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Topical Microsponge Gel: A Novel Carrier For The Management Of Pain And Inflammation

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Abstract –

The most common drug for treatment of pain and inflammation is NSAIDS. But this NSAIDS having the hazardous side effect on oral administration. To overcome the side effect of NSAIDS and to reduce the frequencies of dosing of formulation we need to prepare the formulation with prolong release. This can be achieved by preparation of microsponge of dexibuprofen in carbopol gel. Microsponge was prepared using Eudragit RS 100 as polymer and method was adapted the quasi emulsification solvent diffusion. Microsponges were incorporated in carbopol 940. Microsponge and microsponge containing gel were evaluated for Production Yield, Encapsulation Efficiency, Particle size analysis, Morphology, physiochemical parameter, drug release, analgesic and anti-inflammatory activity. From the in-vitro and ex-vivo release studies it was shown that formulation give prolong release up to 24 hours. Good analgesic and anti-inflammatory activity was obtained as compared to marketed formulation. From this study it was found that microsponge gel formulation has safe and potential as alternative dosage form for the application on painful and inflamed condition.

Key words- microsponge, SEM, ex-vivo, analgesic and anti-inflammatory activity.

Introduction

Microsponges are the porous structure polymeric microparticles that are used for prolonged topical administration. They are designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effects, and modify drug release profiles.[1] Now a day, microsponge have been increasingly investigated to achieve targeted and sustained release of drugs. The microsponges i.e. microsponge drug delivery system consist of size 5 -150 µm in diameter & with a typical 25 µm sphere can up to 250000 pores and an internal structure of pore equivalent to 10 ft. in length which provides a total pore volume of 1ml/gm. This kind of system exhibits large reservoir within microspongic structure which can be loaded with the same weight of active ingredient.[2]

For the successful management of pain and inflammation, dexibuprofen was the first choice of drug from the class of "profen" due to the low incidence of unwanted side effects. Dexibuprofen has been tested and proved to be effective as an analgesic, anti-pyretic and anti-inflammatory after systemic administration. When administered as a topical preparation, Dexibuprofen has been shown to be an effective topical analgesic and anti-inflammatory for the relief of mild to moderate arthritic pain, mustratum corneumular pain, backache, sprains, strains, lumbago and fibrositis by virtue of percutaneous absorption.[3] The oral administration of dexibupfrofen have some disadvantages like, Upset stomach, nausea, vomiting, headache, diarrhea, constipation, dizziness, or drowsiness. The disadvantages of dexibuprofen drug may be overcome by loading the drug into topical microsponge.
The rationale for developing Dexibuprofen microsponge gel formulations was to achieve efficacy by direct penetration of the active agent into painful tissue and to reduce the drug concentrations in blood at a low level.

Materials and methods-
Dexibuprofen was obtained as a gift sample from Glochem Industries Limited, Hyderabad, (Andhra Pradesh), India. Eudragit RS100 was obtained from Yarrow Chem. Products, Mumbai. Carbopol 940 was obtained from Loba chemicals Mumbai. Nylon membrane was procured from Hi media, Mumbai. All other chemicals used were of analytical grade and were used without any further chemical modification.

Method of preparation[4]
Microsponges were prepared by the quasi emulsification solvent diffusion method.
Inner phase: It is prepared by dissolving the Eudragit RS100 in ethanol. The drug was added to the solution and dissolved under ultra-sonication at 35ºC for 15mins.
Outer phase: Dissolving PVA in distilled water and the process was carried out at room temperature, then the inner phase was poured into outer phase at room temperature. After emulsification, the mixture was continuously stirred at 500 rpm for 2Hrs. After the formation of Microsponges, the mixture is filtered to separate the Microsponges. The product was washed and dried in oven at 40ºC. For the evaluation of the Drug: Polymer ratio on the physical characteristics of Microsponges. Four different weighing ratio of drug to Eudragit RS100 are 1:1, 1:2, 1:3 and 1:4, 1:6 and different concentration of PVA were taken for preparation of microsponge.

Table 1. Optimum value for preparation of microsponge

Drug Polymer ratio	1:1, 1:2, 1:4, 1:6
Amount of drug	2gm
PVA	0.5-1.0 gm
Inner phase solvent	Ethyl Alcohol
Amount of inner phase solvent	10ml
Amount of water in outer phase	100 ml
Temperature of inner phase	37ºC
Stirring rate	500rpm
Stirring time	2 hours

Preparation of dexibuprofen microsponge loaded gel[5]
Blank carbopol gel was prepared by dissolving 10 gm of Glycerin in water. Then 1 g of carbopol 940 which were mixed so as to get the paste like consistency. After that 90 ml of purified water was added slowly to the mixture of drug, carbopol 940 and glycerin with constant stirring. Finally, triethanolamine was added dropwise to adjust the ph to 6.5–7.5. Air bubbles were allowed to diffuse out of the gel. Then add accurately weighed equivalent amount of optimized microsponge of dexibuprofen having a different drug/polymer ratio was mixed gently and separately with the gel base to obtain 2% w/w of microsponge-loaded gel.

Table 2. Composition of unentraped dexibuprofen gel and microsponge loaded gel

Sr no.	Ingredients (% w/w)	Quantity taken (gm)
1	Dexibuprofen microsponge equivalent to	2
2	Glycerin	10
3	Triethanolamine (pH adjusted to 7.0)	q.s.
4	Methyl Paraben	0.2
5	Propyl Paraben	0.06
6	Water q.s. to	100

Evaluation of dexibuprofen loaded microsponge
Drug Excipient Compatibility Studies Using FT-IR
Fourier transform infrared spectroscopy (FT-IR) was used to study the compatibility of dexibuprofen with the excipients used in formulation. [5,6]
Determination of Production Yield
It was calculated by following equation, [7]

$$\% \text{ of Production Yield} = \frac{\text{Practicle yield}}{\text{Therotical yield (excipients+drug)}} \times 100$$

Drug content and Encapsulation Efficiency
The drug content and entrapment of drug in the microsponges was calculated by the following formulas: [7]

$$\text{Actual drug content (\%)} = \frac{\text{Actual drug content in weighed quantity of Microsponges}}{\text{Weighed quantity of microsponges}} \times 100$$

$$\text{Encapsulation Efficiency} = \frac{\text{Actual drug content in weighed quantity of microsponges}}{\text{Theoretical drug content in microsponges}} \times 100$$

Particle size analysis

Determination of average particle size of Dexibuprofen microsponge was determined by an optical microscope using calibration ocular and stage micrometer under regular polarized light. [7,8]

Morphology Study Using Scanning Electron Microscope

The internal and external morphology and surface topography can be studied by scanning electron microscopy (SEM). [7,8]

Evaluation of dexibuprofen microsponge gel

Visual Inspection

The organoleptic properties such as color, texture, consistency, homogeneity and physical appearance of gel containing microsponges were checked by visual observation.[8]

pH Measurement

Gel formulation pH was recorded using digital pH meter. 5g gel was dispersed in 45ml distilled water at 27ºC and solution pH was measured.[9]

Viscosity Measurement

The viscosity of gel formulation was determined. The viscosity was determined using a Brookfield digital viscometer (DV-E model). [9]

Spreadability Studies

Spreadability of dexibuprofen microsponge gel was measured in terms of diameter of gel circle produced when placed between two glass plates of definite weight. A weighed quantity 0.5 gm gel was placed within a circle of 1cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 gm was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreading of the gels was noted. (Diameter of the spread circle – initial diameter).[10]

Drug content

Accurately weighted portion of gel (100 mg) was dissolved in 15 ml phosphate buffer (pH 7.4) under ultrasonication for 15 minutes and the volume was made up to 50 ml with same solution. After filtration and suitable dilution, samples were assayed spectroscopically (JASCO V-530, Japan) at 223 nm using a calibration curve.[9,10]

***In vitro* release of drug from gel formulations through nylon membrane**

Nylon membrane with pore size 0.2 µm was mounted between the compartments of the diffusion cell. The diffusion cell was maintained at 37°C. The formulation (0.5 g) was gently placed in the donor chamber. At 0.5, 1, 2, 3, 4, 5, 6, 7,8, and 24 h, 2 ml of the solution from the receptor chamber was removed. The aliquots were analyzed for drug content by UV spectroscopy. and replaced immediately with an equal volume of fresh phosphate buffer The cumulative amount of dexibuprofen permeated through nylon membrane was plotted as a function of time. [11]

***Ex-vivo* skin permeation study**

The porcine skin was clamped between the donor and the receptor chamber of Franz diffusion cell. Further procedure was done as per the in vitro drug release through nylon membrane. The cumulative amount of dexibuprofen permeated through porcine ear skin was plotted as a function of time. [11,12]

RELEASE KINETICS OF THE OPTIMIZED FORMULATIONS

The data obtained from *in vitro* release studies of best of one formulation was fitted to various models such as zero order, first order, Higuchi and Korsmeyer Peppas to obtain the kinetic modeling of drug release.[13]

To study the release kinetics the data obtained from *in-vitro* drug release studies were plotted in various kinetic models.

1. **Zero order rate kinetics:** % Cumulative Drug Release Vs Time
2. **First order rate kinetics:** Log Cumulative. % of drug remaining vs time.
3. **Higuchi model**: Cumulative percentage of drug released vs square root of time.
4. **Korsmeyer Peppas model:** Log cumulative % of drug release vs log time.

Anti-inflammatory activity

Anti-inflammatory activity of microsponge gel formulations by inducing rat paw edema by carrageenan.[14, 15] In order to measure paw volume; animals were marked with permanent marker at ankle of their left hind paw to define the area of paw to be monitored. Paw edema was induced by injecting 100 µl of 1% solution of carrageenan (w/v) in normal saline into the plantar surface of the left hind paw. Half an hour after the test formulation (500 mg) was applied on the dorsal area The paw volumes were measured with a plethysmometer at 0 (before administration of carrageenan) and 1, 2, 3, 4 and 24 hours after carrageenan administration, and the change in paw volume in control and drug treated animals was calculated. The % increase in edema at each point for test formulation in comparison to control group was also calculated. The anti- inflammatory activity was expressed as % inhibition of paw edema.

$$\% \text{ edema} = \frac{b-a}{a} \times 100$$

Where,

a = Paw volume measured before producing edema.

b = Paw volume measured at predetermined intervals after producing edema and application of the formulation.

Analgesic activity

Analgesic activity of optimized gel formulations using the Hot Plate analgesiometer.[14,15] This method was used to measure the anti nociceptive effect of test formulations to an acute thermal stimulus. Acute thermal stimuli study was carried out in mice using hot plate analgesiometer. This study was carried out for 1, 2, 3, and 4hrs. Test formulation and marketed formulation was applied on dorsal surface area of mice. Latency to the heat stimulus was measured by the number of times the animal licks one of its paws in cut off time of 15 seconds. The analgesic activity was measured as % maximal possible effects.

$$\% \text{ MPE} = \frac{b-a}{b} \times 100$$

Where,

% MPE= maximal possible effects

b =Latency to respond to thermal stimulus by control.

a = Latency to respond to thermal stimulus by test formulations.

Result and discussion-

Evaluation of dexibuprofen microsponge

Drug Excipients Compatibility Studies Using FT-IR

The result of FT-IR indicate the no interaction between drug and excipients when compared with infrared spectrum of pure drug as all functional group frequencies were present. From this result it is conclude that drug was compatible with selected polymer and it was apparently stable in the microsponges.

Production Yield

It was found that production yield was greatly affected by drug:polymer ratio as well as by concentration of polyvinyl alcohol. Moreover, increase in the drug:polymer ratio resulted into increased production yield. When drug:polymer ratio was 1:1 (F-1), the production yield was very low, i.e. 72.5± 0.13%, while for drug:polymer ratio 1:6 (F-6) it was 91.3± 0.13%. With the low concentration of polyvinyl alcohol (0.5 gm, F-4), the production yield was quite low, i.e. 85.5± 0.05%. As the concentration of polyvinyl alcohol was increased (from 0.5 to 1.0 gm), the production yield was also found to be increased. The reason behind that the diffusion rate of ethanol from concentrated solution to aqueous phase at higher drug:polymer concentration provides the more time for formation of droplet, thereby improving the yield.

Table 3. Production yield of dexibuprofen microsponge

Formulation code	Production yield (%)
F-1	72.5± 0.13
F-2	78.4± 0.11
F-3	83.1± 0.12
F-4	85.5± 0.05
F-5	87.1± 0.03
F-6	91.3± 0.13

*Each value is average of three separate determinations ±SD

Drug content and Encapsulation Efficiency

It was observed that all the batches formulation showed satisfactory drug content. The drug content values varies from 40.67±0.02 % and 83.34±0.01 % (Table 4). The amount of drug entrapped into prepared microsponges was analyzed and it was observed that the encapsulation efficiency was lower that the theoretical value because the drug loading efficiency did not reach 100 %. As per the literature survey, it may be due to dissolution of some drug in the aqueous phase. There is the increase in the drug loading with increase in drug polymer ratio as given in (Table 4). The reason behind the increasing the drug loading due the more polymers is available for the drug entrapment. The encapsulation efficiency was varied from 71±0.12 % to 91±0.08 %.

Table 4. Drug content and encapsulation efficiency

Formulation code	% Drug content±SD	Encapsulation efficiency ±SD
F1	40.67±0.02	71±0.12
F2	56.55±0.22	78±0.08
F3	66.23±0.42	83±0.14
F4	77.52±0.34	85±0.12
F5	78.18±0.08	88±0.12
F6	83.34±0.01	91±0.08

Particle size analysis

Particle size of microsponge of dexibuprofen with different concentration of polymer having varied size due to change in the polymer concentration. The average particle size distribution of formulated microsponges of F-1 to F-8 was found to be in range of 18-35 µm (Table 5). All the batches showed the intact and spherical particle in optical microscopy.

Table 5. Average particle size of Dexibuprofen microsponges

Formulation code	Average particle size (µm) (Mean± SD) n=3
F-1	18.9±1.02
F-2	20.5±1.05
F-3	21.3±1.54
F-4	33.1±1.32
F-5	34.1±1.25
F-6	34.7±1.23

Morphology Study

The images of SEM indicated that microsponges formed were more porous, predominantly spherical and not much entire dexibuprofen crystals were observed visually. Pores were induced by diffusion of solvent from surface of microsponges. The appearance of the particles was such that they were termed as microsponges.

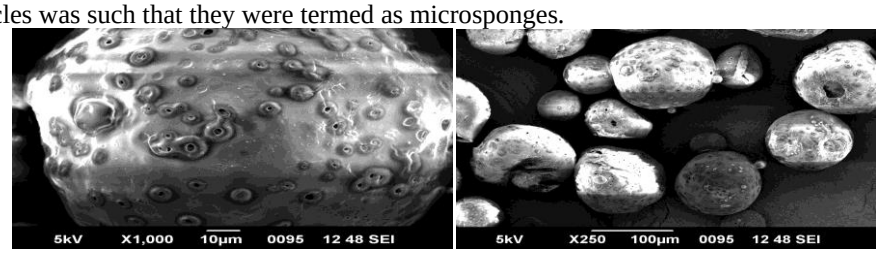

Fig 1. SEM image of dexibuprofen Microsponges

Evaluation of dexibuprofen microsponge gel

All the formulation was evaluated for physiochemical parameters like color, texture and appearance, pH, Viscosity and spredability. The results are shown in table 6.

Time(hr)	% Cumulative Drug Release					
	F-1	F-2	F-3	F-4	F-5	F-6
0	0	0	0	0	0	0
1	1	25.87	24.76	23.45	22.21	23.23
2	2	34.89	33.89	32.76	32.56	34.45
3	3	56.56	55.89	53.87	54.98	52.34
4	4	60.94	58.9	57.38	55.43	56.14
5	5	64.34	63.87	58.35	56.36	54.28
6	6	69.78	68.63	60.34	58.99	60.34
7	7	73.99	72.64	71.45	70.45	64.87
8	8	75.83	73.43	72.57	71.97	66.45
24	24	90.87	88.77	87.56	86.89	85.98

Time(hr)	% Cumulative Drug Release					
	F-1	F-2	F-3	F-4	F-5	F-6
0	0	0	0	0	0	0
1	22.21	18.32	19.56	20.12	18.34	17.32
2	40.09	22.34	23.43	21.21	23.01	22.45
3	52.01	39.78	36.96	34.24	26.43	26.78
4	55.44	40.78	38.56	35.78	35.32	36.45
5	62.01	45.87	43.94	40.83	40.21	42.52
6	64.34	53.45	49.03	48.23	45.32	44.19
7	70.32	57.98	55.43	50.34	53.54	49.23
8	71.83	60.45	56.73	52.84	54.34	53.21
24	80.93	79.67	77.94	78.56	78.54	77.45

Table 6. Physiochemical evaluation of microsponge gel formulation

Diffusion studies

In this seen that there is increase in polymer concentration to drug there is decrease in percentage drug release this may be because of the amount of polymer is less available to encapsulate the drug and there is more drug is available for release. In case of low drug release, there may be formation of thick polymer matrix wall which may cause the longer diffusion path and result in to decreased drug release. It has been reported that with increasing amount of PVA from batches F3-F6, the drug release went on decreasing. This result because of the polymer matrix release drug after swelling and time required for swelling of polymer is directly proportional to stabilizer concentration. The cumulative % drug release data were given in table 7.

Comparing the diffusion study from two type of membrane, the higher values for the diffusion through the nylon membrane compared to animal skin this results might be the variois factors like thickness, composition, type of diffusion of the membrane involved in study. The diffusion through skin is lower than the artificial membrane. This study given the actual concentration of drug in system.

Table 7. Cumulative % Drug Release of Dexibuprofen Microsponge in Different Formulations (Nylon membrane and porcine skin

Parameter	F-1	F-2	F-3	F-4	F-5	F-6
Appearance	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid
Colour	Cloudy Transparent gel	Cloudy Transparent gel	Cloudy Transparent gel	Cloudy Transparent gel	Cloudy Transparent gel	Cloudy Transparent gel
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6.5	6.8	7.0	7.0	6.8	6.8
Apparent Viscosity (cps)	6260	7010	6520	7200	7050	7100
Spreadability (gm/cm/sec)	25.23	27.58	26.41	24.54	24.14	24.51
Total drug content (%± RSD)	99.92±1.23	99.99±1.23	99.1±1.20	99.99±1.33	99.92±1.23	99.97±1.23

Release kinetics of Dexibuprofen microsponge in formulation F-6

The best-fit data in terms of *R2* values are given in Table 8, and the best-fit model selection was based on highest *R2* value. For microsponges prepared with different drug:polymer ratio and different PVA as surfactants, Krosmeyer-Peppas model was more appropriate. In our study, polymer ratio and the concentration of surfactant influenced the dexibuprofen release and majority of formulations released the drug by both Fickian diffusion and the case II transport because the *n* values were ranged from 0.45 to 0.97. Microsponges-loaded gel showed drug release in a control manner, which is the key to reduce side effects associated with topical drug delivery system. Formulation F-6 showed 82.45 % drug release in 24 h and followed Higuchi model of release kinetic.

Table 8. Regression Coefficient (r^2) values of Kinetic model for formulation F-6

Anti-inflammatory activity

percentage edema was suppressed significantly, and at the end of 24 hr, the degree of edema was almost brought back to the baseline value.

The percentage inhibition in edema exhibited by F-6 formulation showed significantly higher at all the time points compared to the control as shown in Table 9. Thus, the microsponge gel formulation was found to be more effective in inhibiting rat paw edema as compared to control.

Table 9. % edema in optimized gel and marketed formulations

	Kinetic drug release		Mechanism of release		
	Zero Order	First Order	Higuchi	Korsemeyer Peppas	
Formulation	Correlation oefficient(r^2)	Correlation coefficient (r^2)	Correlation coefficient r^2)	Slope 'n'value	Correlation coefficient(r^2)
F-6	0.930	0.907	0.957	0.556	0.939

*Control group was treated with saline after induction of edema with carrageenan.

Analgesic activity

The test formulation showed improved anti-nociceptive activity as compared to marketed formulation. As seen from the results, due to the application of the drug formulations, latency to respond to thermal stimuli was increased; these results further support the observations of Carrageenan induced rat paw edema test. It can be concluded, with the experimental conditions of this study, that developed formulations showed superior efficacy compared to the marketed preparation.

Table 10. % maximal possible effect of marketed and microsponge gel formulation

Time(in hrs)	Marketed formulation	F-6
0	0	0
1	33.33	44.44
2	36.11	47.22
3	38.60	55.66
4	45.83	66.66

Conclusion

In this research work dexibuprofen microsponge gel for topical delivery has successfully produced. This delivery system can be utilizes for long term treatment of chronic join pain, muscular pain and disability due to arthritis. The topical drug delivery system can target the inflamed tissue and avoid first pass mechanism, thereby minimizing the side effects of drugs. The prepared formulation of dexibuprofen microsponge in gel can be a novel approach for treatment various type of pain and inflammation and also show sustained release characteristic. The topical application of microsponge formulation for prolonged period at the site of pain can provide more relief to arthritis patient and can avoid gastrointestinal side effect of drug by oral administration.

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