

Synthesis and Characterization of Pharmaceutical salt of Prulifloxacin

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ABSTRACT: The selection of an appropriate salt form for a potential drug candidate is an opportunity to modulate its characteristics to improve bioavailability, stability, manufacturability, and patient compliance. In the present study prulifloxacin was subjected to pharmaceutical acceptable acid fumaric acid. The fumarate salt was characterized by powder X-ray diffraction (PXRD), infrared (IR), differential scanning calorimetry (DSC) and nuclear magnetic resonance spectroscopy (¹H NMR) and subsequently evaluated aqueous solubility and hygroscopicity (DVS). The prulifloxacin fumarate salt shows better thermal characteristics and excellent solubility.

KEYWORDS: Carboxylic acid, fluoroquinolone, antibiotic, solubility.

I. INTRODUCTION

An estimated 50% of all drug molecules used in medicinal therapy are administered as salts. This fact indicates that the salt formation, of a drug substance is a critical step in drug development [1,2]. A drug substance often has certain suboptimal physicochemical or biopharmaceutical properties that can be overcome by pairing a basic or acidic drug molecule with a counter ion to create a salt version of the drug [3]. The process is a simple way to modify the properties of a drug with ionizable functional groups to overcome undesirable features of the parent drug [4]. This fact underlines the importance of salt formation for drugs that are designed, developed, and marketed after a rigorous research and development program.

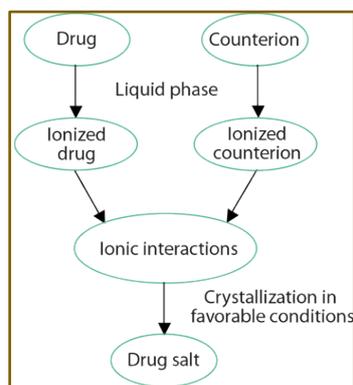
Salts are formed when a compound that is ionized in solution forms a strong ionic interaction with an oppositely charged counterion, leading to crystallization of the salt form [5]. In the aqueous or organic phase, the drug and counterion are ionized according to the dielectric constant of the liquid medium. The charged groups in the drug's structure and the counterion are attracted by an intermolecular coulombic force. During favorable conditions, this force crystallizes the salt form. All acidic and basic compounds can participate in salt formation. However, the success and stability of salt formation depends upon the relative strength of the acid or base or the acidity or basicity constants of the species involved [6].

The salt form is separated into individual entities (i.e., the ionized drug and the counterion) in liquid medium, and its solubility depends upon the solvation energy in the solvent. The solvent must overcome the crystal lattice energy of the solid salt and create space for the solute. Thus, the solubility of a salt depends on its polarity, lipophilicity, ionization potential, and size. A salt's solubility also depends on the properties of solvent and solid such as the crystal packing and presence of solvates [7].

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Scheme 1. Mechanism for salt formation of drugs with salt formers

Prulifloxacin is an oral fluoroquinolone agent with a broad spectrum of activity against Gram-positive and gram-negative bacteria. It is an antibacterial agent used in the treatment of bacterial infections of lower respiratory tract and skin tissues. The functional targets of Prulifloxacin are DNA-gyrase (a type II topoisomerase) and type IV topoisomerase. It acts by promoting cleavage of bacterial DNA. Four drug molecules bind to DNA through hydrogen bonds between the carbonyl group on the quinolone moiety and DNA bases, the two adjacent quinolone rings interact via hydrophobic interactions between the drug molecules. According to Biopharmaceutics Classification System (BCS) (Amidon, 1995) Prulifloxacin is a class II drug i.e. those having low solubility and low permeability. Currently Prulifloxacin is marketed as a 600 mg oral dosage form under the brand name percin. In order to change the physiochemical properties especially to enhance its solubility thereby bioavailability we chose to make pharmaceutical acceptable salt of Prulifloxacin. Good solubility is an important property of an API, which is related to the issues such as bioavailability, biopharmaceutical classification, bio waivers and bioequivalence.

Prulifloxacin was first reported in the US 5086049 patent [8] where it was synthesized from Ulifloxacin. Method of preparation, morphology and physiochemical properties of prulifloxacin salts were not discussed anywhere in the literature. In order to obtain suitable solid form (salt) we have subjected Prulifloxacin with fumaric acid. Fumaric acid was chosen for study as it is used as an acidity regulator and is approved for use as a food additive in the EU, USA [9] and Australia and New Zealand (where it is listed by its INS number 297).

II. MATERIALS AND METHODS

Chemical reagents

Prulifloxacin used was made in-house (>99% purity), fumaric acid was purchased from qualigens and all solvents used were commercial grade.

Characterization methods

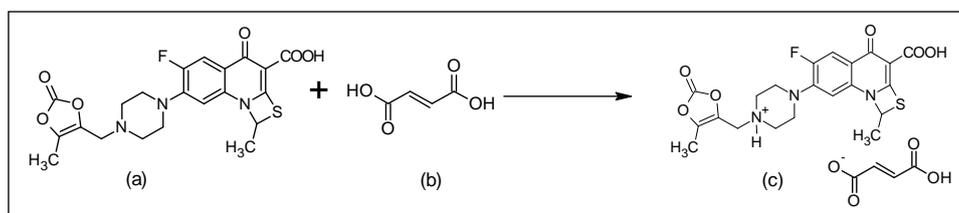
The powder X-Ray diffraction (PXRD) pattern was measured on pan analytical X-ray diffractometer. The samples were scanned from 0 to 40° (2θ). The infrared absorption spectrums (FTIR) were obtained using Perkin Elmer FTIR spectrophotometer. All the samples were compressed into disks with KBr and analysed over the range of 400-4000 cm⁻¹. The ¹H NMR spectra were measured in on a Bruker 400 MHz FT magnetic resonance spectrometer. Samples were dissolved in TFA-d₆. DSC was conducted by using Mettler-Toledo DSc-821e differential scanning calorimeter. Accurately weighed samples (3-5 mg) were placed in hermetically sealed aluminium pans and scanned from 30 °C to 300 °C at a scan rate of 10 °C/min under nitrogen purge. Saturation solubility studies were carried out in water. An excess quantity of sample was added vials containing ultra-pure water. The vials were shaken using magnetic stirrer on water bath at a temperature of 35 ± 2°C until the solution became saturated. After 24 h sample solution withdrawn and filtered through whatman's filter paper. The concentration of the solution was determined spectroscopically using UV spectrometer (UV-1800, Shimadzu) at λ_{max} 280 nm.

Methods

Prulifloxacin (5g) was dissolved in the methanol (35 ml) and dichloromethane (140 ml) mixture at 25-30°C. Solution was filtered through micron filter. Fumaric acid (1.26 g) was added into the filtrate. Stirred at 25-30°C for 2-3 h. Precipitated product was filtered and dried under vacuum at 50°C for removal of solvent afforded white colour crystalline powder. Yield: 5.3 g.

III. RESULTS AND DISCUSSION

Our rationale was that if prulifloxacin is crystallized with fumaric acid, then the resulting complex will contain an ionized drug molecule and the carboxylate counter ion (as shown in Scheme 2) can enhance the solubility of the drug.



Scheme 2. Formation of prulifloxacin fumarate salt (c) from prulifloxacin (a) and fumaric acid (b)

pKa of drug (piperazine base) is 6.25 and first pKa of Fumaric acid is (3.03). Value of Estimated pKa difference between the drug and carboxylic acid (Δ pKa) is 3.22 suggested salt formation based on the rule of three [10]. The calculated pKa differences are greater than three suggested that there should be proton transfer from the carboxylic acid to the piperazine base to give the salt structure.

IV. SPECTRAL ANALYSIS, THERMAL ANALYSIS AND SOLUBILITY

Fig 1 represents the PXRD pattern of the prulifloxacin and prulifloxacin fumarate. Prulifloxacin shows its characteristic PXRD peaks at 12.46, 16.46, 17.98 and 23.96 2θ [11]. The PXRD pattern of prulifloxacin fumarate shows major peaks at 6.4, 11.1, 15.26, 17.29, 18.71, 22.38, 25.40, 29.71 and 30.71 2θ . The characteristic peaks of prulifloxacin and prulifloxacin fumarate were clearly shows that PXRD of product clearly distinguishable.

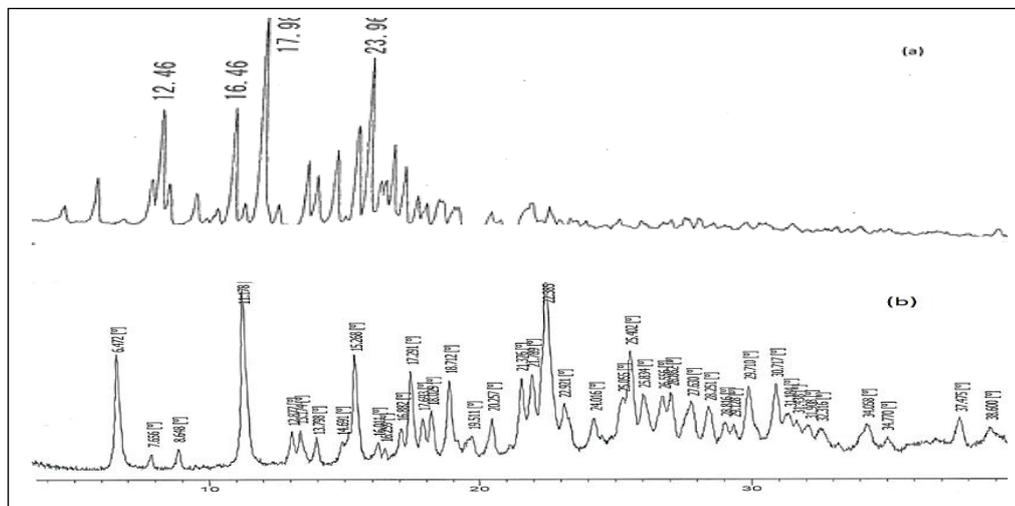


Fig. 1 Powder X-ray diffraction (PXRD) of prulifloxacin (a) prulifloxacin fumarate (b)

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Fig. 2 represents the FTIR spectra of prulifloxacin fumarate. Prulifloxacin fumarate shows stretching vibration of (O-H) at 3437 cm⁻¹, (C=O) stretching vibration of ester at 1814 cm⁻¹, (C=O) stretching vibration of carboxylic acid at 1707 cm⁻¹, stretching vibration of (C=O) of ketone at 1627 cm⁻¹. The intense peaks at around 1707 cm⁻¹ indicate the COOH group is unionized. The broad peaks at 2448 cm⁻¹ is due to the protonated piperazine (NH₂⁺) ionized by the transfer of proton from the acid.

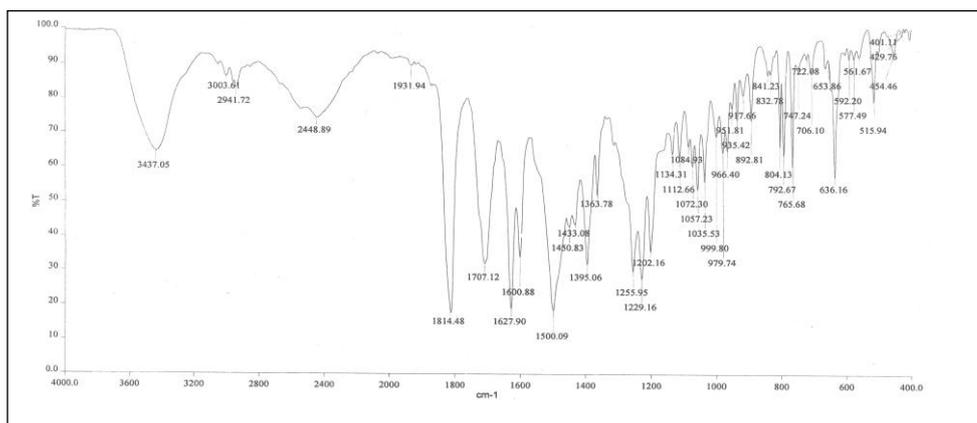


Fig. 2 FTIR spectra of prulifloxacin fumarate

Fig. 3 shows the thermal behaviour of prulifloxacin fumarate. The endothermic event occurred at 245°C. Higher melting temperature of fumarate salt than prulifloxacin (220 °C) [11] without any phase transformation indicates prulifloxacin fumarate is stable compound.

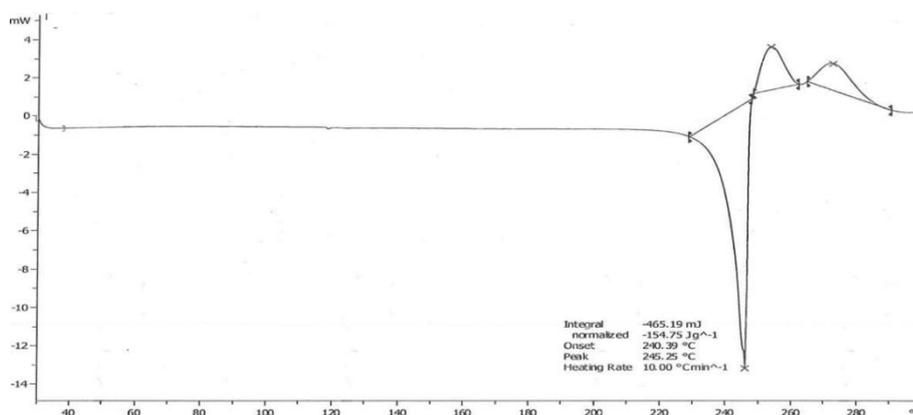


Fig. 3DSC pattern of prulifloxacin fumarate

¹HNMR

The ¹HNMR chemical shift assignments of prulifloxacin fumarate are as follows. δ 7.81 (d, 1H), 6.92 (d, 1H), 6.62 (s, 2H), 6.39 (q, 1H), 3.73 (s, 2H), 3.16-3.69 (m, 4H), 2.54-2.63 (m, 4H), 1.9-2.13 (m, 6H). The signals in ¹H NMR shows the 1:1 ratio of both the components.

Dynamic vapour sorption (DVS)

Dynamic vapour sorption for prulifloxacin fumarate is shown in the figure 4. At 90% RH, the sorbed water by fumarate salt was about 0.38% which suggests that the solid-state form has low hygroscopicity.

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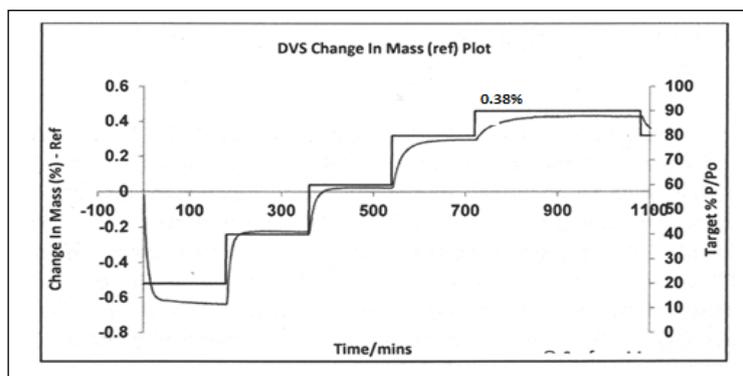


Fig. 4DVS of prulifloxacin fumarate

Prulifloxacin has poor solubility in water hence classified as a BCS class-II drug. Saturation solubility studies of prulifloxacin fumarate were carried out in water at $35 \pm 2^\circ\text{C}$. The aqueous solubility of prulifloxacin fumarate is ($410 \mu\text{g}/\text{ml}$) higher than prulifloxacin ($20 \mu\text{g}/\text{ml}$). The solubility enhancement is good as compared with the reference drug in pure water.

V. CONCLUSION

Prulifloxacin fumarate salt was synthesized and characterised by PXRD, IR, DSC, ^1H NMR Proton transfer from the carboxylic acid to the piperazine base was identified as salt formation sites from the vibrational frequency data. Prulifloxacin fumarate salt shows melting point of 245°C . The results of higher melting temperature and excellent aqueous solubility and less hygroscopicity are encouraging enough to justify the physio chemical problems specially solubility of the original drug prulifloxacin.

VI. ACKNOWLEDGMENTS

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REFERENCES

1. Wermuth, C.G., Stahl, P.H., "Handbook of Pharmaceutical Salts: Properties, Selection and Use"; Wiley-VCH: Weinheim, Germany, 2002, pp 1-7.
2. Berge, S. M., Bighley, L. M., Monkhouse, D. C., "Pharmaceutical salts", J Pharm Sci, 1997, Vol. 66, pp. 1-19.
3. Automated salts and polymorphs screening. http://www.tessella.com/Services/CaseStudies/pdfs/e_GSK_ASAP.pdf, accessed Dec. 15, 2006.
4. Anderson, B.D., Conradi, R. A., "Predictive relationships in the water solubility of salts of a nonsteroidal anti-inflammatory drug" J Pharm Sci, 1985, Vol. 4, Issue 8, pp. 815-820.
5. Bhattachar, S.N., Deschenes, L.A., Wesley, J.A., "Solubility: it's not just for physical chemists", Drug Discov Today 2006, Vol. 11, pp. 1012-1018.
6. Bighley, L.D., Berge, S.M., Monkhouse, D.C., "Presevation of pharmaceutical products to salt forms of drugs and absorption". In: Swarbrick J, Boylan JC, editors. Encyclopedia of Pharmaceutical Technology 13. New York, NY: Marcel Dekker; 1996. pp. 453-499.
7. Florence, A.T., Attwood, D., editors. Properties of the solid state, In: Physicochemical principles of pharmacy, Macmillan Press Ltd: London, 3rd ed., 1998, pp. 5-34.
8. Kise, M., Kitano, M., Ozaki, M., Kazuno, K., Matsuda, M., Shirahase, J., Segawa, J., US patent US5086049.
9. UK Food Standards Agency: "Current EU approved additives and their E Numbers".
10. Sarma, B., Nath, N.K., Bhogala, B.R., Nangia, A., "Synthon competition and cooperation in molecular salts of hydroxyl benzoic acids and aminopyridines" Cryst Growth Des 2009; Vol. 9, pp. 1546-1557.
11. Akai, J., Nishida, H., "crystals of quinoline carboxylic acid derivative solvate", US patent US2007/0149540A1.