ORIGINAL ARTICLE

Development of Parenteral Formulation of Poorly Water Soluble Drugs: The Role of Novel Mixed-solvency Concept

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Abstract

Context: Water solubility is an important molecular property for successful drug development as it is a key factor governing drug access to biological membranes. The solubilization of poorly water soluble drug by facilitated mixed solvency is presented. **Aim:** The aim was to develop the injection formulations of the model poorly water-soluble drug. **Settings and Design:** Trial and error based experimental study. **Materials and Methods:** Mixed solvency approach was used to solubilize the hydrophobic drug. Mixed solvent system prepared using water-soluble hydrotropes (such as sodium citrate and urea) and water-miscible cosolvents, such as polyethylene glycol (PEG) 200, PEG 300, PEG 400, PEG 600, glycerin, propylene glycol, and ethanol. Drug was characterized using ultraviolet, Fourier Transform infrared (FT-IR), and Raman spectroscopy. Solubilizing power (Φ) and the Gibbs free energy of transfer (ΔG^0tr) were determined. Various properties of solution such as pH, viscosity, specific gravity, and refractive index were also studied. **Results:** Desired solubility of drug achieved in a mixed solvent blend AF5, which was more than 200 fold as compared to the solubility in distilled water 0.152 mg/ml. Drug content was found to be more than 98%. FT-IR and Raman spectroscopy results may support intermolecular hydrogen bonding between drug and mixed solvent system. Developed formulation was physically and chemically stable. **Conclusion:** This technique proved a synergistic enhancement in solubility of a poorly water soluble drug due to mixed solvent effect and produces a stable formulation. Mixed solvency concept may reduce the individual concentration of solubilizers and so reduce their toxicity associated with them.

Key words: Formulation, gibbs free energy, poorly water soluble drug, Raman spectroscopy, solubilization

INTRODUCTION

Drug candidates with strong pharmacological activity are increasingly being developed using combinatorial chemistry and high throughput screening.[1] It has been estimated that roughly 40% of all investigational compounds fail development because of poor bioavailability that is often associated with aqueous insolubility.[2] Water insolubility has always been a key obstacle in pharmaceutical formulation, affecting formulation stability, and drug bioavailability. Approaches for achieving complete dissolution of drug often have disadvantages associated with the large quantities of required excipients. There are several techniques to enhance the aqueous solubilities of poorly water-soluble drugs; Hydrotropy is one of such techniques. The term "hydrotropy" has 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.[3-7]

Organic solvents and other cosolvents in parenteral formulations can lead to problems of toxicity, reduced blood compatibility, and injection difficulty because of

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Received: 22-04-2015
Revised: 04-08-2016
Accepted: 18-10-2016

increased vehicle viscosity. The vehicle should contain a minimum amount and low concentration of the co solvent. To significantly enhance the solubility of semi-polar drugs high concentrations of non-aqueous cosolvents may be required.[8,9] Consequently, the concentration and/or the amount of co-solvent and excipients required in the product to maintain the total required dose may be in excess of the level acceptable for injection preparation.

A drug administered in solution is immediately available for absorption and in most cases is more rapidly and efficiently absorbed than the same amount of drug administered in suspensions. Maheshwari has demonstrated the synergistic solubilizing capability due to mixed hydrotropy approach. He is of the opinion that hydrotropy is another type of cosolvency. Instead of using one solubilizer in large concentration for a desired level of solubility, the concentrated solutions made by employment of several solubilizers in small concentrations may show additive or synergistic enhancement in solubility.[10-13]

Maheshwari proposed the concept of mixed solvency. He is of the opinion that all substances whether liquids, gases, or solids possess solubilizing power and hence concentrated aqueous solutions containing various dissolved substances can also improve the solubility of poorly water soluble drugs.[14-16] This mixed solvency concept may be utilized to prepare the concentrated (say 30-40% 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), cosolvents (glycerin, propylene glycol, ethanol, polyethylene glycol [PEG] 200, 300, 400, 600), water soluble solids (PEG 4000, 8000) employing them in small, safe concentrations to solubilize the poorly water-soluble drugs to develop their dosage forms (solutions, syrups, injections, topical solutions, etc.). Therefore, the authors have proposed a mixed-solvency approach for poorly water-soluble drugs.[17-20] In this research, aceclofenac was used as model drug (poorly water soluble drug), it is white crystalline powder, practically insoluble in water, freely soluble in acetone, soluble in ethanol (96%) [Figure 1].[21] The solubilization power of the cosolvents, two solid solubilizers, i.e., urea and sodium citrate system and mixed solvent system were evaluated. The solubilization effectiveness of a solvent is a function of the relative magnitudes of the various solute-solute, solute-solvent, and solvent-solvent interactions. For any given solvent system, the solubilization power gives a quantitative estimate of the solubilization potential of the cosolvent or mixed solvent. The solubilization power (Φ) of each cosolvents and mixed solvent system was determined using following log linear model.

$$\text{Log } S_{mix} = \log S + \Phi V$$

Where S_{mix} and S are the solubilities of drug in solvent mixture and pure solvent, respectively. V is the volume fraction of the co-solvent or mixed solvent and Φ is defined as the solubilization power of the cosolvent or mixed solvents.[22,23]

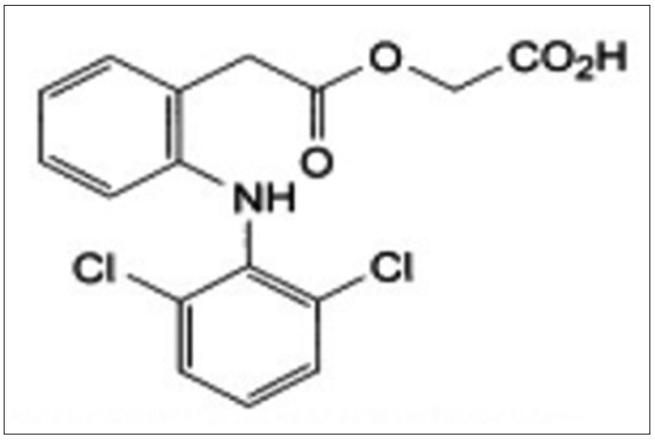

Figure 1: Structure of aceclofenac (as model drug)

The Gibbs free energy of transfer (ΔG^0tr) of drug from pure water to aqueous solutions of excipients was calculated using the following equation

$$(\Delta G^0tr) = -2.303RT \text{ Log } [S_c/S_0]$$

Where S_c is the molar solubility of drug in aqueous mixed solvent system and S_0 is the solubility of drug in pure water. The Gibbs free energy change is an indication of the process of drug transfer from pure water to aqueous mixed solvent. The solubilizing power and the Gibbs free energy of transfer (ΔG^0tr) of drug from pure water to the mixed solvent systems emphasize the solubilization effectiveness and thermodynamics involved during the solubility enhancement respectively.[23,24] Application of the mixed solvency has been employed in the present research work to develop the injection formulations of the model poorly water-soluble drug, aceclofenac. It may reduce the individual concentration of solubilizers and so reduce their toxicity associated with them. It may reduce the total concentration of solubilizers, necessary to produce modest increase in solubility by employing combination of agents in lower concentrations.

MATERIALS AND METHODS

Materials

Aceclofenac was obtained as gift sample from IPCA Laboratories, Ratlam, India. Propylene glycol was purchased from Loba chemie, Mumbai. Urea, Sodium citrate, Glycerin, PEG 200, PEG 300, PEG 400, and PEG 600 were obtained from Merck Chemicals Limited, Mumbai, India. Membrane filter (0.22 µm), (Sartorious, Germany), aluminum seal, glass vials, and rubber plugs (Modern Labs, Indore, India) were also employed in this study. All other chemicals and solvents used were of analytical/high performance liquid chromatography grade.

Estimation of aceclofenac

In the present investigation, ultraviolet (UV) spectrophotometric method was used for the estimation of aceclofenac. The calibration curve of aceclofenac was prepared in distilled water and various concentrations of water soluble solubilizers (hydrotropic agents and cosolvents) at 274 nm using double-beam UV spectrophotometer (UV-1800, Shimadzu, Japan).[5-7,10]

Solubility determination

The solubility of aceclofenac in various solubilizers solutions was determined by equilibrium solubility method. Sufficient excess amount of aceclofenac was added to 10 mL screw-capped glass vials containing 5 ml of aqueous solution of individual solubilizer (35% w/v concentration), buffer of pH 2.5-8 [Table 1 and Figures 2 and 3]. The vials were shaken mechanically for 12 h on mechanical shaker (Lab Hosp, Mumbai, India) at room temperature. The solutions were allowed to equilibrate for the next 24 h. The supernatants of each vial were filtered through Whatman filter paper grade 1 and filtrate after appropriate dilution, analyzed for drug content by UV visible spectrophotometer (Shimadzu-1800) at 274 nm. Solubility was determined in triplicate.[17-20]

Table 1: Solubility enhancement ratio of aceclofenac in different solubilizers at room temperature

Solvents	Solubility ratio	ΔG^0tr $JK^{-1}mol^{-1}$
25% w/v UR	9.80	−5659.29
35% w/v UR	15.66	−6820.39
25% w/v SC	4.42	−3685.12
35% w/v SC	5.61	−4276.43
25% w/v PG	2.73	−2490.16
35% w/v PG	4.47	−3714.46
25% w/v GLY	5.57	−4258.93
35% w/v GLY	7.69	−5059.88
25% w/v PEGTH	5.01	−3996.74
35% w/v PEGTH	7.23	−4906.92
25% w/v PEGTHH	8.15	−5203.94
35% w/v PEGTHH	9.40	−5557.39
25% w/v PEGFH	6.43	−4615.47
35% w/v PEGFH	8.61	−5340.09
25% w/v PEGSH	5.35	−4160.40
35% w/v PEGSH	8.35	−5263.21

UR: Urea, SC: Sodium citrate, PG: Propylene glycol, GLY: Glycerine, PEGTH-PEG 200, PEGTHH-PEG 300, PEGFH-PEG 400, PEGSH-PEG 600

Determination for additive/synergistic effect on solubility in mixed solvent blends

An equilibrium solubility method was used to determine the additive or synergistic effect on solubility. The total strength of all solubilizers was 35% w/v (constant) in all aqueous mixed solvent systems [Table 2 and Figure 4]. The solubility of aceclofenac was determined in these systems.[18-20]

Properties of mixed solvent solutions

Properties of prepared mixed solvent system (solution) such as pH, viscosity, specific gravity, and refractive index were studied [Table 3] using digital pH meter (pHCal, Analab Scintific Instruments Pvt. Ltd. Gujarat, India), Brookfield viscometer (DV-I PRIME, Brookfield Eng. Lab. Inc. USA) pycnometer, and digital refractometer (DG-NXT, Advance Res. Instruments Company, New Delhi, India), respectively.[18-20]

Drug excipients interference studies with UV spectroscopy

To interpret the probable mechanism of solubilization, UV spectral studies of aceclofenac were performed in different

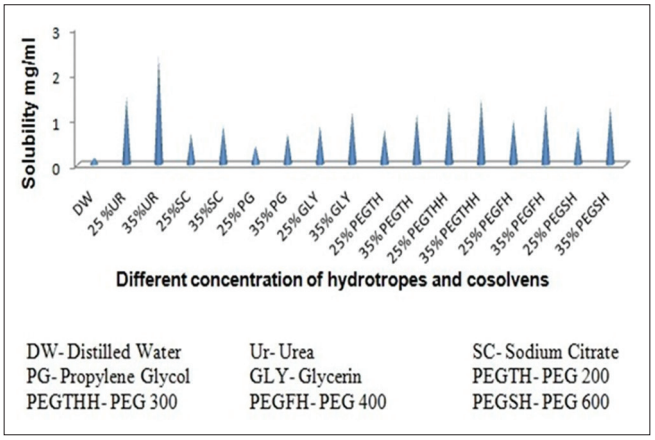

Figure 2: Solubility of drug in hydrotropes and cosolvents

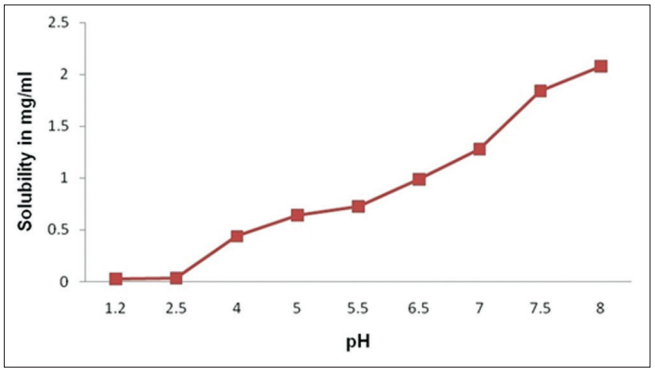

Figure 3: Influence of pH on solubility

Table 2: Mixed solvent for saturated solubility determination of aceclofenac

Blend code	Solvents	Solubility ratio	Solubilization power (Φ)	ΔG^0tr $JK^{-1}mol^{-1}$
AF1	15% SC+5% UR+15% S	147.56	6.19	−12383.37
AF2	10% UR+10% SC+15% S	151.77	6.23	−12453.13
AF3	5% SC+10 UR+20% S	228.35	6.73	−13466.01
AF4	10% SC+5% UR+20% S	137.69	6.11	−12211.75
AF5	8% UR+2% SC+25% S	209.27	6.63	−13249.67
AF6	10% SC+25% S	93.81	5.63	−11260.30
AF7	10% UR+25% S	110.19	5.83	−11659.35
AF8	2.5 SC+2.5 UR+30% S	73.61	5.33	−10659.17

UR-Urea, SC-Sodium citrate, S-Solvent system containing 3, 4 and 5% of each PEG 200, PEG 300, PEG 400, Glycerin and Propylene glycol for 15%, 20%, and 25% concentration, respectively

Table 3: Properties of the selected blends

Experiment blends	pH	Viscosity (cps)	Specific gravity	Refractive index
AF1	7.27	6.18	1.085	1.396
AF2	7.51	6.24	1.090	1.397
AF3	7.42	6.14	1.081	1.396
AF5	7.14	6.12	1.080	1.395

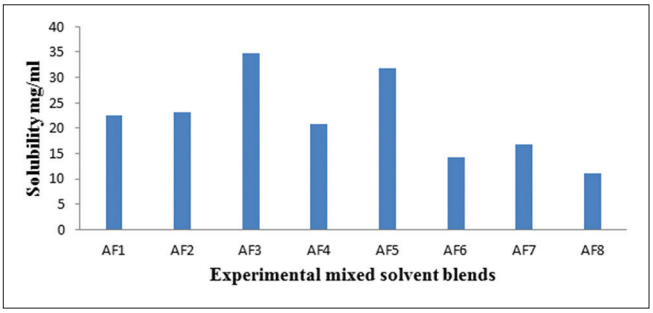

Figure 4: Saturated solubility of drug in mixed solvent system

mixed solvent solutions [Figure 5]. Drug was estimated spectrophotometrically in mixed solvent system and distilled water to observe any interference between drug and excipients by taking respective reagents blank at appropriate wavelength during study.[17-20]

Fourier transform infrared (FT-IR) spectral studies

FT-IR spectra were obtained by means of a FT-IR spectrophotometer (IR Prestige 21, Shimadzu, Japan). The samples were prepared by potassium bromide disc method and measurements were attempted over the range of 400–4000 cm^{-1} [Figure 6] and FT-IR spectrophotometer (IR Affinity-1, Shimadzu, Japan) with single reflection quest ATR. A dropping pipette was used to place the liquid sample over ATR crystal surface and measurements were recorded over the range of 400–4000 cm^{-1} [Figure 7]. Interaction between the components, if any, was indicated by either producing additional peaks or absence of the characteristic peaks corresponding to the drug and solubilizers.

Raman spectroscopic analysis

The Raman scattering technique is a vibrational molecular spectroscopy which derives from an inelastic light scattering process. With Raman spectroscopy, a laser photon is scattered by a sample molecule, and the amount of energy change (either lost or gained) by a photon is characteristic of the nature of each bond (vibration) present and enable a very precise characterization of the molecular structure. Hence, the amount of energy shift for a C-H bond is different to that seen with a C-O bond.

The Raman spectra were recorded by micro-Raman system (Jobin Yvon Horiba LABRAM-HR visible) using Argon as excitation laser source at wavelength 488 nm. Using confocal optics a lateral resolution of 1 μ and an axial resolution of 2 μ can be achieved. An Olympus BX41 microscope (Olympus, Japan) was used with a ×50 magnification lens to focus the sample. 600 lines/mm gratings were used for dispersive geometry; the charge-coupled device (CCD) camera was used as the detector with the spectral resolution of $1cm^{-1}$. Laser power of the source was maintained at 2.5-5 mW throughout all measurement with an accumulation time of 5-10 s. The spectra were collected over the wave number range from 3800-400 cm^{-1}.[25-29] A spectral region between 500-1800 cm^{-1} and 2500-3500 cm^{-1} was selected for raman analysis of aceclofenac in crystalline and solution forms as shown in Figures 8 and 9.

Formulation of aqueous injection

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. The bench was cleaned with 70% v/v alcohol as well as sprayed in the atmosphere. The aseptic room was fumigated using a mixture of 40% formaldehyde and potassium permanganate and the UV lights were switched on for 30 min before formulation of injections and the filling of injections into vials.[17-20]

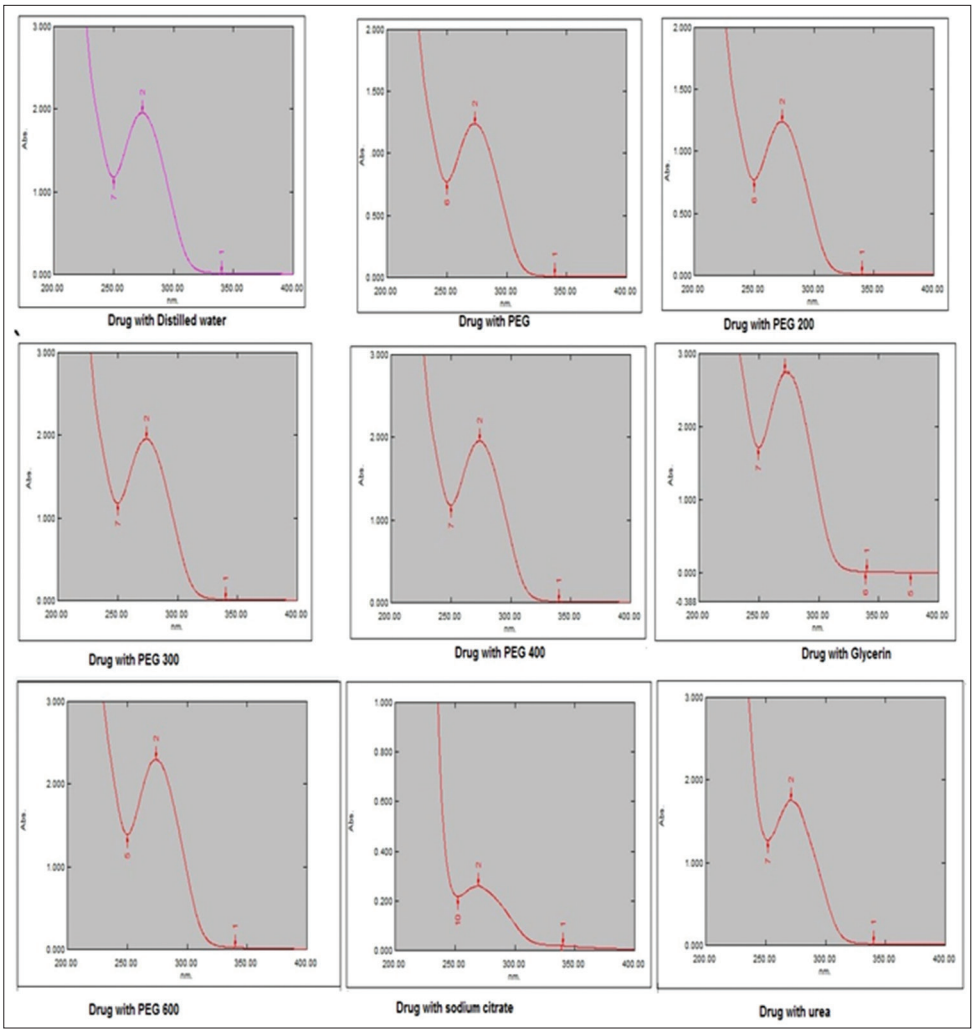

Figure 5: Ultraviolet spectra of drug with different cosolvents

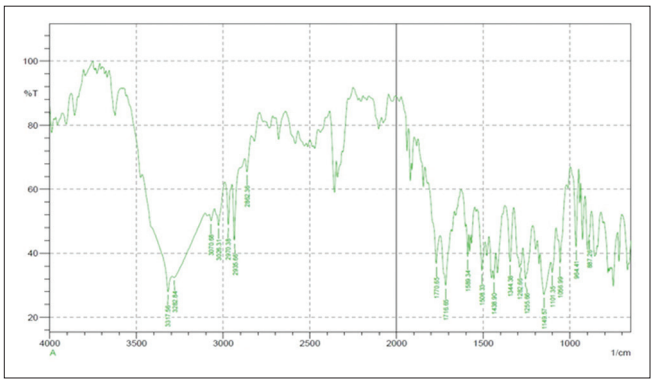

Figure 6: Fourier transform infrared of pure drug

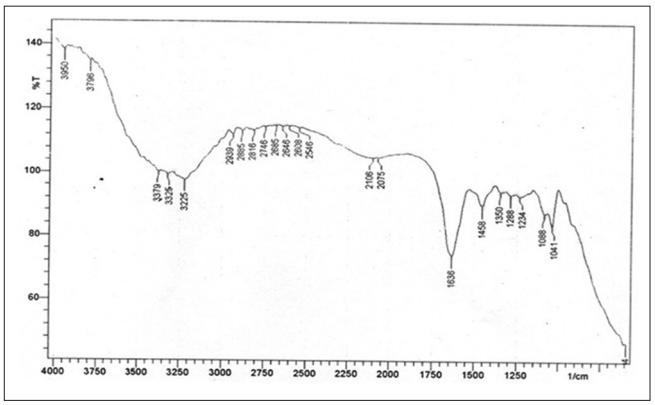

Figure 7: Fourier transform infrared of drug in mixed solvent system

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°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 water to remove any detergent residue and finally rinsed with distilled water. These stoppers were sterilized by autoclaving at 15 lbs pressure (121°C temperature) for 15 min. Finally, the stoppers were rinsed with freshly prepared sterile distilled water and dried in vacuum oven under aseptic condition.[18-20]

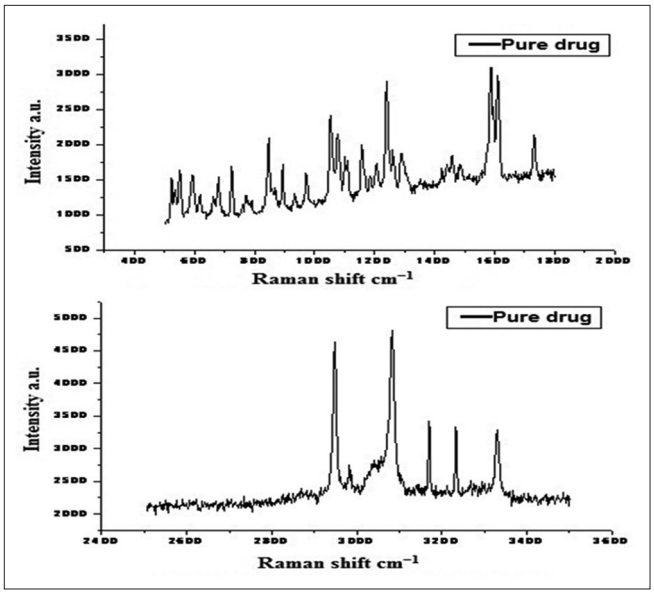

Figure 8: Raman spectra of pure drug

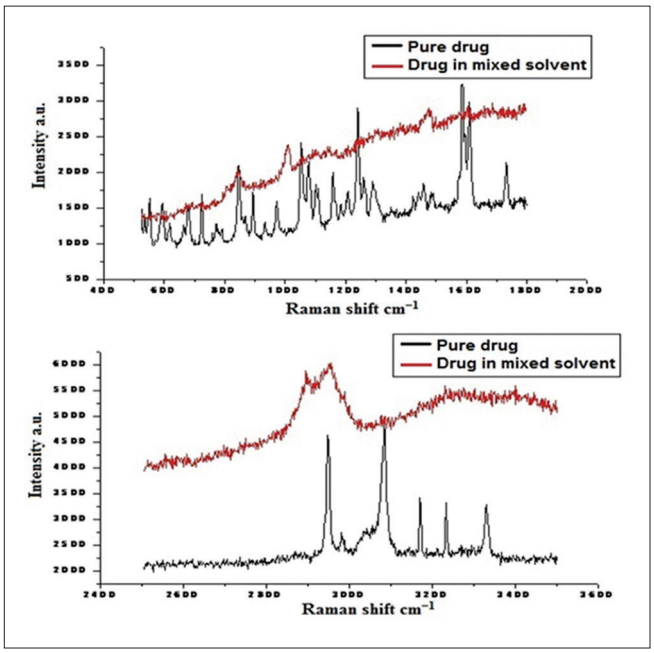

Figure 9: Comparative Raman spectra of solid crystalline drug (black) and dissolved drug (red)

Preparation of aqueous injection

On the basis of solubility data obtained from the final blends of mixed solvent, formulation of aqueous injection of aceclofenac was prepared using mixed solvent system "AF5". This formulation contained 20 mg/ml of drug in mixed solvent system AF5. 0.1% w/v sodium bisulfite was added as an antioxidant. For the preparation of aqueous injection of aceclofenac, 25 ml of distilled water was placed in 50 ml volumetric flask in the aseptic area and weighed amount of hydrotropes, cosolvents, drug and 0.1% w/v sodium bisulfite were added one by one to the flask and shaken for 1-2 h to ensuring the complete dissolution of the former; then the volume was made up to 50 ml with distilled water. Other additives such as 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. These solutions were filtered through 0.45 µm membrane filter (Pall Corporation, USA). The solutions were analyzed spectrophotometrically at 274 nm for drug content after appropriate dilutions with distilled water using the same vehicle as blank after appropriate dilution.[17-20]

Aseptic filtration and packaging

The aqueous injection of aceclofenac was sterilized by filtration through 0.22 µm 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.[17-20]

Characterization

Properties of formulation developed using mixed solvent system

Various properties of the formulation such as pH, viscosity, specific gravity, and refractive index were studied using digital pH meter, Brookfield viscometer, pycnometer, and digital refractometer, respectively [Table 4].

Physical stability studies

The sealed or packed vials of the aqueous injections were visually inspected every day for 30 days against black and white backgrounds to see the changes occurring, if any, in physical appearance of aqueous injection like color, turbidity, pH, etc., [Table 5] on storage at 5°C ± 3°C in a refrigerator, room temperature and 40°°C/75% RH in thermostatically controlled stability chamber (BTI-24A stability chamber, Bio Technics, India).

Chemical stability studies

The injection formulations were subjected to exhaustive chemical stability at 5°C ± 3°C in a refrigerator, room temperature and 40°C ± 2°C/75% RH in thermostatically controlled stability chamber (BTI-24A stability chamber, Bio Technics, India) for a period of 30 days [Table 6]. 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%.[20,30]

RESULTS AND DISCUSSION

According to Biopharmaceutics Classification System aceclofenac is a practically insoluble drug with an aqueous

solubility of <1 mg/ml.[21] The choice of organic solvents used in pharmaceutical parenteral formulations has already been described, when aqueous solutions cannot be considered owing to drug solubility limitations. At the same time, low aqueous solubility also affects quantities of solvents necessary for dissolution. Several solvent systems were tested to achieve complete dissolution of the drug, as summarized in Tables 1 and 2. Poorly water soluble drugs can be solubilized by hydrotropic agents.[3-5] Thus, the hydrotrops and solvents were combined in several ratios in order to improve the aceclofenac solubility. As already described, the aqueous solubility of aceclofenac is 0.152 mg/ml, but when the drug was assayed in different pharmaceutically acceptable hydrotropic agent and cosolvents systems the aceclofenac solubility increased up to 2 mg/ml [Table 1 and Figure 2] and it is probably a result of intermolecular hydrogen bonding between drug and the solvent or cosolvent. The maximum solubility was observed in 35% urea with enhancement ratio of 15.66 and in 35% PEG 300 with enhancement ratio of 9.40. Despite these hopeful results, the solubility of the drug was not sufficient to allow a further development of a parenteral formulation. Therefore, authors decided to explore the most common solvents for injections. It is well-known that PEG of different molecular weights is one of the most useful solubility enhancers, and PEG 200 to PEG 600 are the solvents of choice in liquid formulations due to their good solubilization properties and overall acceptability in terms of side-effect profile. All solubilizers selected for the present study possess a hydrophobic center thus these sites are available for non-bonded and van der Waals interaction with water and drug. The molecules of water join to form cluster together. Solubility of hydrotropic solution could be ranked in decreasing order as urea > sodium citrate and the solubility enhancement ratio 15.66>5.61 respectively. Solubility enhancement for cosolvents could be ranked as PEG 300> PEG400> PEG 600> Glycerine > PEG 200> propylene glycol. The use of hydrotrope combinations yields higher aceclofenac solubility than that of the single hydrotrope. The value of $\Delta G^0<0$ reflects thermodynamically stable product. The ΔG^0tr value provides information about whether the treatment is positive or unfavorable for drug solubilization in an aqueous medium. Negative Gibbs-free energy values indicate improved solubilization, i.e. transfer of drug from pure water to aqueous solution of solubilizers (Mixed solvents).[23,24] The value of Gibbs free energy for cosolvent and solid colvents was observed in range of – 2490.16 to – 6820.39 kJ mol^{-1}, respectively.

Saturated solutions of drug were studied for pH dependent solubility at room temperature. Results showed that aceclofenac was more soluble at alkaline pH than acidic pH. Enhancement in solubility was found 13 folds at pH 8 [Figure 3]. 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. Percentage ionized and hence increases solubility due to increase in pH value.

Mixes solvent system was prepare to determine solubility and about 70 to 200 fold increase in solubility was seen which is may be due to the additive/synergistic effect of the mixed solvents. Combinations of hydrotropic agents with 25% of cosolvent "S" increase solubility by 200 folds, which is well beyond desired concentration shown in Table 2 and Figure 4. Table 2 represented the value of ΔG^0tr for mixed solvent system. For various aqueous formulation blends of mixed solvents (AF1 to AF8) the value of ΔG^0tr are extremely negatives and the value are observe to decrease as increase in solubilizers concentration, demonstrating spontaneous nature of drug solubilization.[23,24]

Table 4: Properties of the developed formulation

Formulation	pH	Viscosity (cps)	Specific gravity	Refractive index
AF5	6.8	6.12	1.081	1.396

Table 5: Physical stability data of formulation AF5

Condition	Physical stability parameter					
	pH		Color		Precipitation	
	Initial	After 30 days	Initial	After 30 days	Initial	After 30 days
5±3°C	6.8	6.8	Colorless	Colorless	No ppt.	No ppt.
Room temperature	6.8	6.7	Colorless	Colorless	No ppt.	No ppt.
40°C/75% RH	6.8	7.1	Colorless	Colorless	No ppt.	No ppt.

Table 6: Chemical stability data of formulation AF5

Formulation code	Temperature	% Residual drug			
		0 Day	7 Day	15 Day	30 Day
AF5	5±3°C	100	99.70	99.52	98.95
	25±2°C	100	99.74	98.41	98.22
	40±2°C/75% RH	100	99.13	98.29	97.85

The structures of drugs and hydrotropes with different centers of different electro negativity might responsible for the intermolecular hydrogen bonding and electrostatic attraction. Hydrogen bonding between the amide group of urea and various negative centers of drug molecule seems to impart aqueous solubility to aceclofenac. Electronegative corboxylate ion and hydroxyl group of sodium citrate impart solubility. By disrupting water's self-association, cosolvents reduce water's ability to squeeze out non-polar, hydrophobic compounds, thus increasing solubility. The developed mixed solvent system produced a clear solution of drug at near neutral or slight alkaline pH (pH 6.9 to pH 7.5), without observing any precipitation phenomenon. The results show that the incorporation of hydrotropes in combination into cosolvent solution yields substantially greater drug solubility than that of hydrotrops as well as cosolvent solution alone. When we increase the concentration of solubilizers there is increase in solubility of aceclofenac but this increased level of solubilizer can have toxic effect. Hence instead of using single solubilizers in large concentration for development of dosage form, a combination of solubilizers in comparatively small concentration increase solubility and the associated toxic effect can be reduced.[14,15,18,19]

The various solution properties of mixed solvent AF1, AF2, AF3, and AF5 were studied because these formulation blends showed solubilization power up to 6.19 – 6.63; that other blends were not selected due to less solubilization power. pH of the formulations is near about neutral and less alkaline which could be less irritant to site of application. The viscosity of all the solutions were increased and deviate with the slight changes or increasing in the concentration while the specific gravity of solutions were again slight deviated because of change in concentration of solubilizers in solutions, the experimental values of refractive index showed that developed formulation blend is homogeneous, single phase clear colorless solution, results represented in Table 3.[17,18]

Most of 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. In case of aceclofenac-mixed solvent system, the values of λmax remain almost same. It can be assume that the minor shift in λmax (274 ± 1 nm), may be because of electronic changes in the structure of drug molecules. UV spectra indicates that there is no interference of λ_{max} of solubilizer/cosolvents with the λ_{max} of drug [Figure 5].[17-19]

The IR spectral analysis of aceclofenac alone showed that the principle peaks were observed at wave numbers of 3317.56 cm^{-1} (N-H stretching vibration for amine), 3282.84 cm^{-1} (O-H stretching), 3026 and 3070 cm^{-1} (aromatic C-H stretching), 1770.64 (C=O stretching of -COOH), 1716.65 cm^{-1} (C=O stretching of COO^{-}) and band observe at 1589 and 1438 cm^{-1} due to N-H and O-H bending, respectively, confirming the purity of the drug as per the established standards. In the IR spectra of the aceclofenac in mixed solvent, the major peaks of aceclofenac were 3379, 3325, 3225 and 1636 cm-^{1} wave numbers. These significant changes indicate the possibility of intermolecular hydrogen bonding between the –NH group of Aceclofenac and –OH group of sodium citrate and PEG200, 400 and 600. A decrease in the intensity of several peak of O-H (s), C-H (s) and –NH group of aceclofenac (at 3317.56 cm^{-1}) may support intermolecular hydrogen bonding between drug and mixed solvent system, thus it can be concluded with some reservation, the absence of any significant change in the IR spectral pattern in the formulations containing the drug and excipients indicated the absence of interaction between the drug and mixed solvent excipients employed for the solubility enhancement [Figures 6 and 7].

Raman vibrational spectroscopy technique can provide a sensitive, relatively quick, non-destructive means of probing molecular structure in solid and liquid.[25,26] From the Figure 8, the dominant bands of the Raman spectrum of crystalline drug appear at 1729 cm^{-1} due to monomeric carboxylic acid. The peak at 1610 cm^{-1} and 1585 cm^{-1} are given by phenyl ring stretching (C-C and C-N) vibrations. The ring breathing vibrations determine also intense bands at 1078 cm^{-1} and 1052 cm^{-1}. The in-plane deformation vibrations of the CH groups of aromatic rings give rise to Raman bands (bending) at 1160 cm^{-1}, 1288 cm^{-1}, 1260 cm^{-1} and 1240 cm^{-1}. The medium intense Raman bands at 524 cm^{-1} and 534 cm^{-1} are determined by the out-of-plane deformation vibrations of the aromatic rings. The vibrations of the CH groups occur in the 840–950 cm^{-1} spectral range of the Raman spectrum. NH stretching vibration generally occurs in the region 3300–3500 cm^{-1}. The peak observed at 3330 cm^{-1} in Raman spectrum is assigned to N-H stretching vibration. The band observed at 3234 cm^{-1} is due to O-H stretching vibrational mode. The band of C-H stretching mode falls in the region of 2946–3170 cm^{-1} for aromatic C-H stretching vibration.

Raman spectra corresponding to the black and red regions of the sample [Figure 9] show characteristic shifts and band shapes due to drug solubilization by mixed solvents. The characteristic prominent Raman bands for liquid formulation were observed at 1731 cm^{-1}, 1610 cm^{-1}, 1586 cm^{-1}. The Raman shift at 840-950 cm^{-1} assign to C-H groups vibrations. The peak observed at 3330 cm^{-1} and at 3234 cm^{-1}, slight shifted toward the lower wave number at 3327 cm^{-1} and 3229 cm^{-1} for N-H stretching vibration and O-H stretching vibration mode, respectively. The significant differences seen between the liquid and solid states was due to the freedom of movement of the O-H, C-O and N-H groups in liquid state, which were not prepared in a lattice arrangement of solid.[27] For understanding the spectra have been divided into the fingerprint regions (500-1800 cm^{-1}) and CH/OH regions (2500-3500 cm^{-1}). Assessment of the spectra shows clear differences between the pure drug and developed

formulation. Spectra in the accompanying [Figure 9] look fairly similar, there are some observable differences. The peak intensity of the crystalline drug is higher as compared to solubilized drug. Solubilized (HYDRATION of drug) form displayed small and broad peak in the phonon region due to the inherent disorder in the sample, while crystalline forms showed distinct, multiple intense peaks arising from a regular periodic arrangement of molecules in crystals and indicative of very ordered and very crystalline in structure.[27] A slight shift in a spectrum occurs when the crystalline form of drug is compared with the solution of drug. Less intense and broadening of peak may be due to electrostatic interaction. Therefore, when the system undergoes a transformation, a clear change in the peak intensity is expected, such changes are clearly observed as wide Gaussian type band and vanishing of crystalline phase, indicating progressive transformation from crystalline to amorphous phase.[28,29] However, in aqueous medium intermolecular changes are relatively higher representing larger spectral differences indicative of hydrogen bonding or electrostatic interaction which restrict the vibrations and leads to decrease in peak intensities.[31]

Blend AF5 was selected and used to develop aqueous parenteral formulation due to its superior and synergistic effect on solubility of aceclofenac with minimum concentration of solubilizers. The various solution properties of formulation were studied; results reveals that pH of the formulations is near neutral and very less alkaline which could be less irritant to site of application. There were no deviations in other parameters like viscosity and specific gravity. One of the most important optical parameters of aqueous formulation is refractive index which represented that developed formulation was one phase homogeneous system [Table 4].

Formulated aqueous injection was subjected to physical stability testing at 5°C ± 3°C, at room temperature and at 40°C ± 2°C. Results of the physical stability study of formulation showed that it remain unchanged in respect of pH, color stability and no turbidity or precipitate formation was observed at different storage conditions. Prepared formulation has shown appreciable physical stability [Table 5]. The data on chemical stability at different temperatures and time intervals are shown in Table 6 which showed that the degradation of aceclofenac follows first order kinetics. The % residual drug content was found to be more than 98% during 1 month studies. Stability studies indicate that developed formulation is quite stable at room temperature.

CONCLUSION

Aqueous insolubility is clearly recognized throughout the pharmaceutical industry as a major problem. Approaches to address this deficiency have been developed, but these are limited by toxicity of the solvents. Aqueous mixed solvent system may offer an alternative that advantageously overcome insolubility problem associated with the hydrophobic drugs and enables drug delivery, potentially via numerous administration routes, including oral and parenteral. Due to drug's very poor aqueous solubility most of the formulations were prepared in a non-aqueous vehicle or organic solvent like dimethyl sulfoxide, or surfactant like Polysorbate 80 including higher concentration of these solvents.

In conclusion, the results of present investigations 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 adoptability for large scale manufacturing. The amount of individual solubilizers required to increase the measurable solubility shall be very high which sometimes shows the toxicity. Therefore, the use of mixed solvents (blends of enumerate solubilizers) which are physiologically compatible, often acts synergistically to improve the solubility and reduces the risk of toxicity. Mixed solvency eliminates the need for including any surfactant in the parenteral dosage formulation with the potential advantage of less toxic reactions. The proposed techniques would be economical, convenient and safe. Thus, this study opens the chance of preparing aqueous formulations of poorly-water soluble drugs. Mixed solvency approach produce a physical stable formulation which result in the administration of low level of cosolvents to the patient, thus reducing or eliminating the effect of cosolvent toxicity and tissue damage. Physical and chemical stability studies showed that the dosage forms could be kept at room temperature for long-term storage. Regarding all the mixed solvent systems proposed, it was worth noting that aceclofenac in mixed solvent system AF5 showed the desirable solubility with minimum concentration of solubilizers.

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. 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.

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Source of Support: Nil. **Conflict of Interest:** None declared.