Formulating and Mechanistic Insights of Microemulsion Based Hydrogel of Taxol Derivative for The Treatment of Cancer

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Abstract: Introduction: Topical drug delivery system is employed to evade the first pass metabolism. It also helps to evading the threats and inconveniences accompanied with intravenous drug administration as well as the settings related to drug absorption including pH changes, enzyme existence, time of gastric emptying are additional benefits of topical formulations. Gellified formulations has tendency to release drug at faster rate as compared with traditional topical remedies like ointments and creams. Gel formulations has many advantages instead of that facing a major limitation is the difficulty in delivery of hydrophobic drugs. So to overcome this drawbacks emulgels are prepared. With this emulgel approach even a poorly water soluble drug can relish the exclusive properties of gels. When enlighten fusion of gel and emulsion are used as tools of drug delivery the dosage form is denoted as Emulgel. In fact, the gelling agent is the integral part of gel within the water phase renovates a classical emulsion into an emulgel.

Objectives: The main objective of present investigation is to formulate the emulgel containing anticancer drug and it will help to quick onset of action improve performance of active pharmaceutical ingredients and reduce the systemic cytotoxicity.
Material and Method: Paclitaxel, HPMC E 15 LV Premium, Liquid paraffin, Tween 80, Span 80, Propylene glycol, Methyl paraben, propyl paraben, Triethanolamine. Emulgel were characterized for drug content, FTIR study, DSC study, Viscosity, Drug content and in-vitro drug release study.
Result and Discussions: The FTIR and DSC spectra revealed that there were no interaction between drug and excipient. The prepared emulgel were opaque in nature having good viscosity. Emulgel are formulated by the magnetically driven stirring technique. The optimize formulation batch shows the rapid release of drug on its topical application. Drug release by diffusion 82.68 % after 6 hours.
Keywords: Emulgel, HPMC, Paclitaxel, Topical drug deliver, microemulsion, hydrogel etc.

1. Introduction

Topical drug delivery encompasses the application of a drug containing formulation to the skin for direct treatment of cutaneous disorder. The topical drug delivery includes the conventional formulations such as ointments, creams and lotions, but these formulations are usually very sticky and may be patient has to face the problem of uneasiness on application. Furthermore, they also have less spreadability and requires application with rubbing. They also exhibit the problem of stability. In order to defeat these problems, the use of transparent gels has increased both in cosmetics and in pharmaceutical preparations [1, 2].

A gel is made up of colloids that is usually 99% by weight liquid, which is restrained by surface tension between it and a macromolecular gelatin fibers network. Gels are developed by entanglement of large amounts of water or hydro alcoholic liquid within a solid colloidal network. Gel formulations is having tendency to release drug at faster rate in comparison with traditional topical dosage forms such as ointments and creams. Furthermore, having many rewards of gels required to face major limitation is their inability to deliver hydrophobic therapeutic moiety. To overcome this restriction an emulsion based approach is being used so that a hydrophobic therapeutic moiety can be successfully integrated and delivered through gels. When gels and emulsions are used in combined form the dosage forms are referred as emulgels. Emulsions possess certain degree of classiness and are easily washable from skin. They also possess great skin penetration power. Emulgels for dermatological use have numerous favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life, bio-friendly, transparent & pleasing appearance [2, 3].

2. Materials and Methods

2.1. Material

Paclitaxel sample was obtained from Neon Pvt. Ltd., Mumbai, HPMC E 15 LV Premium, Liquid paraffin, and Ethanol, Tween 80, Span 20, Propylene glycol Methyl & Propyl Paraben, Triethanolamine were obtained from Molychem Pvt. Ltd.

2.2. Methodology

2.2.1 Formulation table:

Table 1. Composition of different formulation batches (%w/w)

Ingredient	F1	F2	F3	F4	F5	F6
Paclitaxel	1	1	1	1	1	1
HPMC E 15 LV Premium	0.5	1	0.5	1	0.5	1
Liquid paraffin	2.5	5	7	2.5	5	7
Tween 80	0.5	0.5	0.5	0.5	0.5	0.5
Span 80	1	1	1	1	1	1
Propylene glycol	5	5	5	5	5	5
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben	0.01	0.01	0.01	0.01	0.01	0.01
Alcohol	5	5	5	5	5	5
Water	q.s	q.s	q.s	q.s	q.s	q.s
Triethanolamine	Adjusted to pH 6-6.5					

2.2.2. Formulation of paclitaxel microemulsion based hydrogel: Paclitaxel microemulsion based hydrogel formulations batches shown in table 1 were prepared by dispersing HPMC E 15 LV Premium in purified water with constant stirring at a moderate speed, then the pH is adjusted to 6 to 6.5 using Triethanolamine (TEA). The oil phase of the emulsion was prepared by dissolving Span 80 in light liquid paraffin along with ethanolic paclitaxel solution while the aqueous phase was prepared by dissolving Tween 80 in purified water along with methyl and propyl paraben. Both the oily and aqueous phases were separately heated to 70º- 80º C; then the oily phase was added to the aqueous phase with continuous stirring until cooled to room temperature. After preparation of stable emulsion, it is incorporated into gel base in 1:1 ratio under constant stirring with magnetic stirrer [4].

2.3. Characterization [5-8]

2.3.1. Qualitative Solubility Study: It is necessary to determine the solubility of drug and polymers in various solvents which is important while selecting the solvent for the preparation of emulgel with precise particle size.

2.3.2. FTIR Study: FTIR study is conducted to identify the functional groups present in API along with its structural integrity with other formulating agents of emulgel i.e. Paclitaxel. The FTIR analysis of pure drug sample and the blend of drug and excipients of optimized batch was measured using Fourier Transform Infrared spectrophotometer (BRUKER). The quality measures of each formulation ingredient in the mixture was same as that in the optimized batch. The sample of drug and drug polymer blend were then individually mixed with IR grade KBr. This mixture was then scanned over a wave

number range of 4000 to 650 cm-1. The scans were evaluated for presence of principle peaks of drug.

2.3.3. DSC Study: DSC study is carried out to examine the purity of drug as well as its compatibility with excipients used to prepare emulgel. The DSC characterization of drug and its physical mixture were performed by using differential scanning calorimeter (DSC 822c, Mettler Toledo) with a thermal analyzer. The test is carried out using nitrogen flow of 20 ml/min, 2 mg of Paclitaxel sample were taken, and heated at a scanning rate of 50 ºC /min from 20ºC to 250ºC. An empty aluminum pan was considered as standard.

2.3.4. pH: The pH of developed emulgel formulations was determined using digital pH meter. 1g of emulgel was admix in 100 ml distilled water and kept away for two hours. The pH measurement of each formulation was done in triplicate and its average is taken as a final reading.

2.3.5. Rheological Study: The viscosity of prepared formulations was calculated using spindle 64 (Brookfield rheometer) the formulation of which viscosity was to be determined were added in a beaker and rest for 30 min. At the assay temperature ($25^0 \pm 1^0$C) before the measurement were taken. Spindle was lowered perpendicular in to the center of emulgel taking care that spindle does not touch bottom of the jar and rotated at a speed 50 rpm for 10 minutes. The spindled was moved turbulent giving viscosities at number of points along the path. The average of readings were taken in 10 min was recorded as the viscosity of gel.

2.3.6. Drug Content Determination: Paclitaxel content in emulgel was measured by dissolving known quantity of emulgel in solvent (ethanol) by Sonication technique. Absorbance was determined after appropriate dilution at 229 nm by using UV spectrophotometer.

2.3.7. Drug Release Studies: The Franz diffusion cell is used for determination of In-Vitro drug release study. During the study pH 7.4 Phosphate buffer is used as diffusion medium. The whole assembly is kept under the influence of magnetically driven bead of 50rpm speed. For the evaluation of drug release 2ml sample were withdrawn and after its requisite dilution subject to UV spectrometric examination at 229nm.

2.3.8. Particle size: The particle size determination of the optimized formulations was the determined by Zetasizer. Paclitaxel emulgel sample of 1 gm is mixed with water and result is noted.

2.3.9. Spreadability: 0.5 gm of each developed Paclitaxel emulgels sample was taken and forced between two slides (divided into squares of 20 mm sides) and rest for 5 minutes aside. Excess spreading of formulations beyond the marking were not expected. Diameters of widen areas were calculated in centimeters as the value of spreadability.
S = M. L / T
Where, M = wt. tied to upper slide
L = length of glass slides
T = time taken to separate the slides

2.3.10. Extrudability: Extrudability test is carried out to determine the how much force required to extrude the formulation from the filled collapsible tube. The emulgels were filled into crimped, collapsible tubes and the extrudability of the formulation from the packed material was tested.

3. Result & Discussion

3.1. Qualitative Solubility Study

Table 2. Quantitative Solubility Profile of Paclitaxel in different Solvents

Sr. No	Solvent	Inference
1	Distilled Water	Very slightly soluble
2	Ethanol	Freely soluble
3	Acetone	Freely soluble
4	Methanol	Sparingly soluble

3.2. FTIR analysis

The FTIR spectrum of Paclitaxel was recorded using FTIR (cary-630 Agilent technology). The spectrum was recorded over the range of wave no. 4000-650 cm^{-1} the spectra is shown in figure 1 the values of major peaks in FTIR spectrum of Paclitaxel mentioned in table 3 from the observed peak it is clear that Paclitaxel in the pure form.

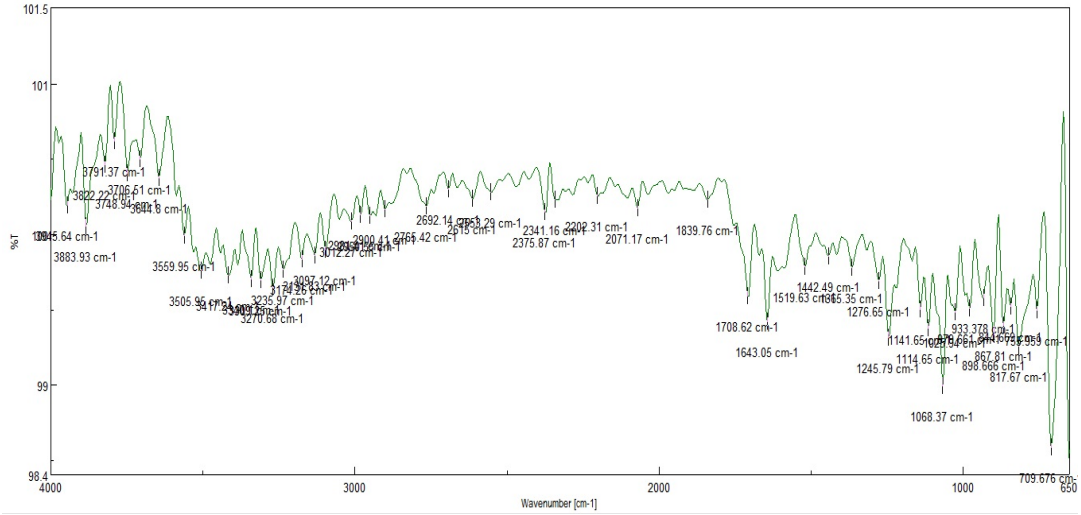

Figure 1. FTIR Spectra of Paclitaxel

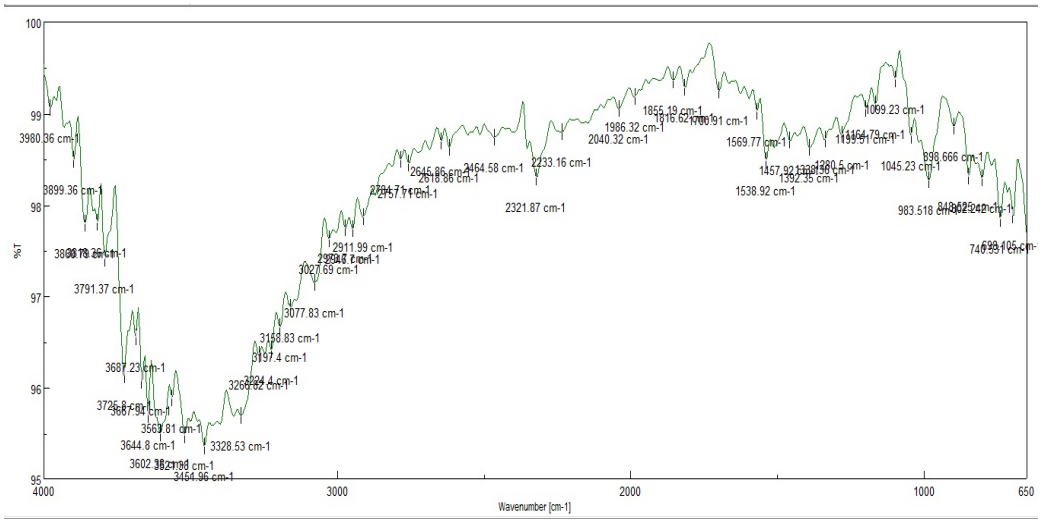

Figure 2. FTIR Spectra of Paclitaxel + HPMC

Table 3. IR values of functional groups of Paclitaxel

Sr. no.	Groups	Peak (wave number) cm^{-1} (observed)	Peak (wave number) cm^{-1} (standard)
1	C-O(Ester)	1839.76	1735-1850
2	C=O (Amide)	1643.05	1640-1690
3	C=O (Ketone)	1708.62	1705-1750
4	OH (Acid)	2900.41	2500-3300
5	N-H (Amide)	3417.21	3200-3500
6	OH (Alcohol)	3559.95, 3270.68	3200-3650

3.3. Determination of λ max

Accurately weighed quantity of 10 mg Paclitaxel and dissolved in 10 ml ethanol (1000 ppm). Used as stock solution from this 1 ml withdrawn and diluted up to10 ml with ethanol (100 ppm) then from this withdrawn 1ml diluted in 10ml (10 ppm). This dilution taken for determination of λ max of Paclitaxel by UV- spectrophotometer in ethanol was found to be 229 nm and it is very close to standard λ max. The UV visible spectrum of Paclitaxel is shown in figure 3 and the value observed is shown in table 4.

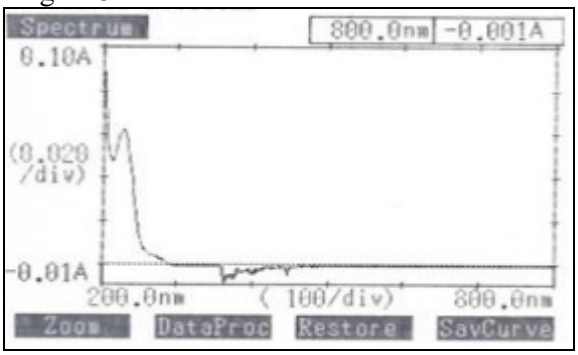

Figure 3. UV- Visible absorption spectrum of Paclitaxel in ethanol

Table 4: Wavelength of maximum absorbance (*λ* max)

Sr.no	Solvent	*λ max (nm)*
1.	Ethanol	229

3.3.1. Standard calibration curve of Paclitaxel in ethanol: Accurately weighed quantity of 10 mg Paclitaxel and dissolved in 10 ml ethanol (1000 ppm). Used as stock solution from the stack solution 1 ml withdrawn and diluted up to 10 ml with ethanol (100 ppm) then from this withdrawn 1 ml diluted in 10ml (10 ppm) from the solution of 10 ppm made the concentrations of 2,4,6,8,10 ppm. The graph of absorbance vs. concentration for pure Paclitaxel was found to be linear in concentration range 2-10 ppm standard calibration curve values of Paclitaxel in ethanol are shown in table 5 and calibration curve shown in figure 4 the R_2 value was found to be 0.996.

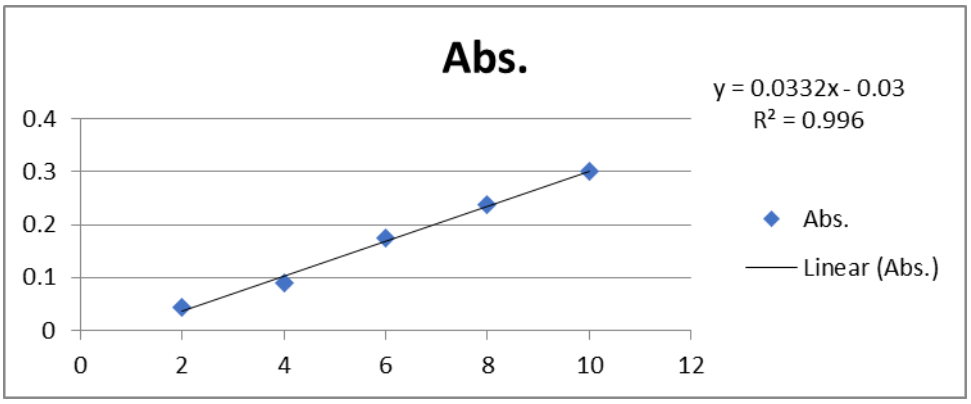

Figure 4. Graph of calibration curve of Paclitaxel in ethanol

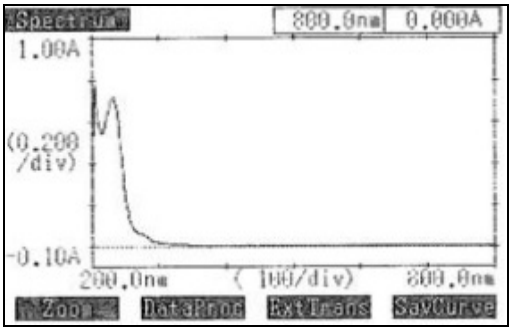

Figure 5. UV- Visible absorption spectrum of Paclitaxel in PBS 7.4

3.3.2. Standard calibration curve of Paclitaxel in PBS 7.4: Accurately weighed quantity of 10 mg Paclitaxel was dissolved in 10 ml ethanol (1000ppm) used as stock solution. From the stock solution 1 ml withdrawn and diluted up to 10ml with PBS 7.4 (100ppm) then from this withdrawn 1ml diluted in 10ml (10ppm). From the solution of 10 ppm made the concentrations of 2, 4, 6, 8, 10 ppm. The absorbance of all the solutions were measured by using UV visible Spectrophotometer at 229 nm and the graph of absorbance vs. concentration for pure Paclitaxel was found to be linear in concentration range 2-10 ppm standard calibration curve values of Paclitaxel in PBS 7.4 are shown in figure 6 the R^2 value was found to be 0.997.

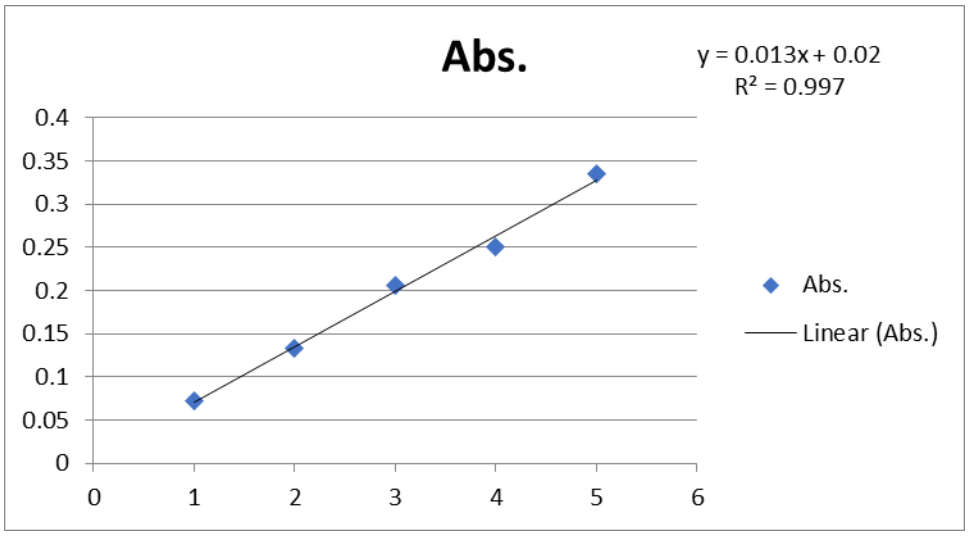

Figure 6. Graph of calibration curve of Paclitaxel in PBS 7.4

3.4. DSC thermogram of Paclitaxel pure drug

Differential scanning calorimetry (DSC) can be applied to analyze and predict physicochemical interaction between components in a formulation thus helps in selecting suitable chemically compatible excipients.

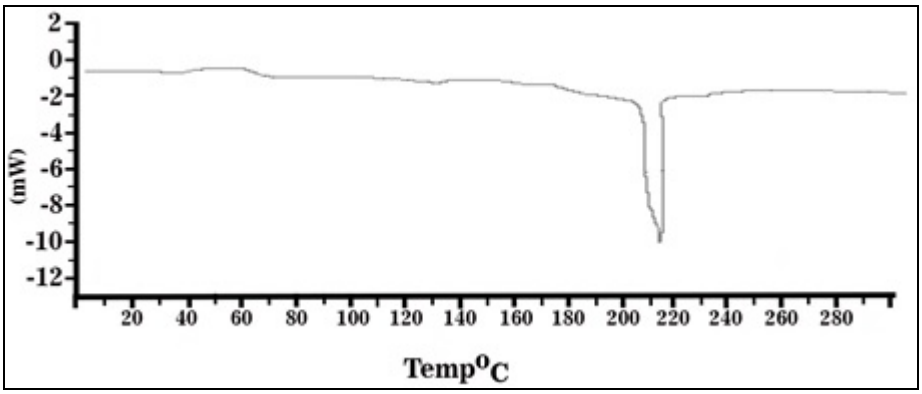

Figure 7. DSC thermogram of Paclitaxel

The average melting point of Modal drug was determined by capillary method and was observed in the range of 216^{0}C–217^{0}C which is with reported melting point. Melting point was confirmation of melting point was done by using Differential Scanning Calorimetry [Mettler Toledo]. In DSC thermogram single sharp endothermic peak at 216^{0}C indicates the desire purity of modal drug sharp peak indicates crystalline nature of API. Thermogram of pure drug shown in figure 7.

3.4.1. DSC thermogram of compatibility studies:

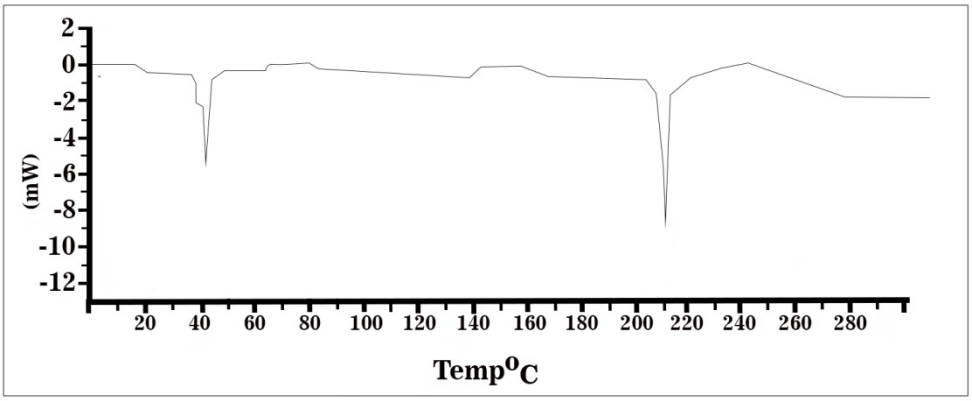

Figure 8. DSC thermograph of Paclitaxel and Polymer

This similarity in peaks indicates the compatibility of Paclitaxel with the gel formulation polymers. Pure drug melting point 216^{0}C and mixture 217^{0}C Similarity in melting point of pure drug and drug polymer mixture indicate purity of API and no interaction and degradation of API in formulation. So the study reveals that there was no any interactivity was found between pure drug sample and formulation excipients shown in figure 8.

3.5. Evaluation of Emulgel

3.5.1. Physical stability: All formulation batches were found to be homogeneous milky emulsions previously while emulgels were found to be off white viscous jelly

preparation. The pH values of all prepared formulation ranged from 6.03 to 6.64 shown in table 5 which are considerable acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5-6.5

Table 5. pH of Paclitaxel Emulgel

Formulation code	P^H
F1	6.20±0.36
F2	6.10±0.35
F3	6.34±0.16
F4	6.64±0.18
F5	6.10±0.23
F6	6.03±0.21

3.5.2. Drug content determination by UV: 1 gm of emulgel was dissolved in 10 ml of ethanol. The volumetric flask was kept for 1 hour and shaken well in a shaker to mix it properly. The mixture was proceeded through filter paper. Withdrawn 1 ml was diluted up to 10 ml with PBS 7.4 the absorbance was measured spectrophotometrically at 229 nm. The drug content determined using standard plot. Following data shown in table 6.

Table 6. Drug content of prepared emulgel

Sr. no.	Formulation code	% Drug content
1	F1	89.59±0.52
2	F2	93.67±0.58
3	F3	88.33±0.85
4	F4	91.77±0.71
5	F5	89.94±0.55
6	F6	90.50±0.43

The average drug content of Paclitaxel emulgel was found to be 90.63%. Drug content values of all F1-F6 formulations were mentioned in table no.6.8 formulation F2 shows very good drug content.

3.5.3. In vitro drug release studies (Franz diffusion cell): Drug release study were performed in Franz diffusion cell were formulation was applied on dialysis membrane and phosphate buffer solution 7.4 was used as diffusion media from this withdrawn 2ml sample at 10 min time interval and diluted with PBS 7.4 and absorbance measured at λ max 229 nm by UV spectrophotometer in all formulation. The in vitro test was performed to ensure uniform and accurate permeability of drug. A good drug permeability was observed among the all emulgel formulations. The drug permeability of all formulations was shown in figure 9.

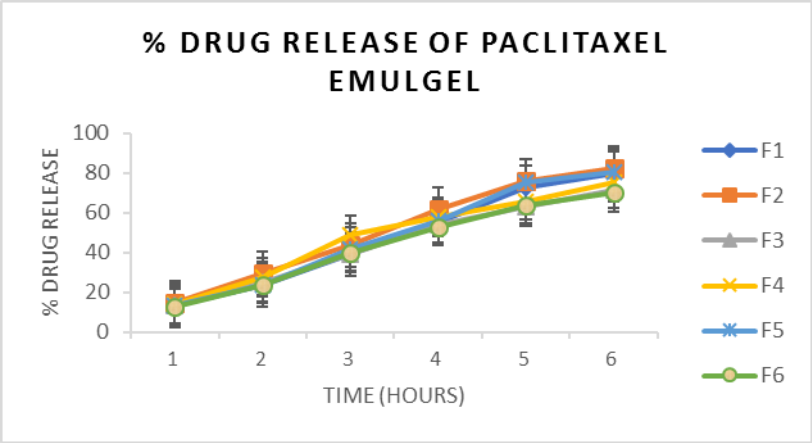

Figure 9. Graph of drug release of formulations F1-F6

3.5.4. Rheological study of Paclitaxel Loaded Emulgel: The viscosity of Paclitaxel emulgel was measured by using Brookfield viscometer (spindled no. 64) at 20 RPM. The corresponding dial readings were noted; Viscosity of all the formulations was shown in table 7. From these results we could observe that increase in conc. of the polymers there is increase in viscosity of emulgel formulation.

Table 7. Viscosity of formulation F1-F6

Formulation	F1	F2	F3	F4	F5	F6
Viscosity (Cp)	5021±0.26	5206±0.41	5340±0.23	5170±0.52	5277±0.29	5390±0.54

3.5.5. Spreadability: A sample of 0.5 g of each developed Paclitaxel emulgels formula was pressed between two slides (divided into squares of 20 mm sides) and left for about 5 minutes where no more spreading of formulation was expected. Diameters of widen circles were calculated in centimeters and were taken as comparative values for spreadability. Evaluation was conducted in triplicate and the average spreadability values were calculated shown in table 8.

Table 8. Spreadability values of formulation F1-F6

Sr. no.	Formulation code	Spreadability cm
1	F1	1.8 ± 0.12
2	F2	1.7 ± 0.31
3	F3	1.4 ± 0.25
4	F4	1.6 ± 0.37
5	F5	1.1 ± 0.14
6	F6	1.3 ± 0.36

3.5.6. Particle size determination: The particle size analysis of the optimized formulations was determined by Zetasizer. 1 gm of Paclitaxel emulgel is dissolved in water and result shown in figure 10.

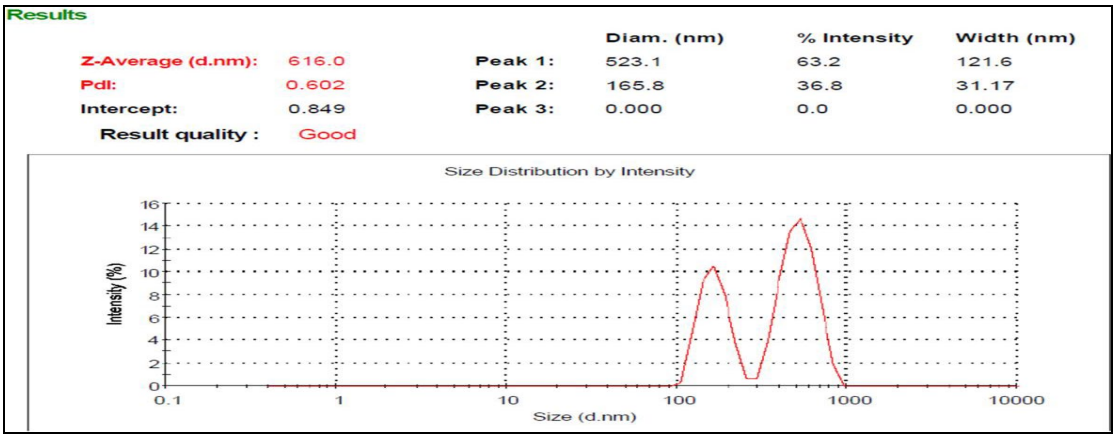

Figure 10. Particle size of Paclitaxel emulgel

The particle size distribution of Paclitaxel emulgel was shown in figure 6.14 the particle size of prepared formulation was determined by using particle size analyzer (Ver. 6.20 Malvern Instruments Ltd.) The average particle size of Paclitaxel emulgel was found to be in standard range up to more than 500 nm so given results obtained particle size 616 nm. Therefore, it was concluding that the emulgel formulation formed are mono dispersed or having uniform size.

3.5.7. Extrudability: During the test, a sealed collapsible tube holding above 20 grams of formulation was constrained firmly at the crimped end and a clamp was applied to prevent any rollback. Extrudability was then calculated by measuring the volume of gel extruded from the tip when a constant load of 1 kg was put over it readings are shown in table 9.

Table 9. Extrudability of formulation F1-F8

Sr. No.	Formulation code	Extrudability
1	F1	Easily extrudable
2	F2	Easily extrudable
3	F3	Easily extrudable
4	F4	Easily extrudable
5	F5	Easily extrudable
6	F6	Easily extrudable

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