IJPSR (2020), Volume 11, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 July 2019; received in revised form, 09 October 2019; accepted, 29 February 2020; published 01 June 2020

SOLID DOSAGE FORM DEVELOPMENT OF DABIGATRAN ETEXILATE MESYLATE WITH INCREASED SOLUBILITY AND DISSOLUTION USING COCRYSTALLIZATION

A. R. Gawade [1] and S. P. Boldhane [*2]

Maeers Maharashtra Institute of Pharmacy [1], Paud Road, Kothrud, Pune - 411038, Maharashtra India.
Development Micro Labs Limited Micro Advanced Research Centre (Marc). [2], No. 58/3, Kudlu Village, Anekal Taluk, Singasandra Post Bangalore - 560068, Karnataka India.

Keywords:

Dabigatran etexilate mesylate, Coformer, Molecular docking, Radar chart, Solution-based evaporation technique, Co-crystals

Correspondence to Author:
Dr. S. P. Boldhane

Sr. General Manager-Formulation, Development Micro Labs Limited Micro Advanced Research Centre (Marc). No. 58/3, Kudlu Village, Anekal Taluk, Singasandra Post Bangalore - 560068, Karnataka India.

E-mail: sanjay.boldhane@rediffmail.com

ABSTRACT: Dabigatran Etexilate Mesylate (DEM), an anticoagulant used to treat and prevent blood clots and to inhibit the occurrence of stroke in people with cardiac arrhythmia. The purpose of this study was to obtain DEM co-crystals by screening eight different conformers and prepare the co-crystals by evaporation technique. Molecular docking was used to identify suitable coformer. Tartaric acid DEM co-crystals have been effectively prepared in the molar ratio of 1:1, 1:2, and 1:3 by solution-based evaporation technique. A 1:1 molar mixture of DEM and tartaric acid was further characterized. Differential scanning calorimetry, Fourier Transform Infrared Spectroscopy (FTIR), Powder X-ray Diffraction (PXRD) and Scanning Electron Microscopy (SEM) were used to characterize manufactured co-crystals. The study confirmed the selection of proper coformer and exhibited enhanced solubility and stability of the DEM co-crystals. Hence, based upon the above results, it revealed that co-crystallization helps in improving the physicochemical properties of the API, altering their chemical structure.

INTRODUCTION: Dabigatran Etexilate Mesylate (DEM) is a salt form of the Dabigatran Etexilate Prodrug **Fig. 1**. It is absorbed and transformed by esterase catalyzed hydrolysis in the liver after oral administration to dabigatran. It is a potent, reversible, univalent direct thrombin inhibitor [1]. It is a BCS Class II drug with absolute bioavailability of 3-7% followed by oral administration. It has a dose of 75 mg, 110 mg, and 150 mg and 12-17 h of elimination half-life [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(5).2899-05

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(5).2899-05

It is prone to the acidic condition and is degraded in the presence of moisture by hydrolytic processes [1, 2]. DEM's aqueous solubility depends on pH with elevated solubility in acidic media and very low solubility in neutral and basic media [3, 4]. It often creates pain and tightness in the chest, swelling of the face or tongue, respiratory or wheezing problems, and feeling dizzy or faint [5].

Due to these physicochemical and bio-pharmaceutical features, attempts are fabricated to improve solubility, bioavailability, and stability. The low water solubility of the active substance is responsible for the risks of low oral bioavailability. In order to enhance therapeutic efficacy, DEM needs an alternative drug delivery system. Co-crystals have recently drawn significant attention in the delivery of drugs by enhancing the drug

physicochemical properties such as melting point, solubility, dissolution rate, stability and bio-availability without changing their chemical structure [6]. Co-crystals are compounds with a stoichiometric ratio of drug substance and co-crystal coformers (CCFs) (1:1, 1:2, 1:3 or *vice versa*) [7]. These co-crystals are combined by non-covalent interfaces like hydrogen bonds, Van der Waals forces and π-π packaging which are robust at room temperature [8, 9, 10, 11]. The co-crystal former is a ballast molecule. Identifying and selecting the appropriate conformer continues the most important factor in the effective co-crystal growth [7]. This technology is explored effectively for the delivery of various drugs such as acyclovir [12], gliclazide [13], piracetam [14], fexofenadine [15], furosemide [16], quercetin [17], baicalein [18], myricetin [19] *etc*. to improve the therapeutic efficacy.

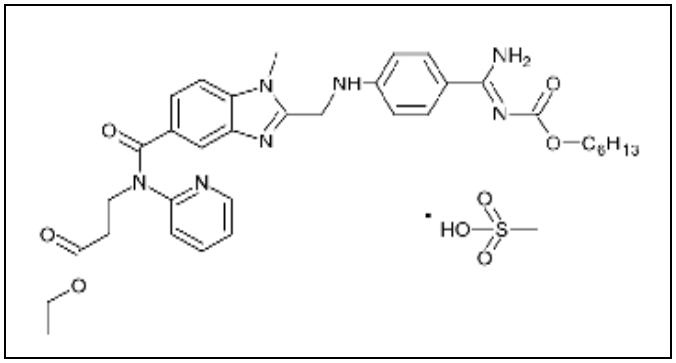


FIG. 1: STRUCTURE OF DABIGATRAN ETEXILATE MESYLATE

The objective of the present paper is to enrich the rate of dissolution, the efficacy of DEM absorption using evaporation techniques. A literature study shows that there have been few DEM formulations reported to date. DEM was therefore chosen as the poorly soluble model drug in this work; the solution-based evaporative co-crystallization method developed DEM tartaric acid pH-independent cocrystals. Fourier transform-infrared spectroscopy, Differential scanning calorimetry, scanning electron microscopy, and powder X-ray diffraction defined the co-crystals produced.

MATERIALS AND METHODS:

Materials: Dabigatran Etexilate Mesylate was obtained from Micro Advanced Research Center (Bangalore, India) as a gift sample. Tartaric acid was collected from Poona Chemical Laboratory. Other reagents have been bought from S. D. Fine Limited Chemicals (Mumbai, India).

Methods:

Molecular Docking and Selection of Conformers: Molecular docking is an effective approach for computer-aided structure-based drug discovery. This strategy predicts the probability of binding and orienting one molecule (API) is connected to a second molecule (coformer) to form a new complex [20]. The strength of the binding affinity between two molecules by means of scoring features can be determined [21, 22]. Based on the literature, eight coformers were selected for the preparation of the DEM co-crystals. The research of molecular docking was carried out on the eight coformers chosen. Furthermore, the radar chart was used to visually compare the quality docking score of coformer information with the advantage of showing multidimensional information without the use of statistical methods. Tartaric acid was verified among the eight coformers based on the potential for interaction type, compatibility, and docking score for co-crystal confirmation with DEM.

Solution Based Evaporative Co-crystallization of DEM: DEM co-crystals synthesis was performed using the solvent evaporation technique. Screening of formation of DEM co-crystals was performed by various coformers in an optimal molar ratio (1:1, 1:2 and 1:3). A mixture of 1:1 DEM and tartaric acid was dissolved in water and continually stirred at 40 - 50 ºC for 15 min. The solvent evaporated at 60-65 ºC when stored for 3 h in a hot air oven. Crystals were triturated in mortar and pestle and stored at room temperature [23, 24].

Characterization of DEM Co-crystals:

Differential Scanning Calorimetry (DSC): A differential calorimeter scanning (DSC7020 thermal analysis system HITACHI) was used for thermal analysis of DEM, DEM co-crystals samples. Powder samples of approximately 2.0 mg were placed in aluminum open crucibles and heated at a rate of 10 ºC/ min up to 400 ºC.

Fourier Transform-Infrared Spectroscopy (FT - IR): FT-IR spectra were registered on a Nicolet iS10 spectrophotometer from 4,000 cm^{-1} to 500 cm^{-1} (Thermo Fisher Scientific, Madison, USA). With 40 scans per spectrum at a resolution of 0.4 cm^{-1}, DEM cocrystals were obtained and analyzed using the DTGS KBr detector.

Powder X-Ray Diffraction (PXRD): DEM, DEM co-crystals XRD patterns were achieved using a Shimadzu XRD-6000X system at ambient temperature (Shimadzu, Japan). Samples with Ni-filtered Cu-K (α) radiations were irradiated at a voltage of 40.0 kV and a current of 40.0 mA. The scanning rate ranged from 3 ºC to 50 ºC over a diffraction angle of 2 ºC/min.

Scanning Electron Microscopy (SEM): Morphologically, DEM, DEM co-crystals were evaluated using scanning electron microscopy (Philips XL-30; Eindhoven, Holland). An aluminum stub was connected to a small piece of double-sided adhesive tape and the powders were sprinkled and distributed over the surface of the stub. Under the argon atmosphere, the samples were sputtered with gold-palladium to make them electrically conductive.

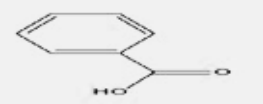
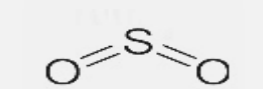
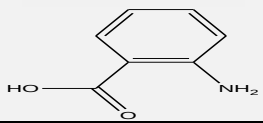
***In-vitro* Dissolution Study:** The dissolution studies were performed in a dissolution apparatus Electrolab, Navi Mumbai using the paddle method in 900 ml of pH 1.2 and 6.8 phosphate buffers at 50 rpm maintained at 37 ± 0.5 ºC. The dissolution medium was added an amount equal to 75 mg of co-crystals and the samples were withdrawn at appropriate intervals. The samples were filtered through Whitman filter paper no. 41, diluted, and spectrophotometrically analyzed at 325 nm.

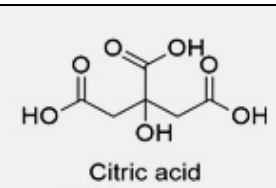
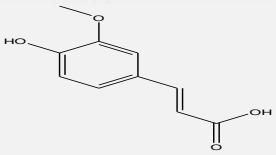
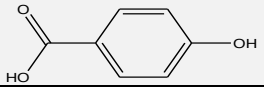
RESULTS AND DISCUSSION: A set of eight coformers with DEM were used in the screening of coformer study. The molecule of DEM structure consists of two aromatic rings (imidazole and pyridine), three carbonyl groups, four aliphatic nitrogen atoms, and three aromatic nitrogen atoms. DEM molecule has three hydrogen bond donors as well as eleven hydrogen bond acceptors due to aromatic nitrogen (N) in imidazole ring, a pyridine ring, and carbonyl groups and significant.

Conformational flexibility; hence it is possible to form co-crystals with certain co-formers [2]. Depending on the ability of interaction, hydrogen bond, docking score, and compatibility tartaric acid were selected as coformer for the preparation of DEM co-crystals **Fig. 2**. The details are summarized in the below **Table 1**. **Fig. 3** represents a visual comparison of docking score in the form of a Radar chart. There are several axes in a radar chart where the information can be plotted. Every axis is one category. The information is displayed on the axis as points. It is possible to join the points belonging to one data series.

A point near the center of an axis shows a reduced value and *vice versa* [25]. Through, the visual comparison from Radar charts and docking scores from molecular modeling, tartaric acid showed the lowest score. Van Der Waals and the electrostatic energy define the interaction between the API and coformer. Higher docking results in the potential for repulsion. In contrast, reduced docking score relates to reduced potential [26].

TABLE 1: MOLECULAR MODELING OF COFORMERS

S. no.	Name	Structure	Docking score	Hydrogen bonding
1	Sodium acetate		-2.108	No hydrogen bond.
2	Benzoic acid		-1.206	No hydrogen bond.
3	Tartaric acid		-3.847	Hydrogen bonding possible
4	Aerosil		-3.5584	Hydrogen bonding possible
5	Anthranilic acid		-3.2290	No hydrogen bonding possible

6	Citric acid	 Citric acid	-2.09101	No hydrogen bonding possible
7	Ferulic acid		-3.75056	Hydrogen bonding possible
8	4 Hydroxy benzoic acid		-3.23360	No hydrogen bonding possible

Solvent evaporation using water as a solvent and tartaric acid as a coformer resulted in adequate DEM co-crystal formation. A preliminary evaluation of cocrystal formation was performed by comparing pure drugs and cocrystals.

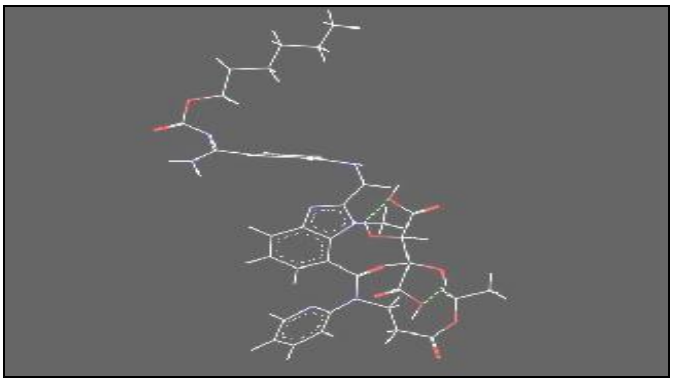


FIG. 2: HYDROGEN BONDING OF DEM- TARTARIC ACID CO-CRYSTALS

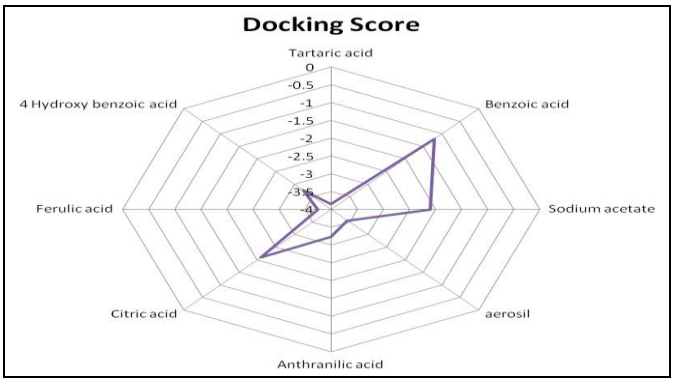


FIG. 3: APPLICATION OF RADAR CHART TO EVALUATE THE COFORMERS

Differential Scanning Calorimetry (DSC): An endothermic peak at 180 ºC represented the melting point of pure drug. A significant difference in the melting point (175.1 ºC) of co-crystals was observed compared to the pure drug (180°C) in **Fig. 4**. This could suggest an interaction between the components, co-crystallization [27].

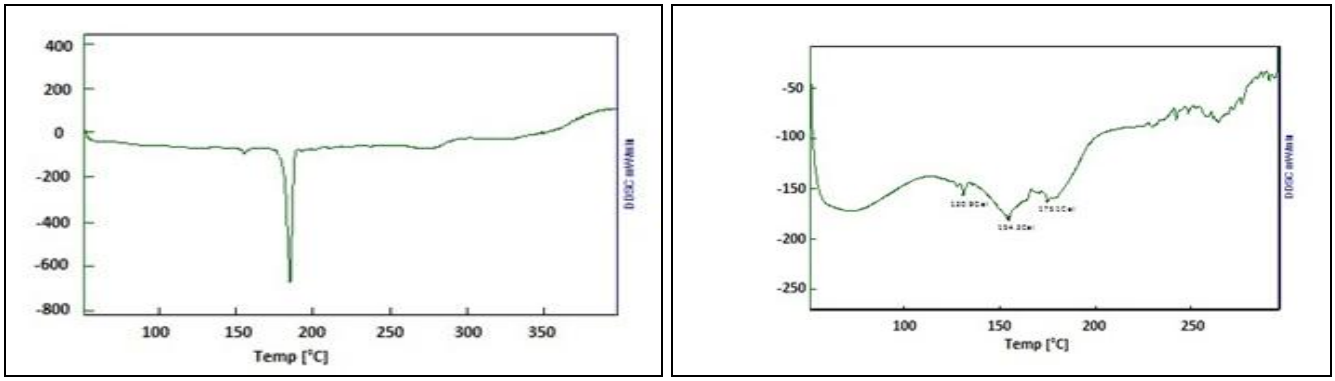


FIG. 4: DIFFERENTIAL SCANNING CALORIMETRY OF A) DEM AND B) DEM CO-CRYSTALS

Fourier Transform-Infrared Spectroscopy (FT - IR): FTIR showed shifts and change in intensity of peaks in DEM, DEM co-crystals as shown in **Fig. 5.** Hydrogen bonding was identified in co-crystals by decreasing O-H peak intensity. A decrease in the frequency of N-H stretching indicates that hydrogen is involved in hydrogen bonding. The extent of frequency decrease and relative band broadening can determine the magnitude of bonding with hydrogen. The role of the degree and strength of the hydrogen bonding is to lower the frequency.

The significant modifications in the carbonyl (C = O) and amine (N-H) stretching area showed the formation of new hydrogen bonds [28, 29].

Powder X-Ray Diffraction (PXRD): X-ray diffract to grams of DEM showed an intense peak at 2 to 20º signifying the crystalline nature of the drug. DEM co-crystals showed no intense drug peaks were observed at 2θ of 20 ºC indicating the existence of the amorphous phase **Fig. 6**. In the case of DEM co-crystals, however, there were no intensive drug peaks at 2θ of 20 ºC showing the presence of the amorphous stage. Decreased intensities and fewer peaks may be due to changes in crystal habit or amorphous form. Reduced crystalline characteristics may result in the enhanced dissolution of DEM compared to pure drug [30].

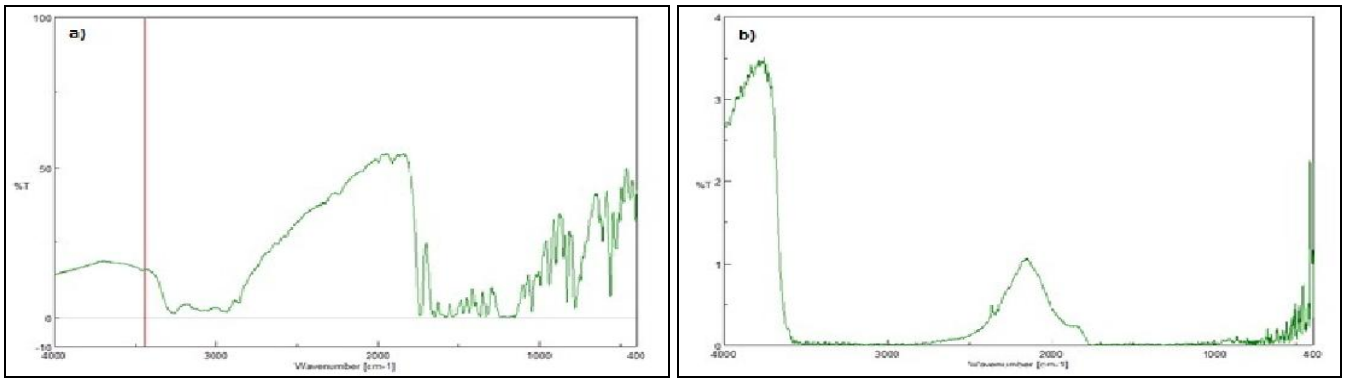


FIG. 5: FTIR GRAPH OF A DEM AND B) DEM CO-CRYSTALS

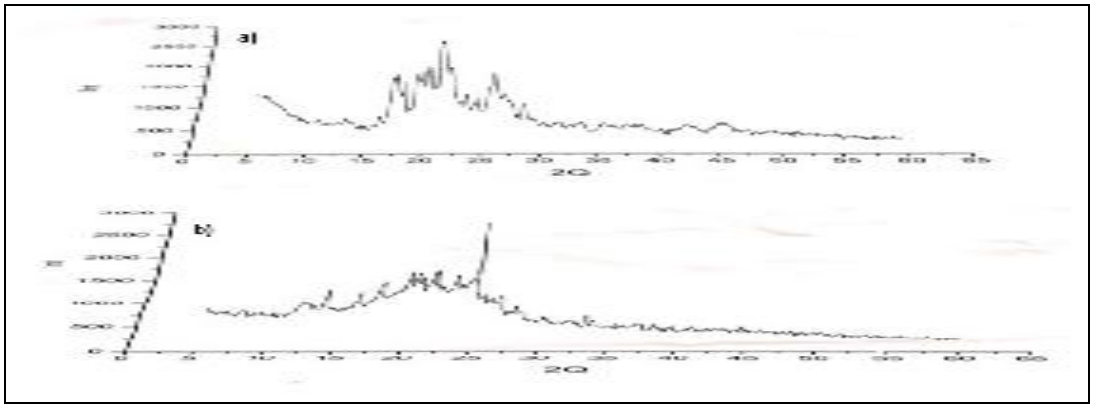


FIG. 6: XRD SPECTRA OF A) DEM AND B) DEM CO-CRYSTALS

Scanning Electron Microscopy (SEM):

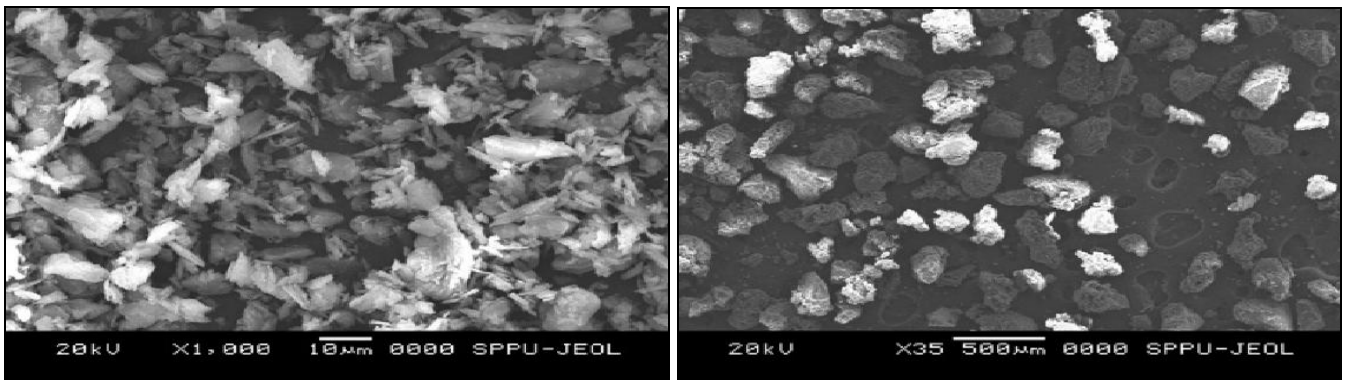


FIG. 7: SEM IMAGE OF A) DEM AND B) DEM CO-CRYSTALS

SEM results reveal that a DEM exhibited irregular shape and the DEM Tartaric acid co-crystals exhibited irregular shape aggregates of crystals **Fig. 7.** Surface morphological properties of drug and crystals confirmed that Dabigatran Etixilate

Mesylate particles crystallized from the solvent system containing tartaric acid as conformer [24, 31].

***In-vitro* Drug Release:** As reported in the literature, DEM solubility depends on pH. It is highly soluble in acidic media and poorly soluble in alkaline media. DEM is also susceptible to acid and undergoes degradation.

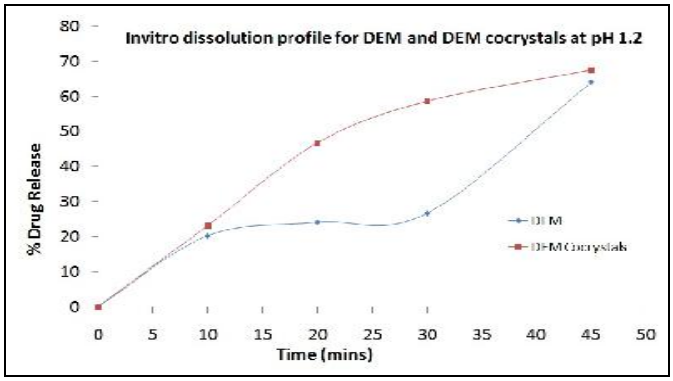


FIG. 8: *IN-VITRO* DISSOLUTION PROFILE FOR DEM AND DEM CO-CRYSTALS AT pH 1.2

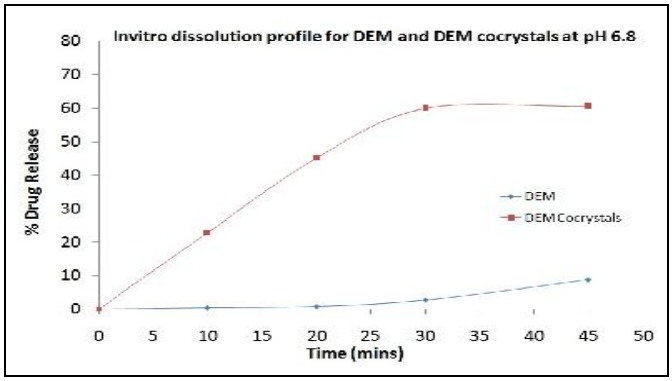


FIG. 9: *IN-VITRO* DISSOLUTION PROFILE FOR DEM AND DEM CO-CRYSTALS AT pH 6.8

Hence, DEM co-crystals were developed by improving the physicochemical properties. The dissolution experiment was performed on DEM and DEM co-crystals at pH 1.2 and pH 6.8 to verify the pH-independent solubility and release of drugs. The DEM and DEM co-crystals dissolution profile is depicted in **Fig. 8** and **9**. The DEM dissolution profile shows that API has a good rate of dissolution in acidic pH (26.49%) as compared to alkaline pH (2.69%) % at 30 min respectively. The complete quantity of DEM dissolved in 45 min was 64.03% in acid pH and 8.71% in alkaline pH. However, DEM co-crystals dissolution rate led to a substantial rise as a function of pH-independent drug release. The quantity of DEM released in the first 30 min was 58.58% and the complete dissolved quantity was 67.5% at pH 1.2. In the first 30 min, the amount of substance dissolved was 59.9% and the drug release in 45 min was 60.54 percent with at pH 6.8 **Fig. 8** and **Fig. 9**.

In addition, higher dissolution of DEM co-crystals can be ascribed to changes in the crystalline pattern, size and shape of co-crystals that escort to increased co-crystal solubility in dissolution media [16, 24, 32].

CONCLUSION: In the present work, prepared DEM co-crystals exhibited excellent physicochemical properties (solubility and dissolution) properties when compared to pure drugs. From the conducted study, we can conclude that co-crystals with tartaric acid prepared by the technique of solvent evaporation of co-crystallization showed an improvement in the solubility and pH-independent drug release, rate of dissolution and stability compared to pure drug.

ACKNOWLEDGEMENT: The authors are grateful to Dr. B. S. Kuchekar Dean, Pharmacy School, Dr. Vishwanath Karad MIT World Peace University, Pune, for offering the required equipment for experimenting.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. WO 2013/124340 Al, Oral Pharmaceutical Compositions of Dabigatran Etexilate.
2. Shankar P, Rajesh N, Prashant P, Hrutuja C and Harsha P: Scientific coformer screening, preparation and evaluation of dabigatran etexilate mesylate co-crystal. Asian Journal of Pharmacy and Pharmacology 2018; 4(6): 810-20.
3. Veenu PM and Krutika KS: Enhanced oral bioavailability and anticoagulant activity of dabigatran etexilate by self-micro emulsifying drug delivery system: systematic development, *in-vitro*, *ex-vivo* and *in-vivo* evaluation. Journal of Nanomedicine and Nanotechnology 2018; 9: 1.
4. Center for Drug Evaluation and Research, Application Number: 22-512, Clinical Pharmacology Review NDA 22-512, Dabigatran.
5. Medication Guide Pradaxa (Pradaxa) (Dabigatran Etexilate Mesylate) capsules.
6. Ranjit T and Bipul S: Drug-drug and drug-nutraceutical cocrystal/salt as alternative medicine for combination therapy. A Crystal Engineering Approach, Crystals 2018; 8(2): 101.
7. Elena IK and Kratochvíl B: Pharmaceutical Co-crystals, Procedia Chemistry 2014; 10: 473 -76.

8. Emily S and John CW: Non-covalent derivatives co-crystals and eutectics, molecules 2015; 20: 14833-48.
9. Xia LD, Jia MC and Tong BL: Pharmaceutical co-crystallization, an effective approach to modulate the physicochemical properties of solid-state drugs. cryst eng comm, 2013; 1-24.
10. Manjunath G, Pradip KM and Deepak C: Co-crystals, a review of recent trends in pharmaceutical and material science applications. Material Science Research India, 2017; (141): 09-18.
11. Anna K, Maria M and Kyriakos K: Pharmaceutical Co-crystals: New Solid Phase Modification Approaches for the Formulation of APIs. Pharmaceutics 2018; 10: 18.
12. Jignasa K and Chirag P: Improvement of physicochemical parameters of acyclovir using co-crystallization approach. Brazilian Journal of Pharmaceutical Sciences, 2016; 52: 4.
13. Reddy M and Anusha P: Development and characterisation of gliclazide cocrystals. International Journal of Pharma and Bio Sciences 2018; 8(1): 22-32.
14. Sonke R: Investigation of the Formation Process of Two Piracetam Co-rystals during Grinding. Pharmaceutics 2011; 3: 706-22.
15. Mounika P: Preparation and characterization of novel co-crystal forms of fexofenadine. International Journal of Innovative Pharmaceutical Research 2015; 6(1): 458-63.
16. Goud N: Novel furosemide co-crystals and selection of high solubility drug forms. Journal of Pharmaceutical Sciences 2012; 101: 2.
17. Umi A: Crystal Engineering of Quercetin by Liquid assisted Grinding Method. Jurnal Teknologi (Sciences and Engineering) 2019; 81: 139-45.
18. Jiaxin P: A nano co-crystal strategy to improve the dissolution rate and oral bioavailability of baicalein. Asian Journal of Pharmaceutical Sciences 2019; 14: 154-64.
19. Mingyu L: Development of a pharmaceutical co-crystal with solution crystallization technology: preparation, characterization, and evaluation of myricetin-proline co-crystals, European Journal of Pharmaceutics and Biopharmaceutics. European Journal of Pharmaceutics and Biopharmaceutics 2016; 10(107): 151-59.
20. Aaftaab S, Khusbhoo J, Sasikala K and Mallika A: molecular docking in modern drug discovery: principles and recent applications. Intechopen, July 2nd 2019.
21. Gaba M, Gaba P, Singh S and Gupta G: An overview on molecular docking. International Journal of Drug Development & Research 2010; 2(2): 219-31.
22. Meng XY: Molecular docking: a powerful approach for structure-based drug discovery. Current Computer-Aided Drug Design 2011; 7(2): 146-57.
23. Maryam KJ: Creating co-crystals: a review of pharma-ceutical co-crystal preparation routes and applications. Crystal Growth and Design 2018; 18: 6370-87.
24. Hilya NI: Improvement of dissolution properties through acyclovir - succinic acid co-crystal using solvent evaporation technique. International Journal of Drug Delivery Technology 2017; 7(4): 304-09.
25. Cavan K: Application of radar chart array analysis to visualize effects of formulation variables on IgG1 particle formation as measured by multiple analytical techniques. Journal of Pharmaceutical Sciences 2013; 12: 102-12.
26. Luca P and Giulio R: Molecular docking: shifting paradigms in drug discovery molecular docking: shifting paradigms in drug discovery. International Journal of Molecular Science 2019; 20: 4331
27. Patrycja S and Marek W: DSC as a screening tool for rapid co-crystal detection in binary mixtures of benzodiazepines with co-formers. Journal of Thermal Analysis and Calorimetry 2018; 133: 785-95.
28. Samsodien H: FTIR, dissolution and anti-viral activity of nevirapine co-crystals. Pharmaceutica Analytica Acta 2017; 8: 9.
29. Dipak DG: Co-crystallization of etodolac: prediction of co-crystallization, synthesis, solid state characterization and *in-vitro* drug release. Marmara Pharmaceutical Journal 2017; 1: 78-88.
30. Prasanthi D and Priyanka K: Formulation and evaluation of liquisolid compacts of dabigatran etexilate mesylate. Indo American J of Pharm Sci 2018; 05(01): 572-83.
31. Setyawan D: Preparation and characterization of artesunate - nicotinamide co-crystal by solvent evaporation and slurry method. Asian Journal of Pharmaceutical and Clinical Research 2014; 7(1): 62-65.
32. Pinki R: Co-crystals formation of clarithromycin with urea: an efficient approach to enhance the solubility and dissolution rate. American Journal of Advanced Drug Delivery 2016; 4(02): 012-20.

How to cite this article:
Gawade AR and Boldhane: Solid dosage form development of dabigatran etexilate mesylate with increased solubility and dissolution using co-crystallization. Int J Pharm Sci & Res 2020; 11(5): 2899-05. doi: 10.13040/IJPSR.0975-8232.11(5).2899-05.