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Research Article

Formulation and Characterization of Nefopam Hydrochloride-Loaded Niosomes for Enhanced Analgesic Drug Delivery

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Abstract

Background: Nefopam Hydrochloride (NF-HCl) is a centrally acting non-opioid analgesic with limited oral bioavailability due to first-pass metabolism. To overcome this limitation and provide sustained drug release, niosomal drug delivery systems were explored.

Objective: To formulate and characterize NF-HCl-loaded niosomes using Span surfactants and cholesterol via Hand Shaking Method (HSM) and Ether Injection Method (EIM), and evaluate their physicochemical and drug release properties.

Methods: Twelve niosomal formulations were prepared using three non-ionic surfactants (Span 20, 40, and 60) in 1:1 and 2:1 molar ratios with cholesterol. The formulations were evaluated for entrapment efficiency (EE%), vesicle size, morphology, zeta potential, in vitro drug release, and drug release kinetics.

Results: Among the formulations, PNF-9 (Span 60:CH, 1:1, EIM) showed the highest EE (58.94%), sustained drug release (58.82% over 8 hours), and a zeta potential of −64.6 mV. Drug release kinetics followed the Korsmeyer-Peppas model with an R^2 value of 0.9965, indicating non-Fickian diffusion. Stability studies confirmed greater retention at 4–8°C.

Conclusion: The optimized NF-HCl niosomal formulation (PNF-9) prepared via EIM using Span 60 exhibited promising characteristics for sustained and targeted analgesic delivery, potentially enhancing therapeutic efficacy and patient compliance.

Keywords: Niosomes, Nefopam Hydrochloride, Span 60, Entrapment Efficiency, Drug Release Kinetics, Ether Injection Method

INTRODUCTION

Niosomes are non-ionic surfactant-based vesicles that have emerged as a promising drug delivery system due to their ability to encapsulate a wide range of drugs, enhancing their bioavailability, stability, and targeted delivery. Structurally similar to liposomes, niosomes are more cost-effective and stable because they are composed of non-ionic surfactants and cholesterol, making them highly suitable for pharmaceutical applications, including analgesic drug delivery[1]. Nefopam hydrochloride, a centrally acting non-opioid analgesic, is used for managing moderate to severe pain. It works by inhibiting the reuptake of serotonin, norepinephrine, and dopamine, providing pain relief without the adverse effects associated with NSAIDs or opioids, such as respiratory depression or gastrointestinal issues[2]. Niosomes consist of an aqueous core surrounded by a bilayer of non-ionic surfactants and cholesterol[3]. The surfactants commonly used include sorbitan esters such as Span 60, Span 40, and Span 20, which offer stability and low toxicity[4], and polyoxyethylene derivatives like Tween 20 and Tween 80, which enhance vesicle permeability and flexibility[5]. Cholesterol stabilizes the bilayer, reduces membrane permeability, and increases encapsulation efficiency[6]. Charge inducers, such as dihexadecyl phosphate (negative) and stearylamine (positive), are added to prevent aggregation and improve vesicle stability[7]. Other additives like antioxidants (e.g., α-tocopherol) may also be included to protect against oxidative degradation[8]. Various methods are available for preparing niosomes. The thin film hydration method involves dissolving surfactants and cholesterol in organic solvents, evaporating them to form a lipid film, and hydrating it with an aqueous drug solution. This produces multilamellar vesicles that can be downsized by sonication or extrusion[9]. In the reverse phase evaporation method, a water-in-oil emulsion is formed and evaporated under reduced pressure, yielding high drug encapsulation efficiency and uniform vesicle size[10]. The ether injection method uses slow injection of surfactant solutions into a heated aqueous phase, where evaporation of ether leads to spontaneous vesicle formation[11]. Microfluidization involves high-pressure mixing of organic and aqueous phases to produce uniform, small-sized niosomes ideal for large-scale production[12]. Sonication applies ultrasonic waves to reduce vesicle size, although care must be taken to avoid drug degradation[13]. Extrusion involves passing vesicles

through membranes with specific pore sizes to achieve uniformity in size and improve control over drug release[14,15]. Encapsulating nefopam in niosomes enables controlled and sustained drug release by fusing with cellular membranes and delivering the drug directly into target cells or tissues[16,17]. This targeted approach enhances the therapeutic efficiency of nefopam, reduces the frequency of administration, and minimizes systemic side effects such as nausea and dizziness. Moreover, niosomal delivery improves patient compliance by maintaining prolonged analgesic effects and avoiding the drawbacks of conventional oral or intravenous administration[18,19]. Therefore, nefopam-loaded niosomes present a novel and effective strategy for pain management, particularly for patients who cannot tolerate opioids or NSAIDs.

MATERIAL AND METHODS

Nefopam Hydrochloride (purity >98%) was procured from Tokyo Chemical Industry (TCI), Japan, via MKJ International, Jaipur (Item Code: N1169-1gm; CAS No. 23327-57-3). The non-ionic surfactants used in the formulation included Span 20 (Sorbitan Monolaurate; CAS No. 1338-39-2; Product No. 056002; Batch No. 070120), Span 40 (Sorbitan Monopalmitate; CAS No. 26266-57-9; Batch No. 071020), and Span 60 (Sorbitan Monostearate; CAS No. 1338-41-6; Batch No. 070916). These surfactants were obtained from Central Drug House (P) Ltd., Daryaganj, New Delhi, under the LBS College of Pharmacy.[20]

Cholesterol (CAS No. 57-88-5; Product No. 12312; Lot No. 1407-7101-1) was purchased from Thermo Fisher Scientific India Pvt. Ltd., Powai, Mumbai. Diethyl ether (CAS No. 60-29-7; Product No. 028132; Batch No. 150422), an organic solvent used during niosome preparation, was also sourced from Central Drug House (P) Ltd., New Delhi.[21]

Phosphate buffered saline (PBS, pH 7.4) was prepared using disodium hydrogen phosphate (Product No. S0400; Batch No. J123D43), potassium dihydrogen phosphate (Product No. P0336; CAS No. 7779-78-0; Batch No. J037F40), and sodium chloride (Product No. S0173; Batch No. J089L20). All PBS reagents were procured from Rankem (Laboratory Chemicals Pvt. Ltd., Tiffany Building, Thane) and Rashtriya Chemicals Corporation, Chandigarh, India. All chemicals and reagents were of analytical grade and used as received without further purification.

Preparation of Niosomes

Two methods were used:

- **Hand Shaking Method (HSM)**: Surfactants and cholesterol dissolved in ether were evaporated to form a lipid film, then hydrated with drug-containing PBS buffer.[22]
- **Ether Injection Method (EIM)**: Organic phase containing surfactants and cholesterol was injected into heated aqueous phase, forming niosomes upon ether evaporation.[23]

RESULTS

Entrapment Efficiency (EE %): Measured by centrifugation and UV analysis at 266 nm.

Table 1: The entrapment efficiency of niosomal formulations (PNF-1 to PNF-6) prepared by the hand shaking method.

Formulation Batch	NIOs	NIOs: CH	Absorbance	Conc.(mg) *Dilution factor	%EE
PNF- 1	Span 20	1:1	0.318	2.832	19.29
PNF- 2	Span 40	1:1	0.434	4.119	27.28
PNF- 3	Span 60	1:1	0.707	6.783	44.38
PNF- 4	Span 20	2:1	0.289	2.624	18.91
PNF- 5	Span 40	2:1	0.469	4.331	28.56
PNF- 6*	**Span 60**	**2:1**	**0.838**	**8.013**	**53.09**

Table 2: The entrapment efficiency of niosomal formulations (PNF-7 to PNF-12) prepared by the ether injection method.

Formulation Batch	NIOs	NIOs: CH	Absorbance	Conc.(mg) Dilution factor	%EE
PNF- 7	Span 20	1:1	0.368	3.379	21.29
PNF- 8	Span 40	1:1	0.577	5.248	36.96
PNF- 9*	**Span 60**	**1:1**	**0.909**	**8.853**	**58.85**
PNF- 10	Span 20	2:1	0.369	3.696	24.35
PNF- 11	Span 40	2:1	0.532	5.165	34.09
PNF- 12	Span 60	2:1	0.773	7.395	49.89

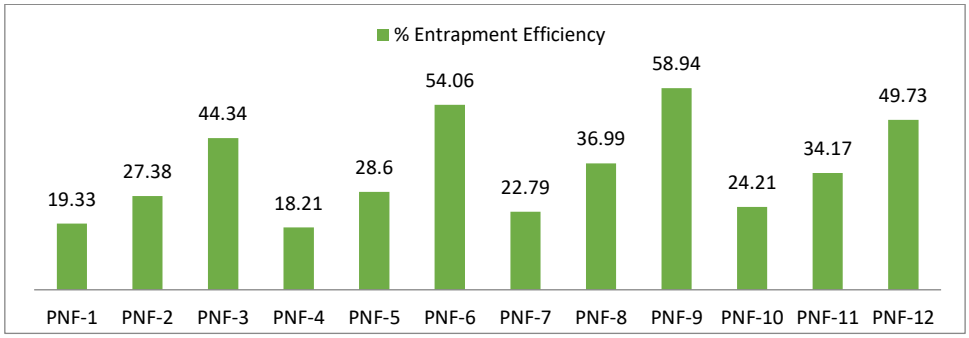

Figure 3: Comparison between EIM and HSM for % EE of all formulations

Morphology and Vesicle Size: Evaluated by optical microscopy and SEM.

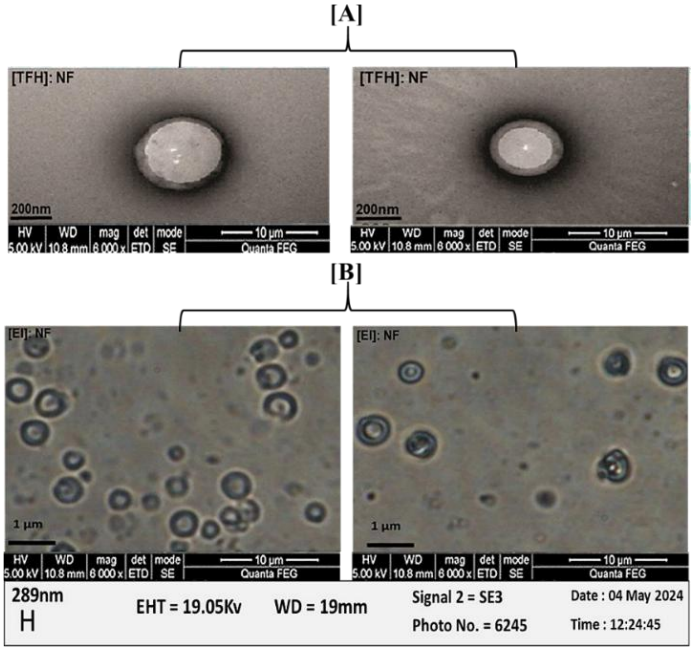

Figure 4: SEM image of niosomes;
[A]. Hand shaking (PNF-6) [B]. Ether injection method (PNF-9)

Zeta Potential: Measured to assess stability.

Figure 5: Zeta potential of niosomes

In Vitro Drug Release: Conducted using Franz diffusion cells for 8 hours.

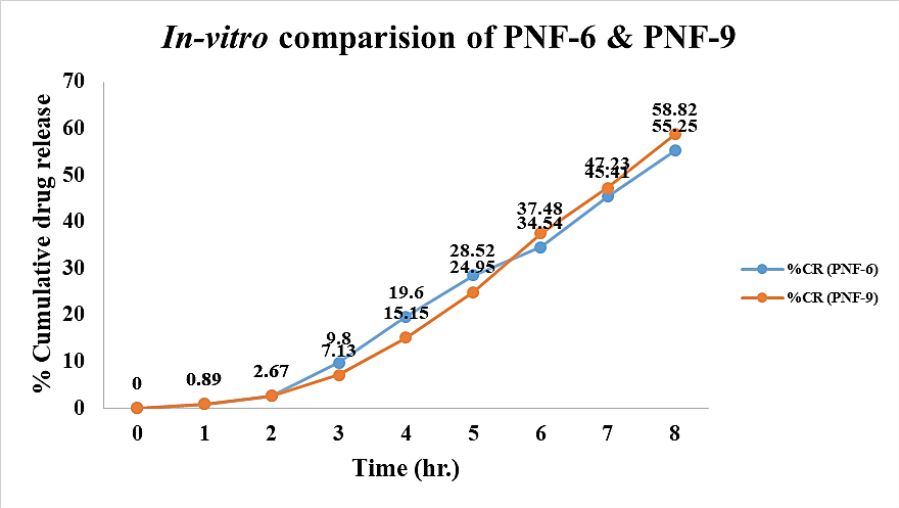

Figure 6: Graphical representation of the *in vitro* comparison of both optimized formulations (PNF-6 and PNF-9)

Table 3: *In vitro* diffusion of NF-HCl niosomes for 8 hours via the HS method

Time (hr.)	Formulation Batch											
	PNF-1		PNF-2		PNF- 3		PNF-4		PNF- 5		PNF- 6*	
	CR	%CR	CR	%CR	CR	%CR	CR	%CR	CR	% CR	CR	%CR
0	0	0	0	0	0	0	0	0	0	0	**0**	**0**
1	0.259	1.67	0.192	0.89	0.289	1.72	0.434	2.89	0.267	1.69	**0.131**	**0.86**
2	0.802	5.29	0.399	2.52	0.623	4.59	0.799	5.29	0.799	5.28	**0.399**	**2.59**
3	1.235	8.79	1.380	9.69	1.198	8.59	1.596	8.83	1.359	8.89	**1.469**	**9.76**
4	2.025	13.42	2.687	17.78	2.036	13.89	2.002	13.42	2.009	13.23	**2.938**	**19.54**
5	2.399	16.52	3.599	24.21	3.086	20.39	2.882	18.69	2.958	19.58	**4.254**	**28.46**
6	3.399	23.21	4.092	26.69	4.485	29.39	3.748	24.89	4.019	26.69	**5.478**	**34.52**
7	4.399	29.39	4.799	32.06	5.729	38.29	4.799	32.12	5.252	34.69	**6.799**	**45.39**
8	5.623	37.39	6.199	41.68	7.082	47.19	6.123	40.91	6.784	45.32	**8.269**	**55.19**

Table 4: *In-vitro* diffusion of NF-HCl niosomes for 8 hours via the EI method

Time (hr.)	Formulation Batch											
	PNF-7		PNF-8		PNF- 9*		PNF-10		PNF- 11		PNF- 12	
	CR	%CR	CR	%CR	CR	%CR	CR	%CR	CR	% CR	CR	%CR
0	0	0	0	0	**0**	**0**	0	0	0	0	0	0
1	0.259	1.87	0.276	1.48	**0.143**	**0.98**	0.213	1.71	0.130	0.78	0.256	1.87
2	0.798	5.45	0.820	5.11	**0.398**	**2.77**	0.822	5.31	0.404	2.59	0.532	3.45
3	1.204	8.00	1.223	8.32	**1.064**	**7.23**	1.190	8.04	0.807	5.23	1.065	7.11
4	1.869	12.76	1.899	12.57	**2.267**	**15.10**	2.001	13.31	1.474	9.67	1.865	12.43
5	2.701	17.76	2.655	17.88	**3.765**	**24.99**	2.822	18.89	2.401	16.21	2.954	19.79
6	3.734	24.85	3.722	24.91	**5.455**	**37.47**	3.741	24.91	3.749	24.89	4.121	27.76
7	4.976	32.98	5.102	33.76	**7.086**	**47.28**	4.999	32.91	5.344	35.56	5.631	37.51
8	6.126	40.54	6.455	43.37	**8.822**	**58.92**	6.581	43.45	7.080	47.32	7.360	49.00

Drug Release Kinetics: Analyzed using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models.

Table 5: R^2 value of drug release kinetics via several mathematical models

Formulation Batch	R^2 Value				
	Zero-Order	First Order	Higuchi Kinetic	Krosmeyer-Peppas Kinetic	Order of Release
PNF-6*	0.9632	0.9107	0.7845	0.9894	Krosmeyer- Peppas kinetic
PNF-9*	**0.9321**	**0.9452**	**0.7307**	**0.9965**	Krosmeyer- Peppas kinetic

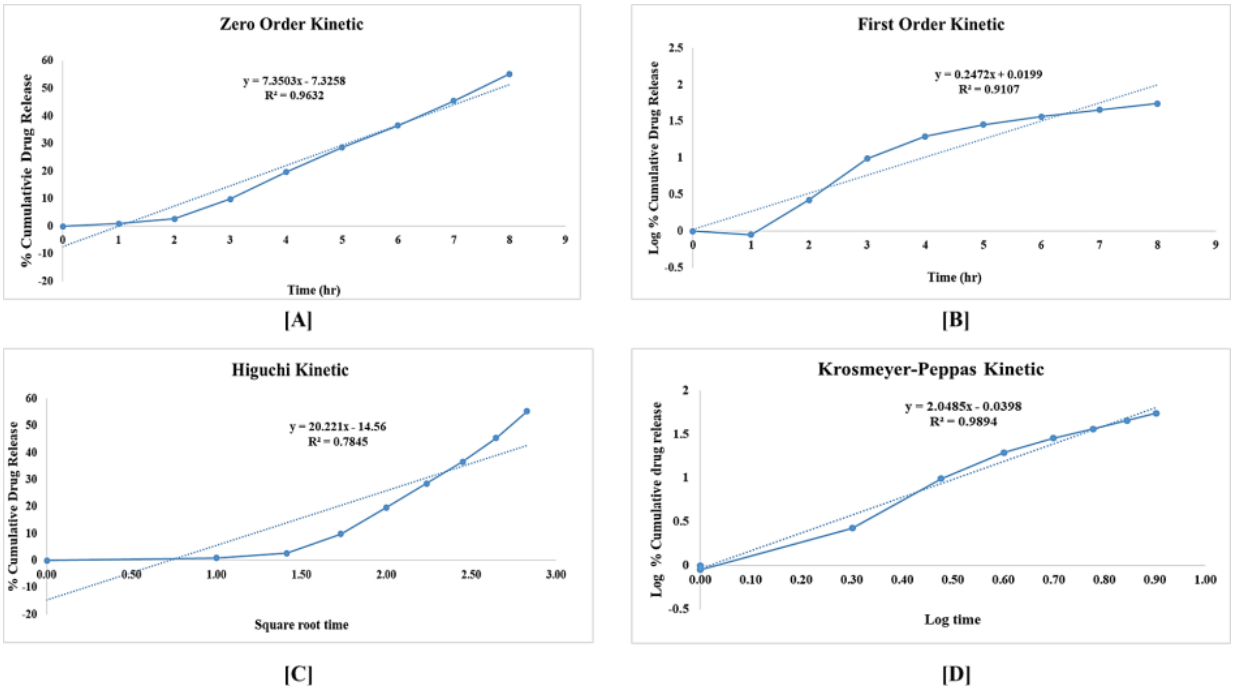

Figure 7: The drug release kinetics of the optimized niosomal suspension (PNF- 6*); [A]. Zero-order kinetics, [B]. First-order kinetic, [C]. Higuchi kinetic and [D]. Krosmeyer-Peppas kinetics

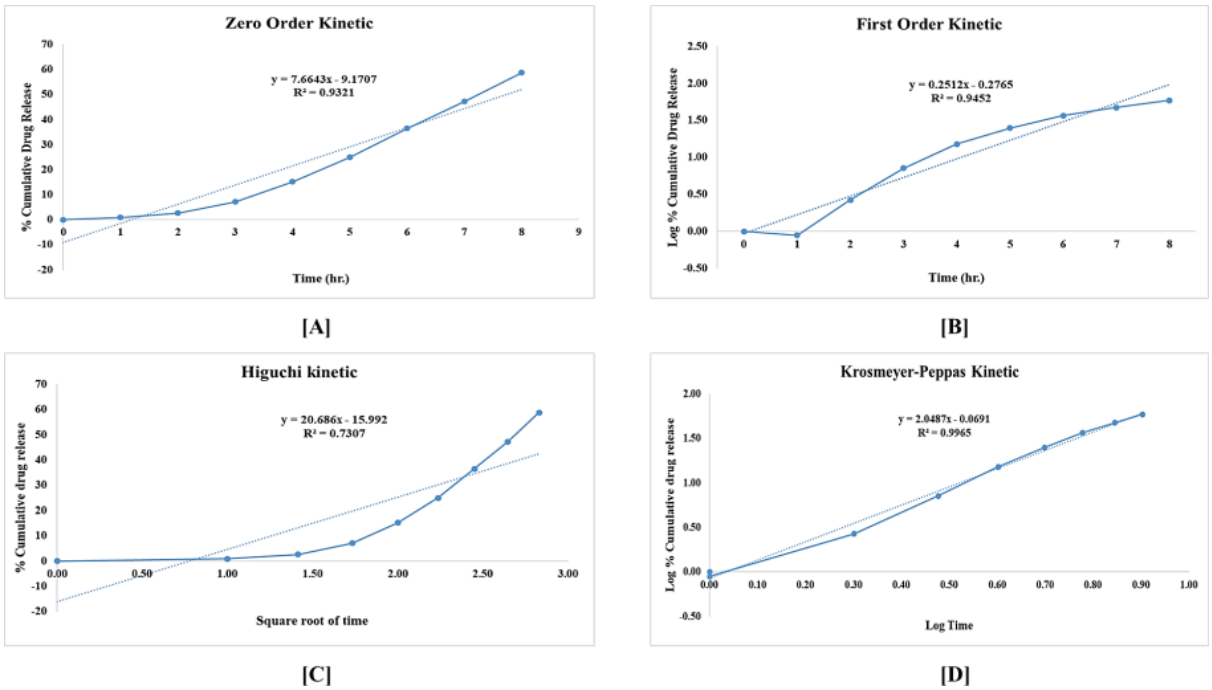

Figure 8: The drug release kinetics of the optimized niosomal suspension (PNF- 9*); [A]. Zero-order kinetics, [B]. First-order kinetic, [C]. Higuchi kinetic and [D].

DISCUSSION

Preformulation and Characterization

NF-HCl was soluble in PBS 7.4, ethanol, and methanol. FTIR confirmed drug-excipient compatibility. Span 60-based formulations exhibited higher EE%, attributed to their lower HLB and longer alkyl chains.

Entrapment Efficiency

EE% ranged from 18.91% to 58.94%. PNF-6 (HSM, Span 60, 2:1) showed 53.09%, and PNF-9 (EIM, Span 60, 1:1) showed 58.94%, confirming higher EE in EIM-prepared formulations.

Vesicle Morphology and Size

Optical microscopy confirmed spherical vesicles. SEM revealed vesicle sizes of ~200 nm (HSM) and ~1 µm (EIM).

Zeta Potential

PNF-6 and PNF-9 showed −41.9 mV and −64.6 mV respectively, indicating better stability in the EIM formulation.

In Vitro Drug Release

PNF-6 and PNF-9 showed 55.19% and 58.92% release respectively over 8 hours. EIM formulations exhibited superior controlled release behavior.

Drug Release Kinetics

Drug release followed Korsmeyer-Peppas kinetics (R^2 > 0.99), indicating a combination of diffusion and erosion mechanisms.

Stability Studies

At 4°C, PNF-9 retained over 81% of its drug content after 9 weeks, while retention dropped significantly at 37°C, suggesting cold storage is preferable.

CONCLUSION

This study aimed to prepare NF-HCl-loaded niosomes to improve entrapment efficiency and sustain drug release. Variations in NIOs and CH with two different methods (HSM and EIM) have been used to improve the entrapment efficiency of niosome drugs. Finally, the highest entrapment efficiency was found to be 57.97% with span 60 involving the EIM with the optimized technique. This will result in a long-lasting effect of the entrapped drug that reduces the adverse effects of dosing frequency and increases the therapeutic effect of the drug. This finding demonstrated that the niosomal drug transport system is a potential carrier of this novel drug transport system.

Author's Contributions

Dr. Garvendra Singh Rathore: Conceptualization, study design, and supervision of the research work.

Ravi Prakash Soni: Experimental work, data collection, and interpretation.

Dr. Vinesh Kumar: Manuscript drafting, literature review, and critical revision.

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Conflicts of Interest: The authors declare no competing interests or conflicts of interest regarding this research.

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