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A novel approach of tramadol hydrochloride ethosomal gel for topical drug delivery system.

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Abstract

The aim of present examination work was to create tramadol hydrochloride based ethosomal gel. Transdermal medication conveyance of the medication through the skin is the best and advantageous arrangement of the medication conveyance. Skin goes about as a significant objective just as a guideline barrier for this Transdermal medication conveyance. Regardless of the numerous benefits of this framework, the significant hindrance is the low dissemination pace of medications across the layer corneum. Despite these deterrents the vesicular framework enters into the skin and applies their activities. Among the different vesicular framework showing great entrance into the skin, ethosomes are the most valuable frameworks. Ethosomes are lipid vesicles containing phospholipids, liquor (ethanol or potentially isopropyl liquor) in generally high focus and water. Ethosomes are delicate vesicles made of phospholipids and ethanol (in higher amount) and water. The size scope of ethosomes may change from several nanometers to microns (µ). Along these lines, it tends to be an obvious end result that ethosomal definition has promising future in powerful dermal conveyance of bioactive specialists.

The communication of medication was precluded by FT-IR contemplates and no incongruence and lipids and surfactant. To upgrade the definition, factor influencing the actual appearance of Ethosomes were researched. Tramadol Hydrochloride loaded Ethosomal gel were formed utilizing soya lecithin by actual scattering strategy and described for molecule size, drug content, dependability, creation yield. Arranged ethosomal gel gave the better physical, morphological with deference grouping of the lipids, surfactant and proportion of lipid and surfactant. Six unique details of ethosomes F1-F6 were formed to acquire the upgraded plan. The (Transmission Electron Microscopy) TEM of ethosomes showed that they were adjusted shape and permeable in surface. *In-vitro* drug arrival of plan demonstrates that detailing F6 was chosen as upgraded definition for fuse into the erythrosomes among every one of the six details and liposome showed drug release in controlled way toward the finish of 10 hours.

Keywords: Tramadol hydrochloride, Ethosomes, *In-vitro* release, Topical gel, skin, Topical drug delivery.

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Introduction

The clinical proof demonstrates that skin gel is a protected and successful treatment choice for use in the administration of skin related illness and utilized for nearby activity to diminish the results related with other ordinary measurements structure. Skin drug organization is a limited medication conveyance framework anyplace in the body through ophthalmic, rectal, vaginal and skin as a skin course. Skin is perhaps the most promptly available organs on human body for skin organization and is principle course of skin conveyance framework. Transdermal medication conveyance of the medications through the skin is the best and advantageous arrangement of the medication conveyance. Skin goes about as a significant objective just as a central obstruction for this skin/ Transdermal medication conveyance. Notwithstanding the numerous benefits of this framework the significant snag is the low dissemination pace of medications across the layer corneum. Disregarding these deterrents the vesicular frameworks enters into the skin and applies their activities. Among the different vesicular frameworks showing great entrance into the skin, ethosomes are the most valuable framework. Tramadol HCl is a narcotic pain relieving which is utilized in the therapy of extreme and constant agony [1,2].

Tramadol HCl is endorsed 3-4 times each day. The continuous dosing of Tramadol HCl prompts the expanded rate of results, non-compliances and improvement of resilience particularly in long haul utilized like osteoarthritis, joint inflammation, post-careful torments and so on Along these lines, to relieve the disservices related with oral treatment, skin ethosomes to expand the better entrance Tramadol HCl [3].

Materials and Methods Introduction

Drug Tramadol Hydrochloride, Carbopol, Methyl paraben and propyl paraben was purchased from Central Drug House (CDH) Pvt. Ltd, New Delhi, India. Soya lecithin and ethanol is obtained from SIGMA-ALDRICH Chemical, Mumbai, RFCL Limited, New Delhi (INDIA) respectively.

Preparation of unsonicated ethosomes

The above preparation consists of ethosomes after stirring on the magnetic stirrer for 5 min. These ethosome are called unsonicated ethosomes. These are now transferred to the sonicator and are sonicated in 3 cycles of 5 minutes each at 4ºC. These are now left aside for 45 minutes.

Formulation chart is shown in Table 1 and flow chart of preparation of ethosome is shown in Figure 1.

Table 1. *Formulation chart.*

S.no	Soya Lecithin	Ethanol (ml)	Propylene glycol (mg)	Tramadol Hydrochloride (mg)	Double distilled
1.	300	2.5	600	100	10
2.	300	3.0	600	100	10
3.	300	3.5	600	100	10
4.	300	4.0	600	100	10
5.	300	4.5	600	100	10

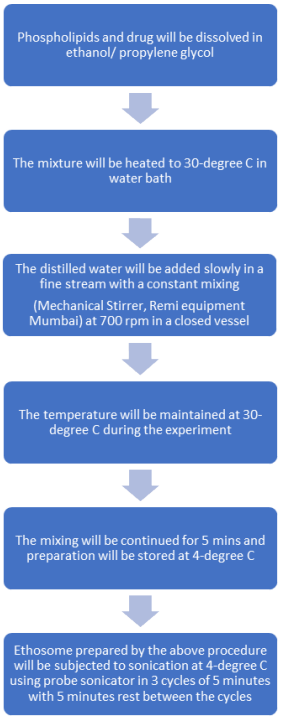

Figure 1. *Preparation of ethosome.*

Preparation of gel base

Ethosomes of Tramadol Hydrochloride were prepared with the use of phospholipids, ethanol, PG and distilled water. Here, phospholipids (soya lecithin) were used as vesicle forming agent, ethanol as penetration enhancer 2% W/W gel base was prepared by dispersing carbapol in distilled water containing 0.2% methyl paraben and 0.02% propyl parabens, using magnetic stirrer. Here, carbapol was used as gelling agent and methyl parabens and propyl paraben were used as preservatives.

Preparation of ethosomal gel in shown in Figure 2 in the form of flow chart.

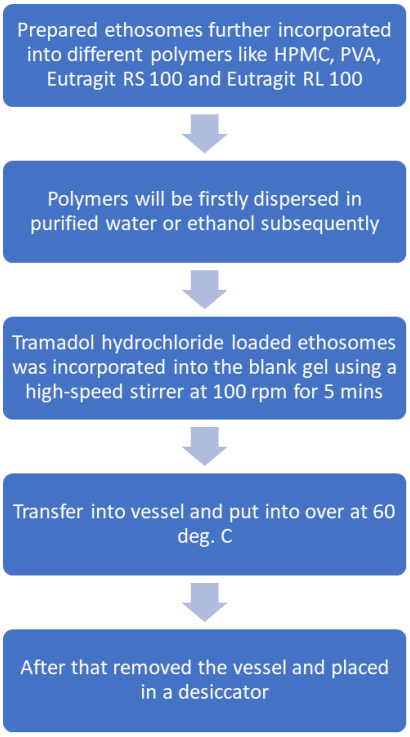

Figure 2. *Preparation of ethosome gel.*

Results and Discussion

Physical characteristics

Tramadol hydrochloride is a white to off white crystalline powdered extract which is pungent in taste (Table 2).

Table 2. *Physical characteristics.*

S.no	Physical characteristics	Observation
1	color	White to off white
2	order	Strong pungent
3	taste	pungent

Melting point range

Dissolving point of medication was controlled by slender combination technique. For this limited quantity of Tramadol hydrochloride was taken in a hair like cylinder shut down toward one side. An advanced softening point contraption was taken and afterward slim cylinder was put in it. Temperature was recorded at which the medication began softening. This was acted in three-fold and a normal worth at noted [4] (Table 3) (Figure 3).

Table 3. *Melting point.*

S.no	Melting point	Reference value	Observation
1	Tramadol hydrochloride	316ºC-320ºC	318ºC

Figure 3*: Melting point apparatus.*

FT-IR study (drug-excipient compatibility studies)

The pure drug and the excipients were mixed separately with IR grade KBr in a ratio of 100:1 and corresponding discs were prepared by applying 5.5 metric tons of pressure in a hydraulic press. The discs were scanned over a wave number range of 4,000-400 cm^{-1}[5].

Medication Excipient similarity was done by utilizing FT-IR spectrophotometer. The FT-IR spectra of unadulterated medication test alone, polymer tests and actual blend with polymer following 15 days for study the any association between them. The FT-IR spectra drug, soya-lectin and both mix combination were shown in Figures 4-6 and Tables 4 and 5.

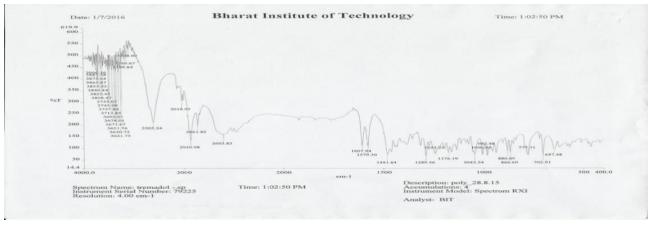

Figure 4*: FT-IR of pure sample of tramadol hydrochloride.*

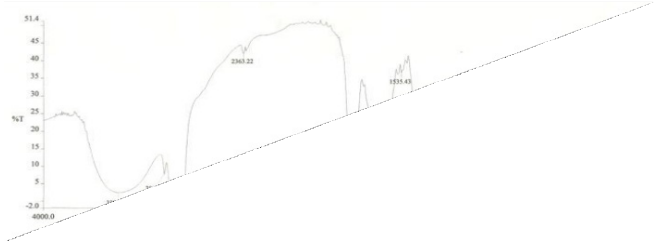

Figure 5*: FT-IR spectra of soya lecithin.*

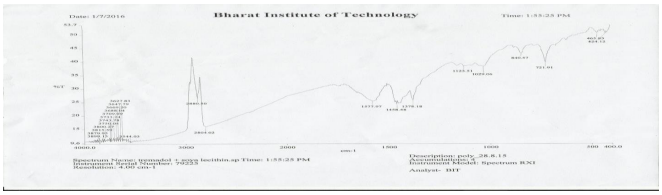

Figure 6*: FT-IR of tramadol+soya lecithin.*

Table 4. *FT-IR studies of pure drug.*

S. no	Functional Group	Reported Frequencies (cm^{-1})	Observed Frequencies (cm^{-1})
1	N-H	3500-3100	3055.70
2	C=O(Carboxylic Acid)	1725-1705	1726
3	OH(Carboxylic Acid)	3400-2400	2461
4	C-H(Aromatic)	3150-3050	3000
5	F	1400-1000	1094.19
6	C-H (Alkane)	3400-2400	2703.5

Table 5. *Drug-Excipients compatibility studies.*

S.no	Region in cm^{-1}	Type of Vibration	Functional group
1	3250	Stretching	C-N
2	2950	Stretching	C-H
3	1380	Stretching	O-H
4	1257	Stretching	C-O

UV spectrum analysis of tramadol hydrochloride

The solution was scanned in the range of 200 to 400 nm to fix the maximum wave length and UV spectrum was obtained (Figure 7).

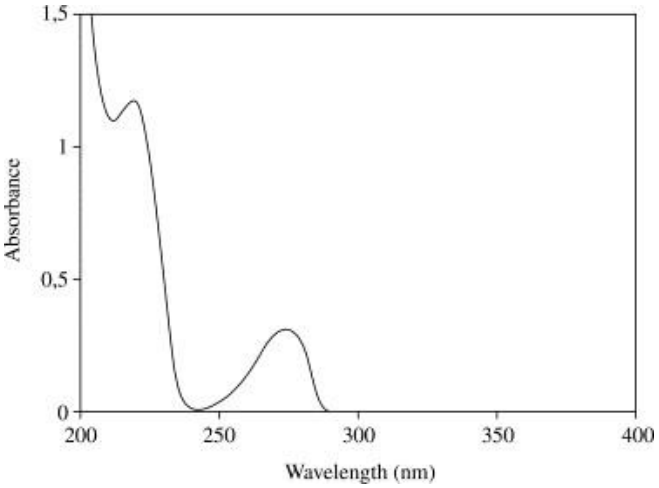

Figure 7*: UV absorption maxima.*

Preparation of standard stock solution of Tramadol Hydrochloride

Exact weighed 100 mg Tramadol hydrochloride and was dissolved in 100 ml phosphate buffer saline of pH 6.8,7.2,7.4 separately where its concentration is 1000 µg/ml, from this stock solution 10 ml was withdrawn and transferred into 100 ml volumetric flask. Volume was made with phosphate buffer saline in order to get standard stock solution containing 100 µg/ml [6].

1. Standard solution of Tramadol hydrochloride prepared buffer saline of pH 6.8.

2. Standard solution of Tramadol hydrochloride prepared buffer saline of pH 7.2.

3. Standard solution of Tramadol hydrochloride prepared buffer saline of pH 7.4.

Standard graph of tramadol hydrochloride

From standard stock solution a series of dilution (10, 20, 30, 40, 50 µg/ml) were prepared using phosphate buffer saline of pH 6.8, 7.2, 7.4 separately. The absorbance of these solutions was measured spectrophotometrically against blank of PBS of pH 6.8, 7.2, 7.4 respectively at 271 nm for Tramadol Hydrochloride [7] (Figures 8-10 and Tables 6-9).

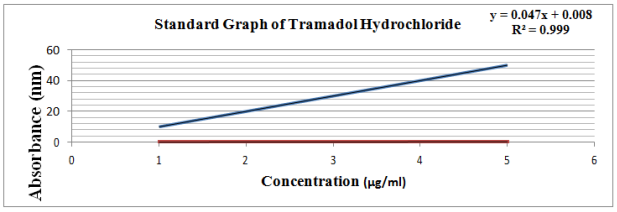

***Figure 8**: Standard graph and regression value of the standard solution with pH 6.8.*

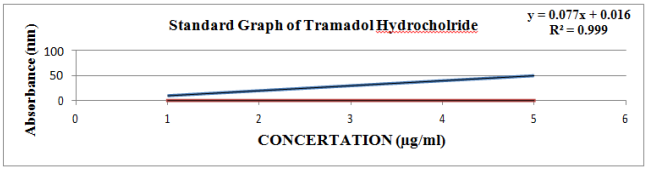

***Figure 9**: Standard solution of tramadol hydrochloride prepared buffer saline of pH 7.2.*

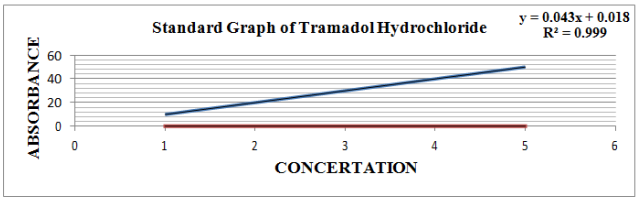

***Figure 10**: Standard solution of tramadol hydrochloride prepared buffer saline of pH 7.4.*

***Table 6.** Standard solution of tramadol hydrochloride prepared buffer saline of pH 6.8.*

S.no	Concentration(µg/ml)	Absorbance(271 nm)
1	10	0.055
2	20	0.103
3	30	0.154
4	40	0.198
5	50	0.246

***Table 7.** Standard solution of tramadol hydrochloride prepared buffer saline of pH 7.2.*

S.NO	Concentration (µg/ml)	Absorbance (nm)
1	10	0.098
2	20	0.167
3	30	0.251
4	40	0.328
5	50	0.406

***Table 8.** Standard solution of tramadol hydrochloride prepared buffer saline of pH 7.4.*

S.no	Concentration (µg/ml)	Absorbance (nm)
1	10	0.063
2	20	0.103
3	30	0.152
4	40	0.195
5	50	0.236

***Table 9.** Evaluation parameter of partition coefficient.*

Solvent	Absorbance	Concentration
Water	4	36.79
n-Octanol	1.865	17.16

Preparation of gel base

2% W/W gel base was prepared by dispersing carbopol in distilled water containing 0.2% methyl paraben and 0.02% propyl parabens, using magnetic stirrer. Here, carbapol was used as gelling agent and methyl paraben and propyl paraben were used as preservatives.

Preparation of ethosomal gel

Ethosomal formulation was subjected to 2000 rpm for 20 min at 40ºC .The sediments were collected and incorporated into the prepared gel base to get ethosomal gel [8].

Evaluation of drug loaded ethosomal gel

pH: The pH of detailing inside the worthy reaches which demonstrate there is no conceivable disturbance up on instillation.

Appearances: Tramadol Hydrochloride is the white crystalline powder (Table 10).

Rheological studies

Viscosity of instilled formulation is an important factor determining residence time of drugs on the target site. Rheological studies were performed using Brookfield Viscometer .Prepared ethosomal gels were evaluated for their viscosities using suitable spindles at varying RPM [9] (Figures 11 and 12) (Tables 11 and 12).

Table 10. *Different characterization parameters of tramadol hydrochloride ethosomal gel.*

S.no	Parameters	Appearance	pH	Gelation Capacity	Gelation Temperature	Drug Content
1	F1	Transparent	7.4	++	37ºC	98.20
2	F2	Transparent	7.0	++	36ºC	101.02
3	F3	Transparent	7.0	++	37ºC	98.34
4	F4	Transparent	6.8	++	35ºC	98.25
5	F5	Transparent	6.4	++	36ºC	101.4

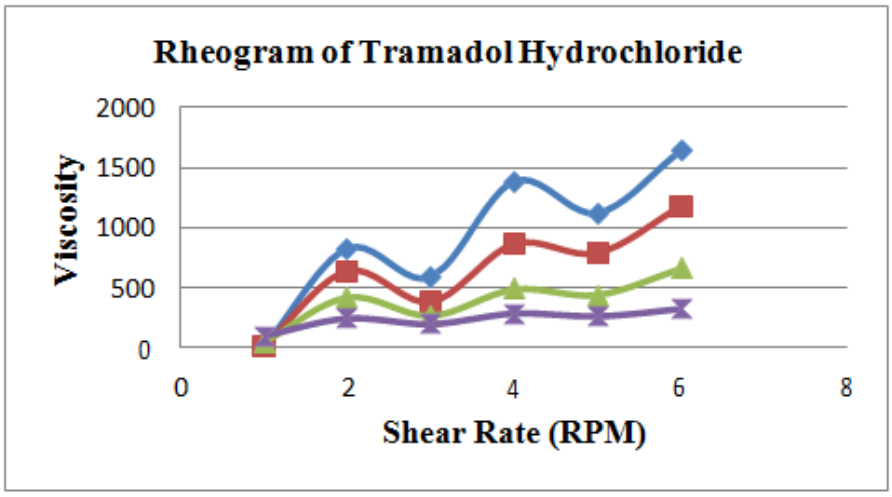

Figure 11. *Rheogram of tramadol hydrochloride before gelation.*

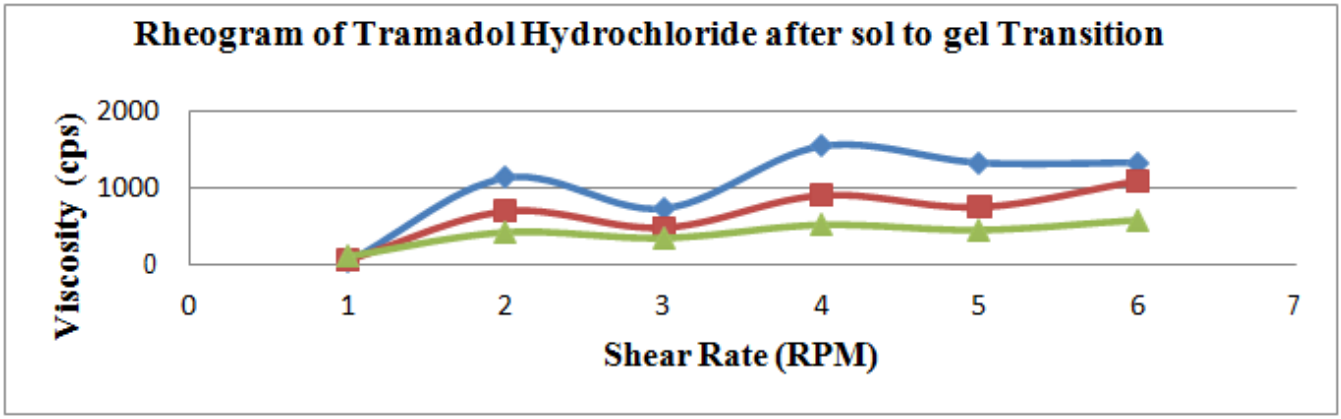

Figure 12. *Rheogram of tramadol hydrochloride after sol to gel transition.*

Table 11. *Shear rate of different formulation before gelation.*

Shear Rate (RPM)	Viscosity of the formulation (cps)				
	F1	F2	F3	F4	F5
10	825	589	1384	1120	1639
20	640	384	865	790	1178
50	420	268	487	441	659
100	245	198	286	263	324

Table 12. *Shear rate of different formulation after gelation.*

Shear Rate (RPM)	Viscosity of the formulation (cps)				
	F1	F2	F3	F4	F5
10	1405	1328	2024	2610	2610
20	1124	725	1540	1324	1324
50	689	475	898	745	1084
100	415	340	512	446	574

Drug entrapment efficiency

To take drug content example take 20 mg of arranged items and put in 25 ml of volumetric carafe and added 10 ml of methanol under sonication for 30 min. the volume was made up to stamp with methanol and afterward went through whatman channel paper no. 41. Consequently arrangement was reasonably weakened with a similar dissolvable and medication content spectrophotometric tested at specific frequency. The rate drug content was determined for all clusters utilizing the condition [10].

$$\text{Drug content}\% = \frac{\text{Theorotically yield}}{\text{Practical yield}} \times 100$$

Evaluation of tramadol hydrochloride efficiency

Stacking effectiveness of ethosomes was dictated by centrifugation strategy. About 2 mg of lyophilized definition was weighed precisely and taken in a 2 ml of miniature axis tube. To it 2 ml of ethanol-PBS blend was taken and plan was suspended by vortex. At that point it was sonicated for few moments and centrifuged at 15000 rpm for 15 min. After centrifugation clear supernatant was gathered, the absorbance of the supernatant arrangement was estimated in UV/VIS Spectrophotometer against the clear at the frequency of 432 nm. The medication content was resolved from the standard bend [11] (Table 13).

$$\%\text{loading efficiency} = \frac{\text{Wt of drug in 1 mg formulation total wt of formulation}}{\text{Total amount of drug for each formulation}}$$

Surface morphology of ethosomal Gel

Imaging technique that provides photographic images and elemental information TEM is useful for characterization the morphology and size of microscopic specimens with particle size as low as Nano meter to decameter.

The sample is placed in an evaluated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomena that, when detected, are used to form images and provides elemental information about the specimens [12] (Figures 13 and 14).

In vitro Diffusion Study

Release kinetics

The energy and component of medication discharge was resolved utilizing distinctive motor models. The active models utilized were zero request as total measure of medication discharge versus time, first request as log aggregate level of medication remaining versus time, Higuchi model as total level of medication discharge versus square foundation of time, Korsmeyer and Peppas as log aggregate percent drug discharge versus log time [13] (Table 14).

In vitro drug release kinetics

In vitro drug release through cellophane membrane

Cellophane film (0.45 μm, got from sigma synthetics) was utilized for this examination. An example of 1 g of the planning was filled in cellophane film recently splashed for the time being in the delivery medium. The stacked film was solidly positioned in disintegration medium (Phosphate support, pH 7.4, 900 ml) at 37ºC ± 0.5ºC. Aliquots every one of 5 ml were removed from the delivery medium at determined time spans. Removed examples were supplanted by equivalent volumes of new delivery medium. The examples were tested spectrophotometrically at λ max 240 nm and the centralization of the medication was resolved from the recently built adjustment bend. Every information point addressed the normal of three judgments. *In vitro* discharge reads were recorded for a 12 hour period (Tables 15 and 16).

Stability studies

Stability Studies are quite possibly the main investigations completed over the span of the improvement cycle. An item steady in the enormous scope of temperature and dampness climate is accepted to an ideal item. As the completed items might be transported into different ecological zones, their strength should be ensured. Every one of the details were exposed to sped up soundness testing. Details were dissected for the visual appearance, lucidity, pH and % drug remaining. 4 weeks of security affirmed that there was no adjustment of the visual appearance, clearness, pH and % drug remaining. a month of steadiness affirmed that there was no adjustment of the visual appearance and lucidity. Anyway slight changes were accounted for in the pH of the plan, yet were in the satisfactory reach (± 0.5). Percent Drug remaining was likewise found acceptable during the security study (Tables 17-20).

Table 13. *Drug entrapment efficiency.*

Formulation code	Absorbance (271 nm)	%Entrapment
F1	0.305	99.01%
F2	0.223	99.25%
F3	0.483	98.3%
F4	1.488	94.5%
F5	0.291	98.9%

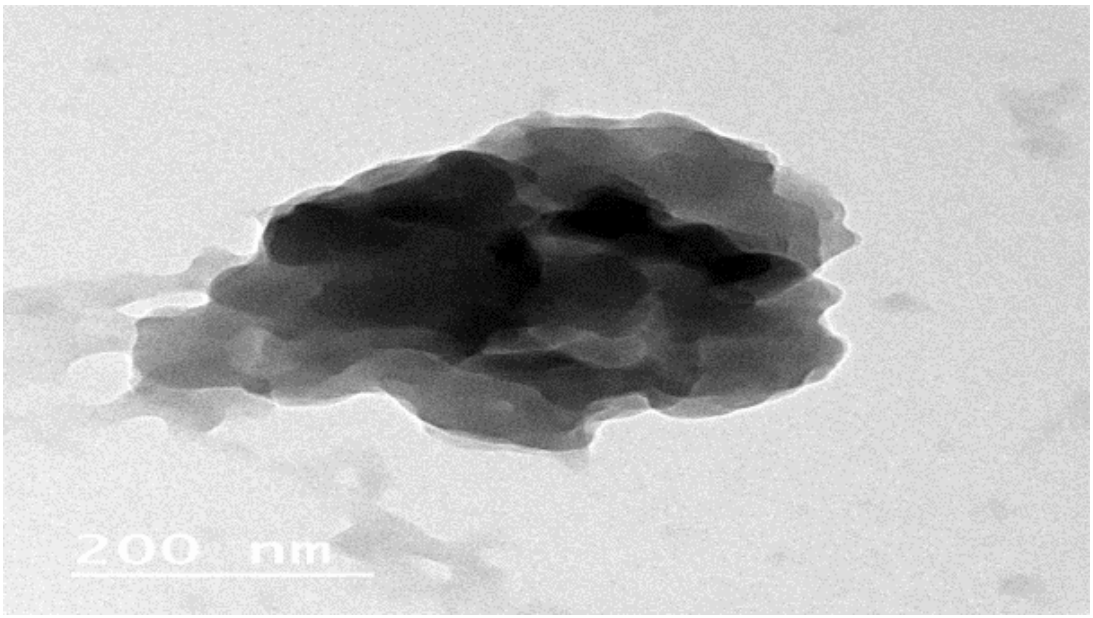

Figure 13. *TEM study.*

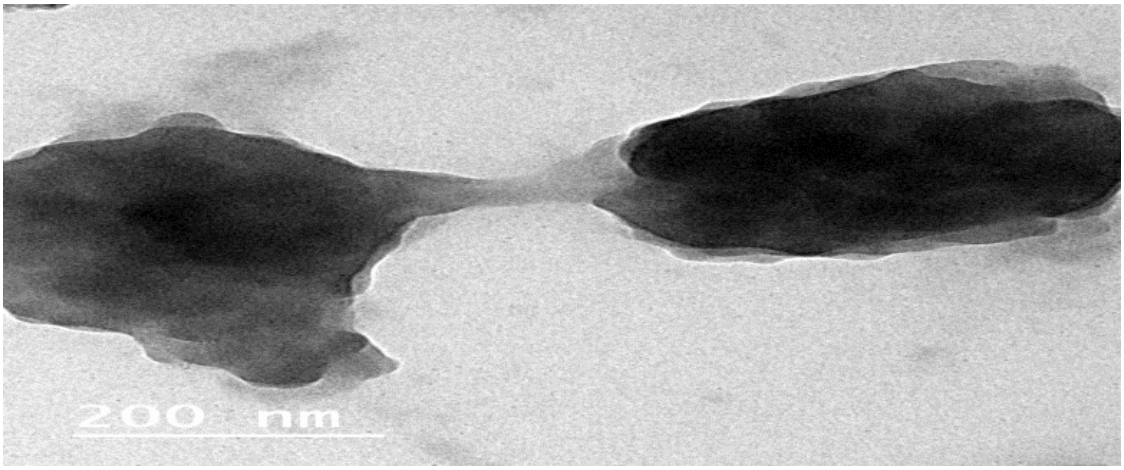

Figure 14. *SEM study.*

Table 14. *In vitro diffusion study.*

Time	F1	F2	F3	F4	F5
1	31.55	21.5	18.29	14.35	13.32
2	43.21	33.56	32.45	26.01	27.23
3	51.56	44.93	40.32	34.27	36.35
4	67.13	55.27	48.10	44.85	44.67
5	75.11	63.16	61.1	54.38	56.59
6	80.24	70.51	69.47	62.34	65.67
7	88.43	79.5	74.86	68.20	70.23
8	91.36	88.87	84.34	78.27	74.86

Table 15. *Release kinetics data for tramadol hydrochloride ethosomal gel preparations.*

Formulation code	Zero order	First order	Higuchi	Korsmeyer peppas		Best fitting model
	R2	R2	R2	R2	n	
1	0.991	0.994	0.947	0.921	0.543	First order
2	0.980	0.993	0.971	0.948	0.648	First order
3	0.960	0.997	0.987	0.941	0.724	First order
4	0.939	0.992	0.996	0.933	0.766	Higuchi
5	0.925	0.994	0.996	0.920	0.820	Higuchi

Table 16. *Skin irritation study.*

Formulation code	Average score		
	DAY-1	DAY-4	DAY-8
Control	0	0	0
Std	0	1.8	3.5
GF2	0	0	1.2

Where, GF2=Gel Formulation

0=no irritation

0.5%=Faint, barely perceptible and slight dryness

1=Definite erythema but no eruption or brain skin

1.5=Well defined erythema with dryness and epidermal fissuring

2=Moderate erythema

2.5=Moderate erythema with barely perceptible edema

3=Sever erythema

3.5=Moderate to severe erythema with escher formation

4=Moderate to server erythema with escher formation and edema extending the applied area.

Table 17. *Stability study result on formulation F1.*

S.no	Number of days	Visual Appearance		Clarity		pH	
		RT	40ºC	RT	40ºC	RT	40ºC
1	0	Transparent	Transparent	Clear	Clear	7.4	7.4
2	7	Transparent	Transparent	Clear	Clear	7.4	7.4
3	14	Transparent	Transparent	Clear	Clear	7.4	7.5
4	21	Transparent	Transparent	Clear	Clear	7.4	7.5
5	28	Transparent	Transparent	Clear	Clear	7.4	7.5

Table 18. *Stability study result on formulation F2, F3, F4, F5.*

S.no	Number of days	F2		F3		F4		F5	
		pH		pH		pH		pH	
		RT	40ºC	RT	40ºC	RT	40ºC	RT	40ºC
1	0	7.0	7.0	7.0	7.0	6.8	6.8	6.4	6.4
2	7	7.0	7.0	7.0	7.0	6.8	6.9	6.4	6.5
3	14	7.0	7.1	7.0	7.1	6.8	6.8	6.4	6.5
4	21	7.0	7.1	7.0	7.1	6.8	6.9	6.4	6.4
5	28	7.0	7.2	7.0	7.2	6.8	6.8	6.4	6.5

Table 19. *Stability study of all formulation at room temperature.*

S.no	Number of days	Percentage Drug Remaining				
		F1	F2	F3	F4	F5
1	0	98.90	101.2	98.34	98.25	101.4
2	7	98.18	101.2	98.32	98.21	101.01
3	14	97.95	100.34	98.28	98.17	100.94
4	21	97.78	100.15	98.24	98.13	100.67
5	28	97.68	99.99	98.16	98.07	100.43

Table 20. *Stability study of all formulation at 40ºC.*

S.no	Number of days	Percentage Drug Remaining				
		F1	F2	F3	F4	F5
1	0	98.20	101.2	98.34	98.25	101.4
2	7	98.13	101.1	98.29	98.19	101.1
3	14	97.87	99.94	98.21	98.15	100.74
4	21	97.73	99.89	98.17	98.09	100.57
5	28	97.64	99.84	98.13	98.03	100.33

Conclusion

It was conclude that Tramadol Hydrochloride ethosomal gel was prepared and evaluated for various parameters like vesicle shape, % drug entrapment efficiency, *in vitro* diffusion study, skin irritation study and stability studies so as to find out the formulation with optimum drug release and efficiency. Formulation 2 was found the best with the spherical vesicle shape and 99.25% drug entrapment efficiency. It showing the non-irritant to the skin when tested on animals and was also stable at 40ºC ± 50ºC and room temperature even after two months.

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