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PREFORMULATION STUDIES OF NEFOPAM HYDROCHLORIDE FOR PHYSICOCHEMICAL CHARACTERIZATION AND COMPATIBILITY ANALYSIS WITH POLY-3-HYDROXYBUTYRATE AND POLY- ε - CAPROLACTONE

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ABSTRACT

The objective of present investigation was to execute physicochemical characterization and compatibility analysis of nefopam hydrochloride (NPH) with poly-3-hydroxybutyrate (PHB) and poly-ε-caprolactone (PCL). Melting point and hygroscopicity assessment of NPH was performed by capillary and Ph. Eur. method, respectively. Residue on ignition (% ROI) was estimated as according to ICH Q4B R1. Drug-excipient compatibility was ascertained by differential scanning calorimetric. Melting point, % LOD and % residue on ignition of NPH was found 260 ± 5 °C, 0.27 % (≤ 0.5%) and 0.07 % (≤ 0.1%), respectively. Log P of NPH as determined by shake flask method was found 3.41. Numerous sharp crystalline peaks in x-ray diffraction pattern of NPH demonstrated highly crystalline characteristics. NPH was found freely soluble in acetone, soluble in water, ethanol, methanol and phosphate buffer pH 7.4 while very slightly soluble in chloroform and dichloromethane. Differential scanning calorimetry concluded compatibility between NPH, PHB and PCL.

KEYWORDS: Poly-3-hydroxybutyrate, Poly-ε-caprolactone, Hygroscopicity, X-ray diffraction, Preformulation

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INTRODUCTION

Nefopam hydrochloride (NPH) is non-opiate, non-sedative and centrally acting analgesic of benzoxazocine class having chemical name 5-methyl-1-phenyl-1, 3, 4, 6-tetrahydro-2, 5-benzoxazocine hydrochloride (Figure 1). It is cyclized analogue of diphenhydramine and has chemical structure analogous to orphenadrin[1-4]. NPH restrains synaptic reuptake of triple neurotransmitters *i.e.* dopamine, nor-epinephrine, and serotonin to deliver analgesic effect. It has been preferred drug for relief of several acute and chronic pains. It is white crystalline powder, scentless, little bitter taste with molecular formula and weight $C_{17}H_{19}NO{\cdot}HCl$ and 289.8 g mol^{-1}, respectively. It is an acidic salt of basic drug with pKa 9.2. It has an optical rotation [α_D^{25}] of about 121° to 128° [5-9].

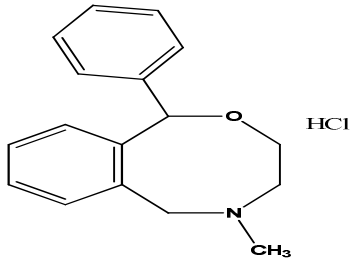

Figure 1
Chemical Structure of NPH

Physicochemical properties of drug that could influence development of an effective dosage form should be investigated prior to formulation. Preformulation is the foremost stage for rational stable, safe and effective product development of an active pharmaceutical ingredient (API). It includes investigation of physicochemical characteristics of drug substance and drug-excipient compatibility [10-12]. The objective was physicochemical characterization of NPH *i.e.* melting point, % loss on drying, residue on ignition, hygroscopicity, solid form identification, partition coefficient, and solubility study. Compatibility of NPH with poly-3-hydroxybutyrate and poly-ε-caprolactone was established using differential scanning calorimetry.

MATERIALS AND METHODS

Materials

Poly ε–caprolactone (average M_w ~ 14,000) and polyhydroxybutyrate were purchased from Sigma–Aldrich Chemie, Gmbh, Steinhelm, Germany. Nefopam hydrochloride was purchased from Hangz Hou–Daying–Chem. Company Ltd., China. Ethanol, methanol, acetone, dichloromethane and chloroform were procured from Loba Chemicals Private Limited, Mumbai, India.

Determination of Physicochemical Characteristics of NPH

Melting Point Using Capillary Method

Melting point is the temperature at which substance initiates to coalesce and gets completely melted. The sufficient quantity of completely dried NPH was introduced into capillary glass tube to form compact column of 2 to 4 mm height. Heat the melting-point apparatus to a temperature 5-10 °C below the expected temperature of melting. Sample tube was placed into slot located on melting point apparatus (Perfit, India) for determination of melting point [13].

Loss on Drying (% LOD)

% LOD was determined as per the method detailed in Indian Pharmacopoeia [14]. About 1-2 gm of NPH was transferred to tared dried weighing bottle. Drug was distributed to depth of approximately 10 mm through gentle sidewise shaking. Glass bottle was placed in drying chamber; stopper was removed and placed adjacent followed by drying at 105° for 3 hrs. Subsequently, glass bottle was cooled in desiccator and reweighed. % LOD was determined using following formula:

$$\%\ \text{Loss on drying (\% LOD)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \qquad \text{Eq. (1)}$$

Where, W_1 is weight of empty weighing bottle; W_2 is weight of bottle with NPH before drying and W_3 is weight of bottle with NPH after drying [15, 16].

Residue on Ignition as Per ICH Q4B (R1)

NPH (1-2 gm) was placed in crucible, weighed accurately and moistened with small amount of sulfuric acid. Subsequently, sample was ignited slowly at low temperature until incineration and allowed to cool. Further, 1 ml of sulfuric acid was added and followed by slow heating till evolution of white fumes disappears. The crucible was transferred to muffle furnace and ignited at 450-550 °C for 3 hrs, allowed to cool in desiccator and weighed accurately [17]. % Residue on ignition was calculated using following formula:

$$\%\ \text{Residue on ignition} = \frac{W_2 - W_1}{W_3} \times 100 \qquad \text{Eq. (2)}$$

Where, W_1 is weight of empty crucible; W_2 is weight of crucible and residue after heating and W_3 is weight of sample.

Hygroscopicity Assessment by Ph. Eur. Method

Hygroscopicity is the phenomenon of retaining water molecules from proximate atmosphere. Moisture sorption has been determined gravimetrically by placing pre-weighed material in a closed desiccator filled with saturated solution of NH_4Cl. NPH (100–300 mg) was transferred into accurately weighed dry plastic petri-plate (w_1), reweighed (w_2) and transferred into desiccator maintained at 25°C ± 2°C temperature and 80 ± 2% RH for 24 hrs. Subsequently, petri-plate was removed from desiccator and reweighed (w_3) [18, 19]. The percentage increase in weight of NPH was calculated using following equation:

$$W_{Ph.Eur} = \frac{W_3 - W_2}{W_2 - W_1} \times 100 \qquad \text{Eq. (3)}$$

Where, $W_{Ph.\ Eur}$ = % total weight gain using Ph. Eur. method. Hygroscopicity of NPH was determined as stated in European Pharmacopoeia (Table 1).

Table 1
Material Categorization as Per Ph. Eur. Method [20]

Material category	Criteria as per Ph. Eur.*
NH	0-0.012% w/w
SH	0.2-2% w/w
MH	2-15% w/w
VH	More than 15% w/w

Weight gain due to moisture sorption at 25°C ± 2°C and 80 ± 2% RH in 24 h.
Ph. Eur.: European Pharmacopeia, RH: relative humidity, NH: non-hygroscopic, SH: slightly hygroscopic, MH: moderately hygroscopic, VH: very hygroscopic.

Solid Form Identification Using X-Ray Diffraction Study

Solid form identification was performed using powder x-ray diffraction (PXRD) pattern of NPH obtained on X-ray diffractometer (Xpert-Pro diffractometer) employing 1.54 A^o CuKα radiations and 1.39 A^o CuKβ radiations. Data was collected over an angular range from 2θ=5° to 2θ=50° in continuous scan mode.

Partition Coefficient (n-Octanol/Water) by Shake Flask Method

Saturated solution of NPH was individually prepared in 25 mL distilled water and n-octanol. Both the phases were transferred in separating funnel followed by shaking on mechanical shaker for 4 hrs. Separating funnel was allowed to stand to establish equilibrium [21, 22]. Aliquots were removed and analyzed by UV spectroscopy. Partition coefficient (P) of NPH was determined using following equation:

$$\text{Partition coefficient (P)} = \frac{\text{amount of NPH in n-octanol}}{\text{amount of NPH in water}} \qquad \text{Eq. (4)}$$

Solubility Study by Equilibrium Solubility Method

Solubility studies of NPH were performed by equilibrating excess amount of drug in water, methanol, ethanol, acetone, phosphate buffer, pH 7.4, chloroform and dichloromethane. Assays were carried out in screw-capped vials and samples were kept in orbital shaker (Remi, India) at 37 °C for 24 hours (to achieve the equilibrium condition). After this interval, samples were filtered through 0.45 µm membrane filter and diluted in volumetric flask with solvent followed by quantification using UV-Vis spectrophotometer [23-25] (Table 2).

Table 2
Values for Estimating Drug Solubility Based Upon USP Definition

Descriptive term	Appropriate volume of solvent in milliliters per gram of solute
Very soluble	Less than 1 part solvent required to dissolve 1 part solute
Freely soluble	From 1 to 10 parts solvent required to dissolve 1 part solute
Soluble	From 10 to 30 parts solvent required to dissolve 1 part solute
Sparingly soluble	From 30 to 100 parts solvent required to dissolve 1 part solute
Slightly soluble	From 100 to 1000 parts solvent required to dissolve 1 part solute
Very slightly soluble	From 1000 to 10,000 parts solvent required to dissolve 1 part solute
Practically insoluble	More than 10,000 parts solvent required to dissolve 1 part solute

Drug-Excipient Compatibility Study by Differential Scanning Calorimetric (DSC)

The thermal analysis of NPH, PHB, PCL and physical mixture (NPH: PHB: PCL in 1:1:1) was performed on DSC 4000 Perkin Almer, Germany using Pyris Software. 5 mg samples were taken in an aluminum pan and heated at a constant rate of 20 $^oC\ min^{-1}$ over temperature range of 25-350 ^oC with nitrogen purging (100 mL/min). The temperature axis and cell constant of

instrument were calibrated using purified indium (99.9 %) as the standard reference material [26].

RESULTS AND DISCUSSION

Determination of Physicochemical Properties of Drug

Melting Point, % LOD and Residue on Ignition

Melting point of NPH was found 260 ± 5 °C which was in compliance with the reported value. % LOD of NPH was found 0.27 % of its weight after being dried at 105°C for three hours which was in compliance with the specification (% LOD ≤ 0.5%). The % residue on ignition of NPH was found 0.07 % which was in compliance with the monograph limit (≤ 0.1%).

Hygroscopicity and Partition Coefficient

Percentage increase in weight of NPH after storage in desiccator at 25°C ± 2°C and 80 ± 2 % RH for 24 h was < 0.2 % which indicated its non-hygroscopic nature. Log P of NPH as determined by shake flask method was found 3.41.

Solid Form Identification

X-ray diffraction pattern of NPH exhibited sharp crystalline peaks at 2θ = 7.7°, 2θ = 12.36°, 2θ = 13.9°, 2θ = 15.75°, 2θ = 16.89°, 2θ = 18.12°, 2θ = 19.4°, 2θ = 19.76°, 2θ = 23.03°, 2θ = 23.7° 2θ = 26.27° 2θ = 35.23° and 2θ = 40.53° which demonstrated highly crystalline features of NPH (Figure 2, Table 3).

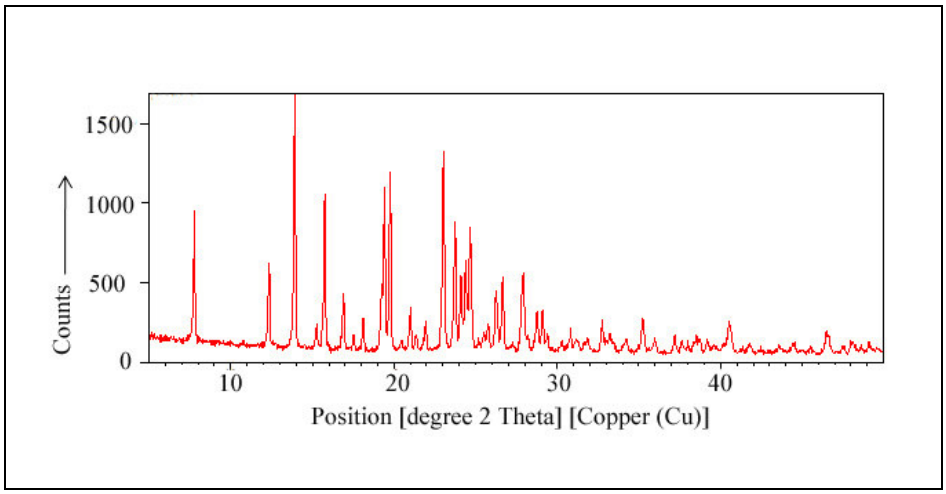

Figure 2
X-ray Diffraction Pattern of NPH Indicating Crystalline Form of NPH

Table 3
XRD Peaks of Nefopam Hydrochloride

Position at degree 2θ	d-spacing (A°)	Relative intensity (%)	Area (degree 2θ)
7.7711	11.3767	50.16	65.07
12.3612	7.41621	67.16	86.07
13.9082	6.36747	100.00	155.67
15.7584	5.62381	62.20	96.83
16.8931	3.7312	45.42	66.74
18.1263	3.6432	29.53	47.39
19.4077	4.57380	64.83	100.92
19.7642	4.49209	71.05	110.61
23.0369	3.86080	79.51	123.78
23.7220	3.75082	50.81	92.28
24.6460	3.61226	49.52	77.09
26.2771	3.39162	23.94	55.90
26.6471	3.34535	29.77	38.63
27.9244	3.19518	31.59	40.98
28.7847	3.10161	15.97	29.00
35.2362	2.54711	12.59	16.33
40.5374	2.22542	11.85	36.89

Solubility Study

Solubility of NPH determined by equilibrium solubility method has been represented in Table 4.

Table 4
Solubility of NPH by Equilibrium Solubility Method

Solvent	Solubility of NPH
Acetone	++++
Water, Ethanol, Methanol	+++
Phosphate buffer, pH 7.4	+++
Chloroform, Dichloromethane	+

+ = very slightly soluble; +++ = soluble; ++++ = freely soluble

Drug-Excipient Compatibility Study

Figure 3 embodied the DSC thermograms of (a) NPH, (b) PCL, (c) PHB, and (d) physical mixture of NPH, PHB and PCL. The DSC thermograms of NPH revealed distinctive endothermic peak at 265.66 ^{o}C corresponding to its melting point (T_m) which signified substantially crystalline characteristics of drug (Figure 3a). PCL and PHB displayed characteristic peak at 68.77^{o}C and 174.15 ^{o}C, respectively, which validated authencity of polymers (Figure 3b and 3c). The distinctive endothermic peaks of drug and polymers persisted noteworthy in physical mixtures which revealed compatibility between drug and polymers (Figure 3d) [27-31].

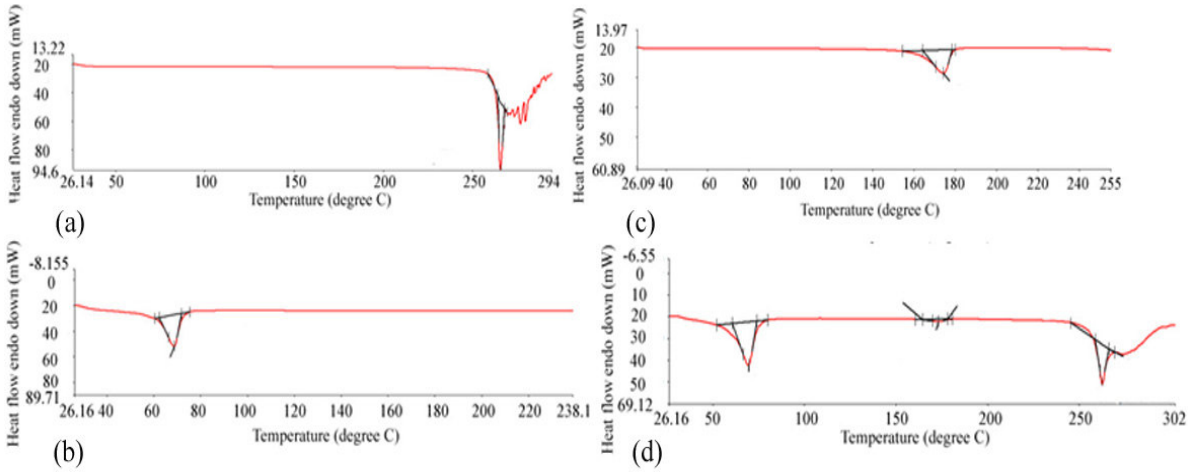

Figure 3

DSC Curves of (a) NPH, (b) PCL, (c) PHB, and (d) Physical Mixture of NPH, PCL and PHB

CONCLUSION

Present investigation illustrated that melting point, % LOD, residue on ignition and log P of NPH were 260 ± 5 °C, 0.27 % (≤ 0.5%), 0.07 % (≤ 0.1%) and 3.41, respectively. Study indicated that NPH was non-hygroscopic and highly crystalline. NPH was found freely soluble in acetone, soluble in water, ethanol, methanol and phosphate buffer pH 7.4 while very slightly soluble in chloroform and dichloromethane. Differential scanning calorimetry concluded compatibility between NPH, PHB and PCL.

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CONFLICT OF INTERESTS

Conflict of interest declared none.

REFERENCES

1. Bilotta F, Pietropaoli P, La Rosa I, Spinelli F, Rosa G. Effects of shivering prevention on haemodynamic and metabolic demands in hypothermic postoperative neurosurgical patients. Anaesthesia. 2001;56:514-9.
2. Crowley LJ, Buggy DJ. Shivering and neuraxial anesthesia. Reg. Anesth. Pain. Med. 2008;33:241-52.
3. Heel RC, Brogden RN, Pakes GE, Speight TM, Avery GS. Nefopam: a review of its pharmacological properties and therapeutic efficacy. Drugs. 1980;19:249-67.
4. Kyung HK, Salahadin A. Rediscovery of nefopam for the treatment of neuropathic pain. Korean. J. Pain. 2014;27:103-11.
5. Rosland JH, Hole K. The effect of nefopam and its enantiomers on the uptake of 5-hydroxytryptamine, noradrenaline and dopamine in crude rat brain synaptosomal preparations. J. Pharm. Pharmacol. 1990; 42:437-8.
6. Girard P, Pansart Y, Coppé MC, Verniers D, Gillardin JM. Role of the histamine system in nefopam-induced antinociception in mice. Eur. J. Pharmacol. 2004;503:63-9.
7. Kim YA, Kweon TD, Kim M, Lee HI, Lee YJ, Lee KY. Comparison of meperidine and nefopam for prevention of shivering during spinal anesthesia. Korean J. Anesthesiol. 2013;64:229-33.
8. Lv M, Wang X, Qu W, Liu M, Wang Y. Nefopam for the prevention of perioperative shivering: a meta-analysis of randomized controlled trials. BMC Anesthesiol. 2015;15:87-97.
9. Verleye M, André N, Heulard I, Gillardin JM. Nefopam blocks voltage-sensitive sodium channels and modulates glutamatergic transmission in rodent. Brain Res. 2004;1013:249-55.
10. Migoha CO, Ratansi M, Kaale E, Kagashe G. Preformulation studies for generic omeprazole magnesium enteric coated tablets. BioMed Res. Int. 2015;2015:307032-41.
11. Bharate SS, Bharate SB, Bajaj AN. Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a

comprehensive review. J Excipients and Food Chem. 2010; 1(3):3-26.
12. Bharate SS, Vishwakarma RA. Impact of pre-formulation on drug development. Expert Opin Drug Deliv. 2013;10(9):1239-57.
13. Killedar SG, Nale AB, More HN, Nadaf SJ, Pawar AA, Tamboli US. Isolation, characterization and evaluation of Cassia fistula Linn. seed and pulp polymer for pharmaceutical application. Int. J Pharm. Investig. 2014;4(4):215-25.
14. Indian Pharmacopoeia, Ministry of Health and family welfare, Government of India, Published by the controller of publications, Delhi, 1996.
15. Chablani L, Taylor MK, Mehrotra A, Rameas P, Stagner WC. Inline real-time near-infrared granule moisture measurements of a continuous granulation–drying–milling process. AAPS PharmSciTech. 2011;12(4):1050-55.
16. Mohamed JM, Bharathidasan P, Raffick MM. Preformulation and development of curcumin magnetic nanosuspension using magnetite (Fe_3O_4) and methyl cellulose. Int. J Pharm. Bio. Sci. 2012;3(4):419-32.
17. ICH Q4B Annex 1(R1): Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Residue on Ignition/Sulphated Ash General Chapter.
18. Murikipudi V, Gupta P, Sihorkar V. Efficient throughput method for hygroscopicity classification of active and inactive pharmaceutical ingredients by water vapor sorption analysis. Pharm. Dev. Technol. 2013;18(2):348-58.
19. Mwesigwa E, Buckton G, Basit AW. The hygroscopicity of moisture barrier film coatings. Drug Dev Ind Pharm. 2005;31(10):959-68.
20. Anonymous. Characters section in monographs. European Pharmacopoeia 6, version 6.8, Section 5.11 ed2010.
21. Baka E, Comer JEA, Tak´acs-Nov´ak K. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound. J Pharm. Biomed. Anal. 2008; 46:335-41.
22. Bharate SS, Kumara V, Vishwakarma RA. Determining partition coefficient (Log P), distribution coefficient (Log D) and ionization constant (pKa) in early drug discovery. Combinatorial Chem. High Throughput Screening. 2016;19:1-9.
23. Dezani AB, Pereira TM, Caffaro AM, Reis JM, Serra CHR. Equilibrium solubility *versus* intrinsic dissolution: characterization of lamivudine, stavudine and zidovudine for BCS classification. Brazilian J Pharm. Sci. 2013;49(4):853-63.
24. The United Stated Pharmacopeia, USP 26, NF 21, 2003.
25. Tiwari G. Biodegradable microspheres for controlled delivery of metronidazole in the treatment of periodontal diseases: formulation consideration. Int. J Pharm. Bio. Sci. 2010;1(2):1-13.
26. Patel KS, Patel MB. Preparation and evaluation of chitosan microspheres containing nicorandil. Int. J Pharm. Investig. 2014;4(1):32-7.
27. Natarajan V, Krithica N, Madhan B, Sehgal PK. Formulation and Evaluation of Quercetin Polycaprolactone Microspheres for the Treatment of Rheumatoid Arthritis. J. Pharm. Sci. 2011; 100:195-205.
28. Bazzo GC, Lemos-Senna E, Gonçalves MC, Pires ATN. Effect of Preparation Conditions on Morphology, Drug Content and Release Profiles of Poly(hydroxybutyrate) Microparticles Containing Piroxicam. J. Braz. Chem. Soc. 2008;19:914-21.
29. Gajra B, Dalwadi C, Patel R. Formulation and optimization of itraconazole polymeric lipid hybrid nanoparticles (Lipomer) using box-behnken design. Daru. J. Pharm. Sci. 2015;23:3-18.
30. Kim JK, Parkb JS, Kim CK. Development of a binary lipid nanoparticles formulation of itraconazole for parenteral administration and controlled release. Int. J. Pharm. 2010;383:209-15.
31. Amin MA, Osman SK, Aly UF. Preparation and characterization of ketoprofen nanosuspension for solubility and dissolution velocity enhancement. Int. J Pharm. Bio. Sci. 2013;4(1):768-80.

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