistency, they intently simulate natural living tissue, more so than any other category of synthetic

biomaterials. Hydrogels can either be chemically durable or they may eventually disintegrate and dissolve (Peppas *et al.*, 2000). Hydrogels are also known as 'reversible' or 'physical' gels if molecular entanglements and/or secondary forces such as ionic, hydrogen bonding or hydrophobic forces play the principal role in forming the linkage. Physical gels are often rescindable and it is achievable to dissolve them by altering the environmental conditions, such as pH and the ionic strength of solution or temperature. In 'permanent' or 'chemical' gels, the linkage of covalent bonds linking distinct macromolecular chains can be attained by cross-linking polymers in the dry state or in solution (Hoffman, 2012). Curcumin (CUR), a constituent of Curcuma longa (Family- Zingiberaceae), chemically known as diferuloyl-methane has been reported to possess anti-oxidative (Srimol et al., 1973), anti-inflammatory (Huang et al., 1988), anticarcinogenic (Rao et al., 1970), and hypocholes- terolemic properties, Some of the novel formulations developed using curcumin include liposomes (Bangham et al., 1974), solid lipid nanoparticles (Tiyaboonchai et al., 2007) transdermal film etc. Following oral administration (up to 8 g per day), it is poorly absorbed, and only the traces of compound appear in blood. It undergoes extensive first-pass metabolism, and hence is a suitable candidate for topical gel formulation. The main objective of this research work is to develop polymeric formulation to be applied in the treatment of skin wounds. Hydrogels are one of the most popular types of wound dressings. In addition to this, hydrogels have been found to promote fibroblast proliferation by reducing the fluid loss from the wound surface and protect the wound from external noxae necessary for rapid wound healing. Hydrogels of natural origin are known to have several advantages over synthetic. There are so many synthetic drugs available for the treatment of wounds but herbal drugs is preferred due to economic and fewer side effects.

Among the formulations useful on damaged skin, hydrogels have shown the superiority as they can provide a moist environment for the wound and at the same time deliver the incorporated drug to the wound. Curcumin and honey have proven wound healing activities. It is envisaged to utilize the properties of both the herbal actives by formulating them in a suitable dosage form for the effective management of wounds.

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MATERIAL AND METHODS

Material

Curcumin was obtained as gift sample from Bioplus Life Science, Banglore, Honey was purchased from Ash Chemie India, Thane, Chitosan was purchased from Himedia, Mumbai and Collagen was purchased from Megma Health Corporation, India. Other materials used in the study (methanol, potassium dihydrogen phosphate, etc.) were of analytical grade. Double-distilled water was used throughout the study.

Methods

Formulation development of curcumin-honey hydrogel

Chitosan (1%) solution was prepared in 0.5% (w/v) acetic acid solution with continuous stirring. The drug and polymers (Curcumin, Honey and Collagen) at different ratios (Table 1) were mixed in previously prepared Chitosan solution by vortexing for 1 h and poured into glass moulds. They were then frozen at −20 °C for 18 h and then thawed at room temperature for 6 h for three consecutive cycles. After three F–T cycles, the hydrogel samples were dried for 6 h at 50°C under vacuum. Theywere then soaked in distilled water for 24 h up to a constant weight to remove the soluble parts. The gels were then dried again at 50°C under vacuum (Cho *et al.*, 2010).

Table 1: List of variables employed in 3^2 factorial designs

Factors	Levels	
	Low (-1)	High (+1)
Curcumin (%)	0.25	
Honey (%)	0.5	1.0
Collagen (%)	0.5	2
Chitosan (%)	1.5	3.0

Final equation in terms of coded factors

Gel strength= $+9.56+0.0213A+1.76B+0.1037C-0.0050AC-0.2125BC-0.1720A^2-0.6945B^2+0.1680C^2$

Viscosity$=+8674.84+342.13A-153.99B+554.06C-0.5500AB-1314.90AC+188.08BC-1245.96A^2-2154.18B^2-137.33C^2$

Spreadability = +6.43++0.0000A+0.0200B+0.8725C

Drug content = +94.96+1.43A-0.3250 B + 0.1000 C -5750 AB -0.4750 ac +0.9750 BC

Characterization of hydrogel pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter

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(Guptal *et al.*, 2010). Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the gel and pH ofselected formulation was measured and readings shown on display were noted.

Measurement of viscosity

Viscosity measurements of prepared topical hydrogel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; the results of viscosity are shown in tableno. 2.

Determination of gel strength

The method by which the properties of polymeric system may be conveniently determined is textureprofile (TA-XT2 Texture analyzer). The experiment was done by placing the gels in standard beakerbelow the probe. In this an analytical probe is then immersed into the sample. The Texture Analyzerwas set to the 'gelling strength test' mode or compression mode with a test-speed of 1.0 mm/s. An acquisition rate of 50 points per seconds and a trigger force of 5 g were selected. An aluminumprobe of 7.6 cm diameter was used for all the samples. The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of g.

Drug content

Accurately weighed amount of gel formulation equivalent to 10mg curcumin of prepared hydrogel formulation was taken in beaker and added 10ml of methanol. This solution was mixed thoroughly and filtered using 0.45µ membrane filter. Then 0.1mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol, this solution was analyzed using HPLC method. Drug content of hydrogel formulations are shown in table 2.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determined by

applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube. The results of Extrudability of gel are shown in table no. 4.

Spreadability

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response (Contreras and Sanchez, 2002; Rao *et al.*, 2009). An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tiedwith weight pan. To determine spreadability, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80g of weight was noted. Good spreadability show lesser time to spread.

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Spreadibility (*g.cm / sec*) *Weight Tied toUpper Slide Lenthmoved on theglass slide*

Timetakentoslide

In Vitro drug diffusion study

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14sq.cms (Gonullu *et al.*, 2015). The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 37 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell.

During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed by developed HPLC method.

RESULTS AND DISCUSSIONS

A hydrogel is a crosslinked network formed from a macromolecular hydrophilic polymer. It is stable upon swelling in water and capable of absorbing a large amount of water, varying from 10% to thousands of times of its own volume. The physical properties, including swelling, permeation, mechanical strength, and surface characteristics, can be modulated through structural modification. Hydrogels based on natural polymers are currently receiving a great deal of interest, and are notable for controlled delivery of bioactive molecules and tissue engineering**.**

The hydrogels of different polymeric blends were obtained by freezing–thawing (F–T) cycle. Regular 3 factor factorial designs for 2 level was employed for screening of significant formulation and process variables involved in the development of hydrogel. Optimization of all process and formulation variables was carried out by 3^2 levels factorial design using Design of expert 12 software (DOE 12 trial version) in the hydrogel formulations. For the optimization, 17 run was designed by Quadratic randomized, Box-Benkon response surface method. The prepared formulations were characterized for viscosity and percentage assay. Curcumin, honey, collagen and chitosan were the key response variables investigated thoroughly for selecting the significant formulation and response variables. Characterization of hydrogel was performed on the basis of pH measurements, Measurement of viscosity, Determination of gel strength, Drug content, Extrudability study, Spreadibility, *In Vitro* drug diffusion study and Stability studies. The gel strength was found for formulation F1 to F17 was found 9.5 to 10.42 g/s respectively. The pH values of the prepared Hydrogel were within acceptable limits of 6.9±0.1-7.2±0.1.The results of viscosity were found between 4868.5cps to 8864.7cps.Drug content is most important in hydrogel

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formulation and the data found are satisfactory. It was found to be 92.32 and 99.12% which shows the good capacity of formulation to hold the drug. The results of Extrudability was found in formulation HGF1, HGF2, HGF3, HGF4 was found 185±4, 192±3, 190±8 and 210±5 g. A modified apparatus was used for determining spreadability. The spreadability was measured on the basis of slip and drag characteristics of the gels and was in the range of 4.3 to 7.1 cm. The gels should have optimum spreadability because very high and very low spreadability values indicate that the application of the gel to the site is difficult. *In-vitro* drug release study of the optimized Hydrogel (HGF1, HGF2, HGF3 and HGF4) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffer pH 7.4 for a period of 24 hours. The data obtained from diffusion studies are summarized in Table 6. The release rate of Curcumin from hydrogel formulation over dialysis membrane was significantly higher than its transport across skin, indicating the barrier properties of skin for drugs. The *in vitro* release data were fitted into different kinetic models viz Zero-order, First order, Higuchi model and Korsmeyer Peppas equation. Hydrogel released drug in controlled release manner over 24 hrs. It was observed from the data as shown that collagen and Chitosan solution concentration affected the percentage release of drug from the dosage form. It was found that cumulative drug release from formulation was reduced insignificantly when Collagen and Chitosan increased. It was due to the fact that with chitosan at concentration of 0.25% and Collagen concentration 1.25% showed good porosity and swelling ratio that allowed the better absorption of buffer media through polymeric network and hence release was prolonged upto 24 hrs. At Collagen concentration increased polymeric structure so formed are somewhat rigid due to increasing viscosity of solution. Hence resist diffusion of buffer media in hydrogel polymeric network.

Table 2: Evaluations of hydrogel formulations of box-behnken design

Formulation	Response 1: Gel Strength (g/s)	Response 2: Viscosity (cps)	Response 3: Spreadability (g.cm/sec)	Response 4: Drug Content (%)
F1	9.5	8675.1	6.53	95.5
F2	9.61	8675.2	6.57	95.3
F3	9.61	8675.4	6.58	95.4
F4	9.5	8674.2	6.55	95.1
F5	9.6	8674.3	6.56	95.2
F6	10.42	6947.7	7.3	96.2
F7	8.1	7185.3	7.1	94.8
F8	10.4	5205.2	6.51	92.8
F9	7.23	6195.1	6.51	95.3
F10	9.54	7084.8	7.1	95.3
F11	9.55	8348.2	7.2	93.5
F12	9.58	8864.7	4.4	97.3
F13	9.57	4868.5	4.3	93.6
F14	10.88	5274.1	6.51	95.3
F15	10.81	5273.1	6.52	93.5
F16	6.6	5277.4	6.54	97.2

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F17	6.5	5274.2	6.53	93.1

On the basis of DOE, four formulations were selected as optimized formulation for preparation of hydrogel because the results of experimental values for composition of gels are more similar to the predicted values and also these are within limit.

Table 3: Experimental results with predicted responses

Formulation	Composition (%) Curcumin/Chitosan/collagen/Honey	Response	Predicted value	Experimental value
HGF1	0.25/2.389/1.191/0.595	Gel Strength (g/s)	9.409	9.5
		Viscosity(cps)	8457.18	8675.1
		Spreadability(g.cm/sec)	5.886	6.53
		Drug content (%)	95.302	95.50
HGF2	0.25/2.250/1.250/0.750	Gel Strength (g/s)	9.564	9.5
		Viscosity (cps)	8674.84	8674.2
		Spreadability g.cm/sec)	6.430	6.55
		Drug content (%)	94.965	95.10
HGF3	0.25/3.000/2.000/0.750	Gel Strength (g/s)	10.471	10.42
		Viscosity (cps)	5462.28	6947.7
		Spreadability(g.cm/sec)	6.450	7.3
		Drug content (%)	95.490	96.2
HGF4	0.25/2.250/0.500/0.500	Gel Strength (g/s)	6.961	6.6
		Viscosity (cps)	6171.3	5277.4
		Spreadability(g.cm/sec)	5.537	6.54
		Drug content (%)	96.165	97.2

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Table 4: Extrudability of HGF1 to HGF4 hydrogel formulations

Code	Extrudability(g)
HGF1	185±4
HGF2	192±3
HGF3	190±8
HGF4	210±5

Average of three Determinations (n=3) mean ± S.D.

Table 5: pH of HGF1 to HGF4 hydrogel formulations

Code	pH
HGF1	6.9±0.1
HGF2	7.1±0.1
HGF3	7.0±0.2
HGF4	7.2±0.1

Average of three Determinations (n=3) mean ± S.D.

Table 6: Regression analysis data of Hydrogel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
HGF1	0.774	0.938	0.922	**0.964**
HGF2	0.608	0.761	0.825	0.937
HGF3	0.649	0.817	0.854	0.949
HGF4	0.793	0.899	0.941	0.957

CONCLUSION

Curcumin, honey, collagen and chitosan were the key response variables investigated thoroughly for selecting the significant formulation and response variables. Characterization of hydrogel was performed on the basis of pH measurements, Measurement of viscosity, Determination of gelstrength, Drug content, Extrudability study, Spreadibility, *In vitro* drug diffusion study and Stability

studies. It was observed from the data as shown that collagen and Chitosan solution concentration affected the percentage release of drug from the dosage form. It was found that cumulative drug release from formulation was reduced insignificantly when Collagen and Chitosan increased.

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