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Research Article

Physico-Chemical Characterization of Diclofenac and Rasagiline Salts and its Relationship for Development of Sublingual Drug Delivery Systems

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ABSTRACT

The current investigation's objective was to study the physicochemical characterization of Diclofenac and Rasagiline salts and its relationship to the development of sublingual drug delivery systems. The selected salts of Diclofenac and Rasagiline were evaluated for crystallinity (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), solubility, in vitro dissolution and taste evaluation using e-tongue. The physicochemical characterization of Diclofenac salts demonstrated that potassium, epolamine and sodium salts of diclofenac showed similar results. Diclofenac free acid is not suitable for this route of administration as solubility was very less in SSF media. Rasagiline mesylate was found more soluble and stable compared to hemitartrate salt. But being a low dose molecule, the impact of dose on the solubility or dissolution was considered negligible. Hence hemitartrate salt was selected as an alternate salt form for development of new sublingual administration.

Introduction

Sublingual dosage form is a pharmacological route of administration in which the drug diffuses into blood through the tongue. Whenever the drug comes in contact with mucous membrane beneath the tongue, it gets absorbed, diffuses through connecting tissues, and enters venous circulation thus avoiding first pass metabolism in liver.[1] Although oral drug delivery is the most chosen route of drug administration in arrears to its versatility, relieve of administration and the utmost patient compliance,[2] there are some disadvantages with swallowing some medicines in certain patient groups, such as geriatric, pediatric and psychiatric patients and low bioavailability due to multiple degrading enzymes in the GI tract and extensive hepatic first-pass metabolism.[3] The sublingual drug administration is often fast and possesses low risk of degradation that might occur in oral delivery routes during the passage through the harsh GI track environment. These dosage forms are convenient for administration to children, elders, mentally impeded and bedridden patients who suffer from dysphagia and hand tremor.[4]

Diclofenac is a derivative of fenamic and an acetic acid class with a secondary amino group (N-H) bridging the two aromatic rings and is responsible for intramolecular H-bonds with chlorine atom of one ring and the carboxyl group of the other ring.[5] Rasagiline mesylate is a selective, II generation, irreversible monoamine oxidase B inhibitor, used for treatment of idiopathic Parkinson's disease.

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Poor solubility for diclofenac and poor permeability for rasagiline causes less bioavailability.[6] Both these drugs are also prone to first-pass metabolism. Hence, to improve their bioavailability, it is proposed to deliver these drugs through sublingual routes.[7] These drugs' different salts were characterized for their physicochemical properties for evaluating their suitability for sublingual delivery.[8] Diclofenac is crystallized with various inorganic and organic cations. From the literature, series of Diclofenac salts were prepared and available as variety organic and inorganic bases.[9]

Materials and Methods

Materials

Diclofenac acid was procured from Aarti drugs, Hyderabad. Diclofenac sodium, Diclofenac potassium and Diclofenac epolamine were purchased from Amoli organics. Rasagiline mesylate and Rasagiline hemitartrate were obtained from Dr. Reddy's Labs, Hyderabad, India.

Methods

The selected salts of diclofenac and rasagiline were evaluated for crystallinity (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), particle size analysis, solubility, partition coefficient, *in vitro* dissolutions, Franz diffusion studies and taste evaluation using e-tongue.

Crystallinity by X-Ray Diffraction (XRD)

Samples of Diclofenac and Rasagiline salts analyzed using an X-ray powder diffraction system (Bruker D8) with Cu Kα radiation operated at 40 kV and 40 mA.[10] The analysis was carried out by fixing the sample's minimum quantity to the quartz zero-background sample plate and placed in the instrument.

Thermogravimetric analysis (TGA)

The TGA samples were analyzed using a Mettler Toledo® instrument TGA/SDTA 851e with the STARe® software, version 6.0. About 10 mg of each sample were analyzed between 25°C - 350°C at a ramp rate of 10°C/ min under nitrogen at a 60 mL/min flow rate.[11]

Differential Scanning Calorimetry (DSC)

The samples analyzed using a Mettler Toledo® DSC821e loaded with STARe® software, version 6.01 with heat flow rate of 30°C/min under a nitrogen flow rate of 50 mL/min into and out of the sample cell in a controlled atmosphere over a temperature range of 30°C to 350°C.[12]

Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of Diclofenac and Rasagiline salts were obtained with FTIR spectrometer (Spectrum Two, Perkin ELMER ES version 10.4.3) KBr pellet technique.[13]

Solubility Studies in Simulated Salivary Fluid at pH 6.5

Diclofenac and Rasagiline salts' solubility was assessed in SSF media at pH 6.5 using the saturation shake-flask technique for 24 hours. A little excess of drug powder added to 250 mL conical flask containing SSF media at 37 ± 0.5°C and stirred for 24 h at 200 rpm speed to attain equilibrium. Post equilibrium was achieved, the excess solid separated by micro-filtration through 0.22 µm Whatman glass microfiber filter (Whatman, UK) and stabilized in carbinol. HPLC determined the drug concentration in the supernatant at maximum absorption wavelength in triplicate.[14]

pH Dependent Solubility Studies

Apart from SSF media, saturation solubility studies for 24 hours were performed in pH 5.5, 6.8, 7.4, 8, 9, and 10 buffers for Diclofenac salts and in 0.1N HCl, pH 4.5 and 6.8 buffers for Rasagiline salts using shake flask method.[14]

Dissolution profile in simulated saliva fluid

In the sublingual cavity, compared with the gastrointestinal tract, dosage form was exposed to minimal physiological agitation and limited volume of saliva i.e., 2 mL to facilitate disintegration and dissolution. From the European Pharmacopeia and Japanese Pharmacopeia or the official compendia, none of the dissolution apparatuses or methods are designed to evaluate the release of drug from a sublingual tablet dosage form under simulated sublingual conditions.

In vitro Franz Diffusion Studies

A diffusion cell (make: Perme Gear, USA, 11.28mm jacketed Franz diffusion cell with o-ring joint, clear glass, 8 mL receptor volume) consists of a 0.45 NVF membrane between the donor chamber and a receptor chamber, with a minimum of 6 units (n = 6 cells) was used for the proposed study. 12.5 mg equivalent dose of diclofenac and 1 mg equivalent dose of rasagiline was placed in donor cell, and 1.5 mL of the pH 6.5 SSF media was added to the donor compartment. The receptor compartment was filled with the receiver fluids (pH 6.5 SSF media) and fixed to a water bath at 37 ± 0.5°C.[15]

Taste evaluation by electronic-tongue

The ASTREE electronic tongue (Make: Alpha moss) consists of 7 sensors, and 1 reference electrode was used for evaluating the taste of all salts for the present investigation. This electronic tongue was fixed to seven lipid membrane sensors labeled based on taste qualities bitterness (three sensors), umami, saltiness, sourness, and astringency and corresponding reference electrodes.[16]

Results and Discussion

Crystallinity by XRD

From the X-ray diffraction pattern (XRD) of diclofenac potassium, prominent diffraction peaks appeared at

6.99°, 25.27°, 26.9° 2θ values, sodium salt showed one characteristic peak at 13.50° (2θ), epolamine salt has high-intensity peaks at 10.9°,15.9°, 21.23° and 25° 2θ values and diclofenac acid showed sharp high-intensity peaks at 10.73°,15.25°,18.8°, 20° and 24° 2θ values. A short or shoulder peaks also appear in all salts at different 2θ values.

XRD pattern of rasagiline mesylate showed main sharp peaks at 9.05°,13.59°,18.15° and 22.78° 2θ values and rasagiline hemitartrate showed sharp high-intensity peaks at 6.793°,12.7°,16.6° and 23.17° 2θ values. The comparative XRD graph of Diclofenac and Rasagiline salts showed that all have crystalline properties with sharp and high-intensity peaks (Figs 1 and 2).

TGA Analysis

The TGA analysis paired with DSC provides information on desolvation/dehydration processes, i.e., to determine the amount of solvent loss in the salt complex. For diclofenac potassium, epolamine, and free acid salts less than 1% water loss was observed up to 70°C confirms the anhydrous forms. For Diclofenac sodium, 12% loss was observed up to 70°C confirms the hydrate forms sodium salt as shown in Figs 3 to 6. For Rasagiline hemitartrate, weight loss was found to be 0.5 % and for mesylate salt to be 0.15 % up to 90°C, as shown in Figs 7 and 8.

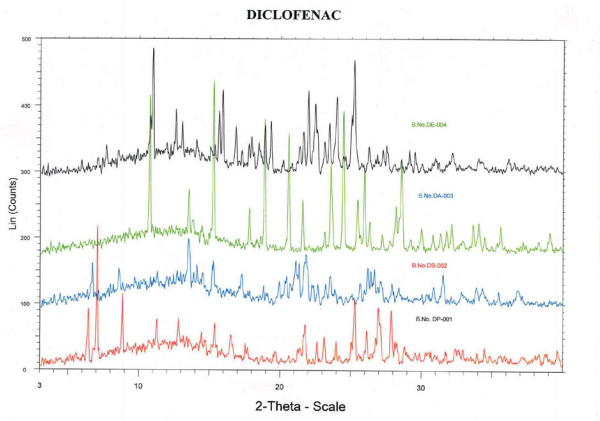

Fig. 1: X-Ray powder diffractograms of diclofenac potassium (DP-001), diclofenac sodium (DS-002), diclofenac acid (DA-003) and diclofenac epolamine (DE-004)

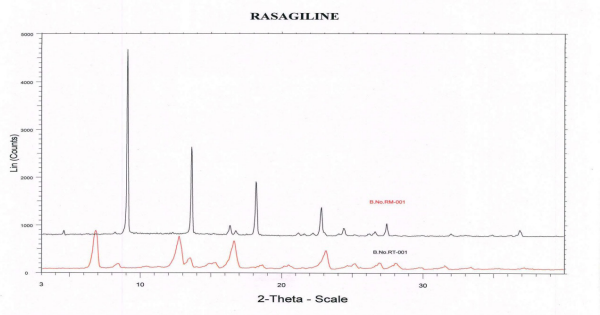

Fig. 2: X-Ray powder diffractograms of rasagiline hemitartrate (RT-001) and rasagiline mesylate (RM-001)

DSC analysis

The DSC chromatogram provides the information of melting point/range, the presence of solvates and/or hydrates, and recrystallizing characteristics of salts. The DSC spectra of Diclofenac sodium showed two endothermic peaks at 65.67°C corresponds to desolvation, and 285.93°C corresponds to the melting point of the crystal form followed by exothermic peak showing the decomposition.

Diclofenac potassium also showed one exothermic peak at 313.68°C, which corresponds to the melting point followed by degradation. DSC of Diclofenac free

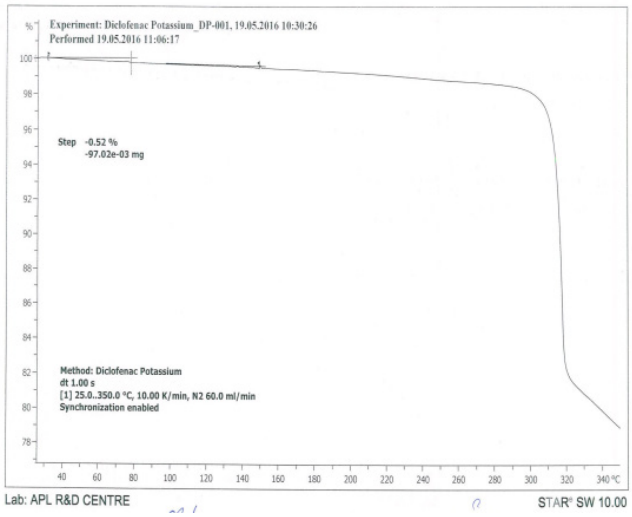

Fig. 3: TGA thermogram of diclofenac potassium

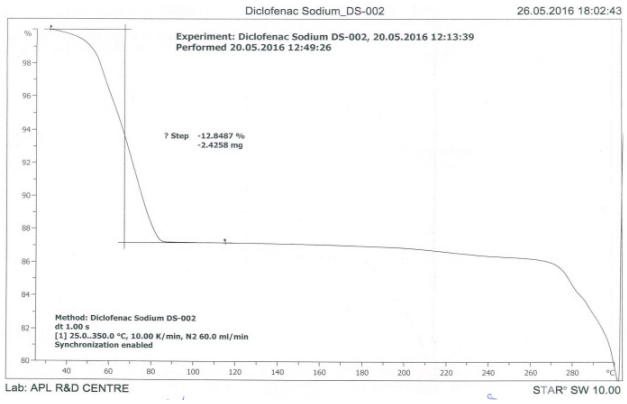

Fig. 4: TGA thermogram of diclofenac sodium

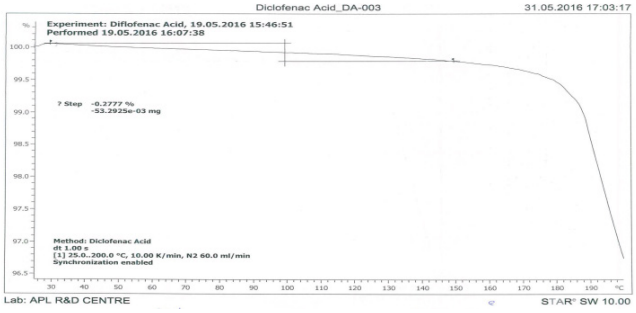

Fig. 5: TGA thermogram of diclofenac acid

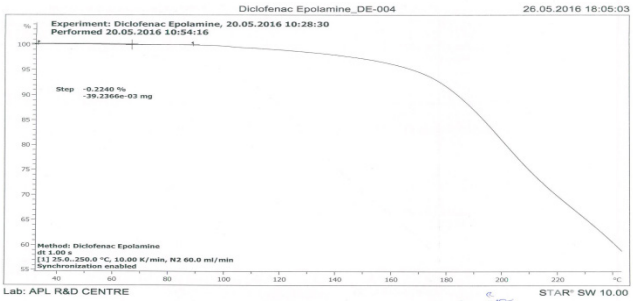

Fig. 6: TGA thermogram of diclofenac epolamine

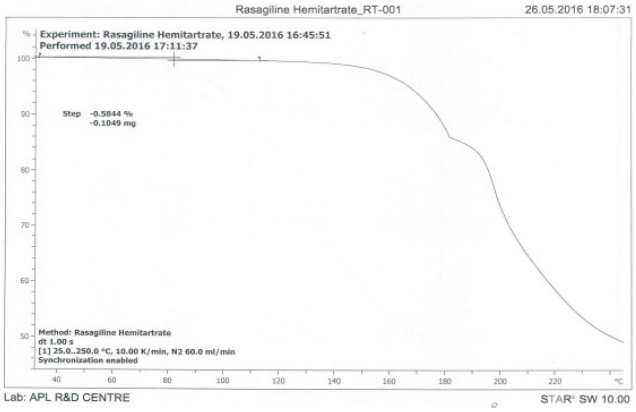

Fig. 7: TGA thermogram of rasagiline hemitartrate

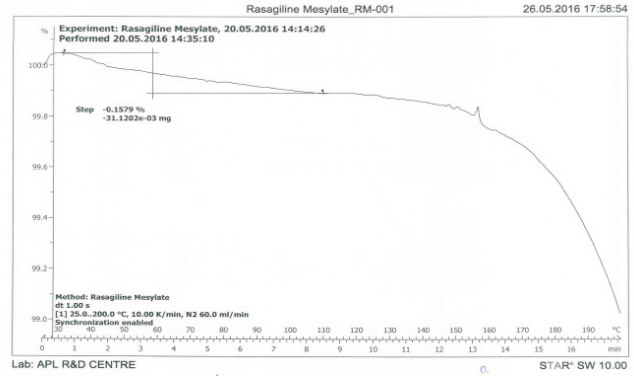

Fig. 8: TGA thermogram of rasagiline mesylate

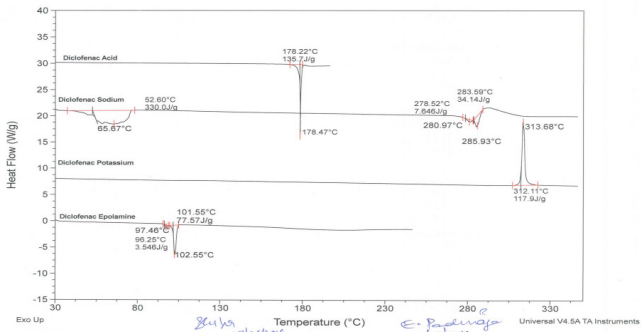

Fig. 9: DSC thermogram of diclofenac acid, diclofenac sodium diclofenac potassium and diclofenac epolamine

acid exhibited one sharp endothermic peak at 178.22°C, indicating the melting point, whereas epolamine showed an endothermic melting peak at 101.56°C with an onset

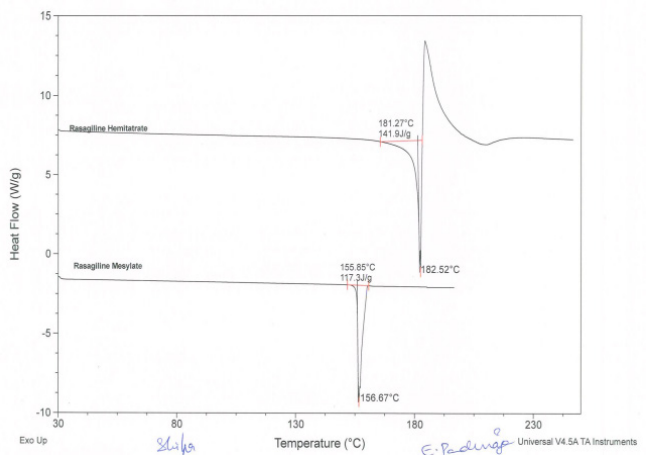

Fig. 10: DSC thermogram of rasagiline hemitartrate and rasagiline mesylate

of the temperature of 96.25°C. Diclofenac salts of sodium, potassium, epolamine and free acid salts showed a sharp endotherm at 280.97°C, 313.68°C, 102.55°C, and 178.47°C, respectively as shown in Fig. 9 describes that the salts are highly crystalline, and the values are matching to melting point in literature.

Similarly, for DSC spectra of Rasagiline hemitartrate exhibited melting endotherm at 182.52°C with onset temperature of 181.27°C followed by exothermic decomposition, and for Rasagiline mesylate showed melting endotherm at 156.67°C with onset temperature of 155.85°C as shown in Fig. 10.

FTIR Spectra

The FTIR spectra of Diclofenac sodium, potassium salt, Diclofenac acid, epolamine salt are shown in Figs 11(a-d) the characteristic peaks of each compound are given in Table 1. The FTIR spectra of pure Rasagiline mesylate and Rasagiline hemitartrate are shown in Figs 12 (a,b) and the corresponding characteristic peaks are given in Table 1.

Solubility in SSF media and Different pH Conditions

Based on saturated solubility study data in SSF media for both salts it was found that epolamine salt shown better solubility (22.24 mg/ml) compared to potassium (17.56 mg/mL) and sodium salts(11.90 mg/ml). Diclofenac acid displayed very less solubility of 0.20 mg/mL. In the case of Rasagiline salts, mesylate (231.00 mg/mL) displayed five times improved solubility than hemitartrate salt (47.30 mg/mL).

As per the pH-dependent solubility studies data shown in Table 2, all three Diclofenac salts showed a similar profile and observed pH-dependent solubility as more solubility above pH 6.8. Whereas for Rasagiline salts as shown in Table 3, mesylate is much more soluble than hemitartrate salt and showed pH-independent solubility across the pH 1 to 6.8.

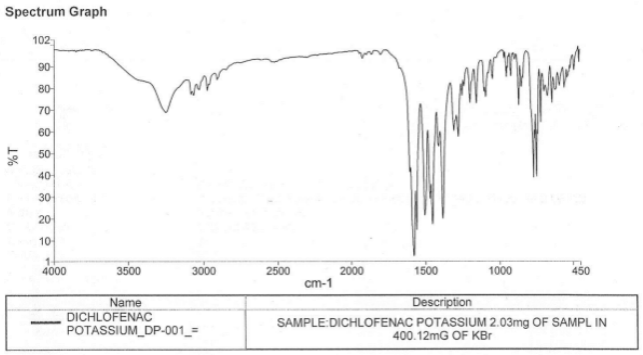

Fig. 11(a): FTIR spectroscopy of diclofenac potassium pure drug

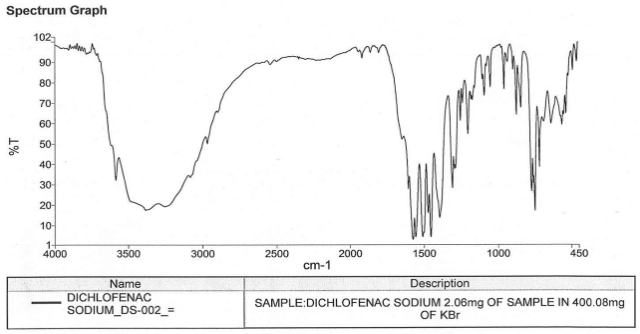

Fig. 11(b): FTIR spectroscopy of diclofenac sodium pure drug

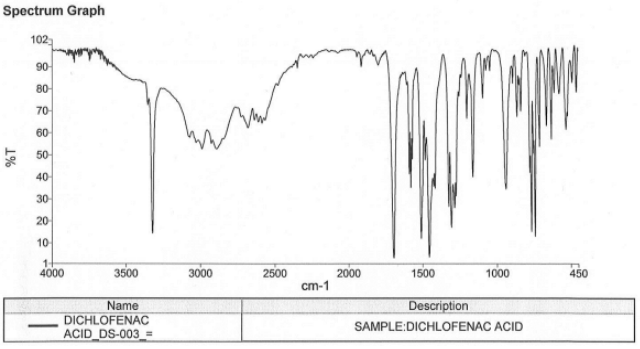

Fig. 11(c): FTIR spectroscopy of diclofenac acid pure drug

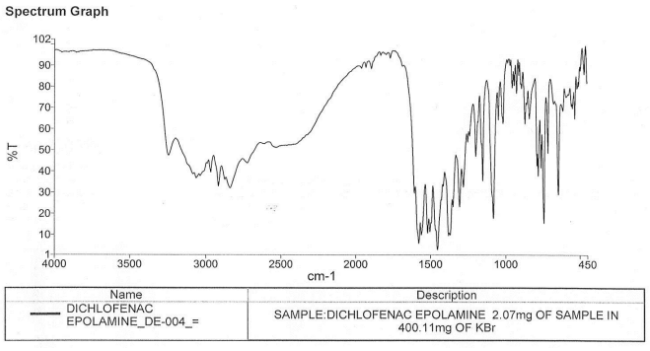

Fig. 11(d): FTIR spectroscopy of diclofenac epolamine pure drug

Dissolutions in Simulated Saliva Fluid

Comparative dissolution profile data generated at 35 rpm in SSF media for Diclofenac and Rasagiline salts show that profiles were quite similar and showed very fast release within 3 min of about 80% for sodium and 85% for potassium and epolamine salts and for rasagiline, both

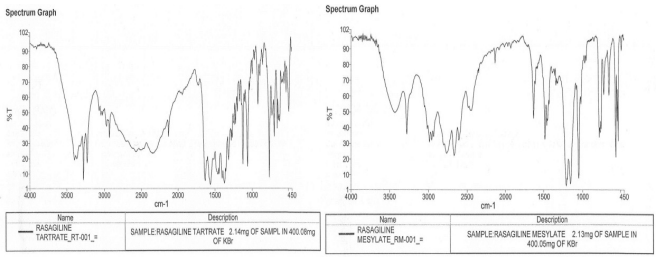

Fig. 12: (a) FTIR spectroscopy of Rasagiline hemitartrate pure drug, (b) FTIR spectroscopy of rasagiline mesylate pure drug

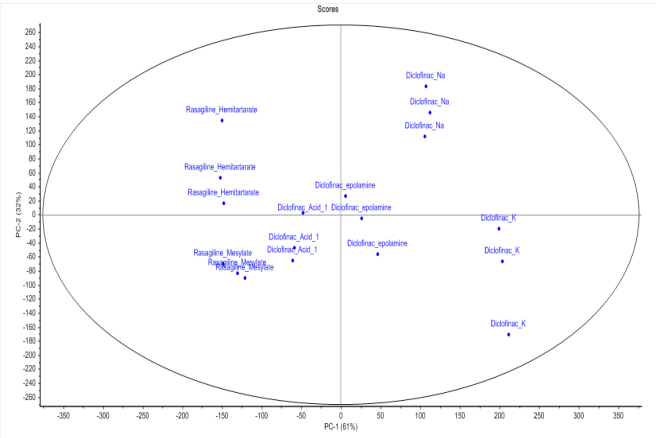

Fig. 13: Bitterness score plot for diclofenac and rasagiline salts

salts showed similar profiles from 3 to 7 minutes. Whereas dissolutions profiles generated at 50 rpm showed complete release within 5 minutes in SSF media for Diclofenac salts.

Diclofenac free poses lower drug release rates of 12% after 7 minutes, indicating the free acid's inferior dissolution in simulated mouth contents compared to the salts. Dissolution profile at 35 rpm showed more discrimination for release as official monographs generally describe drug release techniques do not signify the actual condition of sublingual administration of drugs.

In vitro Franz Diffusion Studies

From *in vitro* drug diffusion studies using membrane filter, it was observed that there is no significant difference in the % drug released within 360 minutes among diclofenac sodium (96%), Diclofenac potassium (97%), and epolamine salts (91%). In contrast, diclofenac acid (3%) form showed very less % drug release. For Rasagiline salts, mesylate salt showed more % drug released compared to hemitartrate initial time points and found comparable after 30 min time point. The hemitartrate salt displayed a release of 99.8% in 240 minutes, and mesylate salt displayed release of 100% in 240 minutes.

Taste Evaluation by e-tongue

The multivariate data evaluation of diclofenac acid and salts of diclofenac and Rasagiline principal component analysis (PCA) showed in Fig. 13, and a map was constructed using output value of bitter taste sensors and aftertaste bitterness sensors. This PCA map also shows the

Table 1: FTIR spectra bands wavelength details of diclofenac and rasagiline salts

Wave number	*Functional group*	*Diclofenac free acid*	*Diclofenac potassium*	*Diclofenac sodium*	*Diclofenac epolamine*	*Rasagiline mesylate*	*Rasagiline hemitartrate*
3400- 3700	O-H				3245	3691,3672	3786
3300-3400	N-H stretching		3250	3387,3360		3431	
3020-3100	Alkene						
2850-3000	Aromatic C-H	3071	2973	2890		3017, 2958	3068,2931
2800-2900	Aliphatic C-H		2906		2724		2970
2200-2500	Nitrile						
2150-2250	Alkyne						
1400-1500 and 1585-1600	C=C stretching aromatic		1407		1431	1627, 1606	
1000- 1350	C-N stretching secondary aromatic		1304,1275	1249, 1235	1196,1250	1076, 1015	1276
1000-1200	C-H plane aromatic bending		2906	1177			1190,
1050-1100	C-X stretching (X=Chloride)						1094
1700-1750	Carboxylate stretching	3353	748, 1572,1199		1574		
1450-1600	Phenyl ring			1575	1651 and 1556		

Table 2: pH dependent solubility data of diclofenac salts

pH of buffer	*Diclofenac potassium (mg/mL)*	*Diclofenac sodium (mg/mL)*	*Diclofenac epolamine (mg/mL)*	*Diclofenac acid (mg/mL)*
5.5	7.43	1.43	4.11	0.01
6.8	76.01	64.88	72.14	0.5
7.4	18.73	14.0	17.4	1.28
8	67.27	52.0	54.21	5.32
9	48.79	41.3	33.45	5.13
10	37.89	30.2	32.56	9.75

Table 3: pH dependent solubility profiles of rasagiline salts

Solvent	*Rasagiline mesylate (mg/mL)*	*Rasagiline hemitartrate(mg/mL)*
Water	613	17.625
0.1N HCl	619	55.40
pH 4.5 acetate buffer	Not done	37.67
pH 6.8 phosphate buffer	602	45.05

variations among rasagiline and diclofenac salts. The major variance is described by I component, the x-axis (PC-1), and a minor part of variance described by II principal component on the y-axis (PC-2). Although PC-1 provides more than 77% of the information, 17% of it is provided by PC-2. The major difference among the samples is carried out wrt PC-1, while additional data acquired by PC-2.

The bitter sensor 3 contains cationic lipids and reacts with anionic substances, while sensors 1and 2 are more precise for cationic substances as they contain anionic lipids. Hence Diclofenac anion will be detected by the bitter sensor 3, while cations of each drug form (epolamine, sodium and potassium) will be detected by sensors 1 and 2. The three bitter sensors determined differences amongst various salts. Further differentiations of the sodium and the potassium salts carried out by astringent and salty taste sensors. Similarly, Rasagiline salts showed much better organoleptic properties compared to Diclofenac salts and only a bitter after taste detected opposite side for hemitartrate and mesylate salts.

Based on the data, the electronic tongue discriminates various taste modalities of Diclofenac free acid and salts of diclofenac and rasagiline and taste matters are thus supposed to be higher for Diclofenac salts than for Diclofenac free acid and Rasagiline salts to develop sublingual formulations.

Based on the physicochemical characterization of diclofenac salts, it was demonstrated that potassium, epolamine and sodium salts of diclofenac showed similar results. Diclofenac-free acid is not suitable for this route of administration as solubility was very less in SSF media. Diclofenac epolamine is generally used in dermal preparations due to its high permeability and low melting point. Even though solubility and taste parameters are satisfactory, even then it is not suitable for the present investigation because of larger molecular size, dose, and anticipated stability-related issues during storage and manufacturability issues due to the low melting point. Hence Diclofenac potassium was finalized choice of salt for further evaluation of sublingual formulation development.

Rasagiline mesylate was found more soluble and stable compared to hemitartrate salt. But being a low dose molecule, the impact of dose on the solubility or dissolution was considered negligible. Moreover, rasagiline hemitartarate is N-1 step for rasagiline mesylate preparation and from the pK_a results of base (7.01), mesylate (7.12), and hemitartrate (7.43) salts at 37°C, it can be assumed that the Rasagiline salt get dissociated and dissolved in biological fluid to Rasagiline base prior the human body takes up the drug. Post uptake, exactly similar Rasagiline molecule is present for the therapeutic effect, independent of the mesylate or hemitartrate origin formulations when administered by oral route in healthy volunteers. Hence hemitartrate salt was selected as an alternate salt form for the development of new sublingual administration.

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