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In vitro evaluation of topical gel prepared using natural polymer

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Abstract

Nimesulide is a second generation non–steroidal anti–inflammatory agent, which is widely used in the long term therapy of rheumatoid arthritis, in alleviating pain and inflammation. But its short half-life (only 3–4 hr), so its causes more fluctuation. After oral administration Nimesulide causes to produces heart burn, nausea, loose motions, pruritus, etc. The present study based on the preparation of bioadhesive topical gel of Nimesulide, so as to avoid all gastric side effects. For the preparation of bioadhesive topical gel natural polymer *aegel marmelos* (plant Bale) was used. Bioadhesive polymers are the agents which increases the contact between the formulation and biological membrane, so as to avoid the fluctuation of formulation and behave as a sustained release formulation. In the present study, prepared bioadhesive topical gel was evaluated with the help of different parameters like drug content, spreadability, extrudability, swelling index study, *in–vitro* drug diffusion study, *in-vitro* drug release kinetic study and *ex–vivo* bioadhesive measurement. On the basis of *in–vitro* drug diffusion study and *ex–vivo* bioadhesive measurement property of gel, we have concluded that natural polymer *aegel marmelos* is the best polymer for the preparation of sustained release bioadhesive topical gel.

Keywords: Topical gel; Bioadhesion; Natural polymer

Introduction

Bioadhesion is the phenomenon between two materials, which are held together for extended periods of time by interfacial forces. It is referred as bioadhesion when interaction occurs between polymer and epithelial surface; mucoadhesion when occurs with the mucus layer covering a tissue. Generally bioadhesion is deeper than the mucoadhesion [1].

Nimesulide is a second generation non–steroidal anti–inflammatory agent, which is widely used in the long term therapy of rheumatoid arthritis, in alleviating pain and inflammation. Its biological half–life have been reported to be 3 to 4 hrs, necessitates multiple daily dosing for maintaining therapeutic effect throughout the day [2]. The oral use of Nimesulide is associated with side effects like gastrointestinal disturbances, epigastric pain, nausea, heartburn, vomiting and diarrhoea. Topical application of the drug prevents these side effects and offers potential advantage of delivering the drug at the site of action [3].

The U.S.P. defines gels as semisolids, either suspension of small inorganic particles or large organic molecules interpenetrated with liquid [4]. Gels are transparent or translucent semisolid formulations containing a high ratio of solvent/gelling agent. When

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dispersed in an appropriate solvent, gelling agents merge or entangle to form a three–dimensional colloidal network structure, which limits fluid flow by entrapment and immobilization of the solvent molecules. The network structure is also responsible for gel resistance to deformation and hence, its viscoelastic properties [5].

In this study, Nimesulide topical gels were formulated using natural bioadhesive polymer and were evaluated with different studies.

Materials and methods

Materials

Natural polymer was extracted from the ripe fruit of Bale (*Aegle Marmelos*) and Nimesulide was obtained as a gift sample from Sun Pharm, Ahmadabad, India. Dimethyl sulfoxide and acetone was purchased from the SD Fine–chemical Ltd., Mumbai and Hydroxy ethylcellulose was purchased from Sisco research Lab (P) Bombay–400093, India. Triethanolamine was purchased from Universal Lab. Pvt. Ltd, Mumbai.

Methods

Extraction of Natural Bioadhesive Polymer

The mucilage from the natural source ripe fruit of bale was extracted following the method of Rao *et al.* In this method, 250 gm natural material obtained from edible fruits, vegetable and starch were soaked in double distilled water and boiled for 5 hrs in a water bath until slurry was formed. The slurry was cooled and kept in refrigerator overnight so that most of the undisclosed portion was settled out. The upper clear solution was decanted off and centrifuged at 500 rpm for 20 minutes. The supernatant was concentrated at 60°C on a water bath until the volume reduced to one third of its original volume. Solution was cooled down to the room temperature and was poured into thrice the volume of acetone by continuous stirring. The precipitate was wased repeatedly with acetone and dried at 50°C under vacuum. The dried material was powdered and kept in desiccators [6].

Preparation of Topical Gel

Gels were prepared by cold mechanical method described by *Schmolka et al.* (1972) [7, 8]. Required quantity of polymer (Natural polymer and Hydroxyethyl cellulose) was weighed and it was sprinkled slowly on surface of purified water for 2 hrs. After which it was continuously stirred by mechanical stirrer, till the polymer soaked in the water. With continuous stirring, triethanolamine was added to neutralize the gel and it maintains the pH of the gel. Now the appropriate quantity of DMSO (Dimethyl sulfoxide) was added to the gel, which behaves as the penetration enhancer, followed by the required quantity of methyl paraben as a preservative. Finally the drug Nimesulide was added to the gel with continuous stirring till drug get dispersed in gel completely. Six formulations of microparticulated intra-vaginal gel were prepared by using Natural polymer and Hydroxyethyl cellulose in different ratio. The prepared gel were packed in wide mouth glass jar covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in dark and cool place [9, 10].

Drug Content Determination

Drug content of gel was determined by dissolving accurately weighed 1gm of gels in 0.1N NaoH. After suitable dilution absorbance was recorded by using UV-visible spectrophotometer (UV – 1700, Shimadzu, Japan) at 392 nm. Drug content was determined using slope of standard curve. [2,11]The drug content was determined by using following equation:

Drug Content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor

Spreadability study of Topical gel

Spreadability was determined by apparatus suggested by *Mutimer et al* (1956) [11] which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of 'Slip' and 'Drag' characteristics of gels [12]. A ground glass slide was fixed on this block. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 Kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability [10-14].

Spreadability was then calculated using the following formula:

$$S = M \times L / T$$

Where, S = is the spreadability, M = is the weight in the pan (tied to the upper slide), L = is the length moved by the glass slide and T = represents the time taken to separate the slide completely from each other.

Extrudability Study of Topical Gel

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow one such apparatus is described by *wood et al* [14].

In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was in triplicate and the average values are presented [15]. The extrudability was than calculated by using the following formula [15]:

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm^2)

Swelling Index Study of Topical Gel

Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared topical gel, 1 gm of gel was taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml 0.1 N NaoH. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows [16, 17]:

Swelling Index (S_W) % = $[(W_t - W_o) / W_o] \times 100$.

Where, (S_W) % = Equilibrium percent swelling, W_t = Weight of swollen gel after time t, W_o = Original weight of gel at zero time.

In-vitro Drug Diffusion Study

Cellophane membrane obtained from sigma chemicals was used for this study. In Kiescary Chien (KC) diffusion cell, 1.0 gm of gel was kept in donor compartment. The entire surface of membrane was in contact with the receptor compartment containing 85 ml of 0.1 N NaoH. The receptor compartment was continuously stirred (100 rpm) using a magnetic stirrer. The temperature maintained was 37 ± 1°C. The study was carried out for 24 hrs with the interval of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. The sample was withdrawn at predetermined period of time and same volume was replaced with fresh 0.1 N NaoH. The absorbance of withdrawn sample was measured at 392 nm to estimate Nimesulide [11].

Drug Release Kinetic Study

To analyze the mechanism of drug release from the topical gel, the release data were fitted to the following equations:

a) Zero – order equation:

$$Q = k_0 t$$

Where Q is the amount of drug released at time t, and k_0 is the zero – order release rate.

b) First – order equation:

$$\text{In } (100 - Q) = \text{In } 100 - k_1 t$$

Where Q is the percent of drug release at time t, and k_1 is the first – order release rate constant.

c) Higuchi's equation:

$$Q = k_2 \sqrt{t}$$

Where Q is the percent of drug release at time t, and k_2 is the diffusion rate constant [18].

Ex–vivo Bioadhesive Strength Measurement of Topical Gel

A modified balance method was used for determining the *ex-vivo* bioadhesive strength [19]. Fresh goat hairless skin was obtained from a local slaughter – house and used within 2 hours of slaughter. The skin was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with 0.1 N NaoH [20,21].

The modified *Patel et al (*2007) [20] method was used for the measurement of bioadhesive strength. The fresh skin was cut into pieces and washed with 0.1 N NaoH. Two pieces of skin were tied to the two glass slide separately from that one glass slide was fixed on the wooden piece and other piece was tied with the balance on right hand side. The right and left pans were balanced by adding extra weight on the left – hand pan. 1 gm of topical gel was placed between these two slides containing hairless skin pieces, and extra weight

from the left pan was removed to sandwich the two pieces of skin and some pressure was applied to remove the presence of air. The balance was kept in this position for 5 minutes. Weight was added slowly at 200 mg/ min to the left – hand pan until the patch detached from the skin surface. The weight (gram force) required to detach the gel from the skin surface gave the measure of bioadhesive strength [19-21]. The bioadhesive strength was calculated by using following:

Bioadhesive Strength = Weight required (in gms) / Area (cm^2)

Results and discussion

Formulation Design of Topical Gel

Topical gels were prepared by using cold mechanical method using Natural Polymer and Hydroxyethyl cellulose in different ratio with other ingredients and solvents as given in Table 1 [10, 11]. All the prepared topical gel formulations contain different drug: polymer ratio and coded as NMG1, NMG2, NMG3, NMG4, NMG5 and NMG6.

Table 1. Formulation Design for the Preparation of Topical Gel

Ingredients	NMG1	NMG2	NMG3	NMG4	NMG5	NMG6
Nimesulide (mg)	100.00	100.00	100.00	100.00	100.00	100.00
Natural Polymer (mg)	100.00	200.00	300.00	-------	-------	-------
Hydroxyethyl cellulose (mg)	-------	-------	-------	100.00	200.00	300.00
Triethanolamine (gm)	0.23	0.23	0.23	0.23	0.23	0.23
Dimethyl Sulfoxide (gm)	2.20	2.20	2.20	2.20	2.20	2.20
Methyl Paraben (mg)	15.00	15.00	15.00	15.00	15.00	15.00
Distilled Water (gm)	up to 100 gm	up to 100 gm	up to 100 gm	up to 100 gm	up to 100 gm	up to 100 gm

Drug Content, Spreadability study, Extrudability study and Bioadhesive strength measurement of Topical Gel

From these data we have found that topical gel prepared from natural polymer having greater drug content, spreadability, extrudability and bioadhesive strength mostly NMG3 as compare to topical gel prepared from Hydroxyethyl cellulose. Table 2 shows the data for the drug content, spreadability, extrudability, and bioadhesive strength measurement of topical gel.

Table 2. Drug Content, Spreadability study, Extrudability study and Bioadhesive strength measurement of Topical Gel

Formulation code	Drug Content (mg/1gm of gel)	Spreadability (gm.cm/sec.)	Extrudability (gm./cm^2)	Bioadhesive strength (gm./cm^2)
NMG1	1.02	13.96	15.97	1.17
NMG2	1.04	14.69	16.43	1.28
NMG3	1.08	14.78	17.58	1.76
NMG4	1.03	13.81	14.09	1.02
NMG5	1.00	12.79	15.31	1.11
NMG6	1.02	11.88	16.03	1.14

Swelling Index Study of Topical Gel

From these data we found, topical gel prepared from natural polymer has greater percent swelling index mostly NMG3 as compare to topical gel prepared from Hydroxyethyl cellulose.

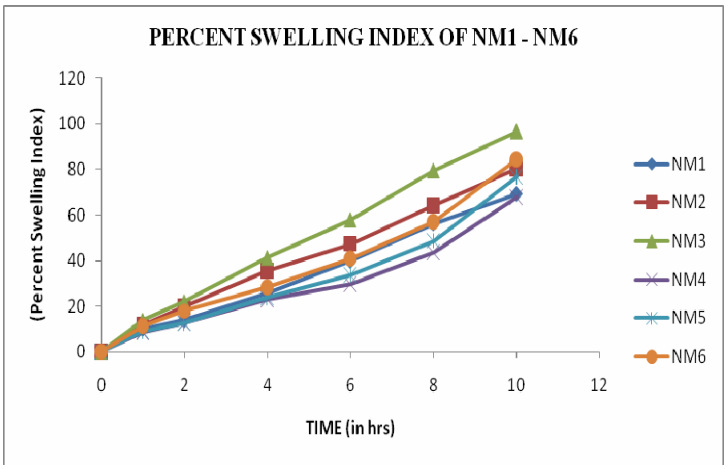

Figure 1. Percent Swelling Index of NM1–NM6

Table 3 shows swelling index study data and figure1 shows graphical representation of swelling index study.

In–vitro Drug Diffusion Study

From these data we have found that the prepared topical gel NMG3 releases 83.09 % of drug over a period of 24 hrs.

Table 3. Swelling Index Study of Topical Gel

Time	Swelling Index (%S_w)					
(hrs.)	NMG1	NMG2	NMG3	NMG4	NMG5	NMG6
1.0	9.98	11.70	13.72	8.67	9.15	11.39
2.0	13.86	19.68	21.84	12.56	12.47	17.84
4.0	25.74	35.43	41.23	22.85	23.59	28.38
6.0	39.87	47.31	57.90	29.71	33.57	40.85
8.0	56.38	64.03	79.46	43.36	48.67	57.07
10.0	69.37	80.37	96.67	67.74	76.73	84.40

Table 4 shows the data for the *in-vitro* drug diffusion study of prepared topical gel. Figure 2 shows the graphical representation of *in-vitro* drug diffusion study of topical gel.

Table 4. *In vitro* Drug Diffusion Study

Time	NMG1	NMG2	NMG3	NMG4	NMG5	NMG6
(hrs.)						
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	10.73	9.04	7.68	14.04	13.77	12.68
1.0	18.77	14.47	13.77	27.85	25.57	23.86
2.0	30.85	25.82	23.57	36.57	34.43	31.39
4.0	41.12	34.32	31.98	48.71	46.94	41.19
6.0	51.33	46.19	43.03	69.23	62.82	59.73
8.0	72.41	53.43	52.17	78.39	76.59	74.82
10.0	85.62	68.82	64.82	88.33	86.95	83.99
12.0	89.22	74.29	73.45	96.62	93.45	90.40
24.0	91.51	86.95	83.09	101.06	98.99	96.06

Drug Release Kinetic Study

Table 5 shows the data for *in-vitro* drug release kinetic study of topical gel. Natural polymer used for the preparation of topical gel is more effective, because it controls the drug greatly in comparison of Hydroxyethyl cellulose (mostly NM3). The r^2 is coefficient of correlation; K_0, K_1 and K_h are the release rate constants for zero-order and first-order and Higuchi plot, respectively.

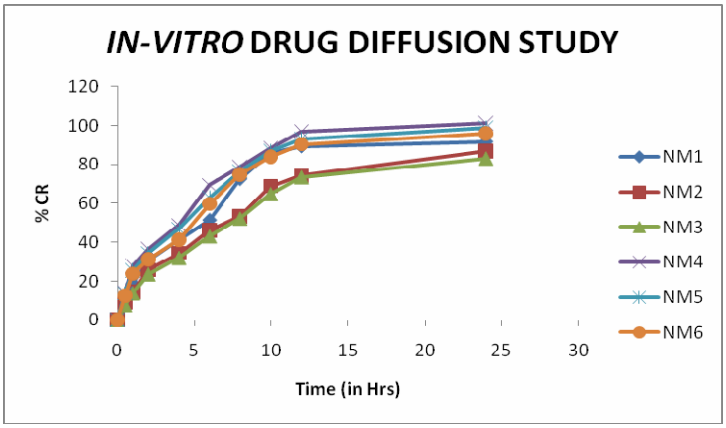

Figure 2. *In–vitro* Drug Diffusion Study of Topical Gel

Conclusion

From the above study we have concluded that the topical gel prepared from the natural polymer having good spreadability, extrudability and bioadhesive strength. So the topical gel prepared from natural polymer will be greatly for making an ideal topical preparation. NM3 has the greater swelling index properties in comparison of others it means topical gel prepared from natural polymer having the greater swelling tendency. From the *In – vitro* drug diffusion study we have concluded that the gel prepared from the natural polymer, controls the release of drug for longer period of time which will be helpful to avoid the more fluctuation and also reduces the cost of therapy.

Table 5. *In-vitro* Drug Release Kinetic study

Formulation	Zero – order		First – order		Higuchi Plot	
Code	r^2	k_0	r^2	k_1	r^2	k_h
NMG1	0.740	4.000	0.740	4.00	0.712	0.348
NMG2	0.836	3.683	0.836	3.683	0.750	0.346
NMG3	0.840	3.573	0.839	3.573	0.796	0.350
NMG4	0.735	4.176	0.734	4.164	0.658	0.336
NMG5	0.749	4.126	0.749	4.126	0.667	0.336
NMG6	0.757	4.065	0.757	4.065	0.688	0.340

From the *in-vitro* drug release kinetic study, we have concluded that the topical gel prepared from the natural polymer releases the drug from gel by following zero-order release kinetic model means natural polymer

plays important role to controls the release of drug from topical gel. At last we have concluded the use of natural polymer for the preparation of bioadhesive preparations will surely be helpful in future.

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