Department of Biopharmaceutics and Pharmacokinetics[1], Faculty of Pharmacy, Ege University, Izmir, Turkey, Research Group on Drug Absorption[2], Department of Engineering, Pharmaceutical Technology Section, Faculty of Pharmacy, Universidad Miguel Hernández, Alicante, Spain; Center For Drug Research & Development and Pharmacokinetic Applications CRO[3], Ege University, Izmir, Turkey

Assessment of fexofenadine hydrochloride permeability and dissolution with an anionic surfactant using Caco-2 cells

E. Gundogdu[1,3], I. Gonzalez Alvarez[2], M. Bermejo Sanz[2], E. Karasulu[1,3]

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Dr. Evren Gundogdu, Department of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmacy, Ege University, Izmir, Turkey
eczaci_evren@hotmail.com

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The purpose of this study was to estimate the effect of the anionic surfactant sodium dodecyl sulphate (SDS) on the permeability and dissolution of fexofenadine hydrochloride (FEX) and the transepithelial electrical resistance (TEER) with Caco-2 cells. The dissolution profile of FEX was evaluated at different pH values (1.2, 3.2, 4.2, 4.5, 5.2 and 6.8) at $37 \pm 0.5\,^{\circ}C$ and chracterized in presence of SDS. The dissolution of FEX was increased in the presence of SDS. For permeability studies, apical to basolateral and basolateral to apical permeability was assesed with various concentrations of FEX (50, 100, 500, 1000 and 5000 μM) and in the presence of SDS. The FEX transport changed with 10 and 50 μM of SDS and the TEER values, after 120 min, decreased. In conclusion, a low and concentration-dependent permeability was found for FEX across the Caco-2 cells. FEX transport increased and TEER decreased with increasing SDS concentrations. These results supports the use of SDS as anionic surfactant in these concentration; SDS can be used safely as permeation and dissolution enhancer for the oral delivery of FEX.

1. Introduction

Fexofenadine hydrochloride (FEX), a second generation non-sedating histamine H_1 receptor antagonist, is an active metabolite of terfenadine. Currently, oral formulations at doses of 60–120 mg/day are available (Lin et. al. 2007; Turker et. al. 2004; Arora et. al. 2002). FEX is essentially unmetabolized by the liver (P450 system). Therefore FEX levels are not affected by drugs that are metabolized by this system, and FEX does not influence the metabolism and levels of these drugs (Susman et al. 1999; Lippert et al. 1995).

Caco-2 cell monolayers are widely used in standard permeability screening assays and for prediction of the oral fraction absorbed because permeability in Caco-2 cell monolayer is well correlated with *in vivo* absorption in humans (Shah et al. 2006). The Caco-2 cell monolayer allows to study the major absorptive mechanisms for drugs, such as passive transcellular and paracellular transport, carrier-mediated influx and efflux mechanisms. This reliable and high-throughput *in vitro* model is also used for screening of drugs, delivery systems, and various excipients and to evaluate their cytotoxicological potential (Artursson et al. 2001; Yamashita et al. 2000; Artursson et al. 1991; Alsenz et al. 2003; Biganzoli et al. 1999; Degim et al. 2004; Hugger et al. 2002; Twiss et al. 1994; Weissenboeck et al. 2004; Foss et al. 2004).

Surfactants are extensively used in pharmaceutical formulations as wetting agents to improve dissolution and absorption of poorly soluble drugs. Low moleculer weight ionic surfactants like SDS, in concentrations that are not toxic to the intestinal mucosa, are probably the most commonly used agents for this purpose (Bhangwant et al. 2002; Davis et al. 1970).

The aim of this study was to evaluate the influence of SDS as anionic surfactant on the FEX transport across the Caco-2 cell system and the dissolution of FEX. Although surfactants can be considered as enhancers to increase intestinal absorption, a major limiting factor can be their potential toxicity to the intestinal mucosa. Thus, in selecting a suitable surfactant for a given formulation, it is important to investigate both the enhancing effect of the surfactant as well as its possible adverse effects on biological barriers. Additionally, the concentration dependence of sodium dodecyl sulphate on FEX intestinal permeability was investigated by monitoring transepithelial electrical resistance (TEER) and quantifying the permeability of FEX across the Caco-2 cell monolayers.

2. Investigations, results and discussion

2.1. Dissolution studies

FEX contains a basic amine and acidic carboxylic acid group (two pK_a values 4.2 and 9.5) (Olse et al. 2006). As a result, the aqueous solubility is pH-dependent. Fig. 1 illustrates the results from the dissolution studies over a period of 180 min. FEX dissolution was considerably slow and incomplete in pH=1.2 due to the salting out effect of the chloride ion. The dissolution of FEX was better at pH 3.2 than at the other pH values as shown in Fig. 2. At pH 3.2 the dissolution and the final concentration were the highest. The F_2 values are shown in Table 1 for all pH values. According to guidance documents (FDA Guidance for Industry 2000), two dissolution profiles are considered similar

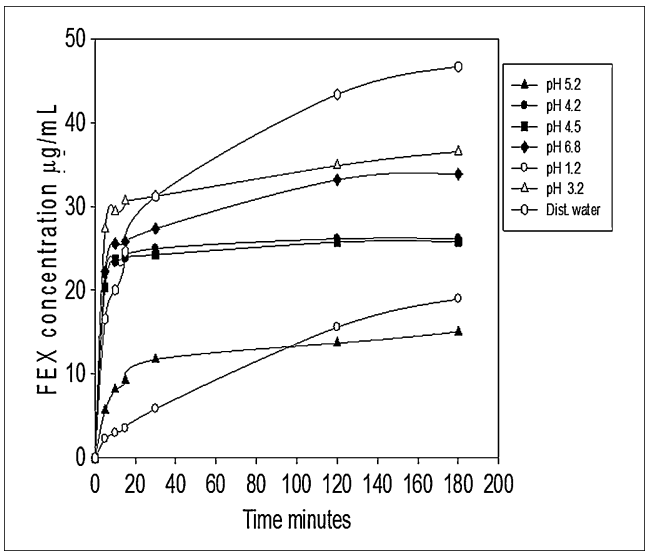

Fig. 1: Dissolution profiles of FEX at different pH values

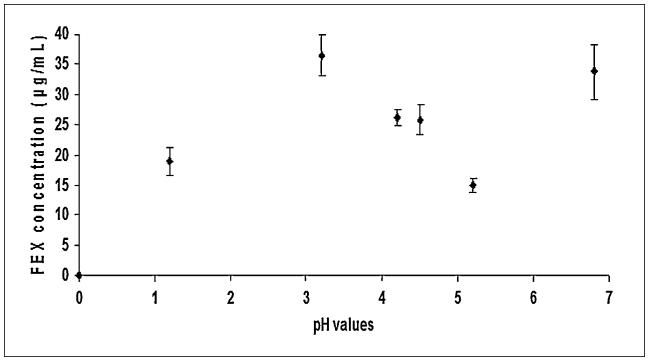

Fig. 2: Comparison of FEX final concentration at different pH values

when the *F*2 value is between 50 and 100. None of the dissolution profiles were similar to the dissolution profile obtained in distilled water confirming the influence of the pH of the medium in the drug dissolution process.

2.2. Effect of SDS on FEX dissolution

The dissolution of FEX was improved using two different concentrations of SDS. Fig. 3 illustrates the dissolution profile of FEX in water and in the presence of two concentrations of SDS. SDS increased the dissolution of FEX in the medium as depicted in Fig. 3. At $37 \pm 0.5\,^{\circ}C$, SDS provided about 1.5- and 2-fold increments in the final concentration of FEX. The improvement of drug solubility by SDS can be explained by two possible mechanisms: improvement of wetting characteristics and micellar solubilization of the drug (Leuner et al. 2000; Damian et al.

Table 1: F_2 values calculated to compare the disolution profile in distilled water versus the different pH values

pH value	F_2 value
1.2	10.17
3.2	32.11
4.2	25.20
4.5	24.67
5.2	11.54
6.8	34.00

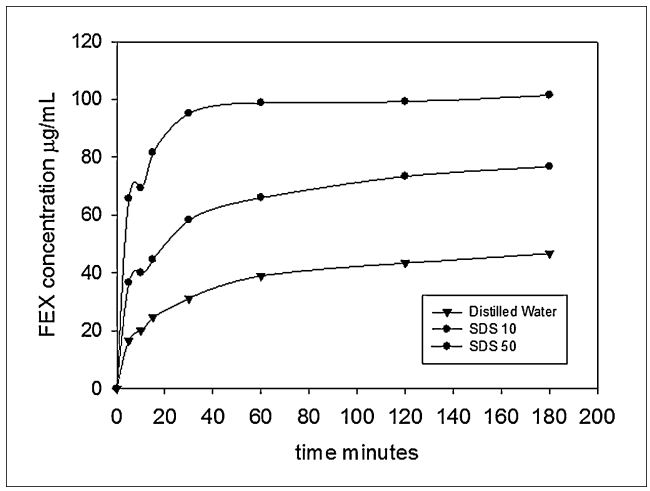

Fig. 3: Dissolution profiles of FEX in distilled water and in the presence of 10 mg and 50 mg of SDS

Table 2: F_2 values to compare the dissolution of FEX in the absence and in the presence of two different amounts of SDS

Amount of SDS (mg)	F_2 value
10	36.11
50	23.88

2000). In this case, the effect of SDS is the wetting improvement as the concentrations of SDS used (140 and 700 µM) were below the critical micellar concentration (8 mM) (Jain et al. 2004). The F_2 values are shown in Table 2 for both SDS concentrations. According to F_2 values, the dissolution profiles of FEX were different at both SDS concentrations when compared with distilled water.

2.3. Permeability of FEX

Figs. 4 and 5 show the transport of FEX across the Caco-2 cell monolayers. For the concentration range investigated (50, 100, 500, 100, 5000 µM) the P_{ab} (the permeability value from apical to basolateral direction) was low. However, the P_{ba} (the permeability values from basolateral to apical direction) was slightly higher than the P_{ab} and both of them were concentration dependent. When the concentration of FEX increased, the the P_{ab}

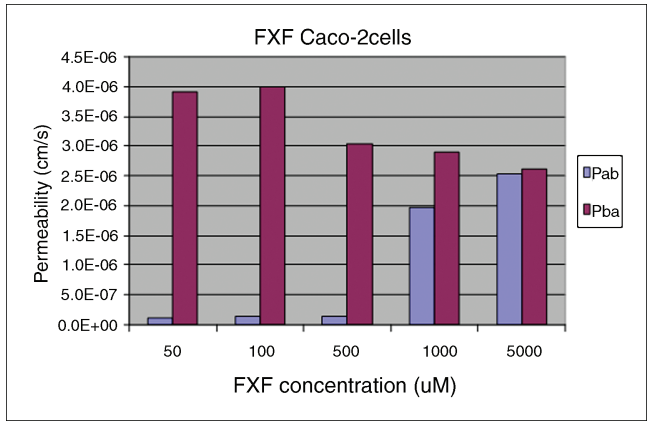

Fig. 4: Permeability values of FEX from basolateral direction to apical direction (P_{ba}) and vice versa (P_{ab}) at different initial concentrations in the donor compartment

Table 3: Permeability value of FEX with different concentrations (± SD)

FEX Concentration (μM)	P_{ab} (cm/s)	P_{ba} (cm/s)	P_{ba}/P_{ab} ratio
50	1.03E-07 ± 1.65E-08	3.91E-06 ± 2.77E-07	38.06
100	1.31E-07 ± 8.38E-08	3.98E-06 ± 1.61E-07	30.30
500	1.41E-07 ± 7.57E-09	3.03E-06 ± 3.48E-07	21.52
1000	19.7E-07 ± 2.34E-08	2.39E-06 ± 1.77E-07	1.48
5000	25.53E-07 ± 2.48E-08	02.61E-06 ± 1.33E-07	1.02
50 + 10SDS	1.35E-07 ± 4.01E-08	35.28E-07 ± 1.39E-07	26.22
50 + 50SDS	170.80E-07 ± 0.18E-07	140.33E-07 ± 0.14E-07	0.82

value increased for from apical to basolateral, this is consistent with the contribution of a secretion transporter and FEX has been demonstrated to be a P-glycoprotein substrate (Shimizu et al. 2006). Additionally, it can be observed that the P_{ba} (from basolateral to apical direction) significantly decreased with the increase of FEX concentration supporting the hypothesis of the contribution of the efflux trasnporter. The permeability values of FEX for both directions and the Pba to Pab ratios are shown in Table 3. At low FEX concentrations the Pba/Pab ratios were higher than 5 confirming the presence of an efflux transporter, while at higher FEX concentrations the carrier is saturated and the ratios become 1.

2.4. Estimation of FEX transport parameters and comparison with other laboratory results

FEX permeability values decreased as concentration was raised in the basal to apical direction while apical to basal permeabilities increased with higher concentrations. This fact is consistent with the presence of a saturable secretion component, as P_{gp}. Model equations (1) – (2) including the passive diffusion component and the Michaelis-Menten component were fitted to the whole dataset of apical to basal and basal to apical permeabilities in order to estimate the passive component and Vm and Km.

$$Peff = Pdif - \frac{Vm}{Km + C} \quad (1,\ \text{apical to basal}) \tag{1}$$

$$Peff = Pdif + \frac{Vm}{Km + C} \quad (2,\ \text{basal to apical}) \tag{2}$$

where P_{eff} is the experimental permeability value, P_{dif} the passive diffusion component and Vm and Km the Michaelis-Menten parameters.

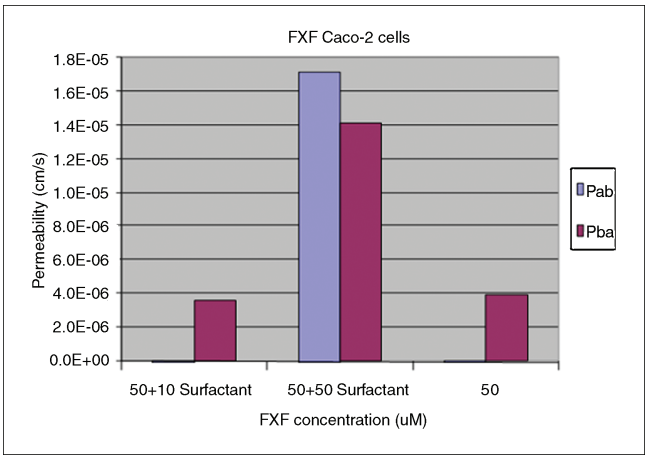

Fig. 5: Permeability values of FEX at 50 μM without additives and in the presence of SDS at 10 and 50 μM

A second model was explored with Eqs. (3) and (4):

$$Peff = Pdif - \frac{Vm}{Km + C} \quad (3,\ \text{apical to basal}) \tag{3}$$

$$Peff = Pdif + \frac{Vm}{A * Km + C} \quad (4,\ \text{basal to apical}) \tag{4}$$

in which "A" is a correction factor that was included to take into account that the binding site of the secretion carrier is located inside the cells so it actually "sees" a different concentration than the donor chamber one. The factor helps to explain why the permeability versus concentration evolution is not symmetrical around P_{dif} in both directions. The change in apparent permeability is more evident in the basal to apical direction because the basolateral membrane represents a lower resistance and when the drug is applied in the basolateral chamber the extra cellular and intra cellular concentrations are more similar than when the drug is placed in the apical chamber. The experimental and predicted permeability values versus the assayed concentrations are shown in Fig. 6 (Gonzalez Alvarez et al. 2008). Fitting procedures were performed with Phoenix WinNonlin Software (Node-Academic Research license to the University Miguel Hernández).

The parameters obtained with both kinetc models are summarized in Table 4. In order to compare both models the sum of squared residuals SSR and AIC values where tabulated.

The residual variances from both fits (sum of squared/degree of freedom) where compared using the Snedecor's F test with an alpha value of 0.05 with Eq. (5):

$$F_{calc} = \frac{(SSR_1 - SSR_2)/(df_1 - df_2)}{SSR_2/df_2} \tag{5}$$

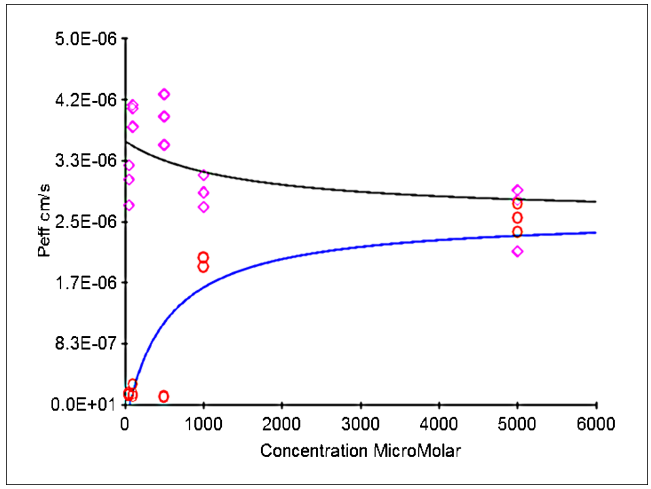

Fig. 6: Apical to basal (circles) and basal to apical (diamonds) permeability values of FEX in each transwell and fitted lines to a model of combined diffusion and Michaelis-Menten transport componetnt with factor correction A

Table 4: Parameters of the fit of the models with passive component an Michaelis–Menten component and goodness of fit indexes (SSR: sum of squared residuals and AIC: Akaike's information criteria)

Parameter	Value	SD	CV%
Pdif cm/s	2.24E-06	8.79E-08	3.92
Km μM	681.5	260.56	38.23
Vm nMol·cm-2·s-1	0.00132	0.000405	30.69
SSR	1.30E-11		
AIC	−1046		
Parameter	Value	SD	CV%
Pdif cm/s	2.57E-06	1.23E-07	4.77
Km μM	513.91	160.38	31.21
Vm nMol·cm-2·s-1	0.001478	0.000391	26.48
A	2.79	0.86	30.82
SSR	9.10E-12		
AIC	−1059		

where SSR_1 is the sum of squared residual of the simplest models and SSR_2 is the sum of squared residuals of the more complex model and df are the degrees of freedom of the fit (number of data points minus number of parameter estimated).

The F calculated was higher than the F tabulated indicating the statistical significance of the more complex model, so the inlcusion of the parameter "A" improves the fit to the experimental data.

In Fig. 7, a comparison of the FEX permeability values obtained in Caco-2 cells in our laboratory and by Petri et al. (2004) is depicted. Slight differences in magnitude could be explained by the different expresion level of the transporters in the cells. Petri el al. (2004) reported for FEX a Vm of 5.21 nmol cm^{-2} s^{-1} and a Km value of 150 μM. That could be an indication of a higher expresion level of P-gp in their cell line (reflected in a higher maximal velocity). It is relevant to characterize the contribution of each transport mechanism in our system (passive 2.5e–6 cm/s versus active 2.4e-6 cm/s (Vm/Km)) in order to establish a hypothesis about the mechanism by which SDS increased FEX permeability in both directions. A inhibition effect over the P-gp transporter would lead to an increase in FEX concentration up to the diffusional value (i.e. 2.5 e–6 cm/s) while as it is shown in the next section, the increase exerted by SDS is much more pronnounced.

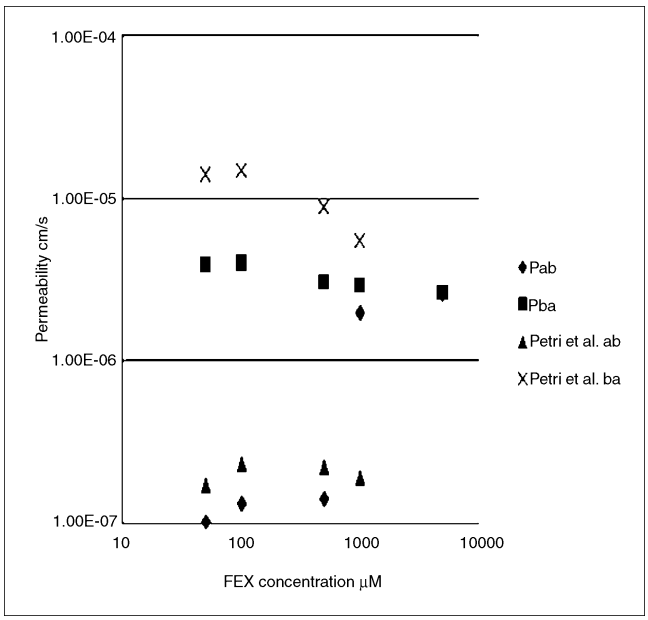

Fig. 7: Comparison of the permeability values of FEX obtained in the present work and the ones reported by Petri at al (28) obtained in Caco-2 cells

2.5. *Effect of SDS on permeability of FEX*

The effect of SDS on the permeability of FEX was investigated. For this study, two different concentations (10 μM and 50 μM) of SDS were used. Fig. 5 shows the effect of SDS on the permeability value of FEX across the Caco-2 cells. The permeability coefficient value of FEX results for both directions are shown Table 3. The permeation of FEX was increased by the presence of SDS. When SDS concentration raised up 10 to 50 μM, the permeability of FEX from apical to basolateral and basolateral to apical direction was significantly increased. As a result, SDS enhanced the permeability of FEX in a dose dependent manner for both side directions. As has been shown in the previous section even if an inhibition of the efflux system cannot be ruled out, the increase in the permeability value has to be explained by another mechanism that could be the enhancement of the paracellular permeation as well as the fluidification of the lipid bilayer.

2.6. *Effect of SDS on TEER value*

SDS is an ionic surfactant that has shown to have an immediate effect on paracellular permeability of Caco-2 cells by decreasing the TEER values, increasing intracellular calcium levels and opening tight junctions (Deli et al. 2008; Anderberg et al. 1992, 1993).

Figs. 8–10 illustrate the TEER value induced by 2 h of SDS at the concentration of 10 and 50 μM with 50 μM FEX. TEER reduction increased when the SDS concentration increased from 10 to 50 μM. It is also interesting to observe that the changing of TEER values upon exposing the cell monolayer to SDS for 2 h showed a linear relationship with permeability values of FEX (P_{ab} and P_{ba}) (Table 5). These results show that the reduction of TEER values can be an important indicator to

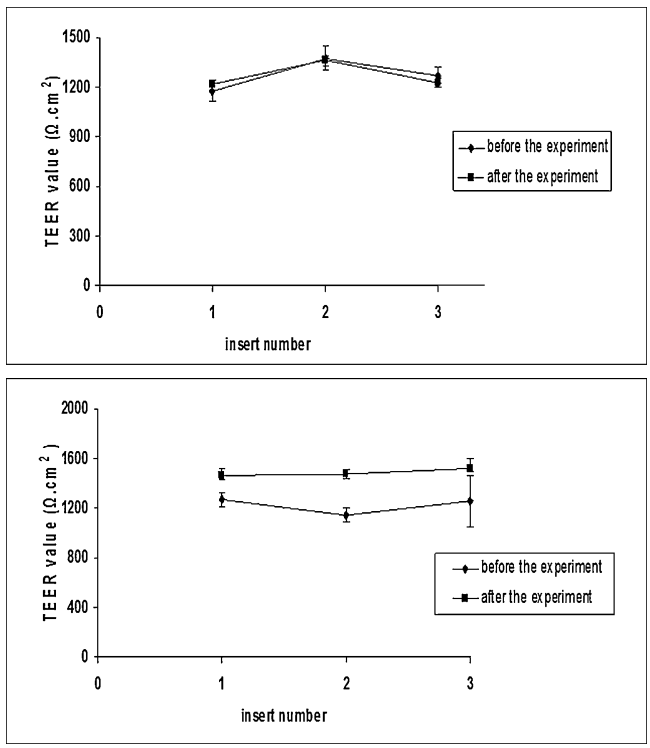

Fig. 8: TEER values of 50 μM FEX from apical to basolateral and opposite direction (mean ± SD)

Table 5: Relationship between the TEER value changing and permeability coefficient of FEX across Caco-2 cell monolayer with SDS (± SD)

FEX and SDS concentration (μM)	P_{ab} (cm/s) (± SD)	%teer change (± SD)	P_{ba} (cm/s) (± SD)	%teer change (± SD)
50 μM FEX	1.03E-07 ± 1.65E-08	2.86 ± 1.59	3.91E-06 ± 2.77E-07	2.54 ± 3.43
50 μM FEX+10 μM SDS	1.3E-07 ± 4.0E-08	6.07 ± 1.37	35.2E-07 ± 1.4E-07	14.62 ± 4.64
50 μM FEX+50 μM SDS	170.8E-07 ± 0.2E-07	12.43 ± 2.45	140.3E-07 ± 0.1E-07	16.51 ± 2.35

predict the permeation-enhancing effect of SDS. The decrease of TEER values is known to specifically indicate the potency of increasing drug permeability. SDS seems to affect the tight junctions and thus more effectively enhance the permeability of FEX. Nevertheless even if a decrease in TEER values was observed in the experiments, the monolayer were not disrupted as the TEER value was above the accepted limit to control monolayer integrity.

2.7. Conclusion

FEX solubility and dissolution are pH dependent as FEX is an ionizable drug with two pKa values. FEX has a low intestinal permeability *in vitro* in Caco-2 cells and the transport of the drug is concentration dependent due to the involvement of P-gp. Nevertheless, the drug also permeates through the paracellular pathway as it has been shown that SDS is able to increase FEX permeability by relaxing the tight junctions and thus decreasing TEER values, while keeping the monolayer integrity at the concentrations assayed. The inhibition of the efflux transporter by the surfactant cannot be ruled out but it is not the only mechanism of enhancement of SDS. On the other hand, the surfactant is also able to increase the drug dissolution rate thanks to the wetting effect. These results have to be considered when designing FEX formulations as the surfactant is able to affect both drug solubility and membrane permeability so eventually an enhanced bioavailability could be obtained with SDS-containing formulations.

3. Experimental

3.1. Materials

SDS was purchased from Sigma Chemical Co. (St. Louis, MO, USA). FEX was a gift from Basel Drug Company (Istanbul, Turkiye). Cell culture reagents and supplies were obtained from GIBCO Invitrogen Co. (United Kingdom).

3.2. Dissolution studies

The dissolution profiles of FEX were determined at six different pH values (pH= 1.2, 3.2, 4.2, 4.5, 5.2, and 6.8). FEX (10 mg) was added in conical flasks which contained 250 ml of the medium studied. Each experiment was performed in triplicate, at least. The flasks were placed in a thermostated water bath at 37.0 ± 0.5 °C agitated at 50 rpm. The samples of 100 μL were taken at certain times (5, 10, 15, 30, 45, 90, 120 and 180 min) and analysed by HPLC. In order to compare the dissolution profiles, F_2 values (Eq. 6) with the percent of drug dissolved at each time point were calculated taking as reference the dissolution profile in distilled water for each pH values.

$$f_2 = 50 + \log\{[1 + (1/n)\sum_{t=1} * n(R_t - T_t)^2]^{-0.5} * 100\} \quad (6)$$

Rt and Tt are the cumulative percentage dissolved at each of the selected n time points of the reference and test product respectively (FDA Guidance for Industry 2000).

3.3. Effect of SDS on FEX dissolution

The effect of SDS on FEX dissolution was determined at two concentrations of SDS. Firstly, 10 mg of SDS were dissolved in 100 mL of distilled water at 37 ± 0.5 °C, then 10 mg of FEX were added to this solution. For the other

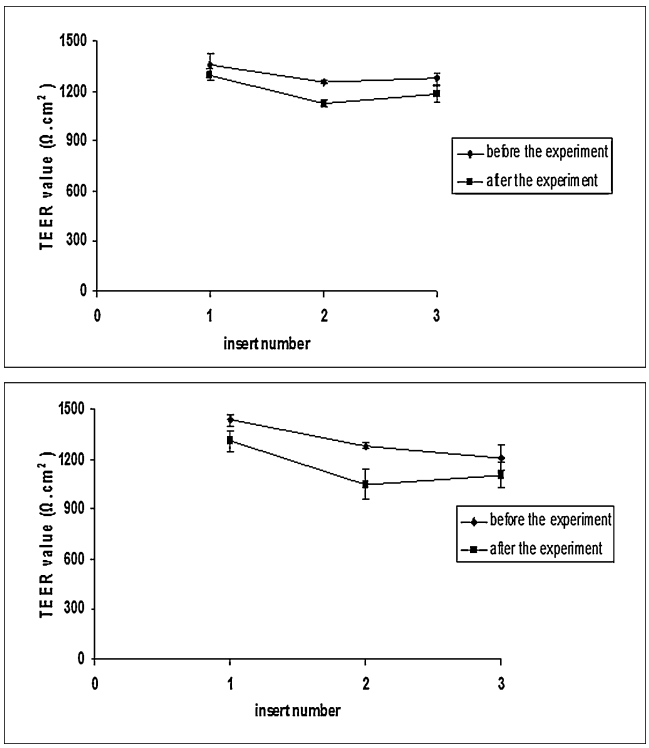

Fig. 9: TEER values of 50 μM FEX with 10 μM SDS from apical to basolateral and opposite direction (mean ± SD)

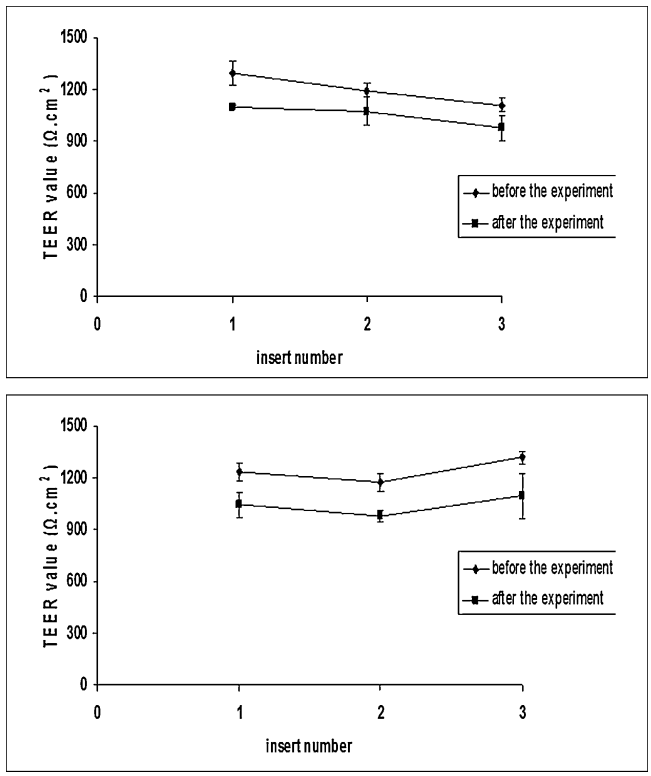

Fig. 10: TEER values of 50 μM FEX with 50 μM SDS from apical to basolateral and opposite direction

SDS concentration, the same process was applied with 50 mg of SDS. The samples of 100 μL were taken at certain times (5, 10, 15, 30, 45, 90, 120 and 180 min) and analysed by HPLC. The dissolution profile of FEX with both SDS concentrations were compared versus the dissolution profile of FEX in distilled water using F_2 values.

3.4. Caco-2 cell cultures

The colonic adenocarcinoma cell line, Caco22, was obtained from the American Type Culture Collection. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM). Cell monolayers were prepared by seeding 4×10^5 cells/well on a 6 wells Transwell insert filter. Cell culture was maintained at 37 °C under 90% humidity and 5% CO_2. Monolayers were used 19–22 days after seeding. The integrity of each cell monolayer was checked by measuring its transepithelial electrical resistance (TEER) with an epithelial voltohmmeter (EVOM, World Precision Instrument, Sarasota, FL, USA) before and after the experiments.

3.5. Permeability studies

The *in vitro* permeability study was developed in Caco-2 cell monolayers grown in transwell inserts with a collogen coated polycarbonate membranes with a pore size of 0.4 μm and a surface area of 4.7 cm^2 in cluster. The cells were maintained at 37 °C in an atmosphere as described above. The medium was replaced every second day for 3 weeks.

Permeability studies were performed in both side directions, from apical to basolateral (A to B) and basolateral to apical (B to A). After washing the Caco-2 cell monolayer twice with prewarmed HBSS medium (pH 7.4), the transport experiments were done by adding the FEX solutions with different concentrations (50, 100, 500, 1000, 5000 μM) to either the apical (AP, 0.5 L) or basolateral side (BL, 1.5 L) while the receiving chamber contained the corresponding volume of transport medium. After shaking at 50 pm for 1 h at 37 °C in a water bath, samples were collected from both sides of Caco-2 cell monolayer and immediately frozen, lyophilized and preserved below −20 °C for subsequent HPLC analysis.

When SDS was used in the transport experiments, it was placed in both sides of the cell monolayer. Four serial samples of 200 μL each were taken at 30 min intervals in receiver side and two more samples in the donor side at the begining and at the end of the experiment.

In order to study the effects of SDS, FEX permeability was determined in the presence at two different concentrations of SDS (10 and 50 μM).

Apparent permeability values (P_{app}) for each substance were calculated according to Eq. (7):

$$Papp = \frac{dQ}{dt}\frac{1}{ACo60} \tag{7}$$

where P_{app} is the apparent permeability (cm/s), dQ/dt is the permeability rate, A is the diffusion area of monolayers (cm^2), and C_0 is the initial concentration of the drug in the donor compartment (Lin et al. 2007).

3.6. Effect of SDS on TEER value during FEX permeability studies

The transport medium was HBSS containing 5 mL HEPES (pH = 4). In general, the measurement of TEER values was performed as follows: Prior to starting the experiments, fully differentiated cell monolayers were washed twice with pre-warmed HBSS/Hepes pH = 7.4 and the cells were equilibrated under the pH conditions of the experiment for 30 min at 37 °C.

The integrity of the Caco-2 cell monolayers was ensured by TEER measurements before and after the FEX permeability experiments by Millipore® voltohmmeter with various concentrations of SDS (10 and 50 μM) and 50 μM FEX for 2 h.

3.7. HPLC analysis of the samples

The samples were analysed by HPLC with fluorescence detection (excitation λ = 220 nm, emission λ = 290 nm) using a mobile phase (v/v) 60:40 acetonitrile:potassium dihydrogen phosphate buffer (pH=3.5) at a flow rate of 0.8 mL/min and the injection volume was 50 μL. A novapack C_{18} (Waters®) cartrigde-type column was used. The method was validated in the range of assayed concentrations and the accuracy and precision were adequate (less than 5% of relative error and less than 10% of coefficient of variation).

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