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ORIGINAL ARTICLE

Methoxsalen Emulgel for Topical Application in the Vitiligo Treatment

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ABSTRACT
Methoxsalen is a psolaren used in treatment of pigmentary skin disorders. Emulgel is a combination of emulsion and gel have emerged as a most promising drug delivery approach for the delivery of hydrophobic drugs. Research was carried out with objective of formulation and evaluation of emulgelincarporated with hydrophobic drug Methoxsalen using Carbapol 934 as a gelling agent. The emulsion was prepared by screening various surfactants, co-surfactants and oils. They were selected on the basis of maximum solubility of drug in them. Selected surfactant, co-surfactant and oil were taken and a pseudo ternary phase diagram constructed with the help of software Chemix. The emulsion was generated from a stable microemulsion point and then mixed into the gel base. The formulations were studied for various parameters such as rheological studies, spreading coefficient studies, in vitro release study, irritation studies. The optimized formula was tested for its irritation test. As a result, it can be stated that Methoxsalen topical emulgel causes less irritation than the marketed product.
KEY WORDS: *Vitiligo, Emulgel, Methoxsalen, Topical drug delivery system.*

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INTRODUCTION

Vitiligo is a chronic skin disorder in which areas of the skin lose their colour. When skin pigment cells die or become unable to function, this condition develops. Worldwideincidenceof1% has been reported. The incidence of vitiligo is found to be 3to4%inIndia. Gujarat and Rajasthan have highest prevalence that is 8.8%. [1, 7]

Emulgels are formed when gels and emulsions are mixed to generate dosage forms.[13,14] They are a hybrid of emulsion/microemulsion and gel, as the name implies.A gelling ingredient in the aqueous phase transforms a traditional emulsion into an emulgel. Various medications are delivered to the skin using both oil-in-water and water-in-oil emulsions.[15]. Emulgelsare elegant and are easy to washed off from skin. They also have a good penetration through the skin. Emulgels for dermatological usage are thixotropic, greaseless, readily spreadable, emollient, nonstaining, water soluble, have a longer shelf life, are biofriendly, translucent, and have a pleasant look.[15].Severalantivitiligo drugs are available as topical formulations on the market (e.g., creams, ointments, and lotions for the purpose of local dermatological therapy). One of these Antivitiligo agents is Methoxsalen.

Methoxsalen is used in the treatment of vitiligo and psoriasis. Methoxsalen possesses poor water solubility and hydrophobicity; hence such drugs pose problems in a topical drug delivery. As a result, a microemulsion-based gel proved to be a suitable option for Methoxsalen solubilization. An essential consideration in the development of emulgel dosage forms is batch design and batch optimization. The aim of this article is to develop an emulgel system of Methoxsalenusing Virgin Coconut Oil (VCO) as oil, Tween 80 as surfactant, PEG 400 as co-surfactant and Carbopol 934 as a gelling agent.[16]

MATERIAL AND METHODS

Materials: Methoxsalen (Fig.1) was purchased from B. P. Agrochemical Uttarakhand, India.Virgin Coconut Oil (Oil) was purchased fromHerbs Nutriproduct Pvt. Ltd, Mumbai., India. Tween 80 was purchased from Merck specialitiespvt. Ltd. Mumbai, India. Ethanol was purchased from Changshu Yang yuan chemicals, China. All additional chemicals and reagents were of A.R.grade, purchased commercially, and used as received.

Screening of Surfactant and Co-surfactant

Various surfactants are screened like Span 20, Span 80, Tween 20 and Tween 80.Tween 80 was selected for further formulation. Surfactants were selected depending on drug solubility. Co-surfactants, on the other hand, were chosen based on their capacity to generate stable emulsions with relevant surfactants at low concentrations. Hence,PEG 400 was chosen as a co-surfactant for emulgel preparation.[15]

Construction of Phase Diagrams

Different combinations of oil, water, and surfactant/co-surfactant were chosen by analyzing drug solubility in various components.The titration approach was used to create pseudo-ternary phase diagrams for oil, surfactant : co-surfactant, and water.. The oil and S-mix mixtures were titrated with water in dropwise manner at various weight ratios ranging from 1:9 to 9:1. Pseudo ternary phase diagram was constructed by titrating with three different ratios of surfactant and co-surfactant (1:1, 1:2, 1:3) until it turns to turbid. Following the discovery of the microemulsion region in phase diagrams, components for microemulsion formulations were chosen at desirable ratios to form the microoemulsion.[6,7]Ratio of oil Smix and water was selected as per ternary diagram (fig. 1).

Formulation of Microemulsion:

VCO was chosen as the oil phase based on the solubility investigations. Surfactant and co-surfactant were chosen as Tween 80 and PEG 400, respectively. Distilled water was used as an aqueous phase. Drug was dissolved in different ratios of oil, surfactant, and co-surfactant in the appropriate amount of oil, surfactant, and co-surfactant. To the above mixture, distilled water was added. Surfactant and co-surfactant were gradually added while stirring continuously, resulting in a transparent and homogeneous Microemulsion.[4]

Characterization of Microemulsion

Dispersion Stability Study: The objective of dispersion stability is to evaluate the phase separation. In the stability studies, formulation selected was subjected to stress tests like heating cooling cycle, centrifugation.

Transmittance test

To assess the transparency and clarity of the Microemulsion this test was performed. Using a UV spectrophotometer, the transparency of the microemulsion was tested at 650 nm with 0.1N HCL as a blank.[8,12]

Determination by the formula:- Absorbance = -log (% T / 100)

Determination of Viscosity and pH

The microemulsion prepared were evaluated for the viscosity by Brook Field viscometer (LV2, Brookfield Inc., USA) and pH by pH meter. The results are shown in table 3.

Particle Size and Zeta Potential Measurement

Dynamic light scattering method using Zetasizer ver.7.12 (Malvern Instruments Ltd.) was used to determine the particle size and zetapotential of the optimised microemulsion.

Formulation of Emulgel

Selection of Surfactant/Co-surfactant Ratio for Formulaton of Emulgel.

The pseudo ternary phase diagrams of different Surfactant/cosurfactant (1:1, 2:1, 3:1) ratio gives microemulsion region. These microemulsion regions are compared and regions which ratio gives the more area for microemulsion formation was selected for further preparation of an emulgel.

Selection of Gelling Agents

Various gelling agents were screened such as hydroxypropyl methyl Cellulose (HPMC), Carbopol 940, Carbopol 934, for formation of gel, in different concentrations of 0.75%, 1% 1.5%. The gel was prepared by adding the known quantity of gelling agent into the water and this mixture was stirred for 10 min with the mechanical stirrer the end point of the gel formation was formation of transparent and clear gel with semisolid consistency as shown in Table no 5 [15].

Procedure for Preparation of Emulgel[16]

Accurate quantity of **Methoxsalen** was weighed as per the formula.Propyl paraben was dissolved in oil.Methoxsalen was added to the oil, surfactant, and co-surfactant combinations at various ratios, as shown in the table above. Drop by drop, a suitable amount of water containing Methyl paraben was added to the mixture, stirring constantly with a magnetic stirrer. Due to constant stirring of mixtures,

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microemulsions containing Methoxsalen were formed spontaneously. Gel was prepared separately by adding **Carbopol** (1%) with constant stirring in water to form homogenous dispersion into water.In above prepared gel, microemulsions were added and finallyTriethanolamine (TEA) was added to adjust the requiredpH. Creamyemulgel or microemulgel formulation was obtained.

Optimized batch of microemulsion was selected for further study. Selected batch was formulated in Emulgel. The emulgel prepared were compared with the VoltarenEmulgel for characteristics like viscosity, pH and spreadbility. One batch from these Emulgelwas selected which was evaluated and optimized. Further optimized batch was compared with marketed formulation of drug. (Melanocyl ointment) (fig no 4)

Evaluation of Emulgel:

Physical Appearance

Color, homogeneity, and consistency of the created emulgel compositions were visually evaluated. [15, 16]

pH Determination

A 10% dispersion of the formulation was prepared in distilled water, and the pH was measured with a pH meter (Lab India) which was previously standardized with pH 4 and pH 7 standard buffers, as indicated in Table 9.[16]

Rheological Study

The viscosity of emulgel formulation was determined at 250C using a Brookfield viscometer equipped with the spindle S64as shown in Table no 9. [15, 16]

Spreadability

The spreadability of formulation was tested using apparatus which consisted of two glass slides (10 × 10 cm), where one glass slide was fixed to the wooden board and the other one was movable, attached to a thread that passed through a pulley and carried a weight. Between the two glass slides, 1gm of formulation was placed.To remove entrapped air between the slides and provide a uniform film of the formulation, a 100 gram weight was allowed to rest on the upper slide for 1 to 2 minutes. The weight was removed, and a pull was applied to the top slide by placing a 45gm weight over the pulley. The time required for moving slide to travel premarked distance (10 cm) was noted. The results provided an indication of the relative spreadability of various formulations. It's a term used to describe the area over which gel spreads easily when applied to skin. A formulation's medicinal efficacy is also determined by its spread value. Spreadability is measured by the time it takes two slides to separate from the emulgel in seconds.[15, 16]Spreadability of an emulgel was determined by locally fabricated apparatus and by using formula

$$S = M. L / T$$

Where, M = weight tied to upper slide

L = length of glass slides

T = time taken to separate the slides

Drug Content Determination

Uniformity of content in emulgel was determined by spectrophotometer. Methoxsalen content in emulgel was measured by dissolving 1gm of emulgel formulation in ethanol by sonication. Absorbance was measured after suitable dilution at 248 nm using UV-VIS spectrophotometer. [16]

***In-Vitro* Diffusion Study**

Diffusion study was carried out utilising diffusion cells with egg membrane (fabricated locally). at 37 ± 0.1°C.Beaker was filled with 100ml of buffer (pH 7) which acts as receptor fluid. Externally powered magnetic beads were constantly stirring the receptor fluid.1gm emulgel was accurately inserted in the cylindrical hollow tube, one end of which was sealed with egg membrane. It acts as a donor compartment. The aliquot (5 ml) were collected at suitable time intervals of 60min up to 6hr. Sample was analyzed by UV at 248 nm after appropriate dilutions. To determine the overall amount of drug released at each time interval, cumulative corrections were done.[15, 16]

Stability Studies:

The stability studies were carried out as per the ICH guidelines (Q1R2). For 3 months, the prepared emulgel formulations were stored in high-density polyethylene bottles away from light at 40 °C±2°C and75%RH± 5%RH. After storage, The samples were examined for physical appearance, pH, and drug release after storage.

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RESULTS AND DISCUSSION

Screening of surfactant:

The solubility of Methoxsalenin VCO was found to be (28.±0.3mg/ml). Among surfactant, tween 80 showed solubility (31.0±0.9).PEG 400showedhighest solubility among the co-surfactants (39.10±0.6mg/ml).Phase diagrams was constructed to determine the microemulsion existence zone.From the pseudo phase diagrams, it was determined that the microemulsions with a tween 80 and PEG ratio of 3:1, as shown in fig. 1, had the highest micro emulsion zone.

Dispersion Stability Studies

The objective of dispersion stability study was to assesthe phase separation. In the stability studies, formulation selected was subjected to stress tests like heating cooling cycle, centrifugation. It was observed that all formulations were stable, clear liquid, no phase separation under stress condition; this confirms the liquid formulations were stable for the storage. Microemulsions are stable formulation composed of fixed proportion of oil, surfactant, co-surfactant and water which does not tends to show any phase separation after multiple changes in the centrifugation.. Given in table no.1After centrifugation for 20 minutes at 3000 RPM all the formulations were still stable, clear liquid, no phase separation occurred under stress conditions. This proves that the formulations are thermodynamically stable. [8]

Transmittance test:

Results for transmittance were mentioned in table no.2. It was noted that the transmittance was found in same range for all batches.No major difference observed in transmittance with change in S mixture of 4 batches.[8]

3.2.4 Determination of Zeta potential viscosity and pH:

FTIR study suggests there is no interaction between the drug and the used excipients. Microemulsions were evaluated forviscosity and pH and the results are shown in table 2. The viscosity of four formulations was found to be between 150 and 190 cps, and after varying the RPM, it was determined that the system of all microemulsion formulations was shear thinning system.That instance, a sudden increase in resistance causes viscosity to drop. But with respect to time the viscosity of the formulations was remained stable and no major fluctuations were observed in it.[7]

Particle Size, Polydispersity index and Zeta Potential Measurement

From the four batches batch 4 was selected as optimum on the basis of globule size (Figure 1), The particle size and zetapotential of the optimized microemulsionare given in figure 2; 3 and in table 3. Required globulessize inthe nanoemulsionwas in between the range of 100-500nm, the polydispersity index should be as close as possible to 1 and the zeta potential should be within the range of -15 to +20 mV. Thus, value for PDI 0.212 is better dispersity index for globules.

Evaluation of Emulgel:

Color, homogeneity, and consistency of the prepared emulgel were visually evaluated and reported in table 8,9. Spreadability value of E2 batch is mentioned in table 5 and table 8 and its compaired with marketed Voltaren and found better speradability results around 40.90 gm.cm/sec whereas marketed emulgel was showing it 37.5 gm.cm/sec as mentioned in table 6. Drug content of 99.4±0.15was found for optimized batch.

In-Vitro Diffusion Study

The *in vitro* permeation rates of Methoxsalen from various emulgel were determined to evaluate the effects of the formulation factors. The in vitro % release for the optimized batch was found to be 90.65% after 6 hrs. of release study.(Table no 8 and 9)

Stability Studies: Results of stability study are mentioned in table no 10. It showed no significant difference in period of assessment at 40 °C±2°C and75%RH± 5%RH.

CONCLUSION

Microemulsion system increases surface area of exposure and enhances solubilization of hydrophobic drug.It enhancesavailable surface area and dissolution rate of drug in the formulation.The in vitro % release for the optimized batch was found to be 90.65% after 6 hrs. of release study.Emulgel is good approach for topical delivery.Drug release from the emulgel was shown to be increased and extended when compared to the marketed formulation. Furthermore, penetration and physical appearance of emulgelwere considered to be in more acceptable form. The result of stability study indicates no significant difference between the parameters tested before and after the stability studies.

Table1 Centrifugation stability of Microemulsion

BATCH NO	**OBSERVATION**
ME1	No phase separation
ME2	No phase separation
ME3	No phase separation
ME4	No phase separation

Table 2 Physicochemical Parameters of Microemulsion

Batch Label	Viscosity (cps)	pH	% Transmittance
B1	190.7±0.947	6.05±0.1	96.71
B2	176.3±0.943	6.1 ±0.2	96.87
B3	162.3±0.473	6.02 ±0.04	96.82
B4	151.6±0.9427	6.4 ±0.22	96.91

Table 3 Evaluation of micro emulsion for PDI, Droplet size and Zeta potential

Sr. No	Batch Label	Conc. of Oil (%)	Conc. of S. Mix (%)	Droplet Size (nm)	PDI	Zeta Potential
1	B1	47.65	31.77	**663.7**	**1.000**	**-16.5**
2	B2	36.86	36.86	**512.9**	**0.02**	**-0.348**
3	B3	28.82	43.23	**460.7**	**0.168**	**-25.5**
4	B4	20.64	48.16	**402.5**	**0.212**	**-15.1**

Table 4 Formulation of Emulgel with different conc of Carbopol 934

Ingredients	**% w/w (E1)**	**% w/w (E2)**	**% w/w (E3)**
Methoxsalen	0.75	0.75	0.75
Oil	20.64	20.64	20.64
S-mix	48.16	48.16	48.16
Water	31.2	31.2	31.2
Gelling agent	**0.75**	**1**	**1.5**
Methyl paraben	0.03	0.03	0.03
Propyl paraben	0.01	0.01	0.01
Triethanolamine	q.s	q.s	q.s

Table 5Comparison of emugels with marketed Emulgel (Voltaren)

BATCHES	**Viscosity(cp)**		**Spreadability (gm.cm/sec)**
	(10RPM)	20 RPM	
(E 1) 0.75%	27870	26500	50
(E 2) 1%	31200	30400	40.90
(E 3) 1.5%	35500	33100	32.14
Marketed emulgel (Voltarenemulgel)	32400	31300	37.5

Table 6 Comparison of % CDR

Time (hr)	**(E1) 0.75%**	**(E2) 1%**	**(E3)1.5%**
1	26.56±0.492	25.07±0.268	25.76±0.327
2	39.20±1.21	40.009±1.04	34.52±0.34
3	53.63±0.869	50.8±0.226	49.55±0.308
4	61.56±0.337	67.56±0.13	67.38±0.137
5	82.71±0.526	81.79±0.218	78.74±0.203

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Table 7 Optimized formula

Ingredients	(E2) % w/w
Methoxsalen	0.75
Oil	20.64
S-mix	48.16
Water	31.2
Gelling agent	1
Methyl paraben	0.03
Propyl paraben	0.01
Triethanolamine	q.s

Table 8 Evaluation of optimized batch

Parameters	Results
Appearance	White and homogeneous
pH	6.08±0.01
Viscosity(cp)	31200±0.5
Spreadability (gm.cm/sec)	40.90±0.005
Cumulative drug release (%)	90.65±0.88
Drug content (%)	99.4±0.15

Table 9 Comparison of %CDR of optimized Emulgelbacth with marketed formulation of Methoxsalen

Time (hr)	Emulgel	Marketed ointment
	% CDR	
1	26.19±1.30	15.29±1.11
2	41.59±0.90	27.82±1.416
3	53.03±0.22	38.01±0.53
4	76.80±1.33	53.58±0.429
5	83.58±1.09	61.6±1.648
6	90.65±0.88	79.69±0.7

Table 10Stability testing of Emulgel

Formulation	Appearance	pH	% drug content	%drug release
0 month	White, creamy and homogeneous	6.43± 0.02	98.96 ±1.7	90.65±0.88
Optimized batch	White, creamy and homogeneous	6.74± 0.02	98.01 ±0.2	90.45± 0.61

Fig 1 ratio selected

2:1

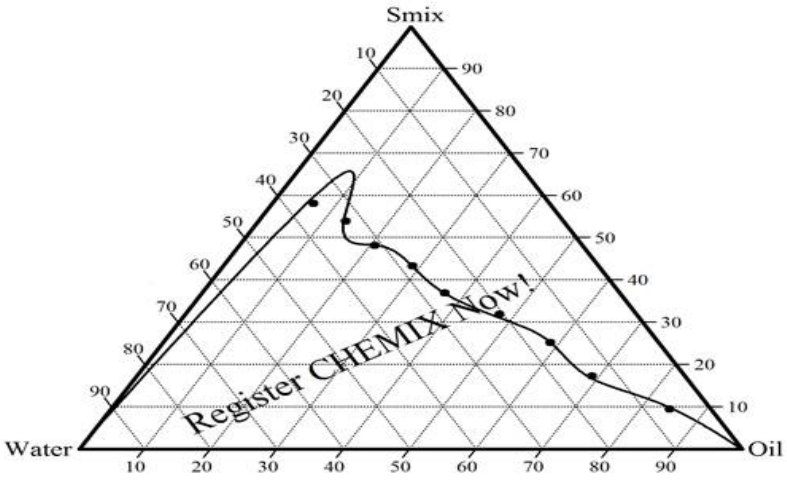

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Fig 2 Optimized batch globule size:

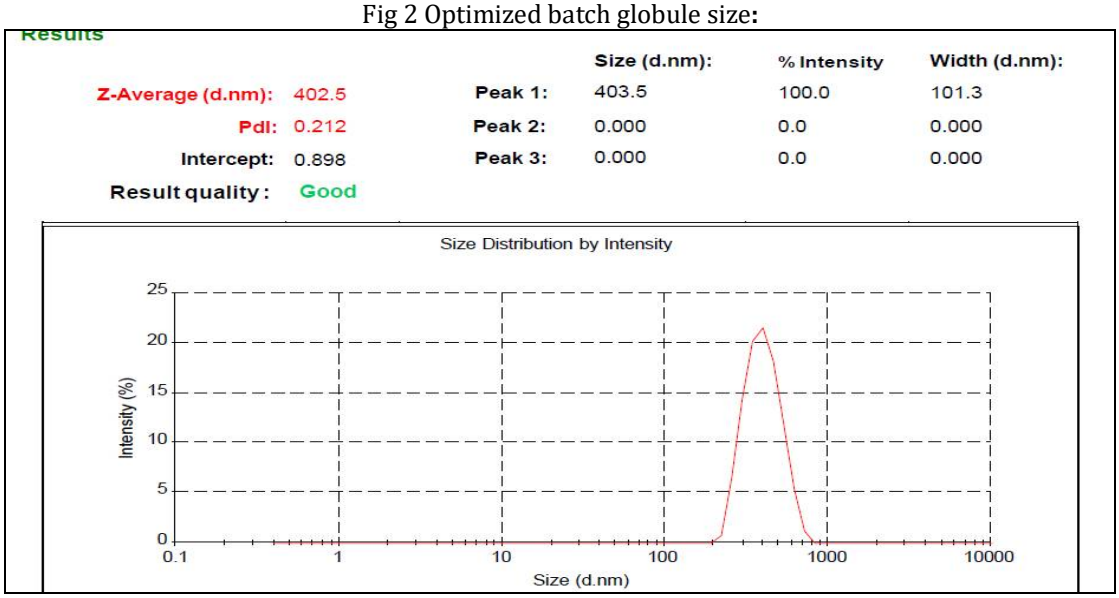

Fig 3 Optimized batch Zeta potential:

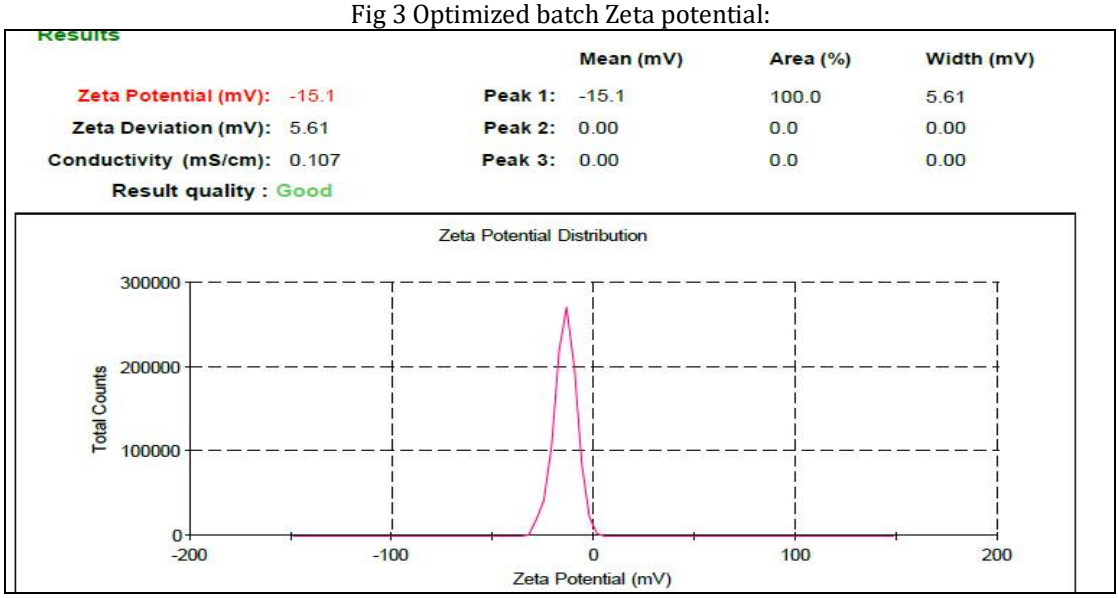

Fig 4 Comparison of % CDR with marketed formulation

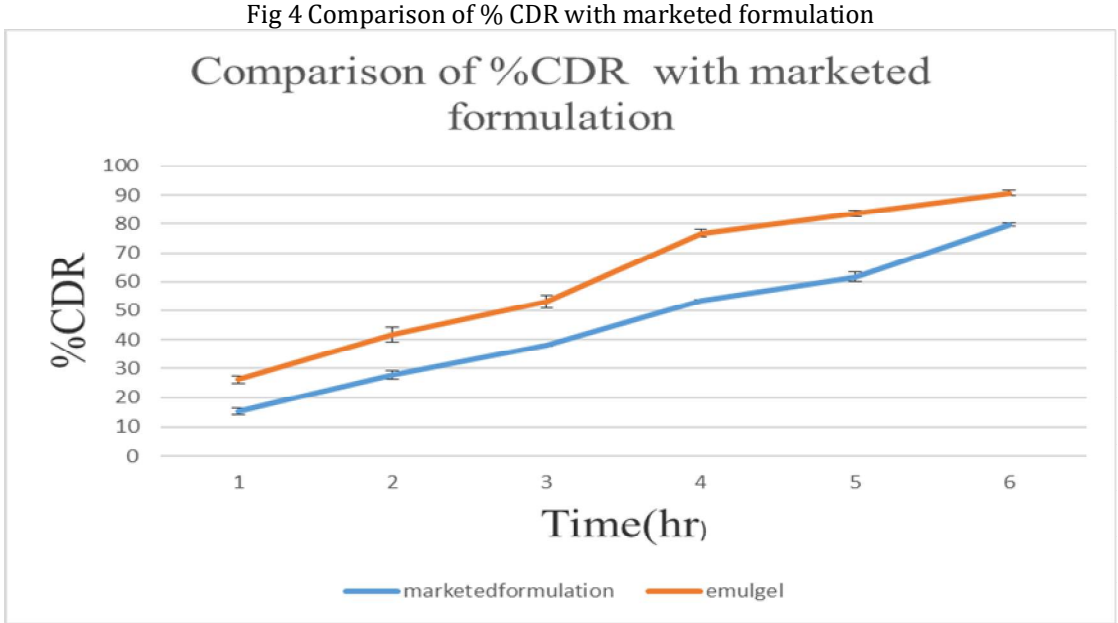

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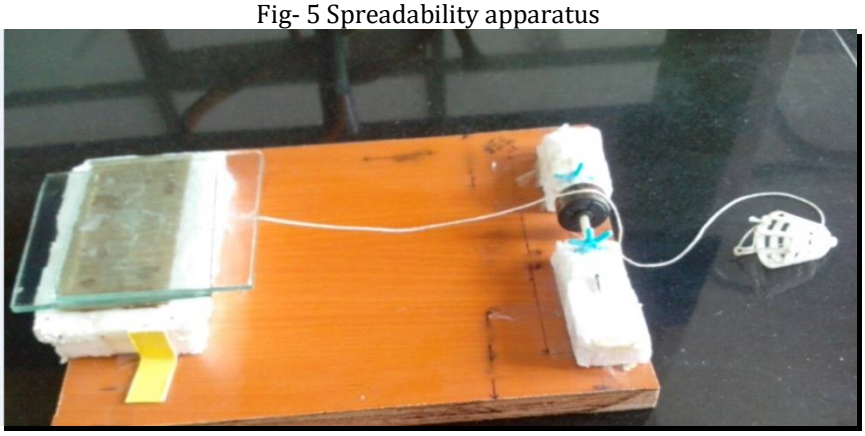
Fig- 5 Spreadability apparatus



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