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Solubility of a poorly soluble immunosuppressant in different pure solvents: Measurement, correlation, thermodynamics and molecular interactions

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ABSTRACT

The objective of this research work was to measure the solubility of mycophenolate mofetil (MPM) in ten different pure solvents at temperatures $T = 298.2$ K to 318.2 K and pressure $p = 0.1$ MPa. The solubilities of MPM in mole fraction were obtained maximum in ethyl acetate (9.28×10^{-2}) and minimum in water (4.16×10^{-6}) at "$T = 318.2$ K" and similar trends were also observed at each temperature investigated. Higher solute-solvents molecular interactions were observed in MPM-ethyl acetate and MPM-Transcutol. Apparent thermodynamic analysis indicated an endothermic and entropy-driven dissolution of MPM in each solvent investigated.

1. Introduction

Mycophenolate mofetil (MPM) (Fig. 1; IUPAC name: 2-(morpholin-4-yl)ethyl (4E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoate; molecular formula: $C_{23}H_{31}NO_7$; molar mass: 433.49 g mol^{-1} and CAS registry number: 128794-94-5) occurs as a white to beige colored powder [1–3]. It is 2-morpholinoethyl ester of mycophenolic acid (MPA), a transplant, antirheumatic, a major immunosuppressive agent and inosine monophosphate dehydrogenase inhibitor [1]. MPM undergoes rapid hydrolysis to give MPA which actually exerts its effects *in vivo* [4]. It is an antimetabolite and potent immunosuppressive agent used as adjunctive therapy in prevention of allograft rejection and in the treatment of serious autoimmune disorders [2,4]. It has been recommended for the treatment of an autoimmune disease (lupus) which is characterized by systemic inflammation and organ damage, where the efficacy of MPM was accomplished partly by attenuating the inflammatory and stimulatory capacity of dendritic cells [5,6]. According to USFDA database, MPM has been reported as practically insoluble in water [7]. The pK_a values for MPM have been reported as 5.6 for the morpholino group and 8.5 for the phenolic group [1,7]. However, the apparent partition coefficient of MPM in 1-octanol/water system has been reported as 238 [7]. It is an innovator product of Roche Diagnostics GmbH which is marketed under the trade name of CellCept® for the treatment of autoimmune disorders [7]. It is commercially available in the form of tablets, capsules and oral suspensions. However, its hydrochloride salt is commercially available as intravenous injection. Due to its poor aqueous solubility, the development of liquid dosage forms of MPM is very difficult. The solubility data of poorly soluble drugs in neat/pure "aqueous and organic solvents" are important in various industrial process such as "purification, recrystallization, preformulation studies and formulation development" of such drugs [8–13]. Hence, the solubility of MPM must be determined properly in these solvents. Apelblat and van't Hoff models are commonly used computational models for the correlation/curve fitting of experimental solubility data of solutes in pure solvents with calculated solubility data [13–16]. Therefore, these models were applied for the correlation of experimental solubility data of MPM with calculated ones. Some formulations approaches such as tablets, enteric coated tablets, capsules, suspensions, nanoparticles, nanosuspensions and nanogels have been investigated for the evaluation of drug delivery potential, dissolution and bioavailability of MPM [5,6,17–25]. The solubility (as mole fraction) of MPM in water at ambient temperature ($T = 298.2$ K) has been reported as 1.79×10^{-6} [7]. However, the solubility data of MPM in any organic solvent have not been reported so far in literature. Therefore, the objective of this research work was to measure the solubility of

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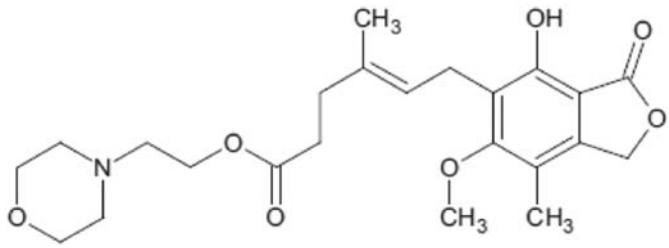

Fig. 1. Molecular structure of MPM (molar mass: 433.49 g mol^{-1}).

MPM in ten different pure solvents including water, ethanol, Transcutol, polyethylene glycol-400 (PEG-400), propylene glycol (PG), ethylene glycol (EG), isopropanol (IPA), *n*-butanol, ethyl acetate (EA) and dimethyl sulfoxide (DMSO) were measured at temperatures T = 298.2 K to 318.2 K and pressure p = 0.1 MPa. These solvents have been categorized as the eco-friendly solvents which are being utilized for various industrial applications such as purification, pre-formulation studies and dosage form design of pharmaceuticals/drugs in pharmaceutical industries [26,27]. Therefore, the investigated solvents were selected in this work. Apparent thermodynamic analysis on measured solubility data of MPM was also performed for the evaluation of dissolution behavior of MPM. The activity coefficients were calculated in order to evaluate the molecular interactions between solute and solvent molecules. The solubility data of MPM generated in this work would be useful in various industrial processes such as "purification, recrystallization, drug discovery and formulation development" of MPM.

2. Experimental

2.1. Materials

(*RS*)-MPM, Transcutol® [IUPAC name: 2-(2-ethoxyethoxy) ethanol] and ethyl alcohol (IUPAC name: ethanol) were obtained from "Roche Diagnostics GmbH (Mannheim, Germany)", "Gattefosse (Lyon, France)" and "Scharlab SL (Barcelona, Spain)", respectively. IPA (IUPAC name: isopropanol) and *n*-butyl alcohol (IUPAC name: *n*-butanol) were obtained from "Sigma Aldrich (St. Louis, MO)". EG (IUPAC name: 1,2-ethanediol), PG (IUPAC name: 1,2-propanediol), PEG-400 (IUPAC name: polyethylene glycol-400), EA (IUPAC name: ethyl ethanoate) and DMSO (IUPAC name: dimethyl sulfoxide) were obtained from "E-Merck (Darmstadt, Germany)". Water (specific conductivity was <1.0 μS cm^{-1}) was collected from "Milli-Q water purification unit". The information about these materials is presented in Supplementary Table 1 (Table S1).

2.2. Analysis of MPM

"Waters Acquity H-class Ultra-Performance Liquid Chromatography (UPLC)" system coupled with a Waters diode-array-ultra-violet detector (DAD-UV) by Acquity "UPLC (Waters, MA)" was used for the analysis of MPM in solubility samples. The chromatographic system includes quaternary solvent manager, sample manager (Acquity, UPLC Waters) with injection capacity of 10 μL and a column heater. The elution of MPM was performed on "Acquity UPLC BEH™ C_{18} column (2.1 × 50 mm, 1.7 μm, Waters, USA)" maintained at T = 323.2 K. The reported liquid chromatographic method was used for the analysis of MPM contents with slight modifications [21]. In reported method, HPLC-UV technique was used but in the present work, UPLC-UV method has been used. The mobile phase was consisted of 82% methanol and 18% 0.02 M phosphate buffer (containing 0.1% triethylamine and pH of the buffer was adjusted to 4.0 by using 85% orthophosphoric acid) which was pumped at an isocratic flow rate of 0.17 mL min^{-1}. The injection volume was 5 μL and column oven temperature was maintained at T = 323.2 ± 2 K. MPM was detected by UV-detector at 214 nm. The retention time of MPM was 0.922 min. The "EMPOWER software" was used to control the UPLC-UV system as well as for data acquisition and processing.

The standard solution of MPM was prepared in the concentration of 500 μg g^{-1}. From this standard solution, the serial dilutions were made on mass/mass basis in order to obtain the concentration in the range of (0.5 to 400) μg g^{-1}. The calibration curve was plotted between the concentration of MPM (μg g^{-1}) and peak area obtained from UPLC analysis. The calibration curve of MPM was observed linear in the concentration range of (0.5 to 400) μg g^{-1} with coefficient of determination (R^2) of 0.9965. The regressed equation for calibration data was obtained as peak UPLC area = 158,746 ∗ concentration + 964,750. The proposed UPLC method for the analysis of MPM was validated well in terms of "linearity, precision, accuracy, sensitivity, selectivity and robustness".

2.3. Solid state characterization of MPM

The solid state characterization of MPM was performed using "Differential Scanning Calorimetry (DSC)". DSC analysis was performed for the investigation of different thermal parameters and the possibility of polymorphic transformations of MPM. This analysis was performed both on pure MPM (initial material) and equilibrated MPM. The equilibrated MPM was recovered from equilibrium sample (water) by slow evaporation of water [28]. DSC analysis on pure and equilibrated MPM was performed using "DSC-8000 Instrument (Perkin Elmer, USA)". DSC instrument was equipped with chiller (T = 253.2 K) and autosampler. The calibration of instrument was carried out using pure indium at T = 283.2 K to 773.2 K. For DSC analysis, a mass of accurately weighed 5.0 mg of pure and equilibrated MPM was taken to an aluminium pan and sealed hermetically. DSC thermogram of pure and equilibrated MPM was recorded under a nitrogen purge of 20 mL min^{-1} at a heating rate of 10.0 K min^{-1} with the temperature range from 303.2 K to 423.2 K.

2.4. Measurement of MPM solubility

The solubility of pure MPM in ten different pure solvents including water, ethanol, IPA, EG, PG, *n*-butanol, EA, DMSO, PEG-400 and Transcutol was measured using an isothermal method at "T = 298.2 K to 318.2 K" and p = 0.1 MPa [29]. The excess quantity of pure MPM was added in known quantities of each pure solvent in triplicates manner. Each solute-solvent mixture was vortexed and transferred to the "Biological Shaker (Julabo, PA)" at 100 rpm for 72 h. After 72 h, each solute-solvent mixture was taken out from the shaker and allowed to settle MPM solid particles for 24 h [13]. After 24 h settling of MPM particles, the supernatants were carefully taken, diluted suitably with mobile phase and subjected for the analysis of MPM content by the proposed UPLC method at 214 nm. The concentration of MPM (μg g^{-1}) in solubility samples was determined from calibration curve of MPM. Then, the experimental mole fraction solubilities of MPM (x_e) were calculated using Eq. (1) [12,28]:

$$x_e = \frac{m_1/M_1}{m_1/M_1 + m_2/M_2} \tag{1}$$

Here, the symbols m_1 and m_2 are the masses of pure MPM and respective pure solvent (g), respectively. The symbols M_1 and M_2 are the molar masses of MPM and respective pure solvent (g mol^{-1}), respectively.

3. Results and discussion

3.1. Solid state characterization of MPM

DSC analysis on pure and equilibrated MPM was performed in order to evaluate different thermal parameters and the possibility of