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Solubilization of Poorly Water Soluble Drug Using Mixed Solvency Approach for Aqueous Injection

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript

Original Research Article

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ABSTRACT

Aim: 1) To increase water solubility of indomethacin drug using mixed solvency approach. 2) To employ use of non toxic solubilizers for increasing solubility of a poorly water soluble drug.
Study Design: Trial and error based experimental study.
Place and Duration of Study: School of Pharmacy, Devi Ahilya Vishwavidyalaya, UTD, Takshashila Campus, Indore, India and College of Pharmacy, IPS Academy, Indore, India between Jan. 2011 and June. 2012.
Methodology: By making mixed solvent (40%) blends of selected water soluble substances from the hydrotropes (urea, sodium benzoate, sodium citrate, nicotinamide); water-soluble solids (PEG- 4000, PEG-6000); and co-solvents (propylene glycol, glycerine, PEG-200, PEG-400, PEG 600) solubility studies were performed with indomethacin (model drug). On the basis of solubility studies formulation was developed. The solubilized drug and prepared formulation were characterized by ultraviolet and infrared techniques. Various properties of solution such as pH, viscosity, specific gravity and surface tension were studied. The developed formulation was studied for physical and chemical stability.

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Result: Aqueous solubility of drug in case of selected blends ranged from 14.55 mg/ml – 19.96 mg/ml (as compared to the solubility in distilled water 0.0464 ± 0.007 mg/ml). The enhancement in the solubility of drug in a mixed solvent containing 10% sodium benzoate, 5% sodium citrate and 25 % S cosolvent (25% S cosolvent contains PEG200, PEG 400, PEG600, Glycerine and Propylene glycol) was more than 400 fold. Prepared formulation F10 show appreciable physical and chemical stability.
Conclusion: The results of this study provide guidance for developing an injectable product and strategies for improving solubility as well as stability of poorly water soluble drug using the concept of mixed solvency. The proposed technique is economical, convenient and safe. The application of mixed solvency approach in the development of formulations shall prove a boon for pharmaceutical industries.

Keywords: Solubility; Indomethacin; aqueous injection; mixed solvency; nsaids; polyethylene glycol; sodium citrate.

1. INTRODUCTION

The formulation of solutions presents many technical problems to the industrial pharmacist. A growing number of new therapeutic molecules are limited by low or erratic bioavailability due to poor water solubility. Because of the clinical demand for new and more efficacious anti-cancer, antiviral, and anti-infective drugs, many of these new drugs may be formulated for injection. A large percentage of drugs currently in preclinical and clinical development for injectable administration are considered poorly water soluble. Developing an injectable formulation and drug product for poorly water soluble compound can be challenging because there is no one best approach [1-4].

Special techniques are required to solubilize poorly water-soluble drugs. Solubility of drug can be increased by variety of contemporary methods such as hydrotropic solubilization, solid dispersions, inclusion complex formation, altering the pH and using cosolvents but excess amount of these agents may have adverse effects. The term “hydrotropy” have been used to designate the increase in aqueous solubility of various poorly water-soluble compounds due to the presence of a large amount of additives. Concentrated aqueous hydrotropic solutions of urea, nicotinamide, sodium benzoate, sodium salicylate, sodium acetate and sodium citrate have been observed to enhance the aqueous solubility of many poorly water-soluble drugs [5-9].

The solubility of weak electrolytes and non polar molecules can be increased by the addition of water miscible solvent in which the drug has good solubility. This process is known as “Cosolvency”. It is proposed that the cosolvent system work by reducing the interfacial tension between predominantly aqueous solution and hydrophobic solute [10].

Cosolvent solubilization is particularly important for parenteral dosage form where it is desirable to incorporate the required dose as true solution in the smallest possible volume of liquid. Hydrotropes and cosolvents are used in 13% of FDA approved parenteral products. [11-13].

Poorly soluble drugs usually possess hydrophilic-hydrophobic balance favorable to their permeation through GI membranes so that dissolution becomes the decisive factor in the bioavailability of drugs. Solubilization of insoluble drugs has been extensively studied to

overcome difficulties encountered during pharmaceutical formulation. Most commonly used excipients to improve the solubility of a non polar drug in aqueous media are buffers, hydrotropes, surfactants, cosolvents and complexing agents. These can be used either alone or in combination. Recently, the synergism of two or three techniques has drawn particular interest [14-16].

Based on large number of experiments on solubilization of poorly water-soluble drugs, authors are of the opinion that hydrotropy is another type of cosolvency technique and all water soluble substances whether solids, liquids or gases have solubilizing properties. Maheshwari et al. have shown application of hydrotropy and mixed hydrotropy in titrimetric and spectrophotometric estimations of a large number of poorly water-soluble drugs precluding the use of organic solvents [17-19]. Melted (temperature less than 100ºC) Poly-ethylene glycol (PEG)-4000, PEG-6000 and PEG-8000 dissolves diclofenac sodium (melting point: 283ºC), thus, acting as solvent for the drug [20]. Melted urea (M.P.: 132-135ºC) dissolves diclofenac sodium (M.P.: 283ºC). Melted ibuprofen (M.P.: 78ºC) dissolves diclofenac sodium (M.P.: 283ºC), salicylic acid (M.P.: 159ºC) and niacinamide (M.P.: 132ºC), which again shows that melted ibuprofen acts as solvent for diclofenac sodium, salicylic acid and niacinamide, respectively. In supercritical fluid technology, liquefied carbon dioxide acts as solvent for many insoluble substances. These points indicate that all types of substances possess some solvent character [20-21].

This mixed solvency concept may be utilized to prepare concentrated (say 40% w/v or so, in strength) combined aqueous solutions of various water-soluble additives from the categories of so called, hydrotropes (sodium benzoate, sodium ascorbate, sodium citrate, niacinamide, urea), co-solvents (glycerin, propylene glycol, ethanol, PEG 200, 300, 400, 600), water soluble solids (PVP, PEG 4000, 6000, 8000,), and cyclodextrins (beta cyclodextrin, HP beta cyclodextrin). These are employed in small and safe concentration to solubilize poorly water soluble drugs to be developed into suitable dosage forms (solutions, syrups, injections, topical solutions etc). The authors propose mixed solvency approach for poorly water soluble drugs (Fig. 1).

In the present investigation, the poorly water-soluble drug indomethacin is selected as model drug for formulating aqueous injection using mixed solvency approach [22-27]. 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 is a white to pale yellow crystalline powder with almost no odor. it is water insoluble in nature [28-29].

The poor aqueous solubility and wettability of the drug give rise to difficulties in formulation of parenteral solutions and may lead to a variable bioavailability. The present study was aimed to investigate the effect of mixed solvent system on the solubility of indomethacin, and to attempt formulation as aqueous injection. The feasibility of preparing injection was examined, suitable blend with good solubility profile selected and parenteral dosage form was formulated. Investigations related to chemical interaction were done by UV spectrophotometery and by Fourier transform infrared spectroscopy. Finally, the short-term physical and chemical stability study was conducted.

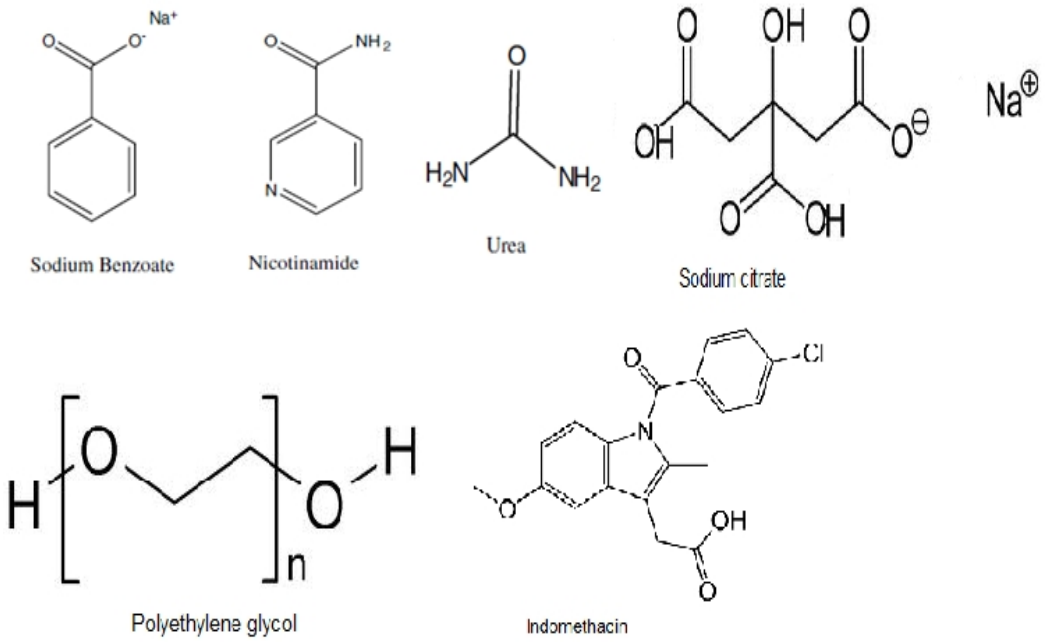

Fig. 1. Structure of Indomethacin and water soluble agents used for mixed solvency

2. MATERIALS AND METHODS

2.1 Materials

Indomethacin was obtained as gift sample from Neon Laboratories, Mumbai, India. Nicotinamide and Propylene glycol were purchased from Loba Chemie, Mumbai. Sodium benzoate, Urea, Sodium citrate, Glycerin, PEG 200, PEG 400, PEG 600 and PEG 6000 were obtained from Merck Chemicals Limited, Mumbai, India. All other chemicals and solvents used were of analytical/HPLC grade.

2.2 Experimental

2.2.1 Estimation of indomethacin

The calibration curve of indomethacin was prepared in distilled water and various concentrations of water soluble agents (hydrotropic agents and co-solvents) and estimated at 320 nm using double-beam UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) [17-20,26].

2.2.2 Solubility determination

Solubility of indomethacin in various solutions was determined by equilibrium solubility method. Excess amount of drug was added to 5 ml screw-capped glass vials containing buffers of pH (1.2 -10.0), aqueous solution of hydrotropic agents and different concentration of solubilizers (Table 1 and 2). The vials were shaken for 12 hr on mechanical shaker (Lab Hosp, Mumbai, India) at room temperature. The solutions were allowed to equilibrate for the

next 24 hr. The solution was transferred into eppendorf tubes and centrifuged for 5 min at 2000 rpm. Supernatants of each vial was filtered through 0.45µ membrane filter (Pall Corporation, USA) and analyzed for drug content spectrophotometrically at 320nm after suitable dilutions. The study was performed in triplicate [10, 22-26].

Table 1. pH dependent solubility of indomethacin at room temperature

Buffer Solutions	Solubility* (mg/ml)	Enhancement Ratio
Distilled Water	0.0464 ±0.007	–
Hydrochloric acid buffer pH 1.2	0.0121±0.004	0.260
Hydrochloric acid buffer pH 2.2	0.0138±0.011	0.297
Acid phthalate buffer pH 4.0	0.360 ± 0.014	7.758
Phosphate buffer pH 5.8	0.461 ± 0.009	9.935
Phosphate buffer pH 8.0	1.911 ± 0.007	41.18
Alkaline borate buffer pH 9.0	2.660 ±0.012	57.32
Alkaline borate buffer pH 10.0	3.768 ±0.006	81.20

** Average of 3 determinations*

Table 2. Solubility enhancement ratio of indomethacin in Different solubilizers at room temperature

Solvents	Solubility* (mg/ml)	Enhancement Ratio
Distilled water	0.0464±0.007	--
20 % Sodium Benzoate	17.226±0.011	371.25
40 % Sodium Benzoate	30.857±0.008	665.021
20 % Urea	3.883±0.005	83.685
40 % Urea	8.632±0.014	186.034
20 % Nicotinamide	14.216±0.017	306.379
40 % Nicotinamide	26.418±0.006	569.353
20 % Sodium Citrate	0.316±0.012	6.810
40 % Sodium Citrate	0.704±0.016	15.172
20 % w/v Propylene Glycol	0.198±0.014	4.26
40 % w/v Propylene Glycol	0.546±0.008	11.767
20 % w/v Glycerine	0.442±0.004	9.525
40 % w/v Glycerine	1.147±0.006	24.719
20 % w/v PEG 6000	0.548±0.009	11.810
40 % w/v PEG 6000	1.230±0.015	26.508
20 % w/v PEG 200	0.412±0.016	8.879
40 % w/v PEG 200	1.029±0.004	22.176
20 % w/v PEG 400	0.497±0.009	10.711
40 % w/v PEG 400	1.203±0.007	25.926
20 % w/v PEG 600	0.612±0.010	13.189
40 % w/v PEG 600	1.522±0.014	32.801

** Average of 3 determinations*

2.2.3 Influence of pH on solubility and stability

Samples of saturated solution of drug were kept at different pH for 30 days and analyzed for the drug content at 1, 7, 14 and 30th day to obtain the % of degradation and thereby to find the pH of maximum stability of indomethacin [22-26].

2.2.4 Method of determination for additive/synergistic effect on solubility in blends

An equilibrium solubility method was used to determine the additive or synergistic effect on solubility. The total strength of all solubilizers was 40% w/v (constant) in all aqueous mixed solvent systems (Table 3). The solubility of indomethacin was determined in these systems [20-21,26].

Table 3. Mixed solvent (total concentration 40%, constant) for saturated solubility determination of indomethacin

Blend Code	Solvents	Solubility* (mg/ml)	Enhancement Ratio
A1	Distill water	0.0464±0.007	----
F1	15% SB+25% S	5.698± 0.007	122.801
F2	15 %UR+25% S	1.507±0.011	32.478
F3	15%N+25% S	2.279±0.008	49.116
F4	15% SC+25% S	10.588±0.006	228.189
F5	10%SB+5%UR+25% S	8.235±0.012	177.478
F6	10%UR+5%N+25% S	5.073±0.009	109.331
F7	10%N+5%SC+25% S	14.558±0.007	313.750
F8	10%SC+5%SB+25% S	17.867±0.004	385.064
F9	10%SB+5%N+25% S	12.205±0.009	263.038
F10	10%SB+5%SC+25% S	19.963±0.007	430.237
F11	10%N+5%UR+25% S	15.330±0.011	330.387
F12	10%N+5%SB+25% S	16.029±0.014	345.452

** Average of 3 determinations*

SB- Sodium benzoate, UR- Urea, N- Nicotinamide, SC- Sodium citrate, S- Solvent system containing 5% of each PEG 200, PEG 400, PEG 600, Glycerin and Propylene glycol

2.2.5 Properties of mixed solvent solutions

The solution properties of mixed solvents such as pH, viscosity, specific gravity and surface tension were studied using digital pH meter, Brookfield viscometer, pycnometer and stalagmometer respectively (Table 4) [22-26].

Table 4. Properties of the optimized blends

Experiment Blends	pH	Viscosity (cps)	Surface tension (dynes/cm)	Specific gravity
F7	7.51	6.04	54.22	1.054
F8	7.19	6.15	55.84	1.012
F10	7.08	6.12	59.24	1.008
F11	6.94	6.14	55.38	1.049
F12	7.13	6.09	56.42	1.044

2.2.6 Drug excipient compatibility studies

2.2.6.1 UV spectral studies

To study the possible spectroscopic changes in the structure of drug in presence of different solubilizing agents and to interpret the probable mechanism of solubilization, UV spectral studies of indomethacin was performed. (Figs. 2,3,4,5,6,7,8,9,10,11 and 12) [22-26].

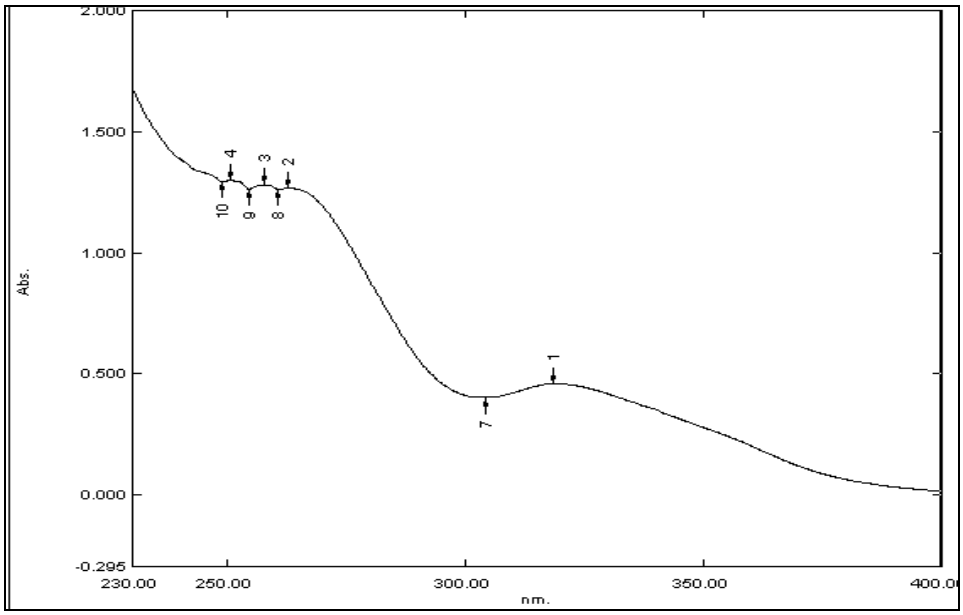

Fig. 2. UV spectra of Indomethacin in water

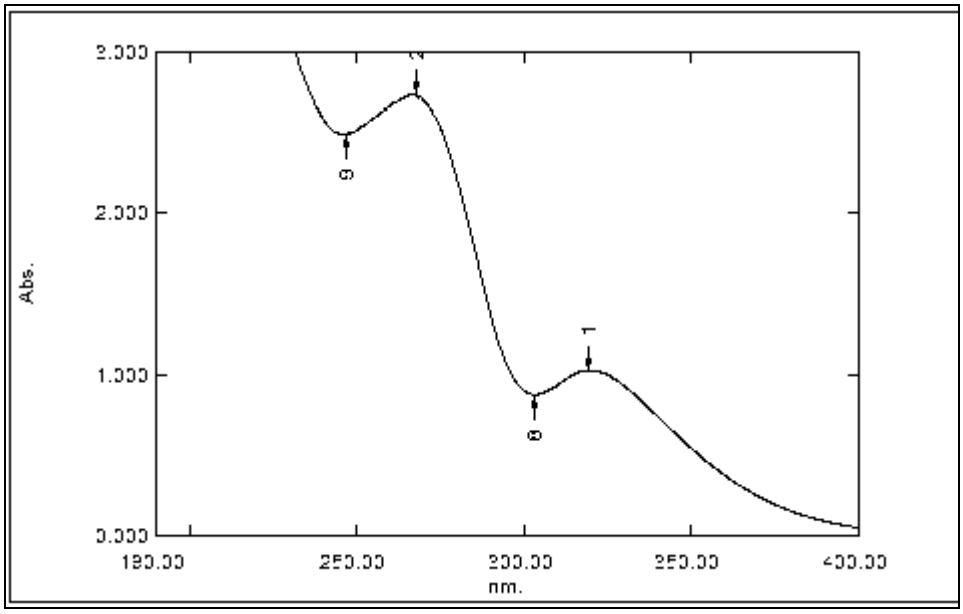

Fig. 3. UV spectra of Indomethacin in Glycerin

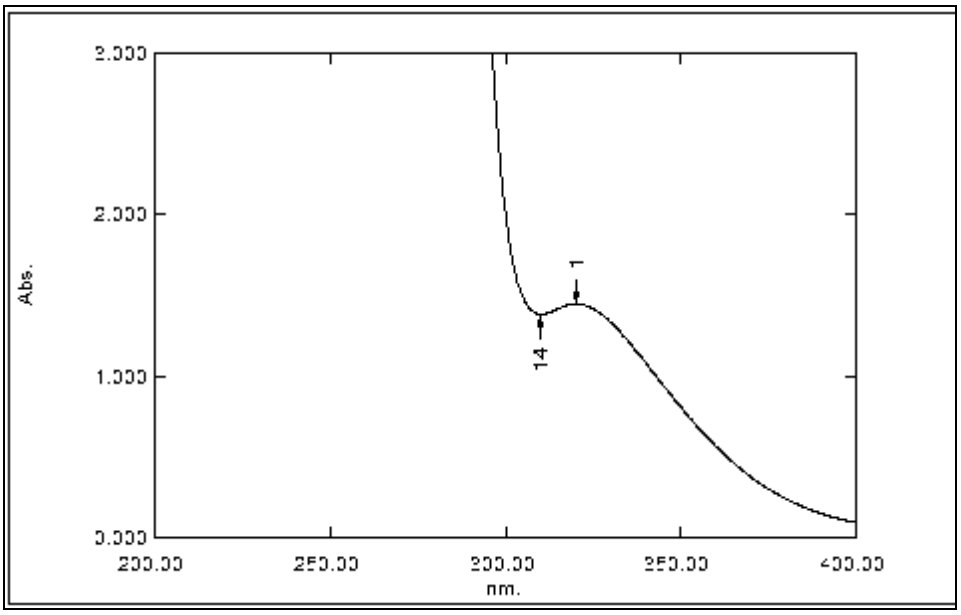

Fig. 4. UV spectra of Indomethacin in Nicotinamide

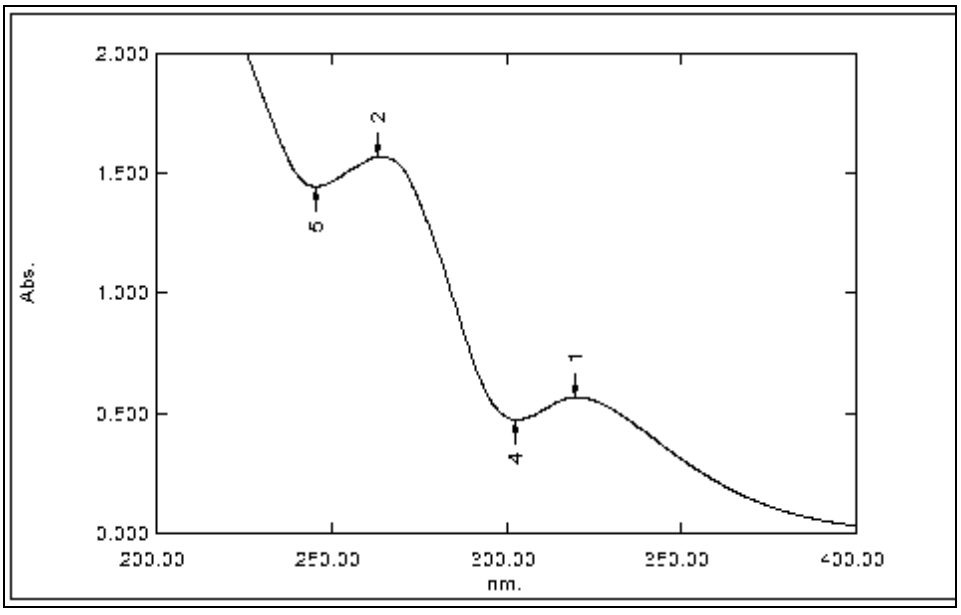

Fig. 5. UV spectra of Indomethacin in PEG 200

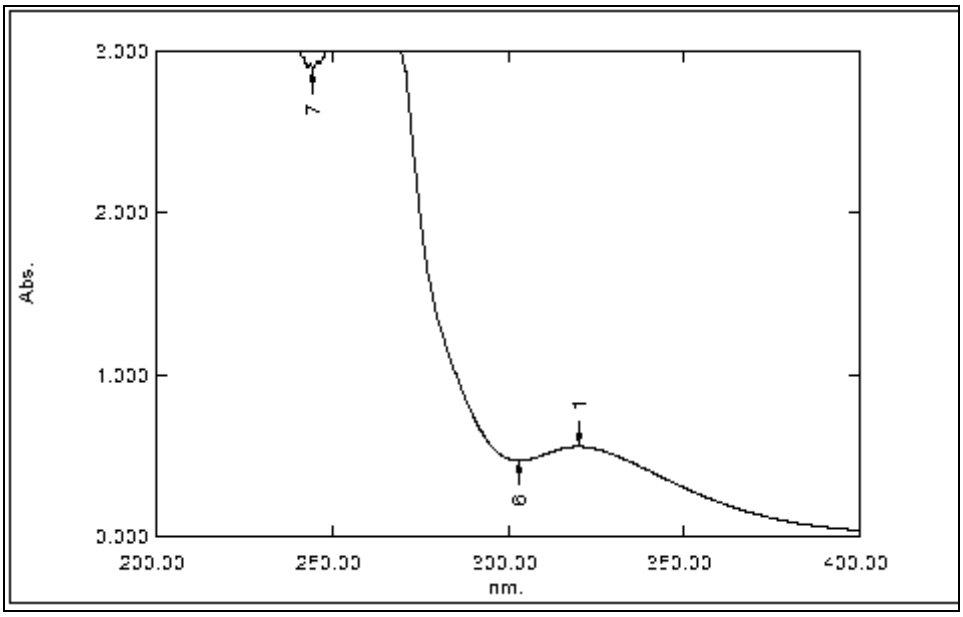

Fig. 6. UV spectra of Indomethacin in PEG 400

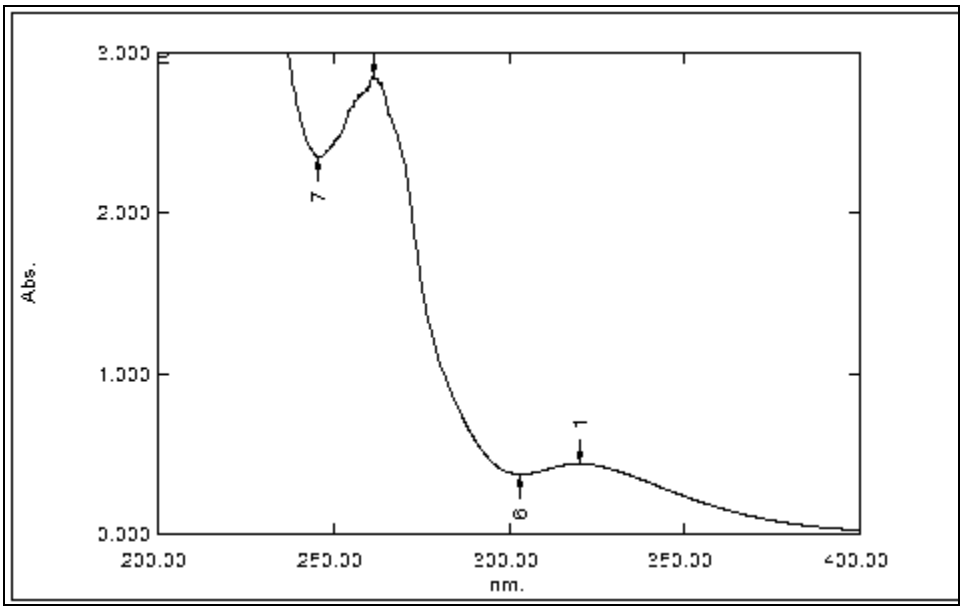

Fig. 7. UV spectra of Indomethacin in PEG 600

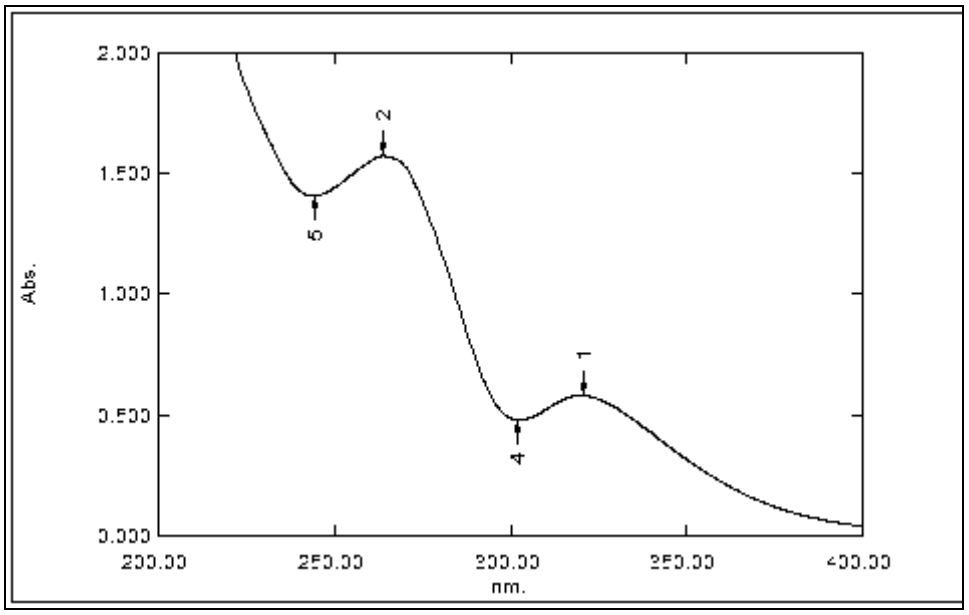

Fig. 8. UV spectra of Indomethacin in Propylene Glycol

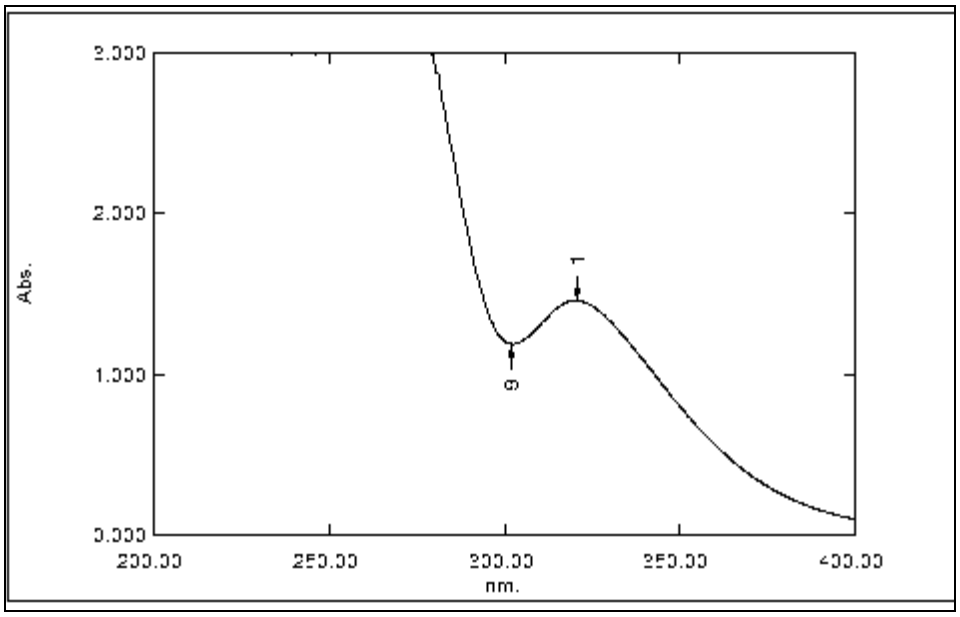

Fig. 9. UV spectra of Indomethacin in PEG 6000

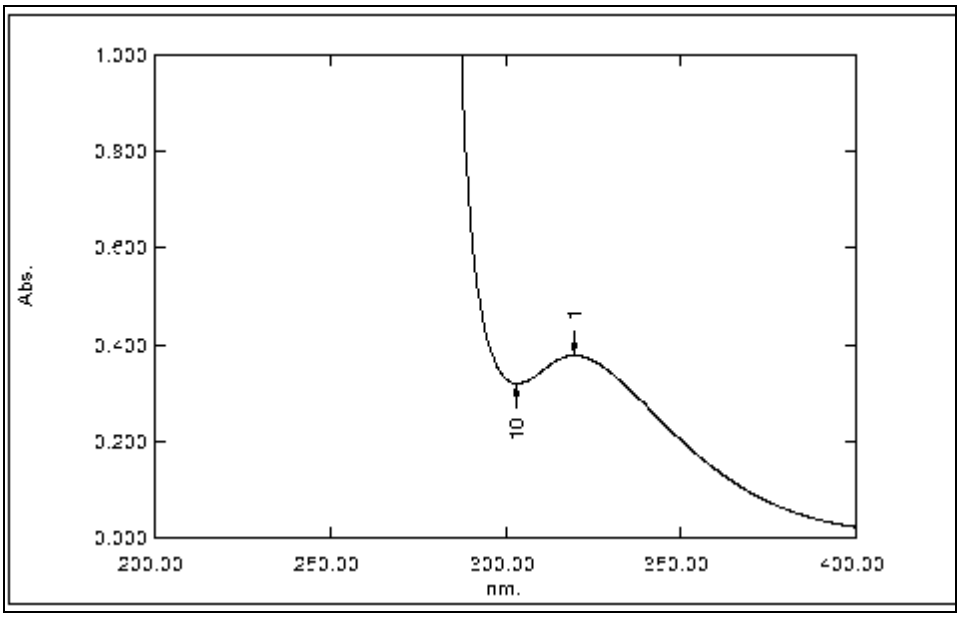

Fig. 10. UV spectra of Indomethacin in Sodium Benzoate

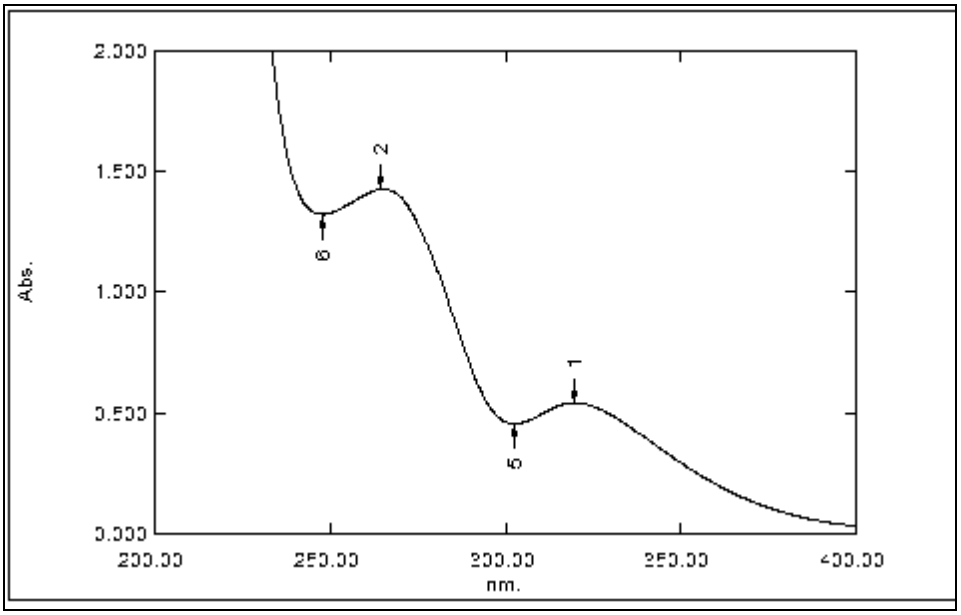

Fig. 11. UV spectra of Indomethacin in sodium citrate

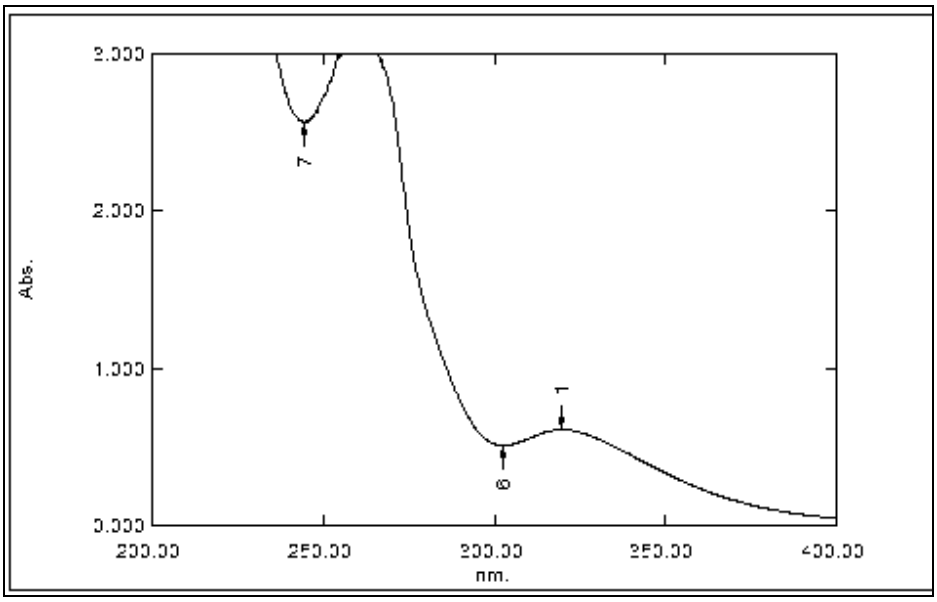

Fig. 12. UV spectra of indomethacin in Urea

2.2.6.2 Fourier transform infrared (FTIR) spectral studies

FTIR spectra were obtained by means of a FTIR spectrophotometer (FTIR – 8300S, Shimadzu, Japan). The samples were prepared by mixing of drug and potassium bromide in 1:100 ratio and measurements were attempted over the range of 400–4000 cm^{-1} (Figs. 13 and 14) [22,26].

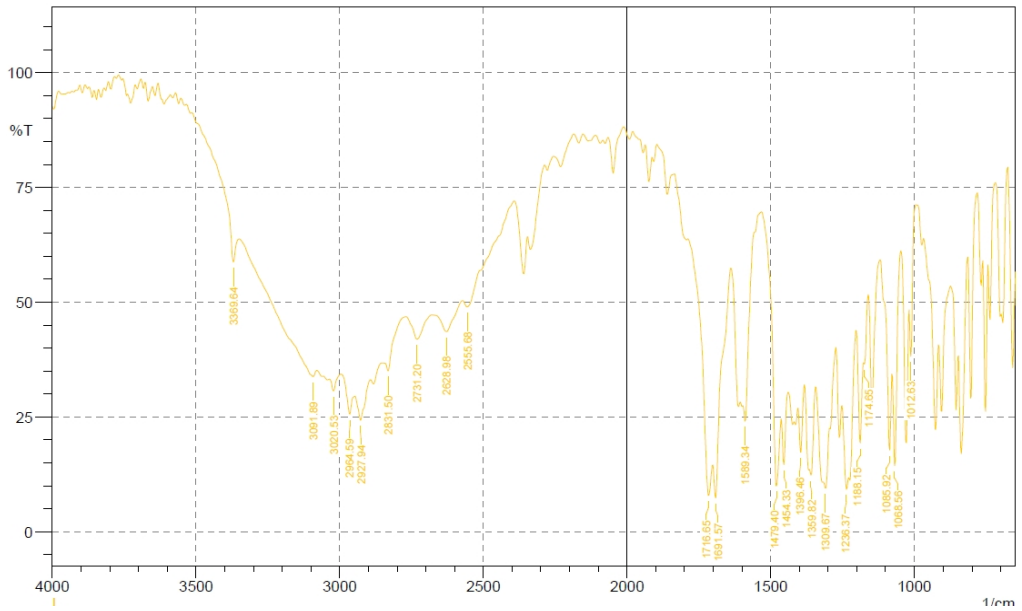

Fig. 13. FTIR spectrum of indomethacin pure drug

Fig. 14. FTIR spectrum of indomethacin with all excipients

3. FORMULATION OF AQUEOUS INJECTION

3.1 Preparation of Aseptic Area

The walls and floor of aseptic room were thoroughly washed with water and then disinfected by mopping with 2.5% v/v Dettol (Reckitt Benckiser India Ltd., Kolkata, India) solution [23-25)]. The bench was cleaned with 70% v/v isopropyl alcohol which was also sprayed in the room. The aseptic room was fumigated using a mixture of formaldehyde and potassium permanganate and the UV lights were switched on for 30 min prior to formulation of injections and the filling of injections into vials [23-27].

3.2 Treatment of Packing Material

Amber color glass vials of 2 ml capacity were washed several times with water then finally rinsed with distilled water. All these vials were placed in an inverted position and sterilized by dry heat in an oven at 160 ºC 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 water to remove any detergent residue and finally rinsed with distilled water. These stoppers were sterilized by autoclaving at 121 ºC and 15 lbs pressure for 15 min. Finally, the stoppers were rinsed with freshly prepared sterile distilled water and dried in vacuum oven under aseptic conditions [23,26-28].

3.3 Preparation of Aqueous Injection

On the basis of solubility data obtained from the final blends of mixed solvent, formulation of aqueous injection of indomethacin was done using mixed solvent system ‘F10’. This formulation contained 12.5 mg/ml indomethacin in mixed solvent system ‘F10’ (10% Sodium

benzoate, 5% sodium citrate, 25% w/v solvent system 'S'). Sodium bisulfite (0.1% w/v) 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. [23-27]

For the preparation of aqueous injection of indomethacin, 45 ml of solvent system 'F10' was placed in 50 ml volumetric flask, weighed amount of indomethacin and 0.1% w/v sodium bisulfite were added and shaken for 1 hour to ensure complete dissolution. pH of the solution was adjusted to 6-7.5 with 0.1 N HCl and 0.1 N NaOH solution and volume was made up to 50 ml. These solutions were filtered through 0.45 µ membrane filter (Pall Corporation, USA). The solutions were analyzed spectrophotometrically at 320 nm for drug content after suitable dilutions taking water for injection as blank [23-27].

3.4 Aseptic Filtration and Packaging

The aqueous injection of indomethacin was sterilized by filtration through 0.2 µ disposable membrane filter fitted in a holder of 5 ml glass syringe and the pressure on the piston was adjusted. After filtration, the preparation was packed by the sterilized air tight rubber closure and labeled. The final packed vials were sterilized by autoclaving at 15 lbs/sq. inch (121ºC) for 15 min [24-28].

4. CHARACTERIZATION OF AQUEOUS INJECTION

4.1 Stability Study

4.1.1 Physical stability studies

The sealed or packed vials of the aqueous injections were visually inspected each day for 30 days against black and white backgrounds for changes in color and turbidity on storage (room temperature, 5ºC±3ºC in refrigerator, and 40 ºC/75% RH in thermostatically controlled ovens) [26,30].

4.1.2 Chemical stability studies

The injection formulations were subjected to exhaustive chemical stability studies at 5ºC±3ºC in a refrigerator, room temperature and 40ºC±2ºC/75% RH in thermostatically controlled ovens for a period of 30 days. The formulations were analyzed spectrophotometrically initially and at particular intervals to calculate the drug content. The percent residual drug for each injection formulation at different time intervals as well as at different temperatures was calculated considering the initial drug content for each formulation to be 100% (Table 6). From the chemical stability data, the shelf life of formulation was calculated. [25-27,30].

5. RESULTS AND DISCUSSION

5.1 Estimation of Indomethacin in Distilled Water

Results of equilibrium solubility showed that indomethacin has very limited solubility in distilled water (0.0464 mg/ml) which is well below the target solution concentration of 1 mg/ml [23,26].

5.2 Influence of pH on Solubility

Saturated solutions of drug were studied for pH dependent solubility and stability at room temperature and analyzed for drug content. Results show that indomethacin has more solubility in alkaline pH. This may be due to the presence of carbonyl and carboxylic acid functional groups on indomethacin which render it acidic. Enhancement in solubility was found 80 folds at pH 10 [22, 26, 31].

One of the major factors responsible for dissolution of an organic compound is its ability to dissociate into ionic species, which depends on the pH of the media and Percentage ionized and hence increases solubility due to increase in pH value [32]. Drug was stable at all pH range. The drug content was found to be more than 98% after stability duration of 30 days (Table 1)

5.3 Solubility Determination in Hydrotropic Agents and Co-solvents

The hydrotropes selected for the study (nicotinamide, sodium benzoate, urea and sodium citrate) possess hydrophobic center which can interact due to large surface area and a mobile electron cloud. These sites are available for non-bonded and Van der Waals interaction with water and drug. Water molecules join together to form cluster. For solubilization, the ionized hydrotropes break this association and use the ion–dipoles of water for solvation [33].

The solubility of indomethacin in different hydrotropic agents and co-solvents is shown in Table 2. The maximum solubility was observed in 40 % sodium benzoate with enhancement ratio of 665.021. Solubility enhancement for 40 % hydrotropic solution could be ranked in decreasing order as Sodium benzoate> nicotinamide> urea> sodium citrate and the solubility enhancement ratio 665.021 > 569.353 >186.034 >15.172 respectively. Solubility enhancement for co-solvents could be ranked as PEG 600 > PEG 6000 > PEG 400 > Glycerine> PEG 200> Propylene Glycol. The use of hydrotrope combinations yields higher indomethacin solubility than that of the single hydrotrope. The higher the hydrotrope concentration in the solution the more drug could be solubilized [23].

On increasing the concentration of solubilizers, the solubility of indomethacin increases but this leads to enhanced toxic effect of the solubilizing agents. Investigations reveal that incorporation of hydrotropes in combination into cosolvent solution increases drug solubility better than cosolvent solution alone. Hence, instead of using a single solubilizer in larger concentration for development of dosage form, a combination of solubilizers in comparatively smaller concentrations increase solubility and the associated toxic effects can be reduced [26].

5.4 Solubility in Mixed Solvents

Mixed solvent system was prepared to determine solubility and about 100 to 400 fold increase in solubility was seen which may be due to the additive/synergistic effect of the mixed solvents. Combination of 10% sodium benzoate, 5% sodium citrate and 25% of cosolvent “S” increase solubility by 430 folds, which is well beyond desired concentration shown in Table 3. The structures of drugs and hydrotropes with different centers of different electro negativity might be responsible for the intermolecular hydrogen bonding and electrostatic attraction. Nicotinamide formed hydrogen bonding with oxygen of the carbonyl

groups of the drug and lowered the wave number of its carbonyl stretch. This imparted aqueous solubility through various hydrogen bonding centers on hetero atoms with non-bonded electron pair on it. 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. Hydrogen bonding between the amide group of urea and various negative centers of indomethacin molecule seems to enhance aqueous solubility to indomethacin. Electronegative corboxylate ion and hydroxyl group of sodium citrate increase solubility of drug. By disrupting self-association of water, cosolvents reduce the squeezing out of non-polar hydrophobic compounds and increase solubility. Most of the cosolvents have hydrogen bond donor and/or acceptor groups as well as small hydrocarbon regions. Hydrotropic agents produce weak ionic interaction, which indicates that their hydrophilic hydrogen bonding groups ensure water miscibility to a greater extent [26].

5.5 Properties of Mixed Solvent Solutions

The solution properties of mixed solvents were studied for selected blends F7-F8 and F10-F12 which showed solubility enhancement ratio upto 300-400 fold (Other blends were not selected due to less solubility enhancement ratio). Results reveal that pH of the formulations is less alkaline so it would be less irritant at the site of application. Viscosity of solutions increased as increasing concentration of hydrotropes leading to the formation of aggregates, change in specific gravity and surface tension [34]. Change in specific gravity might occur due to increase in partial molal volume upon aggregation [31]. The decrease in surface tension was due to self association as hydrotropes are not surface active agent [22]. Results showed in Table 4.

5.6 UV spectral Studies

In case of indomethacin-mixed solvent system, a minor shift in λmax (320 ± 0.5 nm) was observed, which may be due to electronic changes in the structure of drug molecules. This is not indicative of any complex formation between drug and hydrotrope molecules, as the complex formation can be evidenced by formation of new chromophores (by merging of two peaks to generate a common peak). Small additional peaks of solubilizers and cosolvent were also observed in UV spectra which indicates that there is no interference of solubilizer/cosolvents with the absorbance of the drug (Figs. 2-12).

5.7 Fourier Transform Infrared (FTIR) Spectral Studies

To check the integrity of the drug in the formulation an IR spectral study was carried out. The spectra obtained from IR studies (at wavelength 4000 cm^{-1} to 400 cm^{-1}) showed that there are neither major shifts nor any loss of functional peaks between the spectra of drug and polymer. IR of drug with all solubilizers was compared with that of the pure drug to see whether there was any change in the structure or interaction with the polymers. The solubilized product with mixed solvent shows the two carbonyl stretches of indomethacin shifted to lower wave number than the carbonyl stretch of pure compound, that is 1716.65 and 1691.57 to 1670.35 and 1595.13. This proves hydrogen bonding and London force between aromatic rings which impart aqueous solubility to drug. The IR spectrum for indomethacin shows peaks at 3,342.6 and 1,670.5 cm^{-1}, corresponding to carboxylic O−H and C=O stretch respectively. The O–H stretching frequency of the carboxylic acid group of

indomethacin observed at around 3,350.5. Pattern of C=O stretch vibrations is typically observed in the range of 1600-1750 cm^{-1}. Peak at 1691 cm^{-1} is assigned to benzoyl vibration. Peak at around 754 cm^{-1} represent halogen (below 1000) and aromatic overtone was observed at 1000-2000 cm^{-1} (Figs. 13 and 14) [22, 26].

5.8 Physical Stability Studies

Blend F10 was selected and used to develop aqueous parenteral formulation owing to its superior and synergistic effect on solubility of indomethacin. Formulated aqueous injection was subjected to physical stability testing at 5ºC±3ºC, at room temperature and at 40ºC/75% RH. Results of the physical stability study of formulation F10 showed that it remain unchanged in respect of pH and color. No turbidity or precipitate formation was observed at different storage conditions, showing appreciable physical stability (Table 5) [23, 26].

Table 5. Physical stability data of formulation F10

Condition	Physical stability parameter					
	pH		Color		Precipitation	
	Initial	After 30 days	Initial	After 30 days	Initial	After 30 days
Refrigeration (5ºC±3ºC)	7.08	7.09	Colorless	Colorless	No ppt.	No ppt.
Room Temperature	7.08	7.14	Colorless	Colorless	No ppt.	No ppt.
40ºC/75%RH	7.08	7.17	Colorless	Colorless	No ppt.	No ppt.

5.9 Chemical Stability Studies

The indomethacin content was also found to be within the pharmacopoeial limits in all the formulations. The data on chemical stability at different temperatures and time intervals are shown in Table 6. The degradation of indomethacin follows first order kinetics. The time required for the 10% degradation of drug for formulations was calculated. Results show that the prepared formulation F10 had a shelf life of 7-8 months at room temperature [25,27-30].

Table 6. Chemical Stability Data of Formulation F10

Formulation Code	Temperature	Concentration* mg/vials				Degradation
		0 Day	7 Day	15 Day	30 Day	
F10	5ºC±3ºC	49.82± 0.004	49.70± 0.011	49.52± 0.007	49.42± 0.009	0.803%
	25±2ºC	49.82± 0.004	49.64± 0.013	49.47± 0.017	49.25± 0.011	1.145%
	40ºC±2ºC /75% RH	49.82± 0.004	49.53± 0.009	49.29± 0.014	49.05± 0.014	1.54%

** Average of 3 determinations*

6. CONCLUSION

The present investigation showed the possibility of aqueous injection of poorly water soluble drugs using combination of various solubilizers and hydrotropic agents which acts

synergistically at very low individual concentrations. Hence, toxicity and safety related issues may not raise concern and would suggest their adaptability for large scale manufacturing.

Stable aqueous injection of indomethacin and other poorly water soluble drug could be successfully developed using the concept of mixed-solvency. These combinations eliminate the need for including any surfactant in the parenteral dosage formulation with the potential advantage of less associated toxicity. The amount of individual solubilizers required to increase the measurable solubility may be very high which sometimes shows the toxicity. Therefore, the use of mixed solvents (blends of enumerate solubilizers) which are physiologically compatible and often acts synergistically will improve the solubility and reduce the risk of toxicity.

The proposed technique is economical, convenient and safe. Thus, this study opens the chance of preparing aqueous formulations of poorly-water soluble drugs and the chemical stability of drug remains unaffected. Mixed solvency approach produce a physical stable formulation with low level of cosolvents to the patient, thus reducing or eliminating the effect of cosolvent toxicity and erythrocyte damage.

The present investigation focuses on the application of mixed-solvency concept to discourage the use of organic solvents in formulation and analysis to a great extent.

Thus, it can be concluded that with the carefully designed experimental technique, solubility of poorly water soluble drug can be improved using “mixed solvency” approach. The application of mixed solvency approach in the development of formulations shall prove a boon for pharmaceutical industries because the quantities of water soluble solubilizers present in the blends can be selected at safe level (well below their toxic levels) for a modest increase in solubility of a water-insoluble drug.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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