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European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 701–714
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Solubilization of indomethacin using hydrotropes for aqueous injection

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Received 2 June 2006; accepted in revised form 15 June 2007
Available online 26 June 2007

Abstract

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that exhibits analgesic, antipyretic and anti-inflammatory activities. It is practically insoluble in water. The effect of various hydrotropes such as urea, nicotinamide, resorcinol, sodium benzoate and sodium *p*-hydroxy benzoate on the solubility of indomethacin was investigated. The solubility enhancement of indomethacin by the hydrotropes was observed in decreasing order as sodium *p*-hydroxy benzoate > sodium benzoate > nicotinamide > resorcinol > urea. In order to elucidate the probable mechanism of solubilization, various solution properties of hydrotropes such as viscosity, specific gravity, surface tension, refractive index and specific conductance of hydrotropic solutions were studied at 25 ± 2 °C. Each solubilized product was characterized by ultraviolet, infrared, powder X-ray diffraction and differential scanning calorimetry techniques. The hydrotropic solubilization of indomethacin at lower hydrotrope concentration may be attributed to weak ionic interactions while that at higher hydrotrope concentration may be due to molecular aggregation. Aqueous injectable formulations using sodium *p*-hydroxy benzoate, sodium benzoate and nicotinamide as hydrotropes were developed and studied for physical and chemical stability.

Keywords: Hydrotropic solubilization; Indomethacin; Aqueous injection; Cyclo-oxygenase (COX)

1. Introduction

Indomethacin is an acidic non-steroidal anti-inflammatory agent. It is a non-selective inhibitor of cyclo-oxygenase (COX) 1 and 2 enzyme that participates in prostaglandin synthesis from arachidonic acid. Indomethacin for injection has great implications for patient care. It is labeled for closing hemodynamically significant patent ductus arteriosus (PDA) in premature infants weighing 500–750 g when medical management is ineffective after 48 h and there is clinical evidence of respiratory distress, continuous heart murmur, hyperactive precordium, cardiomegaly, or pulmonary plethora (by chest radiography) [1]. Although not a labeled use, indomethacin for injection has also been used prophylactically within the first 24 h of life to prevent the development of symptoms in premature infants with PDA [2] and to reduce the occurrence of intracranial or intraventricular hemorrhage in very-low-birth-weight neonates [3–5].

Indomethacin is practically insoluble in water (0.02 mg/ml), which precludes its use in injection formulations. Thus, indomethacin for injection had been on shortage due to manufacturing difficulties [6]. Hydrotropic solubilization of a wide variety of drugs has been demonstrated [7–15] in the literature and a number of mechanisms have been reported [16–27]. The present study was aimed to investigate the effect of hydrotropes on the solubility of indomethacin, and to attempt formulation in aqueous injection [28–30]. Formulations were also studied for physical and chemical stability [31,32]. The chemical structures of drug and various hydrotropes used in this study are shown in Fig. 1.

2. Materials and methods

2.1. Materials

The gift sample of indomethacin was provided by M/S Signa Pharma Pvt. Ltd., Kanpur, 208 012 UP, India. Urea,

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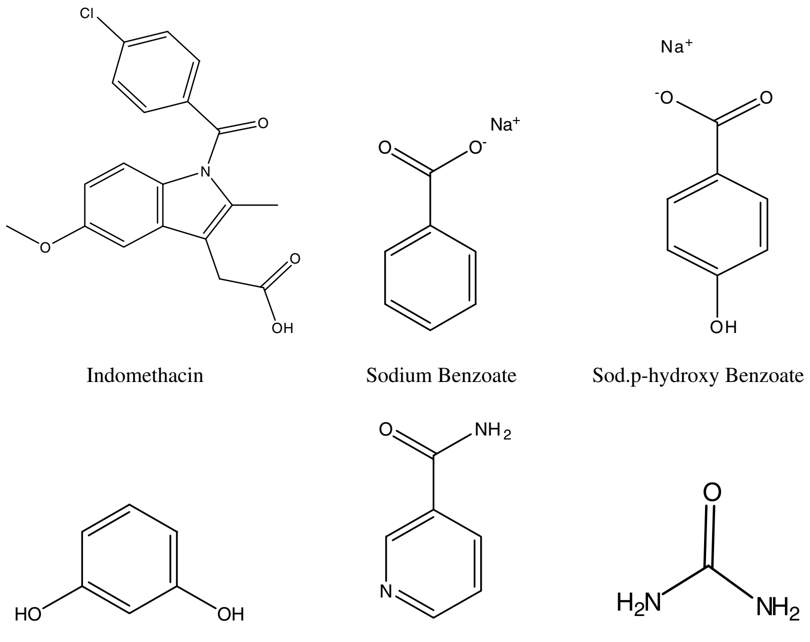
doi:10.1016/j.ejpb.2007.06.013



Indomethacin Sodium Benzoate Sod.p-hydroxy Benzoate

Resorcinol Nicotinamide Urea

Fig. 1. Chemical structures of indomethacin and various hydrotropes.

nicotinamide, resorcinol, sodium benzoate and *p*-hydroxy benzoic acid all AR grade were purchased from CDH, Mumbai, India.

2.2. Estimation of indomethacin

In the present study, UV spectrophotometric method [33] was used for the estimation of indomethacin. The calibration curve of indomethacin was prepared in distilled water at 319.5 nm using double-beam spectrophotometer (UV-1701, Shimadzu, Japan).

2.3. Solubility study

An excess quantity of indomethacin was added to screw-capped 10 ml glass vials containing the different aqueous systems viz. distilled water, buffers of pH 2.5–8 (pH is maintained during vortexing) and hydrotropic solutions of different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 2.0 M) in water. The vials were shaken vigorously for 15 min on a touch type vortex mixer (Jyoti Scientific Industries, Gwalior 474 009, India) and then the solutions were allowed to equilibrate with mechanically shaking and intermittent vortexing for 72 h at 25 ± 2 and 37 ± 2 °C in a rotary flask shaker and shaker water bath (Jyoti Scientific Industries, Gwalior 474 009, India). After completion of 72 h each vial is centrifuged for 10 min at 2000 rpm. The supernatant of each vial was filtered through 0.45 μm membrane syringe filter (Sonar Axiva, Axiva Sichem Pvt. Ltd., Delhi, India), filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 319.5 nm against respective hydrotrope solution diluted accordingly as blank. The solubility of indomethacin was determined in triplicate.

2.4. Properties of hydrotropic solutions

The various solution properties of hydrotropes such as pH, viscosity, specific gravity, surface tension, refractive index, conductance and diffusion rate were also studied in an attempt to reason out the increase in solubility of indomethacin with increase in hydrotrope concentration [34,35].

2.5. UV spectral studies

In order to interpret the probable mechanism of solubilization, UV spectral studies of indomethacin were performed in different hydrotropic solutions to study the possible spectroscopic changes in the structure of indomethacin in presence of different hydrotropes.

2.6. Fourier transform infrared (FTIR) spectral studies

FTIR spectra were obtained by means of a FTIR spectrophotometer (FTIR – 8400 s, Shimadzu, Japan). The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulation of 20 scans and a resolution of 4 cm^{-1} over the range of 400–4000 cm^{-1}.

2.7. Thermal analysis

Differential scanning calorimeter (DSC) curves were obtained with a Pyris-6 DSC (Pyris series, Perkin Elmer, USA). The samples were prepared by placing 5 mg samples into an aluminium pan, which was covered and crimped for analysis. Samples were desiccated over calcium chloride for 24 h prior to assay in an effort to remove surface absorbed water. Thermographs were analyzed qualitatively by examining both the peak temperature and the endothermic transition contour. The nitrogen flow rate was 20 ml/min and the heating rate was 5 °C/min over the range of 40–300 °C.

2.8. X-ray powder diffraction

X-ray diffraction patterns were obtained at room temperature (25 °C) using a D-8 Advance, Bruker-AXS Diffractometer (Germany). Samples were exposed to CuKα radiation at scanning rate 2°/min with step size 0.050°, step time 1.5 s over scanning range 3.000–120.000° of the diffraction angle 2θ; the generator was set to 40 kV and 30 mA.

2.9. Formulation of aqueous injection

On the basis of solubility data obtained, three formulations of aqueous injection of indomethacin namely IPHB, ISB and INMD were prepared, which contained 1 mg/ml of indomethacin in 1 M sodium *p*-hydroxy benzoate, 1.2 M sodium benzoate and 1.2 M nicotinamide solution, respectively [36,37]. In all the three formulations, 0.1% w/v sodium metabisulfite was added as an antioxidant. Other additives like chelating agent and buffering agent were not included in these formulations as they might lead to change in the solubility behavior and upset the basic solubility enhancement ratio. For the preparation of aqueous injection of indomethacin, about 40 ml of distilled water was taken in 100 ml borosilicate glass beakers and placed on a magnetic stirrer. Then weighed amounts of hydrotrope; sodium metabisulfite (0.1% w/v) and indomethacin were added into the beaker one by one after ensuring the complete dissolution of the former. pH of the solution was adjusted to 6.9 ± 0.2 with either *p*-hydroxy benzoic acid, benzoic acid, hydrochloric acid or sodium hydroxide solution then the volume was made up to 50 ml with distilled water. The contents of these beakers were stirred for additional 1 h on the magnetic stirrer for complete solubilization and equilibration then flushed with nitrogen for 15 min. These solutions were filtered through 0.45 µm membrane filter (Sonar Axiva, Axiva Sichem Pvt. Ltd., Delhi, India). The solutions were analyzed spectrophotometrically at 319.5 nm for drug content after appropriate dilutions with distilled water using the same vehicle as blank after appropriate dilution.

2.9.1. Treatment of packaging material

Clear glass vials of 2 ml capacity were washed several times with de-ionized water jet then finally rinsed with distilled water. All these vials were placed in a perforated stainless steel box in inverted position and sterilized by dry heat in an oven at 180 °C for 2 h. Rubber stoppers used for plugging the vials were first shaken in 0.2% liquid detergent solution for 2 h, then washed several times with de-ionized water to remove any detergent residue and finally rinsed with distilled water. These stoppers were immersed in double strength hydrotrope and sodium metabisulfite solution and then sterilized by autoclaving at 15 lbs pressure (121 °C temperature) for 20 min. Finally, the stoppers were rinsed with freshly prepared sterile distilled water and dried in vacuum oven under aseptic condition.

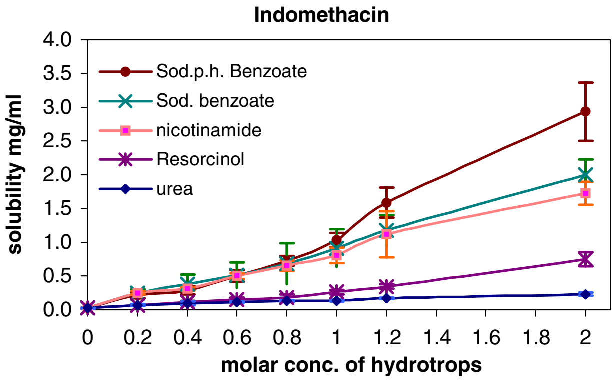


Fig. 2. Comparative equilibrium solubility of indomethacin in various hydrotropes.

Table 1
pH-dependent solubility (mg/ml) of indomethacin at different temperatures

S.No.	pH	Solubility[a] mg/ml ± SD		Enhancement ratio	
		At 25 ± 2 °C	At 37 ± 2 °C	At 25 ± 2 °C	At 37 ± 2 °C
1	2.45	0.0030 ± 0.0002	0.0031 ± 0.0002	0.1185	0.0997
2	3.02	0.0029 ± 0.0002	0.0032 ± 0.0003	0.1165	0.1033
3	4.04	0.0053 ± 0.0003	0.0057 ± 0.0004	0.2129	0.1834
4	4.98	0.0136 ± 0.0009	0.0158 ± 0.0008	0.5432	0.5111
5	6.01	0.3203 ± 0.0206	0.3640 ± 0.0206	12.8247	11.7924
6	6.56	0.8845 ± 0.0618	1.0318 ± 0.0658	35.4174	33.4289
7	7.07	3.1244 ± 0.1759	3.3944 ± 0.1934	125.1131	109.9793
8	7.62	3.5417 ± 0.2815	3.7381 ± 0.3468	141.8257	121.1152
9	7.96	3.6399 ± 0.3085	3.9100 ± 0.3263	145.7581	126.6832

[a] Values are means ± SD of three observation.

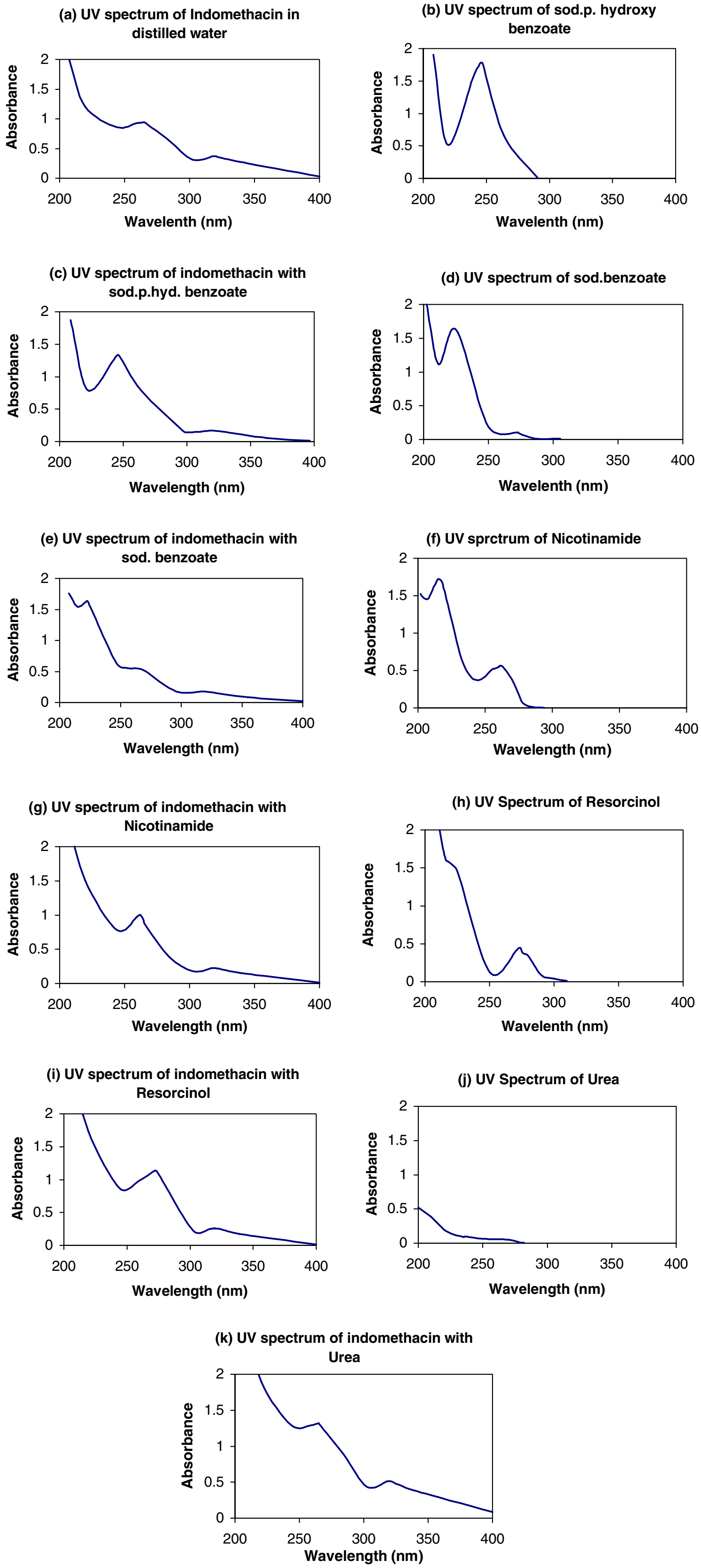


Fig. 3. UV spectrum of (a) indomethacin in distilled water, (b, d, f, h, j) various hydrotropes in distilled water, (c, e, g, i, k) indomethacin in various hydrotrope solutions.

2.9.2. Preparation of aseptic area

The walls and floor of aseptic room were thoroughly washed with detergent solution and water and then disinfected by mopping with 2.5% v/v Dettol® (Reckitt Benckiser India Ltd., Kolkata, India) and 2.5% v/v Savlon® (Johnson & Johnson Ltd., Mumbai, India) solution alternatively. The laminar airflow bench was cleaned with the same disinfectant and 70% v/v isopropyl alcohol was sprayed onto the surfaces as well as into the atmosphere. The aseptic room was fumigated using a mixture of formaldehyde (40%) and potassium permanganate and the UV lights were switched on overnight prior to filling of injections into vials.

2.9.3. Aseptic filtration

The aqueous solutions of indomethacin were prepared as above and sterilized by filtration through 0.2 μm membrane syringe filter (Axiva, Delhi, India) previously sterilized by autoclaving.

2.9.4. Flushing with nitrogen gas

The sterile vials were pre and post flushed with sterile nitrogen during filling with sterile aqueous solution of indomethacin, stoppered immediately and sealed with 13 mm aluminium seals.

2.10. Stability studies

2.10.1. Physical stability studies

The sealed vials of the formulations were visually inspected every day for 45 days against black and white backgrounds to see the changes occurring, if any, in physical appearance of aqueous injection like color, turbidity, precipitation, etc., on storage at 4 ± 2 °C in a refrigerator, 25 ± 2 (RT), 37 ± 2, 45 ± 2 and 60 ± 2 °C in thermostatically controlled ovens. For freeze thaw cycling the vials were kept alternately at 45 ± 2 and 4 ± 2 °C for 24 h and shaken every day for 10 min on a mechanical shaker.

2.10.2. Chemical stability studies

The formulations were subjected to exhaustive chemical stability at 4 ± 2 °C in a refrigerator 25 ± 2 (RT), 37 ± 2, 45 ± 2 and 60 ± 2 °C in thermostatically controlled ovens for a period of 45 days. The formulations were analyzed spectrophotometrically initially and at intervals of 1, 3, 7, 14, 21, 30 and 45 days to calculate the drug content. The percentage drug remaining for each formulation at different time intervals as well as at different temperatures was calculated considering the initial drug content for each formulation to be 100%. From the Arrhenius plots, the K values at 25 °C were determined by extrapolating the graph. The time period required for 10%, degradation of drug ($t_{10\%}$) for each formulation was calculated.

3. Results and discussion

The results of solubility studies at different pH indicated that indomethacin was more soluble at alkaline pH than acidic pH. This may be due to the acidic nature of indomethacin. The aqueous solubility of indomethacin was increased up to 145 times at pH 7.96 at 25 ± 2 °C (Table 1). The solubility of indomethacin was found to increase up to 117.5 times at 25 ± 2 °C in 2 M sodium *p*-hydroxy benzoate solution. It is evident from the solubility data of indomethacin that by increasing the temperature from 25 ± 2 to 37 ± 2 °C, the solubility of drug was increased, showing that solubilization of indomethacin was endothermic. The solubility enhancement ratio of different hydrotropes could be ranked in decreasing order as: *sodium p-hydroxy benzoate > sodium benzoate > nicotinamide > resorcinol > urea* as shown in Figs. 2 and 3 and the solubility enhancement ratio for 2 M hydrotrope concentration was found to be 117.5 > 64.5 > 49.4 > 30.0 > 9.3, respectively (Table 2). Phase solubility diagram (Fig. 2) was Ap type; this shows that increase in total solubility of drug was not linear function of hydrotropic concentration. This positive deviation from linearity is due to formation of water soluble complexes of higher order between drugs and solubilizer, typical of hydrotropism. The solubilization seemed to be continuous phenomenon as solubility increased even at very low concentrations of ligand. The critical hydrotropic concentration, at which hydrotropic action begins, therefore was not determined.

The higher solubility of indomethacin in presence of one hydrotrope over other can be explained on the basis of Poochikian and Gradock's explanation [38]. The

Table 2
Solubility enhancement ratio of indomethacin in hydrotropic solutions at 25 ± 2 °C

S.No.	Conc. of hydrotropes (M)	Solubility enhancement ratio[a] of indomethacin				
		Urea	Nicotinamide	Sod. benzoate	Sod. *p*-hydroxy benzoate	Resorcinol
1	0.0	1.000	1.000	1.000	1.000	1.000
2	0.2	2.732	9.915	10.054	8.609	2.660
3	0.4	3.721	12.464	15.192	11.427	4.402
4	0.6	4.488	20.087	20.500	20.170	6.092
5	0.8	5.150	26.137	27.382	28.979	7.246
6	1.0	5.458	32.298	36.623	41.414	10.444
7	1.2	6.867	44.816	47.044	63.560	13.708
8	2.0	9.291	69.065	80.076	117.499	30.043

[a] Values are means of three observation.

hydrotropes selected for the present study (nicotinamide, resorcinol, sodium benzoate and sodium *p*-hydroxy benzoate) possess a hydrophobic center having the parent benzene nucleus (Fig. 1) which can interact due to a large surface area and a mobile electron cloud known as an aromatic sextet. Thus, these sites are available for non-bonded and Van der Waals interaction with water and indomethacin. The molecules of water join to form cluster together. For solubilization, the ionized hydrotropes break this association and use the ion–dipoles of water for solvation.

The increasing hydrotrope concentration results in unassociated form of water to make cluster of the hydrotrope by hydrogen bonding and non-bonding interactions at the various centers of drug molecule. Thus, charge delocalization along with an increase in π-cloud area on hydrotropic molecule would account partially for difference in apparent drug solubility in presence of various hydrotropes [38].

The UV absorption spectra (Fig. 3) of indomethacin in various hydrotrope solutions showed a slight shift in $\lambda_{\max}$ (319.5 ± 0.5 nm), which can be due to minor

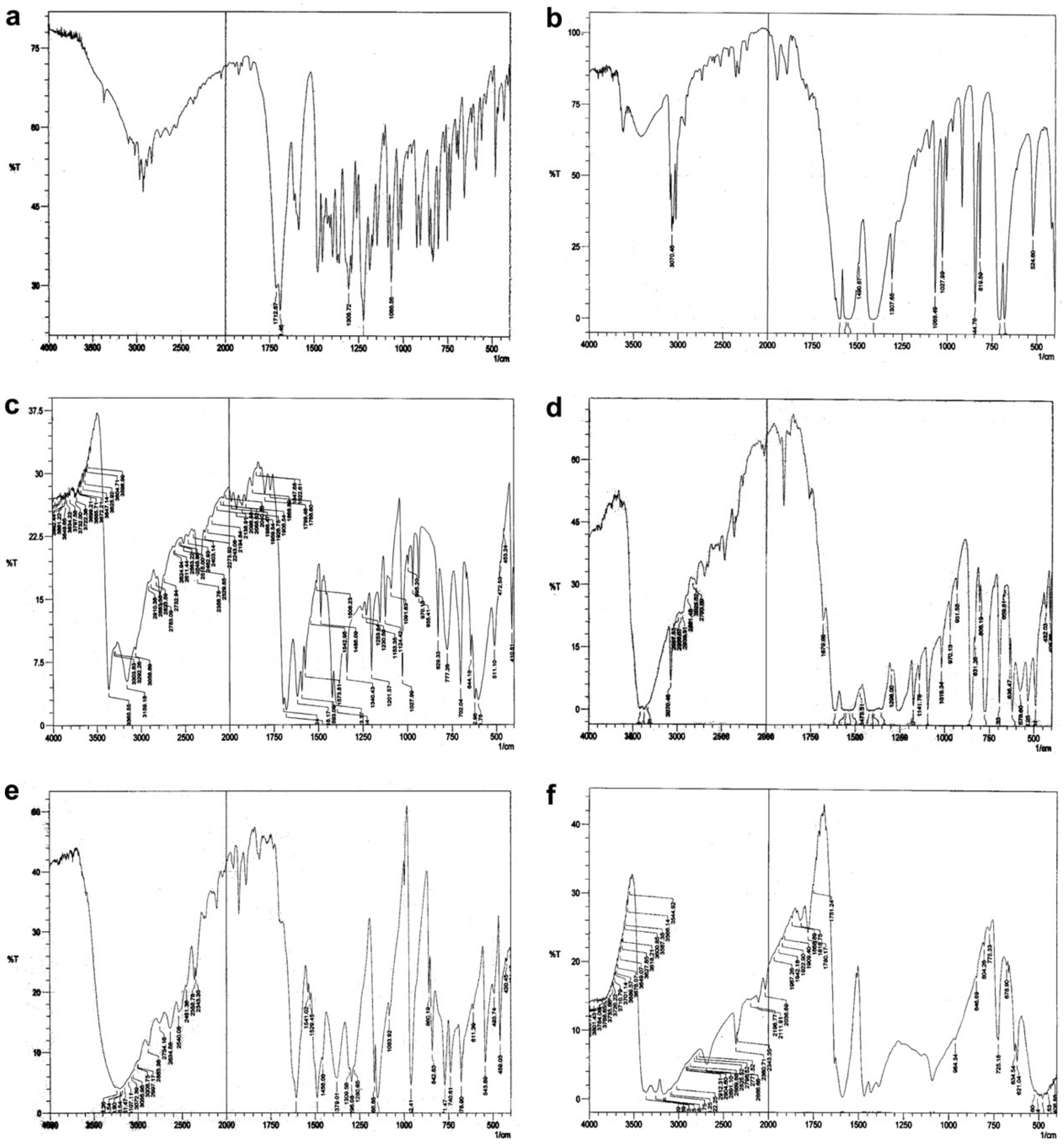


Fig. 4. FTIR spectrum of (a) indomethacin, (b) sodium benzoate, (c) nicotinamide, (d) sodium *p*-hydroxy benzoate, (e) resorcinol, (f) urea, (g) indomethacin–sodium benzoate (PM), (h) indomethacin–sodium benzoate (solubilized), (i) indomethacin–sodium *p*-hydroxy benzoate (PM), (j) indomethacin–sodium *p*-hydroxy benzoate (solubilized), (k) indomethacin–nicotinamide (PM), (l) indomethacin–nicotinamide (solubilized), (m) indomethacin–urea (solubilized), (n) indomethacin–resorcinol (solubilized).

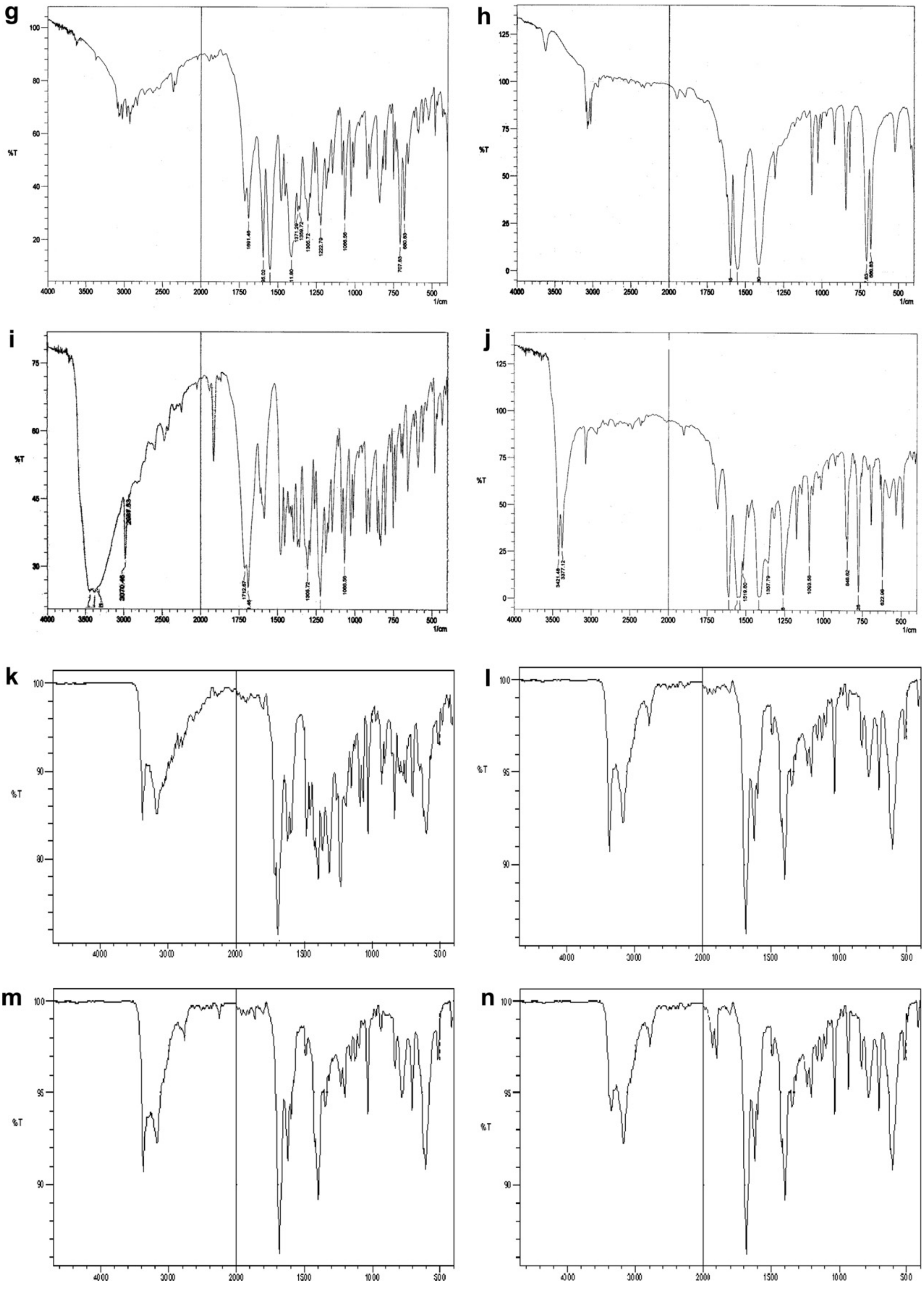


Fig. 4 (*continued*)

electronic changes in the structure of drug molecules. This is not indicative of any complex formation between drug and hydrotrope molecules, because the complex formation can be evidenced by formation of new chromophores (by appearance of a new peak or merging of two peaks to generate a common peak). No evidence of strong complex formation was obtained from FTIR spectral analysis (Fig. 4). Patterns of physical mixture (PM) and dried solubilized form show approximately superimposition of spectra of hydrotropes and indomethacin. However, there is slight shift in the peaks and peak intensity of indomethacin when it is in solubilized form. These changes may be due to weak interactions between indomethacin and hydrotrope molecules due to weak hydrogen bonding, ion–dipole bond formation or London forces of attractions (Fig. 5).

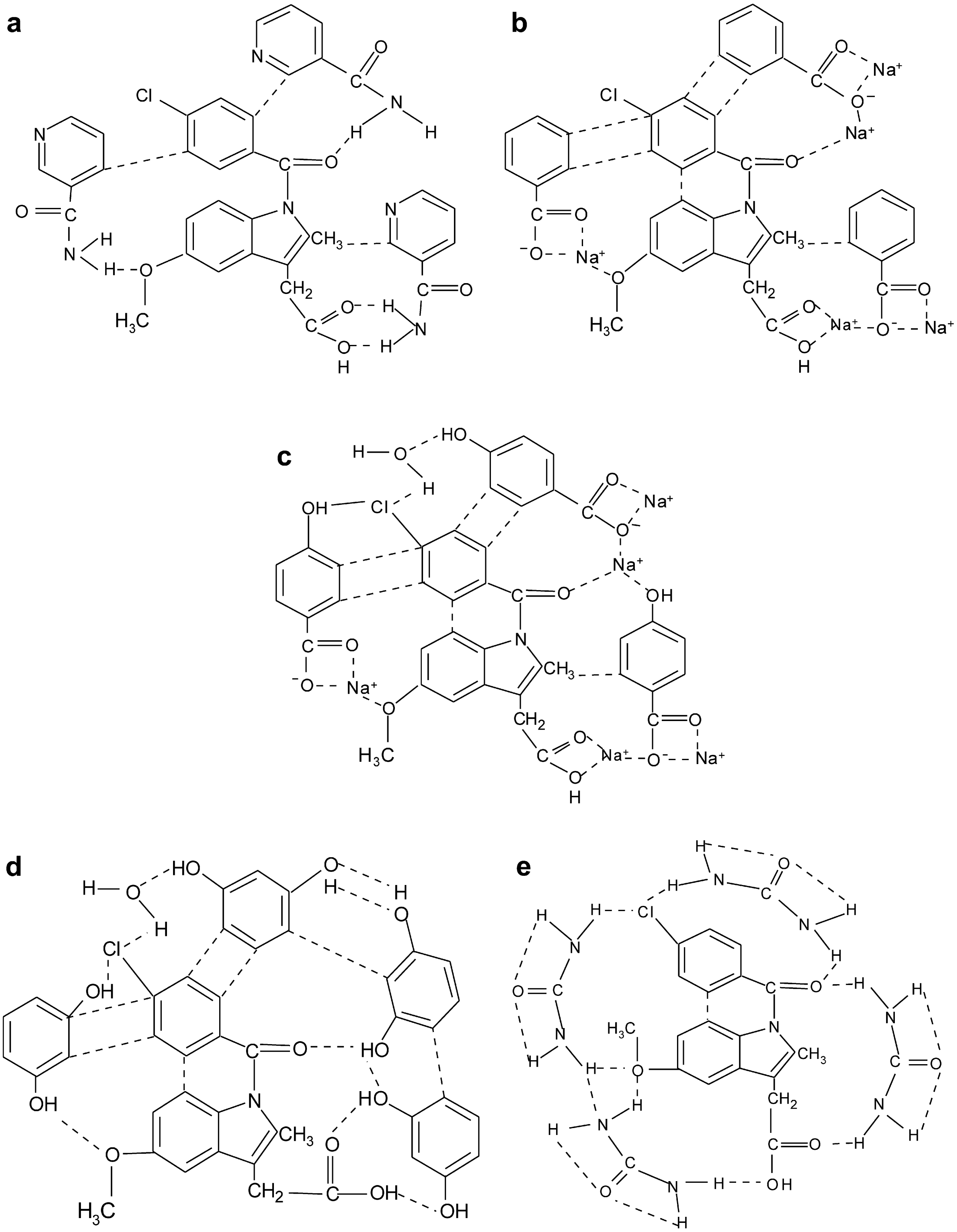


Fig. 5. Possible interaction of (a) indomethacin with nicotinamide, (b) indomethacin with sodium benzoate, (c) indomethacin with sodium *p*-hydroxy benzoate, (d) indomethacin with resorcinol, (e) indomethacin with urea in aqueous solution.

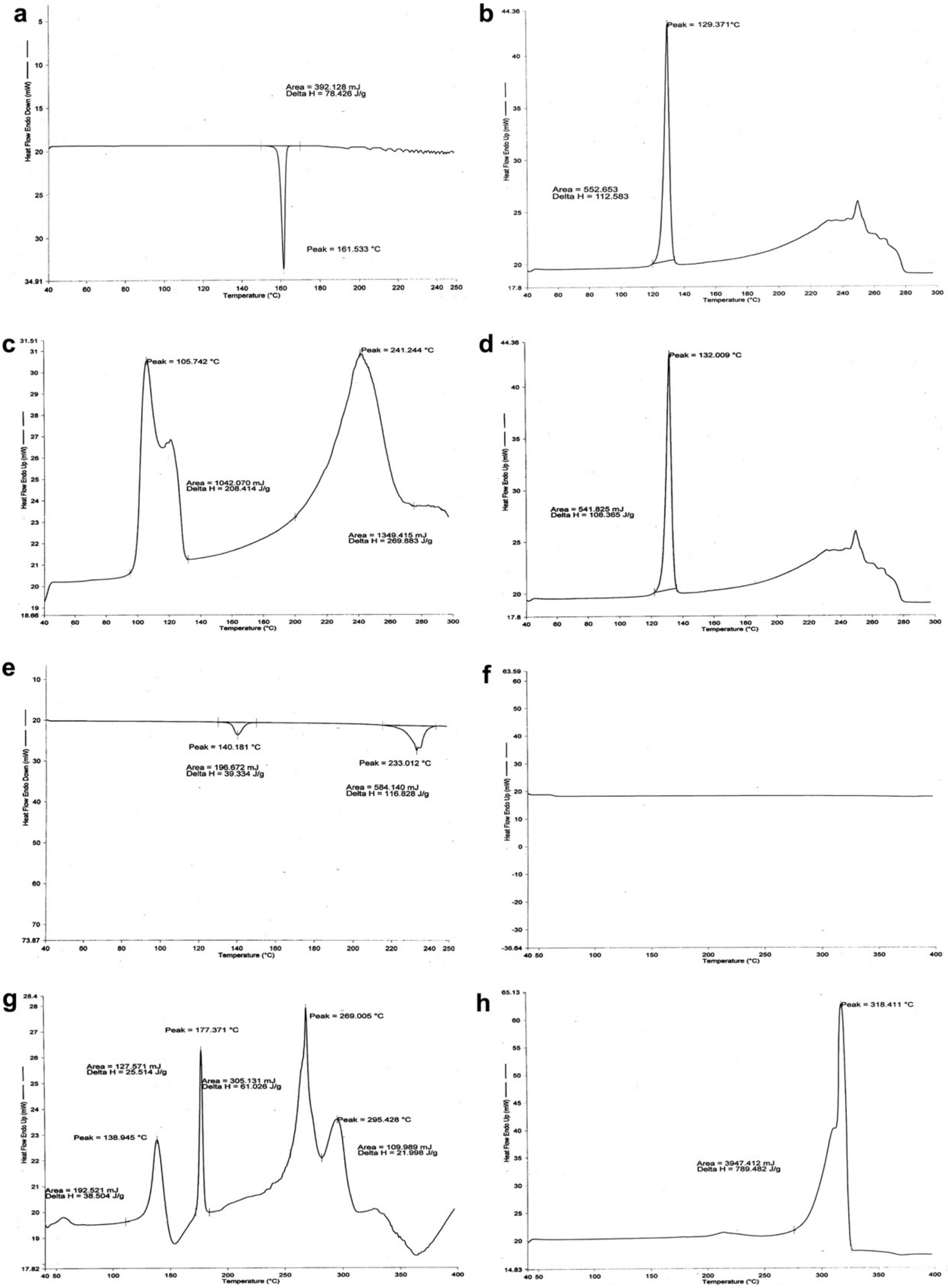


Fig. 6. DSC curve of (a) indomethacin, (b) nicotinamide, (c) indomethacin–nicotinamide (PM), (d) indomethacin–nicotinamide (solubilized), (e) indomethacin–sodium benzoate (PM), (f) indomethacin–sodium benzoate (solubilized), (g) indomethacin–sodium *p*-hydroxy benzoate (PM), (h) indomethacin–sodium *p*-hydroxy benzoate (solubilized).

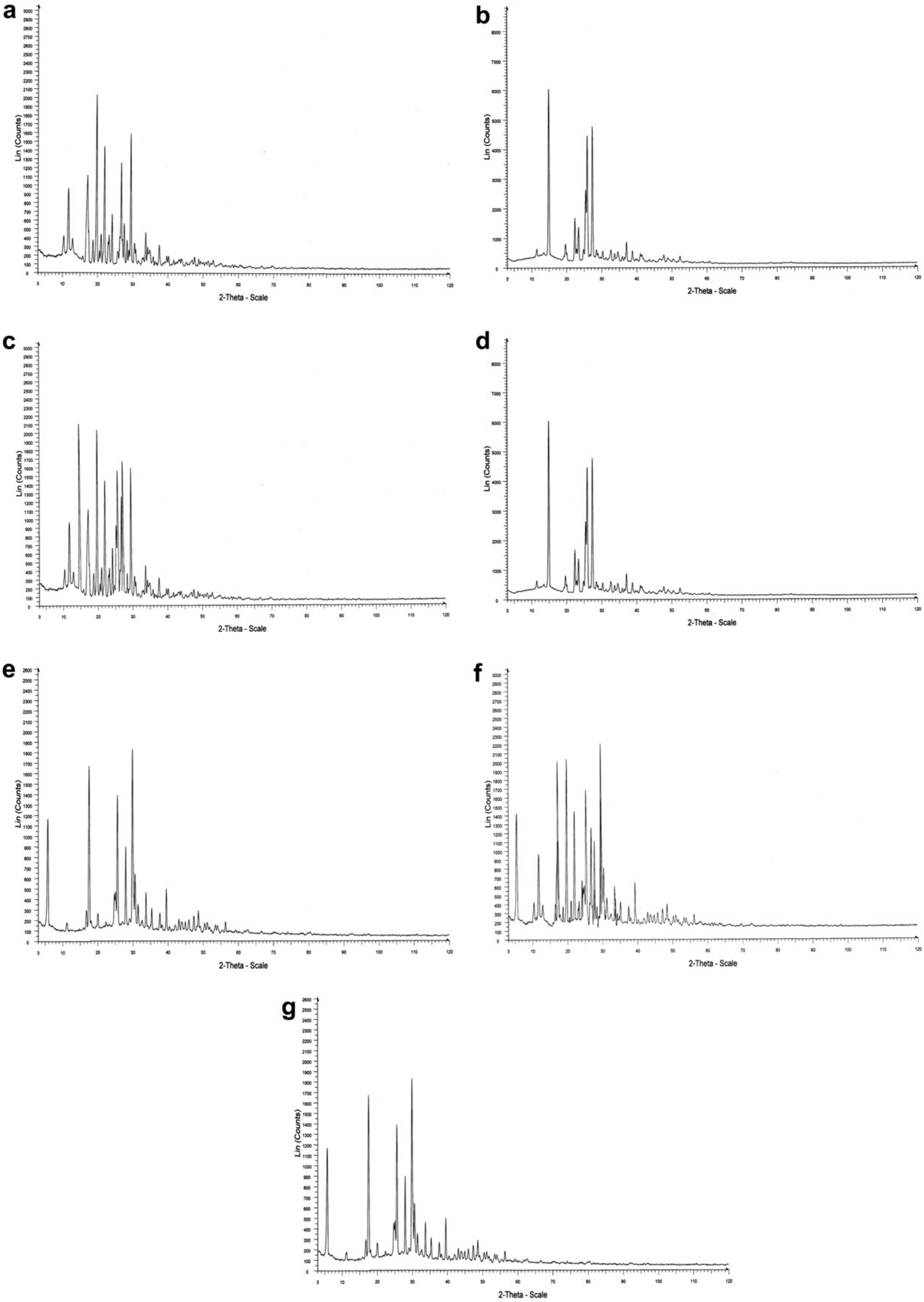


Fig. 7. XRPD of (a) indomethacin, (b) nicotinamide, (c) indomethacin–nicotinamide (PM), (d) indomethacin–nicotinamide (solubilized), (e) sodium *p*-hydroxy benzoate, (f) indomethacin–sodium *p*-hydroxy benzoate (PM), (g) indomethacin–sodium *p*-hydroxy benzoate (solubilized).

The solubilized product of indomethacin with nicotinamide shows the carbonyl stretch of indomethacin molecule at lower wave number (1680.23) than the two carbonyl stretches of pure indomethacin, i.e., 1712.67 and 1619.46. This proves that nicotinamide formed hydrogen bonding with oxygen of both the carbonyl groups of the drug and lowered the wave number of its carbonyl stretch. This way nicotinamide imparted aqueous solubility in it through various hydrogen bonding centers on hetero atoms with non-bonded electron pair on it. Further there is possibility of London forces acting between the non-polar parts of both the molecule (Fig. 5a). The solubilized product with sodium benzoate shows the two carbonyl stretches of indomethacin shifted to lower wave number than the carbonyl stretch of pure compound, i.e., 1712.67 and 1691.46 to 1596.95. This reveals that sodium ion from sodium benzoate formed ion–dipole bond with carbonyl group of indomethacin. Further London forces between aromatic rings (non-polar parts) and hydrogen bonding between polar parts also play a role in imparting aqueous solubility enhancement of indomethacin by sodium benzoate (Fig. 5b).

In case of indomethacin with sodium *p*-hydroxy benzoate hydrogen bonding between hydroxyl group of sodium *p*-hydroxy benzoate and carbonyl group of indomethacin and ion–dipole interaction between sodium ion and indomethacin seems to play a major role. The FTIR spectrum shows two carbonyl group peaks of indomethacin shifted from 1712.67 and 1691.46 to 1596.95 due to interaction with sodium ion of sodium *p*-hydroxy benzoate. The spectrum also showed hydroxyl group stretch of pure sodium *p*-hydroxy benzoate shifted from 3415 to 3326 peaks at 1552. Fig. 5c shows various centers of hydrogen bonding, ion–dipole interaction and London forces of interaction. Resorcinol forms hydrogen bonding through its two hydroxyl groups to various centers at indomethacin thus covering it with polar sheath and imparting it aqueous solubility (Fig. 5d). Hydrogen bonding between the amide group of urea and various negative centers of indomethacin molecule seems to impart aqueous solubility to indomethacin (Fig. 5e).

The DSC curve of pure indomethacin showed an sharp endothermic peak at 161.533° (Fig. 6), attributed to melting, indicating that the bulk powder is γ-form and high quality crystalline powder [39,40]. The curves of solubilized form of indomethacin with nicotinamide showed two peaks at 132.009° and 249.705° and the curves of solubilized form of indomethacin with sodium *p*-hydroxy benzoate showed peaks at 318.411°. No peaks were observed in the solubilized form of indomethacin with sodium benzoate. Thus, in all three solubilized forms no endothermic peak attributed to melting of indomethacin was observed in DSC curve, which indicates alteration of crystalline nature of indomethacin.

X-ray powder diffraction (XRPD) patterns of indomethacin show many different peaks, i.e., 11.577°, 16.969°, 19.550°, 21.806°, 26.581° and 29.336° (Fig. 7), which are characteristic of γ-form [39,40], indicating that the bulk powder was a high quality crystalline powder. While the diffraction patterns of solubilized forms of indomethacin with nicotinamide or sodium *p*-hydroxy benzoate show lesser degree of crystallinity and disappearance of characteristic peaks of indomethacin, which suggest that the crystalline nature of the drug is changed or it is converted to amorphous form and the peaks (crystallinity) are shown only by respective hydrotropes. The diffraction patterns of solubilized form of drug with sodium benzoate are completely diffused indicating a new amorphous solid phase. These results confirm the findings of DSC and suggest there is no complex formation.

The plots of specific gravity versus hydrotrope concentration showed a negative deviation (Fig. 8) that indicates an increase in partial molal volume upon aggregation, and this increase in volume may be due to expansion of the hydrocarbon portion of the molecule or its partial removal from the high compressive force of water [41].

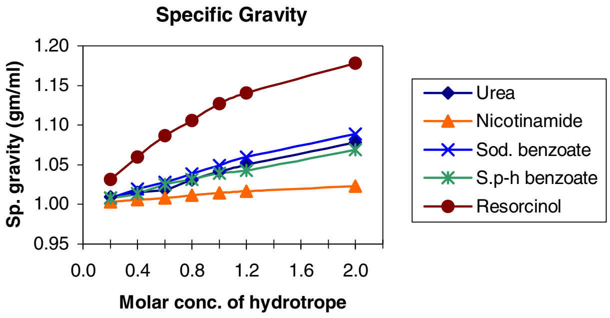


Fig. 8. Plots of specific gravity versus molar hydrotrope concentration.

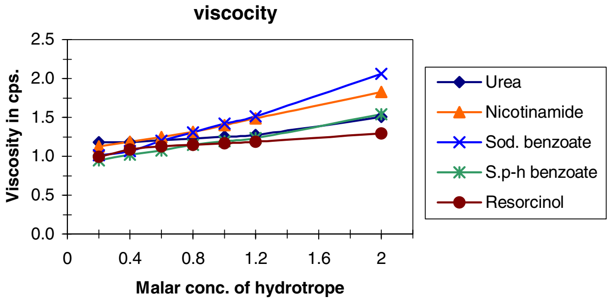


Fig. 9. Plots of viscosity versus molar hydrotrope concentration.

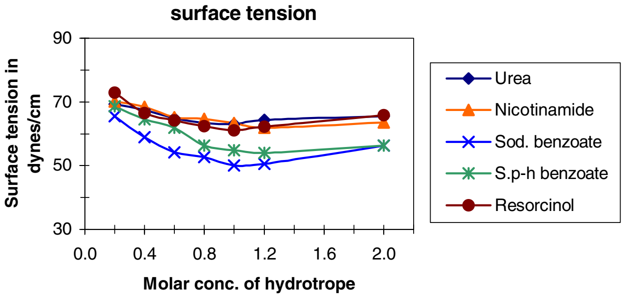


Fig. 10. Plots of surface tension versus molar hydrotrope concentration.

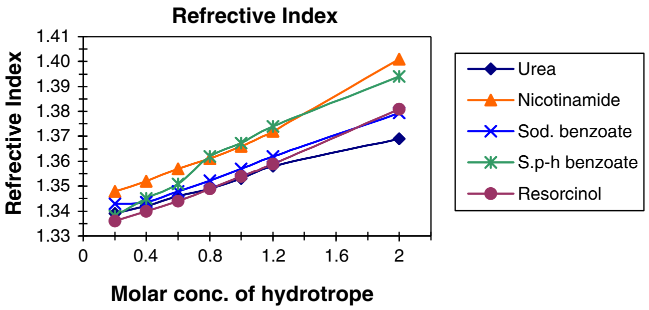


Fig. 11. Plots of refractive index versus molar hydrotrope concentration.

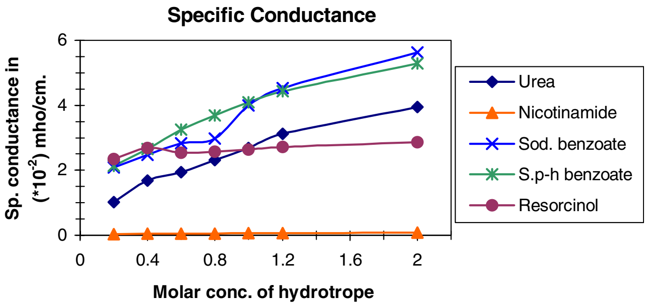


Fig. 12. Plots of specific conductance versus molar hydrotrope concentration.

The positive deviation in the viscosity plots (Fig. 9) indicates that aggregate formation is associated with an increase in viscosity of hydrotrope concentration, which is in agreement with the self-association of phenolic compounds [42]. The surface tension plots (Fig. 10) showed a moderate decrease in surface tension on increasing the hydrotrope concentration as hydrotropes are not surface active agents [34,43]. The plots of refractive index versus hydrotrope concentration (Fig. 11) showed negative deviation. The deviation from linearity in specific conductance plots (Fig. 12) is strongly indicative of molecular aggregation [44]. It was revealed from different studies that at lower hydrotrope concentration, there were weak ionic interactions while at higher hydrotrope concentration, the molecular aggregation seems to be the possible mechanism of hydrotropic solubilization [34,35,45–48].

The solubility at higher hydrotrope concentration is result of different kind of interaction between drug and ligand molecules. Both hydrophobic and donor acceptor forces (which are frequently larger in magnitude) may be involved as controlling factors in higher order water soluble complex formation. The results of spectrum study suggest charge transfer interaction is not determinant of complex formation. The investigation revealed involvement of ligand hydrophobicity as an important factor in complexation and stacking hydrotropes through its planar-

Table 3
Chemical stability data of formulations

Temperature (°C)	Formulation	Percent indomethacin remaining[a] at time interval of							
		0 day	1 day	3 days	7 days	14 days	21 days	30 days	45 days
4 ± 2	IPHB	100.00	99.56	99.61	99.48	99.38	99.15	98.83	98.61
	ISB	100.00	99.94	99.76	99.74	99.44	99.41	99.06	98.92
	INMD	100.00	99.83	99.76	99.46	99.47	99.23	99.15	98.99
25 ± 2	IPHB	100.00	99.42	99.15	98.88	98.61	97.99	97.45	96.23
	ISB	100.00	99.83	99.73	99.42	98.91	98.53	98.16	97.53
	INMD	100.00	99.49	99.42	99.12	98.99	98.64	98.23	97.82
37 ± 2	IPHB	100.00	99.33	98.90	98.56	97.68	96.83	95.59	93.65
	ISB	100.00	99.68	99.33	98.84	98.26	97.73	96.85	95.86
	INMD	100.00	99.33	99.05	98.83	98.42	97.75	97.03	96.34
45 ± 2	IPHB	100.00	99.25	98.88	98.26	96.87	95.79	94.42	91.83
	ISB	100.00	99.27	99.14	98.42	97.67	96.82	95.88	94.23
	INMD	100.00	99.22	98.97	98.56	97.86	97.14	96.21	94.87
60 ± 2	IPHB	100.00	98.99	98.56	97.99	96.15	94.36	92.17	88.23
	ISB	100.00	99.13	98.87	97.99	96.46	95.16	93.24	90.75
	INMD	100.00	99.19	98.87	98.03	96.88	95.66	93.94	91.54

[a] Average of three observations.

Table 4
Degradation rate constant and shelf life of formulated products

Formulations	Degradation rate constant K (days^{-1}) × 10^4 at different temperatures					Shelf life (day) as at 25 ± 2 °C	Shelf life (day) by Arrhenius plot
	4 ± 2 °C	25 ± 2 °C	37 ± 2 °C	45 ± 2 °C	60 ± 2 °C		
IPHB	2.70	7.59	13.66	18.05	26.48	137	110
ISB	2.43	5.55	9.12	12.44	21.03	187	185
INMD	2.08	4.35	7.66	10.83	18.88	239	227

ity, and strongly supports the assumption that ligand hydrophobicity [49] is directly related to the degree of hydrotropic solubilization. The studies suggest solubilizers exert their hydrotropic action not via direct interactions with solute, but rather by altering the nature of water as a solvent. Finally, it can be concluded that the solubilization of drugs cannot be attributed to complexation. The hydrotrope self-association significantly plays a role in solubilization mechanism. Additionally, high concentration of hydrotropic conjugation with self-association, change in the solvent nature of water and hydrotropic system which behaves as a co-solvent system in which one component is solid in solution rather than liquid.

The physical stability study showed that except for a slight to moderate color change in formulations which are stored at 45 or 60 °C, all other formulations remain unchanged in respect of color stability and no turbidity or precipitate formation was observed under different storage conditions. The change in color may be due to accelerated degradation of indomethacin at higher temperature in presence of oxygen that may get entrapped during fabrication despite precautions. The data on chemical stability at different temperatures and time intervals are shown in Table 3 from which it can be inferred that the degradation of indomethacin follows first order kinetics. K values, i.e., decomposition rate constant of formulation were obtained by the slope of the curves plotted between log of % age drug remaining versus $1/T$ and are presented in Table 4. The time required for the 10% degradation of drug for each formulation at 25 °C was calculated by the K value as well as by the extrapolation of Arrhenius plot. The results show that the prepared formulations had a shelf life of 137–239 days (calculated from the K value obtained by storage data at 25 °C) and 110–227 days (calculated from the K value obtained by extrapolation of Arrhenius plot of storage data at elevated temperatures) which can be further enhanced by incorporation of appropriate additives, processing in an inert gas environment and improving storage condition, i.e., keeping in cool and dark place.

Acknowledgements

The authors thank M/S Signa Laboratory, Kanpur, UP, India, for providing the gift sample of drug and Head, Institute of Pharmacy, Bundelkhand University, Jhansi, 284 128 UP, India, for providing necessary facilities to carry out these investigations.

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