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# Preparation and Evaluation of Stable Nonionic Surfactant Vesicular System for Tramadol HCl

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## ABSTRACT

The study was aimed at developing and optimizing a stable nonionic surfactant vesicular system known as niosomes for Tramadol HCl in order to improve its release properties and bioavailability. In the study different composition of non ionic surfactants and lipids were used to prepare nonionic surfactant vesicles formulation. The formulations were evaluated for entrapment efficiency, particle size, zeta potential, viscosity and in vitro drug release in 12 hrs. The in vitro permeability of the drug was evaluated by using dialysis membrane. The results of the in vitro drug release / permeability in 12 hrs shows better release in comparison with pure drug and marketed formulation. The results support the potential for nonionic surfactant vesicular system as an alternative to conventional delivery of Tramadol HCl.

**Keywords:** Non ionic vesicular system, Tramadol HCl , In vitro release, Permeability.

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## INTRODUCTION

Nonionic surfactant vesicles which are known as niosomes are microscopic lamellar structures formed from the mixture of nonionic surfactants and lipids in the form of cholesterol or lecithin which is hydrated in aqueous media [1]. It was observed in the literature that, the vesicular size in niosomes is generally around 0.025 µm to 0.15 µm. The larger unilamellar vesicles of size more than 0.25 µm can be labeled as only nonionic surfactant vesicle rather than niosomes. The large size of vesicles may give more stable dispersion and may encapsulate more amount of drug (hydrophilic) than small sized vesicles. Niosomes can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials. It is reported to attain better stability than liposomes. It can prolong the circulation of the entrapped drugs. Niosomes improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.[2]

Tramadol hydrochloride is a synthetic centrally acting aminocyclohexal analgesic that acts as an opioid agonist with selectivity for the µ receptor.[3] It inhibits reuptake of norepinephrine and serotonin which appear to contribute to the drugs analgesic effect.[4] This drug is used as an effective agent for moderate to severe chronic pain.[5,6] The half life of the drug is about 4-5 h and the usual oral dosage regimen is 50 to 100 mg every 4 to 6 h with a reported maximum dosage of 400 mg/day. To reduce frequent administration of dosage form and to improve patient compliance, a sustained-release formulation of this drug is desirable. The drug is freely water soluble therefore selection of agents for release modification is critical step in the formulation development of this drug.[7] Frequent dosing schedule is the reason for poor patient compliance, increased side effects and tolerance development, especially, in long term use in conditions like arthritis, osteoarthritis, arthralgia, postoperative surgical pains *etc*.[8-10] Thus a modified release form for Tramadol HCl is needed and stable non ionic surfactant vesicles contains lipids may prove to be safe and effective alternative for existing immediate release form of Tramadol HCl. In the present study lipids like soya lecithin and cholesterol are used and the non ionic vesicles are stabilized by using Sorbitan fatty acid esters and/ or Polysorbates to form a stable and convenient delivery of Tramadol HCl.

## MATERIALS AND METHODS

Tramadol HCl was obtained as gift sample from Trio Remedies Pvt. Ltd. Ahmadabad, Gujarat, India. Span 60, Tween 80, Diethyl ether, Methanol, potassium dihydrogen ortho phosphate, sodium hydroxide was obtained from Loba chemie Pvt. Ltd. Mumbai, India. All the reagents, chemicals and solvents used in the study are of AR grade.

### Evaluation of raw materials

Identification and standardization of drug and other excipients used in the study were preformed as per the monographs available in the literature.

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## Preparation of Non ionic surfactant vesicular system (NSV's)

NSV's containing Tramadol HCl were prepared by modified ether injection method. The method includes the selection of nonionic surfactant like sorbitan fatty acid esters (span 60),polysorbates (tween 80), and lipids like cholesterol, soya lecithin. Drug was dissolved in solvent mixture. The resulting solution was slowly injected using microsyringe at a rate of 1ml/min into 15 ml of hydrating solution phosphate buffer (pH 7.4) The solution was stirred continuously on magnetic stirrer and temperature was maintained above 60ºC. As the lipid solution was injected slowly into aqueous phase, the vaporization of solvent takes place, resulting in spontaneous vesiculation and formation of nonionic surfactant vesicles. Different batches of nonionic surfactant vesicles were prepared in order to optimize the preparation. The nonionic surfactants and lipids were taken in different proportions. Table No.1.

**Table no.1: Composition of surfactant and lipid for preparation of nonionic surfactant vesicles.**

| Sr. No | Formulation code | Drug (mg) | Surfactant (mg) | | Lipid (mg) | | Drug: surfactant: lipid |
|---|---|---|---|---|---|---|---|
| | | | | | Cholesterol | Soya lecithin | |
| 1 | F1 | 100 | Span 60 | 100 | 100 | - | 1:1:1 |
| 2 | F2 | 100 | | 200 | 100 | - | 1:2:1 |
| 3 | F3 | 100 | | 100 | - | 100 | 1:1:1 |
| 4 | F4 | 100 | | 200 | - | 100 | 1:2:1 |
| 5 | F5 | 100 | Tween 80 | 100 | 100 | - | 1:1:1 |
| 6 | F6 | 100 | | 200 | 100 | - | 1:2:1 |
| 7 | F7 | 100 | | 100 | - | 100 | 1:1:1 |
| 8 | F8 | 100 | | 200 | - | 100 | 1:2:1 |

## Characterization of NSV's

## Physicochemical properties

The physicochemical properties of drug including organoleptic properties like appearance odor, taste, melting point were studied. The Tramadol HCl is White and crystalline powder, is odorless, and has Bitter taste. The melting point of Tramadol HCl was found in the range of 181° to 183°C. Tramadol HCl is highly soluble in polar solvents with pKa value 9.41.

## Vesicle size and Shape analysis

All batches of NSV's (F1-F8) have been characterized for vesicle size under motic microscope (B1 Advanced ) at 40x and 100x objective lens. The vesicle size and morphology of nonionic surfactant vesicles were determined by using motic software.[11] Figure No.1. The results showed that the maximum vesicle size obtained was 0.99 µm and the minimum size was

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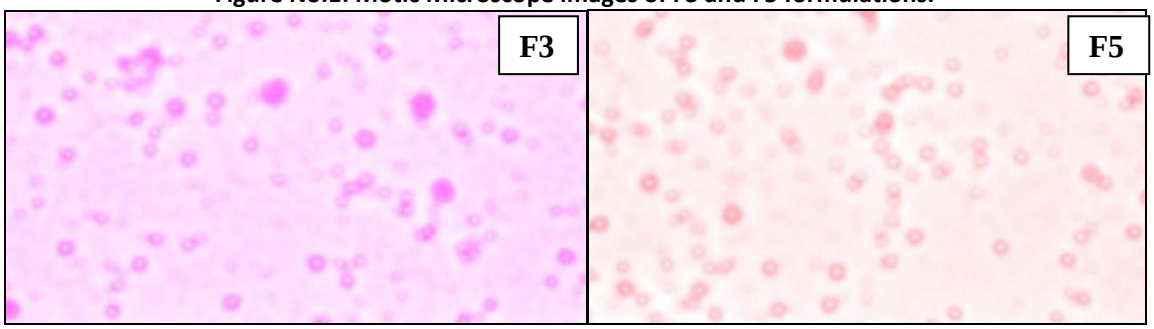
0.1 µm. The shape of the vesicles was found spherical with large unilamellar type. The mean diameters observed with standard deviation are as given in Table No.2.

**Table no.2: Evaluation parameters of nonionic surfactant vesicles.**

| Sr. no. | Formulation code | Entrapment efficiency* | Drug content* | Particle size* | % Cumulative *In-vitro* Drug Release* |
|---|---|---|---|---|---|
| 1 | F1 | 39.34±0.69 | 99.02±0.34 | 0.34±0.15 | 97.53±0.16 |
| 2 | F2 | 64.05±0.34 | 99.88±0.45 | 0.33±0.17 | 89.68±0.03 |
| 3 | F3 | 88.26±0.55 | 101.35±0.71 | 0.34±0.18 | 101.45±0.18 |
| 4 | F4 | 67.85±0.04 | 101.99±0.05 | 0.33±0.18 | 92.37±0.16 |
| 5 | F5 | 87.06±0.26 | 101±0.26 | 0.33±0.18 | 99.45±0.30 |
| 6 | F6 | 57.48±0.32 | 103.37±0.15 | 0.33±0.17 | 56.71±0.04 |
| 7 | F7 | 62.83±0.16 | 112.88±0.49 | 0.33±0.19 | 86.11±0.10 |
| 8 | F8 | 51.37±0.20 | 99.83±0.28 | 0.34±0.18 | 71.254±0.07 |

*indicates average ± standard deviation (n=3).

**Figure No.1: Motic Microscope images of F3 and F5 formulations.**




## Drug Content

For the drug content determination 1ml of NSV's dispersion was dissolved in 100 ml phosphate buffer pH 7.4 with the help of bath sonicator (Biomedica BM 1599) and then filtered and analyzed by UV spectrophotometer (Shimadzu 1800) at 271 nm. The % Drug content was calculated by equation (Eq$^{n}$ No.1). The results showed that the drug contents were in the range of 99.83 to 112.01% . Table No.2.

$$\% \text{ Drug content} = \frac{\text{Drug content in dispersion } \mathbf{x}\ 100}{\text{Theoretical amount of drug}} \quad \text{Eq}^{n} \text{ no.1}$$

## Entrapment efficiency

For the entrapment efficiency total solution of NSV's were centrifuged. Supernatant liquid and the precipitate were separated. 1 ml of supernatant and 10 mg of precipitate was dissolved in 100 ml of phosphate buffer 7.4 with help of bath sonicator (Biomedica BM 1599) and filtered to analyze by UV spectrophotometer (Shimadzu 1800) at 271 nm. Entrapment

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efficiency was calculated by following equation (Eq$^{n}$ No.2). The results showed that the maximum entrapment of 88.26% for F3 and lowest entrapment of 39.34 % for F1 formulations. Table No.2.

$$\% \text{ Drug entrapped} = \frac{\text{Drug content in precipitate } \mathbf{x}\ 100}{\text{Theoretical amount of drug}} \quad \rightarrow \text{Eq}^{n} \text{ no.2}$$

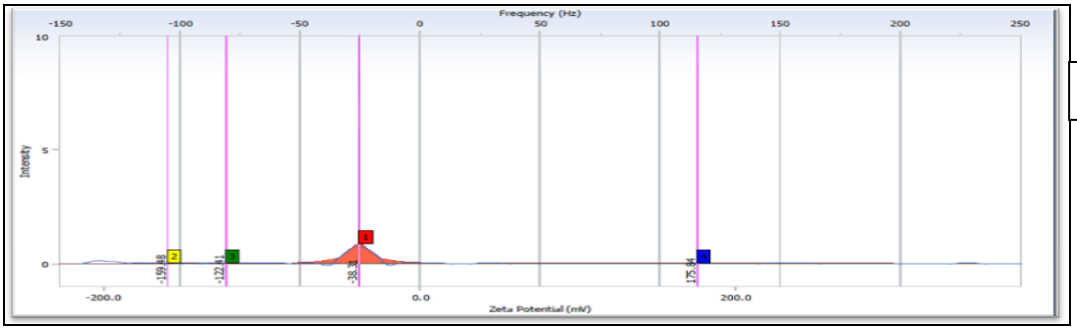
**Vesicular surface charge**

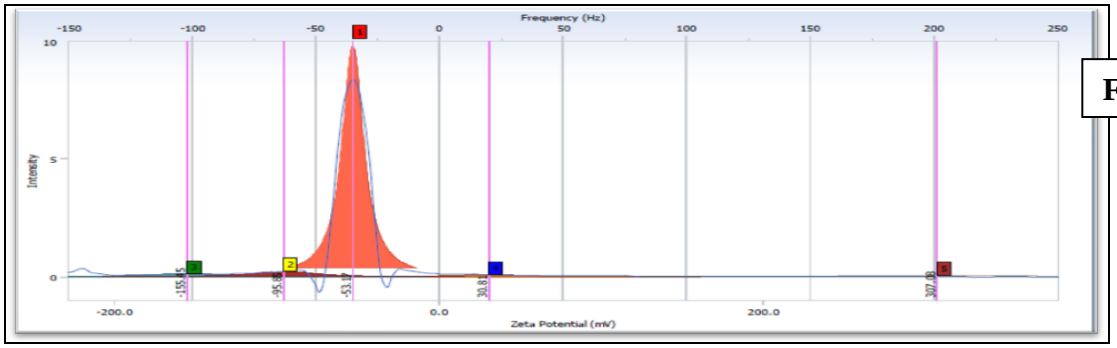
Colloidal properties of the prepared NSV formulations were studied for the magnitude of zeta potential. The suitably diluted NSV dispersion was taken for the study. The zeta potential is estimated by electrophoresis light scattering and laser Doppler velocimetry method. The temperature was set at 25$^{0}$C. Charge on vesicles and their mean zeta potential values with standard deviation of measurements were obtained directly from the measurement. The zeta potential results are shown in Figure No.2. and Table No. 3.

**Table no. 3- Zeta potential and conductivity measurement of optimized formulations.**

| Sr. no. | Parameters | F3 | F5 |
|---|---|---|---|
| 1 | Zeta Potential (mV) | -38.31 | -53.17 |
| 2 | Mobility (cm²/Vs) | -3.004e-004 | -4.146e-004 |
| 3 | Conductivity (mS/cm) | 0.0122 | 0.0126 |
| 4 | Doppler shift (Hz) | -25.24 | -34.85 |

**Figure No.2: Zeta potential of nonionic surfactant vesicles formulation F3 and F5**



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### *In vitro* release studies

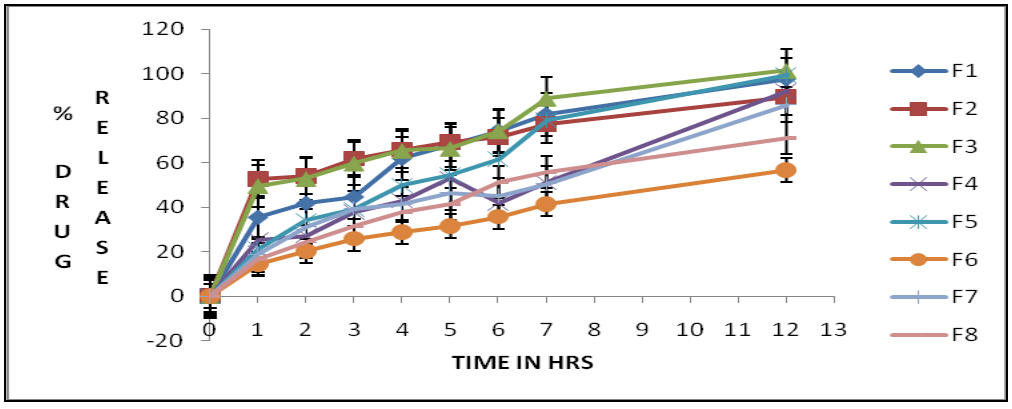
The release of Tramadol HCl from NSV formulations were determined using membrane diffusion technique. The NSV formulation equivalent to 10mg of drug was placed in a glass tube of diameter 2.5cm with an effective length of 8cm that was previously covered with soaked osmosis dialysis membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 100ml of phosphate buffer (pH 7.4), which acted as receptor compartment. The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touching (1-2mm deep) the surface of diffusion medium. The temperature of receptor medium was maintained at 37±1 $^0$C and agitated at 100rpm speed using magnetic stirrer. Aliquots of 1 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed at 271 nm in Double beam UV-VIS spectrophotometer using phosphate buffer (pH 7.4) as blank. The drug release/permeability of optimized formulations were compared with pure drug and marketed formulation. The graph of % drug released was plotted against time in hr. The results are given in Table No.4 and Figure No.3.

**Table no. 4: comparison of optimized formulations with pure drug and marketed formulation**

| Time in hrs | Plain drug* | Marketed formulation* | F3* | F5* |
|---|---|---|---|---|
| 0.30 min | 29.13±0.19 | 26.38±0.26 | 15.89±0.12 | 5.24±0.13 |
| 1 | 43.64±0.22 | 35.79±0.28 | 26.31±0.23 | 11.28±0.12 |
| 1.5 | 58.59±0.26 | 47.31±0.34 | 38.26±0.07 | 15.19±0.19 |
| 2 | 69.23±0.05 | 58.29±0.26 | 49.59±0.05 | 21.12±0.08 |
| 3 | 81.15±0.11 | 71.25±0.04 | 59.75±0.04 | 34.01±0.03 |
| 4 | 100.29±0.06 | 87.57±0.78 | 68.68±0.02 | 39.02±0.04 |
| 5 | - | 99.18±0.12 | 74.39±0.07 | 49.76±0.10 |
| 6 | - | 101.29±0.01 | 79.34±0.18 | 54.42±0.30 |
| 7 | - | 102.35±0.1 | 84.24±0.12 | 61.58±0.42 |
| 8 | - | - | 88.84±0.07 | 79.12±0.18 |
| 12 | - | - | 101.45±0.13 | 99.45±0.09 |

*indicates average ± standard deviation (n=3).

**Figure No. 3: *In-vitro* release of Tramadol HCl nonionic surfactant vesicles ( F1-F8)**



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## Viscosity

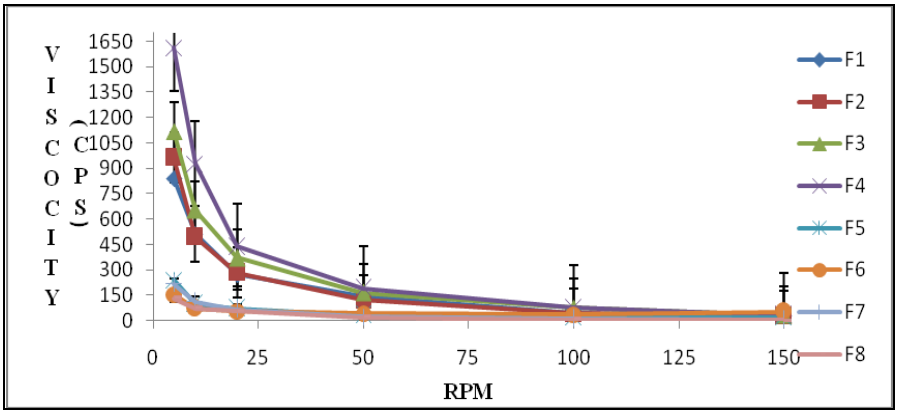
The viscosity measurements were carried out by using Brookfield programmable RVDV-II+ pro model (Brookfield Eng. Lab., Inc.USA). The formulations samples were placed in small sample adapter. Temperature was maintained in the range of $30^0C \pm 2^0C$. The study was performed at various RPM (from 5-150). The rheological behavior of the NSV's formulations were studied given in the Table No.5. Figure No. 5

**Table no. 5. : Viscosity of nonionic surfactant vesicle formulation at different RPM.**

| RPM | F1* | F2* | F3* | F4* | F5* | F6* | F7* | F8* |
|---|---|---|---|---|---|---|---|---|
| 5 | 841.6±7.6 | 963.0±9.8 | 1117.6±6.8 | 1611.7±12.5 | 239.7±4.5 | 155.5±1.0 | 216±5.2 | 132.7±2.5 |
| 10 | 510.0±10.0 | 495.3±5.0 | 653.3±6.1 | 927.6±7.5 | 99.1±1.0 | 73.2±1.0 | 112.3±2.5 | 73±2.6 |
| 20 | 274.6±12.6 | 283.6±3.2 | 371.3±3.2 | 437.3±6. | 72.3±2.5 | 55.2±3.6 | 65.5±1.0 | 54.6±4.5 |
| 50 | 142.3±11.2 | 122.5±2.6 | 163.7±5.1 | 190.6±2.0 | 32.3±2.5 | 42.1±2.0 | 22.7±2.0 | 21.3±1.5 |
| 100 | 45.2±10.5 | 43.3±3.0 | 76.3±3.9 | 74.3±4.0 | 22.3±1.5 | 37.0±2.6 | 11.7±0.7 | 7.5±0.5 |
| 150 | 26.4±5.5 | 28.3±0.7 | 32.3±3.0 | 32.6±2.3 | 19.9±2.8 | 24.2±1.0 | 7.3±0.5 | 3. 7±0.3 |

*indicates average ± standard deviation (n=3).

**Figure No. 5: Viscosity of formulations at different RPM (F1-F8**




## Stability study

Stability studies were carried out as per the ICH guidelines. Stability study was performed on samples stored at 2 to 8°C at periodical intervals of 15, 30 and 45 days. All NSV formulations studied has shown good stability and no significant change in vesicle size and percent entrapment was observed. [12, 13] Table No 6.

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**Table no. 6: Characterization of nonionic surfactant vesicles kept for stability study.**

| Batch code | Vesicle size range(µm)* | | | Entrapment efficiency (%)± S.D.* | | |
|---|---|---|---|---|---|---|
| | After 15 days | After 30 days | After 45 days | After 15 days | After 30 days | After 45 days |
| F3 | 0.91±0.28 | 0.68±0.25 | 0.64±0.56 | 85.29±0.19 | 81.48±0.94 | 77.29±0.78 |
| F5 | 0.82±0.33 | 0.60±0.64 | 0.58±0.11 | 83.64±0.04 | 79.28±0.13 | 74.91±1.39 |

*indicates average ± standard deviation (n=3).

## RESULTS AND DISCUSSION

Among the various methods available for the preparation of NSV's, modified ether injection method was used. The entrapment efficiency of nonionic surfactant vesicles prepared at varied concentration of surfactants, lipids was found in the range of 39.34 - 88.26%.

The effect of amount of surfactant and lipid was found to be affecting entrapment efficiency. The similar effect was observed on the drug content in the formulation F3 and F5 has showed better entrapment efficiency, viscosity, and stability. The vesicular sizes of the nonionic surfactant vesicles were measured by using motic microscope with calibrated eyepiece micrometer. The average vesicular size of nonionic surfactant vesicles of all the formulations was measured in the range of 0.33±0.15µm to 0.99±0.17µm. The result suggested that nonionic surfactant vesicles prepared were of uniform size and spherical in shape as shown in Figure No.
1. It has seen that large unilamellar vesicles were formed which are uniformly distributed in the formulation.

In all the NSV's prepared with span 60 and soya lecithin, a linear relation between amount of surfactant and lipid with entrapment efficiency was observed. The encapsulation efficiency of nonionic surfactant vesicles was governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Cholesterol with tween 80 improves the fluidity of the bilayer membrane and improves the stability of bilayer membrane. *In Vitro* drug release (permeability) of various formulations of NSV were found in the range of 56.71% to 101.91% for formulations F1-F8. From the release profile, viscosity and vesicular size formulation F3 and F5 were optimized and were taken for stability study and were further compared with pure drug and marketed formulation for release characteristics. Formulation F3 showed the % drug released 101.91% and for formulation F5 showed 99.45% in 12 hrs. The % drug release for pure drug is 100.29% in only first 4 hrs. and for marketed formulation it is 100.25% in 7 hrs. The results showed the better and optimal release pattern of optimized formulation as compared to pure drug and marketed formulation. Table No. 4, Figure No. 4.

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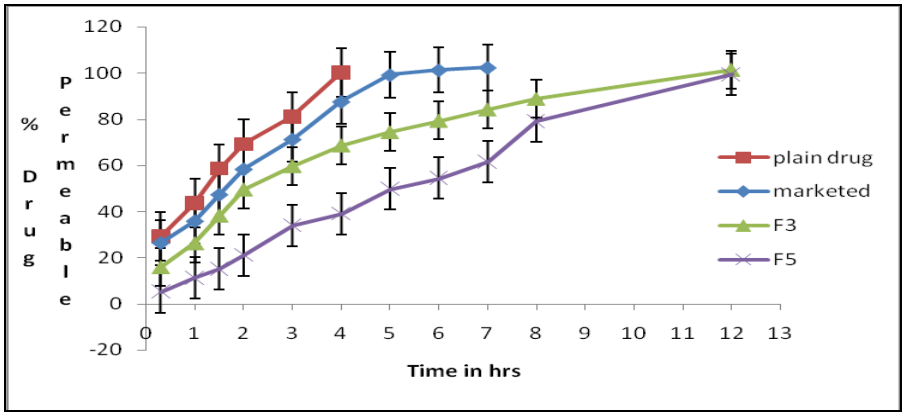
**Figure No. 4: comparison of optimized formulation with pure drug and marketed formulation.**




## CONCLUSION

The present study demonstrated the successful preparation of Tramadol HCl nonionic surfactant vesicles (NSV's). A modified release NSV's of water soluble drug using hydrophilic-hydrophobic polymeric blend can be prepared by modified ether injection method with better yield of production, drug content, and better encapsulation efficiency. All NSV's formulations have good morphological and vesicular size characteristics. It could be revealed that Tramadol HCL nonionic surfactant vesicles NSV's formation had different characters and also demonstrated a better sustained release behavior up to 12 hrs in optimized formulation in comparison with pure drug and marketed formulation. NSV's form can be the promising alternative to the conventional Tramadol HCl products.

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## REFERENCES

[1] Malhotra M, Jain NK. Indian Drugs 1994;31(3), 81.
[2] Jain NK, Controlled & Novel Drug Delivery, 1st edition, published by CBS Publishers & Distributors, New Delhi; 2004;292 .
[3] Hennies HH, Friderichs E. Arzneimittel For Schung 1988;38:877-880.
[4] Poulsen L, Arendt – Nielsen L, Brosen K, Sindrup SH. Clin Pharmacol Ther 1996;60:636-644.
[5] Osipova NA, Novikov GA and Beresnev VA. Curr Ther Res Clin Exp 1991;50:812-821.
[6] Sunshine A and Olson NZ. Clin Pharmacol Ther 1992;51:740-746.
[7] Gonjari D. Drug Discove Ther 2009;3(4):176-180.
[8] Obaidat AA and Obaidat RM. Eur J Pharm Biopharm 2001;52:231– 235.

[9] Tiwari SB and Murthy TK. AAPS PharmSciTech 2003;4(3):1–6.
[10] Scott LJ and Perry CM. Drugs 2000;60(1):139–176.
[11] Dahiya NK, Rao R, Nanda S. J Pharm Biomed Sci 2011; 5: 1-8.
[12] Kapadia R, Khambete H, Katara R, Ramteke S. J Pharm Res 2009; 2: 745-751.
[13] Pandey VP, Deivasigamani K. J Pharm Res 2009; 2: 1330-1334.