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Exploring the correlation between ibuprofen solubility and permeability in intestinal disease conditions

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ABSTRACT

In this study, physicochemical properties of Ibuprofen were evaluated using HPLC-DAD system to examine the effect of pH on the solubility and intestinal permeability of Ibuprofen. Ibuprofen is still one of the most used and safest non-selective NSAIDs among those approved. Ibuprofen solubility is dramatically affected by pH changes. There is not enough data to show if pH dependent water solubility of ibuprofen will affect its absorption/bioavailability. For this purpose, lipophilicity parameters (logkw) of ibuprofen were determined at various pH ranges using HPLC-DAD system and the results were compared with in-vivo intestinal permeability results obtained where a perfusion medium having different pH values used (pH 3.9, 4.9 and pH 7.4). The results confirmed that the acidic pH of the perfusion medium increased the permeability of ibuprofen,

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but in basic pH values (pH 7.4) the permeability was relatively lower. The permeability results were in correlation with determined lipophilicity.

Keywords: Ibuprofen, intestinal permeability, HPLC diffusion coefficient, acid dissociation constant, drug absorption

INTRODUCTION

Nonsteroidal anti-inflammatory drugs [NSAIDs] are a drug class FDA-approved for use as antipyretic, anti-inflammatory, and analgesic agents. These effects make NSAIDs useful for the treatment of muscle pain, dysmenorrhea, arthritic conditions, pyrexia, gout, migraines, and used as opioid-sparing agents in certain acute trauma cases[1,2]. Ibuprofen (IBU) is still one of the most commonly used and safest non-selective NSAIDs among others, and its use for various diseases beyond its primary purpose has been reported[3-6]. These are necrosis, bronchopulmonary dysplasia, cardiovascular diseases, neurodegenerative diseases and cancer prevention[7-11]. The other interesting thing about IBU is its common usage as an OTC medicine[9,12]. However, some studies show that IBU is not safe when it is used over-dose or in regular daily usage[9,13-16]. A basic search on Rxlist [www.rxlist.com] shows that there are more than fifty different formulations of IBU marketed in the USA and it is easy to reach as an OTC for a wide population. The chemical structure of IBU is shown in Figure 1.

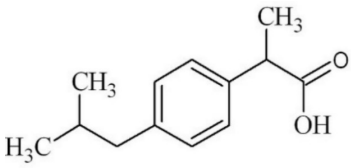

Figure 1. Chemical structure of Ibuprofen

A study published in 2005 by Savolainen et al. about the brain delivery of IBU presents that IBU concentration in both plasma and brain reached a steady state within 6 h. This study proves that limited brain penetration prevents the possible usage of IBU in treating or preventing neurodegenerative disorders such as Alzheimer's disease[17]. Mainly, these studies concentrated on the clinical aspects of NSAID use and its impact on gastrointestinal complications.

The main focus of our current study is to evaluate the use of an NSAID in cases involving certain gastrointestinal complications. These complications include conditions such as inflammatory bowel diseases. This evaluation is essential because the pH level in the bowels undergoes an acidic shift under such cir-

cumstances. This shift in pH can potentially result in unexpected pharmacokinetic profiles for IBU. These unexpected profiles may occur due to changes in the permeability of this weakly acidic drug. Essentially, the intraluminal pH of the gastrointestinal tract is variable. In healthy subjects, it is from highly acidic pH in the stomach (pH 1.5 – 3.5) to about pH 6.0 in the duodenum. The pH gradually increases in the small intestine from pH 6.0 to about pH 7.4 in the terminal ileum. Although the pH drops to 5.7 in the caecum, it gradually increases, reaching a pH 6.7 in the rectum[18]. However, as mentioned, intraluminal pH may vary in a disease state. For example, very low intraluminal colonic pH was reported for ulcerative colitis (pH 3.0 – 4.5) which is a form of inflammatory bowel disease[19]. The pKa value for IBU is 4.9 and in the disease conditions where the pH of the column is dramatically lower in comparison to the normal conditions, the solubility of the IBU is affected. When the solubility is affected, we assume that the permeability, and by that way, the pharmacokinetic profile will also be changed.

As a group working on pharmacokinetics and analytical method development, we primarily focused on the changes in the intestinal permeability of IBU in a case of a gastrointestinal complication. In this paper, an intestinal permeability study was performed in two different conditions and one of them refers to the healthy intestine [pH: 7.4 and 5.9] and the other [pH 3.9] refers the inflammatory bowel disease. pH 5.9 and pH 3.9 also refer the pKa ± 1 for IBU (pKa 4.9). To prove the dramatic solubility changes of IBU [a weakly acidic drug] in various pH, an *in vitro* experimental approach was designed and Log kw [20] for IBU was determined for pH 4.5, 5.5, 6.5 and 7.5. The results obtained from *in vitro* Log kw studies for the solubility of IBU depending on pH of the medium were compared with the results of the intestinal permeability of IBU while simulating a disease condition changing the pH of the colon.

METHODOLOGY

Chemicals

IBU, Metoprolol tartrate (MET), Phenol red (PR) was supplied from Sigma Aldrich (St. Louis, MO, USA). Sodium phosphatedibasic (NaH_2PO_4) and NaOH were from Merck (Darmstadt, Germany), disodium monohydrogenphosphate (Na_2HPO_4), potassium chloride (KCl), Sodium hydroxide (NaCl), Sodium Sulfate (Na_2SO_4), Sodium bi carbonate ($NaHCO_3$), Mannitol, Acetonitrile (ACN), Methanol (MeOH), ortho phosphoric acid (purity > 99%). 1-octanol was obtained from Sigma Aldrich. HPLC grade water was purchased from Carlo Erba (France) and it was used for the preparation of standard solutions and buffers. All solutions were of analytical grade.

Instrumentation

The chromatographic separation of all samples was carried out using an HPLC system (Shimadzu, Nexera–*i*, LC–2040C 3D Model, JAPAN) that was coupled to a Shimadzu Nexera–*i* 2040C 3D Model (Model, JAPAN) UV/DAD detector. Chromatographic separations were carried out on a C18 (150 mm 4.6 mm, 2.7 μm particle size) column (Restek Raptor ™) for *logkw* studies and C18 (250 mm x 460 mm, 5 μm particle size) column (Supelco) with a pH range of 3-6.50 and isocratic separation with a mobile phase consisting of 20 mM phosphate buffer solution (PBS): ACN (55:45, v/v) at a flow rate of 1 mL min^{-1}. This pH range is also used for the assessment of IBU's pKa value and permeability studies. The wavelength of the detector was set to 220 nm and 254 nm, and the retention times were determined automatically by an online computer running Shimadzu LabSolution software. The injection volume was 10 μL. The delivery of the perfusion medium to the jejunum was accomplished using a peristaltic pump. The perfusion medium was administered to the jejunum with the help of a Gilson Minipuls 3 peristaltic pump (USA).

Preparation of the standard stock solutions, buffer solutions, mobile phases and perfusion medium

Standard stock solution of Ibuprofen, metoprolol tartrate and phenol red (1000 μg mL^{-1} in MeOH)

Standard stock solution was prepared by dissolving 25 mg of IBU, 25 mg of PR and 25 mg MET in 20 mL volumetric flasks. All solutions were stored at 4°C in the fridge during experiments. Each standard was prepared in 75% (v/v) MeOH for IBU, MeOH for PR and Milli Q water for MET, respectively.

20 mM Phosphate buffer solution (PBS)

PBS was prepared by dissolving 2.84 g of Na_2HPO_4 in approximately 800 mL water and then increasing the volume to 1000 mL with water once the disodium Na_2HPO_4 solution was completely dissolved.

20 mM PBS:MeOH (from 60:40 v/v to 30:70 v/v) solutions (pH 4.50 –7.50) for log kw determination using HPLC

PBS and MeOH in a range between 60:40 v/v and 30:70 v/v were mixed to reach 500 mL final volume. The pH of the mobile phases (60:40 v/v and 30:70 v/v) was arranged to 4.5, 5.5, 6.5 and 7.5.

Perfusion medium

The perfusion medium contains 25 mM NaCl, 10 mM KCl, 40 mM Na_2SO_4, 20 mM $NaHCO_3$ and 80 mM mannitol. O-phosphoric acid was utilized to modify the pH of the buffer depending on the process. Perfusion medium was made fresh and filtered through a 0.22 μm membrane filter before being used in the experiment.

Procedures

Method optimization

MET which is used as reference standard and PR (zero permeability marker) are commonly used compounds in Single Pass Intestinal Perfusion (SPIP) technique. For this purpose, IBU, MT and PR mixture solution were used for optimization studies. After some preliminary studies, the mobile phase decided to be as (pH 6.61 10 mM Na2HPO4:ACN)/ (45/55,v/v). Flow rate was set at 0.8 ml min^{-1} and wavelength at 220 nm. Column temperature was adjusted 25°C and injection volume was 10 μL. under these conditions, the obtained chromatogram of IBU, MET and PR is shown in Figure 2.

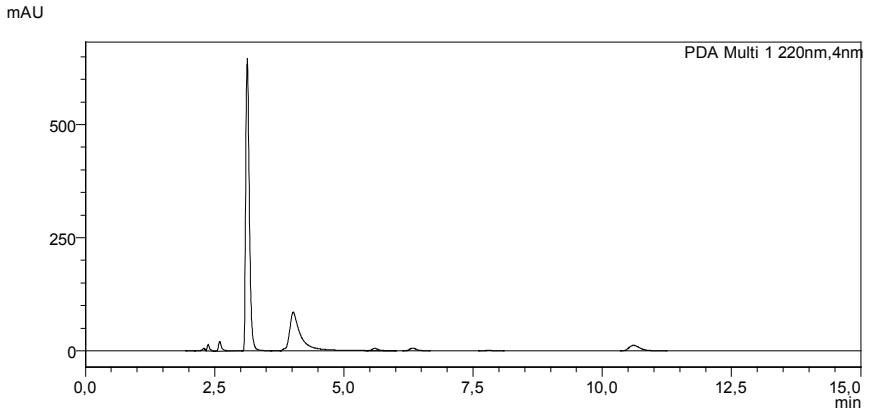

Figure 2. Representative chromatogram of PR, MT and IBU using HPLC-UV/DAD system

System suitability testing (SST) was investigated including resolution (R_s), tailing factor (T), capacity factor (*k'*), asymmetry factor (A_s), selectivity (α) and theoretical plate number (N) to decide optimal conditions for HPLC-UV/DAD method. Obtained SST results was shown in Table 1.

Table 1. System Suitability Testing results of applied method

System Suitability Testing				
Parameter	**IBU**	**MTP**	**PR**	**Recommended value**
Retention time (min)	10.727	3.977	3.149	-
Theorical plate number (N)	5114.1	2875.7	4174.8	N > 2000
Tailing factor (T)	1.406	1.92	1,478	T < 2
Asymmetry factor (A_s)	1.04	1.11	1	0.95 < As <1.2
Capacity factor (k')	3.732	0.754	0.389	k > 2
Resolution (R_s)	18.08	3.509	6.13	Rs > 2
Selectivity (α)	4.94	1.94		α > 1

Method validation

Applied HPLC-UV method was validated according to the International Conference on Harmonization guidelines ICH Q2(R1) to evaluate the quality of the analytical method [21] and also the method was validated for the linearity, LOD, LOQ, accuracy, precision, specificity and robustness. LabSolution software (Shimadzu Corporation) was used to monitor all data and integrated all of the chromatograms. All results were given as mean ± standard deviation for three replicates (n=3) of the samples. MS Excel 2007 was used for data analysis (Microsoft Corporation, USA) for data analysis.

Linearity, LOD and LOQ

The linearity of the IBU, MET and PR was evaluate using nine-point calibration point for each compound within the range of 0.5-80 μg mL^{-1}, 4.0-160 μg mL^{-1}, 7.0-210 μg mL^{-1} respectively. Three calibration sets were prepared and tested in triplicate for each compound. The obtained regression equation values are showed that good linear relationship has been achieved. Linearity, LOD and LOQ values are shown in Table 2. LOD and LOQ were determined using the 3.3 and 10 standard deviations (SD) of the achieved detector response (σ) to slope of the calibration curve (m), respectively. "σ" was calculated using the standard deviation of regression lines' y-intercepts.

Table 2. Linearity, range, LOD and LOQ results

Parameter	IBU	MTP	PR
Equation	y =25890x-4961.8	y =19562x-30960	y =43257x-83916
SE of intercept	184.972	496.366	2630.199
SE of slope	22.704	138.870	164.852
R^2	1.0000	0.9998	0.9996
Range (μg mL^{-1})	1-75	4-160	7-210
LOD (μg mL^{-1})	0.041	0.084	0.348
LOQ (μg mL^{-1})	0.124	0.439	1.053

Accuracy and precision

The method's accuracy was assessed using a recovery test, which compares the theoretical concentration of the chemicals with the experimental concentration. Three different concentrations of IBU (2, 200 and 50 μg mL^{-1}), MET (4, 20 and 80 μg mL^{-1}) and PR (7, 35 and 140 μg mL^{-1}) samples were prepared, and each was analyzed triplicate in same day. Recovery was calculated from the Eq (1).

$$Recovery\ (\%) = (Observed\ amount) - (original\ amount) / (Spiked\ amount) \times 100 \quad Eq\ (1)$$

To show precision of the applied method *intra-day* and *inter-day* variability studies were investigated. The precision data was shown with relative standard deviation (RSD%) which is calculated by Eq (2).

$$RSD\% = SD / Mean \times 100 \quad Eq\ (2)$$

All solutions were injected to the HPLC-UV/DAD system three times on the same day and, three consecutive days. According to the recovery results, the method was found accurate for IBU (98.29-104.17%), MET (97.27-119.85%), PR (98.52-117.70%). Results showed that a good accuracy and precision values are achieved (Table 3).

Table 3. Accuracy and precision results

Compound	Main value (µg mL^{-1})	*intra-day*			*inter-day*		
		Found value (µg mL^{-1})[a]	Precision (RSD %)[b]	Accuracy (recovery %)[c]	Found value (µg mL^{-1})[a]	Precision (RSD %)[b]	Accuracy (recovery %)[c]
IBU	2	2.088 ± 0,008	0.663	104.407	2.083 ± 0.003	0.278	104.17
	10	9.807 ± 0,004	0.076	98.067	9.829 ± 0.011	0.197	98.29
	50	50.202 ± 0,001	0.005	100.403	50.284 ± 0.042	0.145	100.56
MET	4	4.779 ± 0,006	0.234	119.486	4.794 ± 0.083	2.987	119.85
	20	19.427 ± 0,019	0.168	97.136	19.455 ± 0.018	0.157	97.27
	80	81.362 ± 0,608	1.294	101.703	81.159 ± 0.102	0.217	101.44
PR	7	8.222 ± 0,012	0.251	117.457	8.239 ± 0.010	0.211	117.70
	35	34.412 ± 0,061	0.309	98.319	34.482 ± 0.033	0.202	98.52
	140	137.575 ± 0,042	0.053	98.268	138.114 ± 0.223	0.343	98.65

[a] Mean ± Standard Error, [b] RSS, Relative Standard Deviation, [c] Recovery % = [(Observed amount) – (original amount) /Spiked)/] × 100, (n=6).

pKa determination of IBU

Standard stock solution of IBU was prepared as 10.0 µg mL^{-1} s for pKa determination with HPLC at the range of pH 3.00 – 6.50. Based on pH and capacity factor (k') of IBU, a sigmoidal curve was produced. The sigmoidal relationship was used to determine the pKa of IBU.

pKa determination of Ibuprofen using HPLC

Standard solution of 10.0 µg mL^{-1} IBU was investigated in different pH values (3.00, 3.50, 3.85, 4.20, 4.50, 4.85, 5.20, 5.50, 5.85, 6.20 and 6.50) with containing 20 mM PBS: ACN (55:45 v/v) mobile phase. Uracil was used to show dead volume in the analysis. Retention time of IBU was changed by changing pH of the PBS and obtained chromatograms of IBU is given in Figure 3.

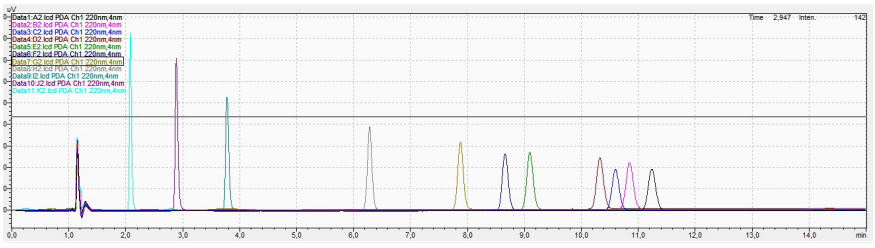

Figure 3. Obtained overlaid chromatograms using HPLC in different mobile phase pH

The data measured from sigmoidal curve for pKa determination were found using derivative of the graphic equation. A sigmoidal curve was constructed between the pH of the solutions and capacity factor (k') of IBU. The pKa of IBU was determined according to the sigmoidal relation.

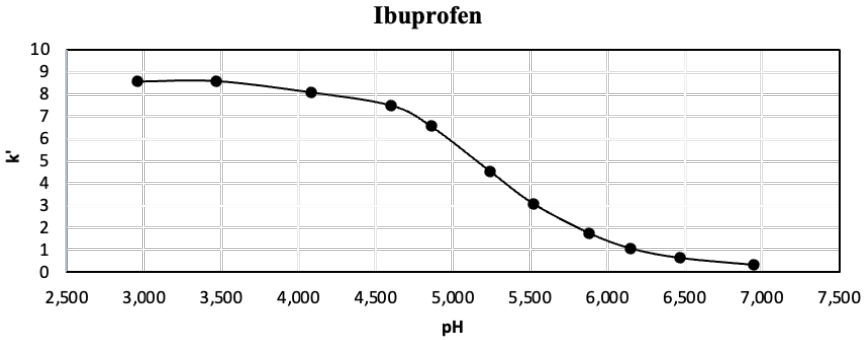

Figure 4. Obtained sigmoidal curve for determination of pK_a value of IBU

The sigmoidal curve was drawn (Figure 4) and pKa values of IBU was calculated as 5.20. Our pKa result is in accordance with the obtained results in the literature[22].

log kw determination

IBU was diluted to 5.0 μg mL^{-1} by using 20 mM PBS: MeOH (from 60:40 v/v to 30:70 v/v) solutions for log *kw* determination at pH 4.50, 5.50, 6.50 and 7.50. The relationship between log k' and methanol concentration in the mobile phase is well known in HPLC theory[20,23]. It is described with Equation 3 where k_w shows the k' value for a compound when aqueous phase is used as eluent, S is the slope of the regression curve, and φ is the volume percentage of methanol in the mobile phase. If the φ is zero, which means that there is no MeOH in the mobile phase and the mobile phase has consisted of only the phosphate buffer, the log k' will be equal to the log k_w.

$$\log k' = \log kw - S\varphi \quad (Eq.\ 3)$$

Intestinal absorption studies

Animals

All animals used in absorption studies were kept and handled according to Anadolu University's Committee on Animal Use and Care's regulations with the protocol number 2019/2. Before each trial, female Sprague Dawley albino rats (250-300 g) were fasted overnight (for around 12-18 h) with free access to tap water.

SPIP protocol and experimental groups

According to previously published research, an *in situ* single pass intestinal perfusion operation was performed[24-27]. It was decided to use pentobarbital (60 mg/kg) as an anesthetic and administer it intraperitoneally. A tiny midline incision (3-4 cm) was made in the abdomen, and 10 cm of the jejunum was isolated and carefully cannulated before the rest of the procedure was completed. The cannulation of the jejunal segment was accomplished using flexible PVC tubing (inlet tubing with an internal diameter (id) of 0.76 mm and exit tubing with an id of 1.70 mm). The perfusion pump was then connected to the tubings (Minipuls 3, USA). To remove any remaining debris, the jejunum was washed with blank perfusion media at a flow rate of 0.4 mL.min^{-1} for 15 minutes at room temperature. Perfusion tests were carried out at four different pH levels as Group 1; pH=7.4, Group 2; pH=5.9 (pH=pK_a+1), Group 3; pH=3.9 (pH=pK_a-1). An initial perfusion solution was perfused through the exposed segment at a flow rate of 0.2 mL min^{-1} for 60 min for each group, comprising the test drug (IBU) and the reference substances MET and PR.

Quantification of the absorption samples

The amounts of IBU and reference substances dissolved in the perfusion medium, that passed through the rat gut were determined using validated HPLC method. The peak areas of IBU, MET and PR were measured at two different wavelengths, 220 nm and 254 nm, respectively. The calibration curves for IBU (1-75 μg mL^{-1}), PR (7-210 μg mL^{-1}), and MET (4-160 μg mL^{-1}) were created by diluting stock solutions of compounds with mobile phase.

Data analysis

To determine the drug's effective permeability (Peff), the C_{out}/C_{in} ratio was corrected for water transport using Equation 4[28].

$$\left[\frac{C_{out}}{C_{in}}\right]' = \left[\left(\frac{C_{out}}{C_{in}}\right).\left(\frac{C_{in.Phenol.Red}}{C_{out.Phenol.Red}}\right)\right] \qquad \textit{(Eq. 4)}$$

$C_{in.Phenol.Red}$ indicates the PR concentration at the inlet, whereas $C_{out.Phenol.Red}$ indicates the PR concentration at the outflow. The effective permeability (P_{eff}) values of the medication were determined through the rat gut wall using the "plug flow" model described in Equation 5[29].

$$P_{eff} = \frac{-Q \ln\left[\frac{C_{out}}{C_{in}}\right]'}{2\pi r l} \qquad \textit{(Eq 5.)}$$

where Q is the flow rate of perfusion solution (mL.sec^{-1}); $\left[\frac{C_{out}}{C_{in}}\right]'$ is the corrected drug concentration ratio of the outlet to inlet concentration (Equation 3). "r" is the radius of the perfused intestinal segment (for jejunum r = 0.2 cm) and "l" is the length of the intestinal segment (cm)[30-32]

The net water flux (NWF) values in the *in-situ* perfusion studies (water absorption and efflux in the intestinal segment) were calculated based on inlet ($C_{in.Phenol.Red}$), outlet ($C_{out.Phenol.Red}$) concentrations of PR and (Q_{in}) the inlet perfusate flux using the following Equation 6:

$$NWF[\frac{\mu L}{h.cm}] = \frac{\left[1-\left(\frac{C_{out.Phenol.Red}}{C_{in.Phenol.Red}}\right)\right].Q_{in}}{l} \qquad (Eq.\ 6)$$

A negative net water flux indicates loss of fluid from the mucosal side (lumen) to the serosal side (blood). A positive net water flux indicates the secretion of fluid into the segment[29].

RESULTS and DISCUSSION

For determination of log kw values, IBU (10 µg mL^{-1}) and uracil solutions (5 µg mL^{-1}) diluted with water were analyzed using the mobile phase containing various ratios [70:30, 60:40, 50:50, 40:60;v/v] of MeOH and 20 mM phosphate buffer solutions (pH 4.5, 5.5. 6.5, and 7.5). The detection wavelength was 220 nm and the injections were performed triplicate. log k' values were extrapolated from binary eluents to 100% aqueous solutions to estimate the log kw values (Table 4). When the equation 1 was used, log kw values were found as 4.26, 3.74, 3.31, and 3.02. Based on the procedure described in experimental section, calculated P_{eff} values are shown in Table 5 and Figure 5.

Table 4. Obtained results for k' values

Mobile phase ratio (%*v-v*) (MeOH-PBS)		k' values			
		pH 4.5	pH 5.5	pH 6.5	pH 7.5
IBU/Uracil	70-30	2.97	1.4	0.97	0.74
	60-40	10.56	4.8	3.18	2.1
	50-50	35.8	14.82	8.6	5.90
	40-60	>85	-	-	-

Table 5. Obtained P_{eff} values of IBU and MTP from SPIP studies

	P_{eff} value(x10^{-4} cm/sn) (Mean±SD)		
Compounds	**pH 7.4**	**pH 5.9**	**pH 3.9**
MTP	0.564 ± 0.384	0.389 ± 0.210	0.277 ± 0.050
IBU	0.816 ± 0.501	1.821 ± 1.297	4.309 ± 0.328

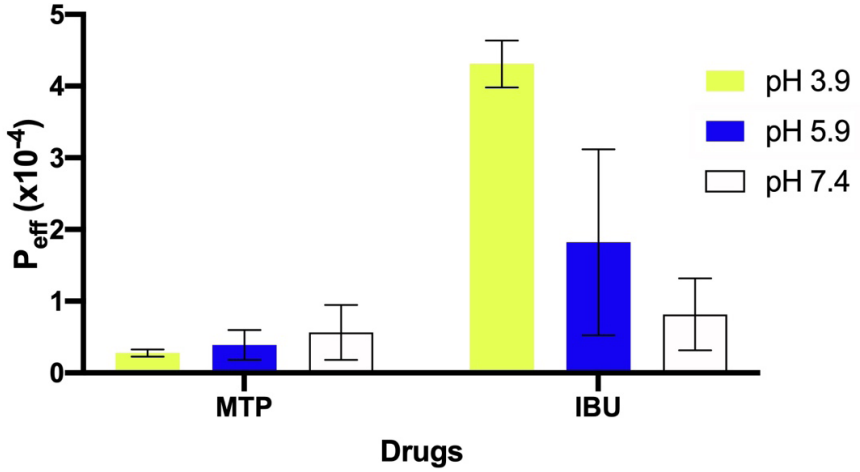

Figure 5. P_{eff} value of Intestinal permeability studies

The results of the *in vivo* intestinal permeability study performed at pH 3.9, 5.9 and 7.4 showed that there was a correlation between pH dependent solubility change of IBU and intestinal permeability values. The relationship was linear for both pH-log kw (y= -0.421x + 6.10, R^2=0.987 where x: pH and y: log kw) and pH-P_{eff} (y=-1.10x + 8.10, R^2=0.975 where x: pH and y: P*eff*) and this situation allowed us to suggest that the permeability of IBU as a result of passive diffusion was directly corelated with the pH of the medium. As it is reported by DrugBank [https://go.drugbank.com/], MET is a basic compound with a pKa value of 9.67 and IBU is an acidic drug and pKa value is 4.9. The reason why the trend of the permeability of IBU was increased and MET was decreased for pH 4.5 to 7.5, respectively can be easily explained by their pKa defining ionization percentage in a different medium having different pH values. The relationship between pH dependent lipophilicity of a compounds and pKa can be described with Log D which is distribution coefficient widely used to measure the lipophilicity of ionizable compounds. The acidic characteristics of IBU (pKa: 4.9) allow its lipophilicity to increase dramatically in acidic medium (pH

3.9), but above its pKa value, its lipophilicity decreases noticeably. This situation was confirmed in log kw studies, since log kw is related to the lipophilicity of a compound as reported in previous studies [33-36]. The basic characteristics of MET (pKa: 9.67) make MET behave the opposite of IBU based on pH changes of the medium. However, the pKa value of MET was relatively higher than the pH of the medium (pH 3.9, 5.9 and 7.4) we used in intestinal permeability studies. This situation caused the change of MET permeability relatively less than the permeability of IBU based on pH changes of the medium. The brief results of our study showed that *in-vitro* consideration of the lipophilicity of the drugs and/or drug candidates ascertains proper information on permeability related to passive diffusion. Since intestinal permeability may change dramatically as a result of the pH changes in the medium, some diseases like inflammatory bowel disease transforming the pH of the colon may cause noticeable changes in pharmacokinetic profiles of drugs. This situation must be considered especially for drugs having pKa values between 3.0 and 7.0.

In this study, the intestinal permeability of an NSAID, IBU, was evaluated where the pH of the perfusion medium varies from 3.9 to 7.4. The acidic pH of the perfusion mediums is to simulate a disease like inflammatory bowel disease causing a dramatic change in the colon in some cases. The results showed that the permeability of IBU changed considerably from pH 3.9 to 7.4. This situation was mainly associated with the changes in the lipophilicity of the IBU. The *in-vitro* results (log kw studies) also confirmed the changes of the lipophilicity using HPLC. Our data suggest that the pH changes in the colon as a result of the disease may cause a difference in the pharmacokinetic profiles of the drug and this situation related to the passive diffusion profile for the drug can be predicted using log kw experiments.

STATEMENT OF ETHICS

Ethical approval has been approved for this research. [All animals used in absorption studies were kept and handled according to Anadolu University Committee on Animal Use and Care's regulations with the protocol number as 2019/2.]

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS CONTRIBUTIONS

Mustafa Sinan Kaynak: Project administration, Funding acquisition Visualization, Formal analysis, Data curation, Investigation, Software, Conceptualization, Methodology, Writing- Original draft, Writing- Reviewing and Editing. Murat Soyseven, Berna Kaval, Mustafa Çelebier, Emrah Akgeyik, Selma Şahin, Göksel Arli: Visualization, Formal analysis, Data curation, Investigation, Validation, Software, Methodology, Writing- Original draft preparation, Writing-Reviewing and Editing.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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