IN VITRO INVESTIGATION OF SODIUM DICLOFENAC ADSORPTION ON SUCRALFATE

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Abstract: Adsorption of sodium diclofenac was investigated in the presence of sucralfate – a cytoprotective agent preventing gastropathy, adverse effect of diclofenac. Evaluation of adsorption was performed by means of a static method in vitro taking into account pH of the environment, temperature, concentration of the investigated agents and the form of sucralfate. Findings obtained prove that sodium diclofenac is adsorbed on sucralfate in all investigated pH ranges and the capability of sucralfate binding depends on its form, temperature and environmental pH. The highest binding was observed at pH 5.0 in the presence of sucralfate, which at this pH has the form of a suspension, while the lowest – at pH 1.5 in the presence of sucralfate in the form of paste. Low values of adsorption temperature of diclofenac as well as the relationship between the level of its adsorption and environmental pH are the dominating factors pointing to the physical and exothermic adsorption.

Keywords: non steroidal anti-inflammatory drug, sucralfate, interaction, Freundlich adsorption isotherm

The treatment of rheumatoid inflammatory conditions requires prolonged administration of non steroidal anti-inflammatory drugs (NSAIDs), which are effective analgesics. The use of NSAIDs leads to the damage of gastric and duodenal mucosa, erosion in 35-60% and ulceration in 10-25% of patients. Prolonged therapy with these agents significantly delays healing of the lesions (1).

Classical mechanism of NSAIDs action consists in blocking the synthesis of endogenous prostaglandins, they damage microvascular endothelium, inhibit angiogenesis (2) and decrease mucosal flow (3), causing damage of the mucous membrane.

Sodium diclofenac exerts anti-inflammatory effect through inhibition of COX-2 activity; however, by inhibiting COX-1 activity it causes unfavorable adverse effects, so-called gastropathies. In order to alleviate the adverse effects of these drugs on mucous membrane, gastroenterologists administer concomitantly gastroprotective agents. Sucralfate, one of them, has a protective effect on gastric mucosa. Its action consists in limiting the access of aggressive factors to the site of lesion on the mucous membrane. This drug stimulates mucous membrane to release gastroprotective prostaglandins PGE₂, PGF₂ and 6-keto-PGF with simultaneous reduction of thromboxane B₂ generation, reveals affinity to the gastric mucosa lesion and provokes local accumulation of EGF, which stimulates tissue regeneration (4).

Clinical observations confirm the effectiveness of sucralfate as a cytoprotective drug. Recent studies by Lazzaroni (4) and Pilotto (5) confirm the effectiveness of simultaneous administration of cytoprotective agents in combination with anti-inflammatory drugs.

Taking into account the capability of sucralfate to form complexes as well as its strong adsorption potentials, which depend largely on environmental pH, the drug may cause delayed or decreased absorption of drugs which were administered concomitantly (6).

Earlier reports (7-8) pointed to adsorptive properties of sucralfate in relation to gastroenterological agents administered simultaneously.

Thus a decision was made to investigate the character of the interaction between sucralfate and the most commonly administered drug, sodium diclofenac, phenylacetic acid derivative, taking into account certain physicochemical factors such as concentration of the adsorbent, pH of the environment, its temperature and the form of adsorbent.

EXPERIMENTAL

Substances

Diclofenac (Polpharma S.A, Poland), sucralfate (KRKA, Slovenia), Venter tabl. (KRKA, Slovenia), hydrochloric acid (POCH, Poland), sodium hydroxide, (Chemical Works Oświęcim, Poland), potassium hydroxyphthalate (Reachim, USSR), potassium bihydrophosphate (POCH, Poland).
Preparation of stock solutions of sodium diclofenac

0.050 g samples of substance were weighed on an analytical balance and subsequently transferred to 100 cm$^3$ volumetric flasks, diluted and made up to the mark with Clark-Lubs buffer solution of pH 1.5, Walpole’s acetate buffer solution of pH 3.6, Sorensen’s buffer solution of pH 5.0, buffer solution of pH 6.4 and buffer solution of pH 7.6.

Preparation of sucralfate in the form of adhesive paste

0.1 g of sucralfate was evenly distributed on the bottom of a 50 cm$^3$ Erlenmeyer flask and 1 cm$^3$ of 0.1 M HCl solution was added dropwise. The solution was stirred manually for 3 min at 30 rpm. The obtained paste was washed with bi-distilled water until the disappearance of chloride ions (9).

Determination of levels of sodium diclofenac

Sodium diclofenac level was determined spectrophotometrically following prior dissolution of the investigated samples in 0.1 M solution of sodium hydroxide at $\lambda = 275$ nm.

The levels of the investigated active agents were found from standard plots described by the equation $y = 0.3304x + 0.0081$. The correlation coefficient was 0.9995 and regression standard deviation $\sqrt{0.037169}$.

Measurements of diclofenac adsorption isotherms

0.1 g portions of powdered sucralfate were added to seven 50 cm$^3$ Erlenmeyer flasks to which aliquots of 0.5, 1, 2, 4, 6, 8 and 10 cm$^3$ of diclofenac stock solution were added. The volumes were made up to 10 cm$^3$ with buffer solution of relevant pH. The flasks were agitated on a rotary shaker at 80 cycles/minute at 4°C, 25°C, 36°C and 50°C for 180 min.

A similar procedure was applied to measurements of diclofenac adsorption in the presence of sucralfate in the form of a paste.

Next, the mixtures were allowed to stand for 30 min, to enable decanting, the solutions were centrifuged and supernatants were used for the determination of levels of the investigated therapeutic agents. The amount of the drug adsorbed on sucralfate was calculated from the difference between the amounts of the investigated drugs determined prior to and after sorption.

Research

Sodium diclofenac adsorption was investigated by static method in concentration ranges from 0.25 mg/10 cm$^3$ to 5.0 mg /10 cm$^3$ using various buffer solutions of pH from 1.5 to 7.6. and temperature from 4 to 50°C. Moreover, the degree of sodium diclofenac ionization and its lipophilic properties in relation to changes in pH of the medium were studied.

Sucralfate occurs in different forms depending on pH of the medium. At pH below 3 it forms polymers in the form of adhesive paste. At pH 3.0-6.5 sucralfate assumes the form of a suspension, while at pH above 6.5 it undergoes hydrolysis (9). Thus adsorption of active substances was inves-
In vitro investigation of sodium diclofenac adsorption on sucralfate

The concentration of active substances was corresponding to average single doses used in the treatment, pH of the buffers corresponded to pH in successive segments of the digestive tract, temperature of the medium corresponded to physiological and dietary conditions. The results of measurements of the amount of bound diclofenac were used to determine Freundlich adsorption isotherms (Fig. 1, 2), Langmuir adsorption isotherms (Fig. 3, 4), to determine the parameters of adsorption isotherm equation (Table 1) and to calculate mean percentage of the adsorbed dose of the drug (Table 2). Adsorption enthalpy was calculated on the basis of graphic analysis of the logarithm of adsorbed amount as a function of reciprocal temperature (Fig. 5) and the equation parameters were determined (Table 3). The effect of temperature on sorption level and enthalpy term (H) was demonstrated for diclofenac in buffer solution of pH 5.0, where the highest sorption ability was observed.

Standard deviations of mean adsorption levels were in the range from 0.12 mg/cm³ to 0.94 mg/cm³ and variation coefficients were from 2.21% to 9.78%. Normal distribution of independent and dependent variables was checked by means of Kolmogorov-Smirnov test, linear correlation coefficients were determined and their significance was tested by means of Hill test. Parameters A and 1/T were found to differ significantly from zero in all the cases. Moreover, the values of constant error A and 1/T were determined by calculating variation coefficients and linear correlations.

RESULTS AND DISCUSSION

Observed impeded absorption of the drugs in the presence of sucralfate can be explained by formation of complexes and adsorption (10).

Table 1. The effect of environment pH on the adsorption of diclofenac sodium on sucralfate.

<table>
<thead>
<tr>
<th>pH 1.5</th>
<th>pH 3.6</th>
<th>pH 5.0</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of diclofenac [mg/cm³]</td>
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<td>Concentration of diclofenac [mg/cm³]</td>
<td>Concentration of diclofenac [mg/cm³]</td>
</tr>
<tr>
<td>X [%]</td>
<td>X [%]</td>
<td>X [%]</td>
<td>X [%]</td>
<td>X [%]</td>
</tr>
<tr>
<td>0.002</td>
<td>0.003</td>
<td>0.022</td>
<td>0.021</td>
<td>0.017</td>
</tr>
<tr>
<td>0.1562</td>
<td>0.1923</td>
<td>0.421</td>
<td>0.142</td>
<td>0.175</td>
</tr>
<tr>
<td>0.004</td>
<td>0.004</td>
<td>0.021</td>
<td>0.021</td>
<td>0.045</td>
</tr>
<tr>
<td>0.003</td>
<td>0.023</td>
<td>0.061</td>
<td>0.021</td>
<td>0.35</td>
</tr>
<tr>
<td>0.012</td>
<td>0.019</td>
<td>0.061</td>
<td>0.021</td>
<td>0.64</td>
</tr>
<tr>
<td>0.251</td>
<td>0.287</td>
<td>0.111</td>
<td>0.112</td>
<td>2.83</td>
</tr>
<tr>
<td>0.0026</td>
<td>0.027</td>
<td>0.061</td>
<td>0.021</td>
<td>0.186</td>
</tr>
<tr>
<td>0.026</td>
<td>0.027</td>
<td>0.061</td>
<td>0.021</td>
<td>0.297</td>
</tr>
<tr>
<td>0.027</td>
<td>0.027</td>
<td>0.061</td>
<td>0.021</td>
<td>0.379</td>
</tr>
<tr>
<td>0.042</td>
<td>0.045</td>
<td>0.061</td>
<td>0.021</td>
<td>0.468</td>
</tr>
</tbody>
</table>

X% – mean percentage adsorbance

Table 2. Parameters of Freundlich isotherm equation of diclofenac sodium sorption from buffer solutions on sucralfate.

<table>
<thead>
<tr>
<th>pH of investigated solutions</th>
<th>1/n ± Δ1/n</th>
<th>k ± Δk</th>
<th>r</th>
<th>Rg. b. std.</th>
<th>% [A]</th>
<th>Co/Cw</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 1.5</td>
<td>1.1302 ±0.054</td>
<td>0.145 ±0.01</td>
<td>0.9892</td>
<td>0.0215</td>
<td>0.31</td>
<td>20.45</td>
</tr>
<tr>
<td>pH 3.6</td>
<td>0.8555 ±0.029</td>
<td>0.56 ±0.02</td>
<td>0.9976</td>
<td>0.0891</td>
<td>28.47</td>
<td>8.12</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>3.3194 ±0.056</td>
<td>0.003 ±0.0009</td>
<td>0.9826</td>
<td>0.0432</td>
<td>90.9</td>
<td>7.33</td>
</tr>
<tr>
<td>pH 6.4</td>
<td>2.1263 ±0.018</td>
<td>0.002 ±0.0001</td>
<td>0.9914</td>
<td>0.1112</td>
<td>99.6</td>
<td>2.01</td>
</tr>
<tr>
<td>pH 7.6</td>
<td>1.3374 ±0.056</td>
<td>0.0022 ±0.0002</td>
<td>0.9876</td>
<td>0.1231</td>
<td>99.97</td>
<td>1.42</td>
</tr>
</tbody>
</table>

1/n, k – Freundlich adsorption isotherm equation constants
r – correlation coefficient
D1/n, Dk – 1/n, k parameters errors in Freundlich isotherm equation
Rg. b. std – regression standard deviation
% [A] – the percentage of dissociated sodium diclofenac molecules
Co/Cw – mean coefficient of oil/water partition Co/Cw-
Diclofenac is adsorbed on sucralfate at all the investigated pH and the sucralfate binding capability is determined by its form, i.e. indirectly, by environmental pH.

At pH 1.5 mean sorption level ranged from 15% to 28% depending on concentration of diclofenac and was the lowest among the samples investigated in acidic medium (Table 2). At pH below 3, increased solubility of aluminium ions is observed as a result of sucralfate dissociation, which leads to the formation of sucralfate polymers in the form of adhesive paste with weak adsorptive properties.

At pH 3.6 and 5.0 corresponding to the environment of a stomach filled with gastric contents, mean sorption level for the highest diclofenac dose was 40.0% and at pH 5.0 it was up to 85.3%. The highest adsorption level at these pH ranges can be explained by physicochemical properties of sucralfate. At pH 3.0-6.5 higher solubility of saccharase sulfate is observed than that of aluminium ions, what is confirmed by mole ratio of aluminium to saccharase sulfate as determined by potentiometric titration. In this situation sucralfate takes the form of suspension with strong adsorption ability.

The greatest difference in the effect of diclofenac concentration on adsorption level was observed at pH 6.4 corresponding to pH of duodenal juice. When investigating the lowest concentration of diclofenac, the level of sorption was 1.4%, while its increase to the highest concentration resulted in adsorption on the level of about 28% - i.e. about 20-fold increase. At pH 7.6 corresponding to the pH of succus entericus, adsorption was 1.75% at the low-

### Table 3. Regression parameters of the formula logA = const-H/2,303 RT for various diclofenac concentrations in the presence of sucralfate in buffer solution at pH 5.0.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Slope</th>
<th>Intercept</th>
<th>Regression coefficient</th>
<th>Regression standard deviation</th>
<th>(\Delta H^*) (J·mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/cm(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>0.6838 ±0.032</td>
<td>-1.455 ±0.01</td>
<td>0.9892</td>
<td>0.0215</td>
<td>13.09</td>
</tr>
<tr>
<td>0.05</td>
<td>0.6606 ±0.029</td>
<td>-0.9031 ±0.02</td>
<td>0.9776</td>
<td>0.0891</td>
<td>12.64</td>
</tr>
<tr>
<td>0.1</td>
<td>0.5966 ±0.026</td>
<td>-0.5966 ±0.007</td>
<td>0.9926</td>
<td>0.0432</td>
<td>11.42</td>
</tr>
<tr>
<td>0.2</td>
<td>0.5767 ±0.011</td>
<td>-0.2455 ±0.0001</td>
<td>0.9798</td>
<td>0.0112</td>
<td>11.04</td>
</tr>
<tr>
<td>0.3</td>
<td>0.5733 ±0.056</td>
<td>-0.1909 ±0.004</td>
<td>0.9897</td>
<td>0.0231</td>
<td>10.97</td>
</tr>
<tr>
<td>0.4</td>
<td>0.5524 ±0.048</td>
<td>-0.0309 ±0.006</td>
<td>0.9137</td>
<td>0.0411</td>
<td>10.57</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5492 ±0.016</td>
<td>0.0208 ±0.001</td>
<td>0.8953</td>
<td>0.0524</td>
<td>10.51</td>
</tr>
</tbody>
</table>

\(\Delta H^*\) values were calculated from the slope of each plot according to Eq. 3

**Figure 3.** Langmuir plot for the adsorption of sodium diclofenac from buffer solutions on sucralfate.

**Figure 4.** Langmuir plot for the adsorption of sodium diclofenac from buffer solution on sucralfate.
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Figure 5. The effect of temperature on the rate sodium diclofenac adsorption on sucralfate in buffer solution at pH 5.0.

The highest concentration of the drug and it reached 4.68% at the highest one. A decrease of adsorption at pH 6.4 and 7.6 may be explained by sucralfate decomposition as a result of hydrolysis at pH above 6.5. The data presented in Table 2 indicate that the level of adsorption depends on the degree of diclofenac ionization and its lipophilic properties. The lower ionization level of the drug, the higher lipophilic properties it has, thus the higher ability to become adsorbed on the surface of sucralfate.

Summarizing the above-presented analysis, it can be stated that the level of adsorption depends on diclofenac concentration and its adsorption on sucralfate increases with increasing concentration. On the ground of diclofenac adsorption data Freundlich adsorption isotherms were plotted as logarithmic functions of the amount of the drug adsorbed by sucralfate mass unit at equilibrium concentration in buffer solutions.

\[
\log \frac{x}{m} = \frac{1}{n} \log c + \log k
\]

(1)

\(c\) - concentration of adsorbent in equilibrium solution (mg/cm³)

\(x\) - mass of adsorbed substance (mg)

\(m\) - mass of adsorbent

\(k, 1/n\) – isotherm equation constants

The value of 1/n parameter in Freundlich equation allows to evaluate the rate of adsorption of a given substance. The value of k constant determines the adsorption capability of sucralfate.

It was also possible to describe diclofenac adsorption on sucralfate by means of Langmuir isotherm:

\[
\frac{c}{x/m} = \frac{c}{k_2} + \frac{1}{k_1 \times k_2}
\]

(2)

\(c\) - concentration of substance in equilibrium solution (mg/cm³)

\(x/m\) - amount of substance (mg) adsorbed by adsorbent mass unit (1 mg)

\(k_1, k_2\) - Langmuir constants

\(k_2\) constant determines the amount of absorbate (mol/g) covering the surface of adsorbent with a monomolecular layer; it is equivalent to the monolayer capacity.

The Freundlich plot is characterized by a higher linear correlation coefficient (r = 0.9892) than Langmuir plot (0.8817). This fact proves that adsorption is a constant function of drug concentration and for this reason monomolecular layer coverage of the surface of adsorbent is excluded.

Thus it may be assumed that diclofenac adsorption on sucralfate may be the effect of mainly physical forces (intermolecular, electrostatic).

The course of Freundlich adsorption curves presented in Fig. 1, 2, indicated a growth of the level of sorption which increased with increasing concentration of the investigated drugs. Mean sorption level obtained at the highest concentration of the investigated drugs was 1.5 to 4.5 higher than that obtained at the lowest concentrations of the investigated substances.

The parameters and the analysis of the course of diclofenac isotherms from buffer solutions (Table 1, Fig. 1, 2) demonstrate differences in the affinity of the drug to sucralfate at various pH of the environment.

Analysis of Freundlich curve parameters presented in Table 2 indicates that adsorption capability (k) of sucralfate in relation to diclofenac assumes the lowest value (0.56) at pH 3.6. Under these conditions sucralfate takes the form of suspension. At pH 1.5, when sucralfate has the form of adhesive paste, its sorption affinity is almost fourfold lower in comparison to that observed for suspension. The parameter 1/n characterizing the intensity of adsorption process assumed the highest value of 3.3194 at pH 5.0.

Statistical analysis was carried out by means of Student’s test. The value p < 0.05 was assumed as statistically significant.

Fig. 5 presents the effect of temperature on...
adsorption of sodium diclofenac on sucralfate in a buffer solution of pH 5.0 according to equation:

$$\log A = \text{const} - \frac{\Delta H}{(2.303RT)}$$ (3)

$A$ – % of adsorbed substance in equilibrium solution (mg/cm$^3$)

$T$ – absolute temperature

$R$ – gas constant (8.314 J/mol·K)

$\Delta H$ – enthalpy term of adsorption

The course of the curves points to a decrease of sorption capability with increasing temperature, which proves the fact that the process of adsorption is exothermic. This relation can be explained by increased solubility of diclofenac at higher temperatures. At the same time solubility of the adsorbent increases and its adsorption surface decreases. As $\Delta H$ is directly proportional to $\Delta S$, the decrease of entropy with increased concentration of the drug results in decreased adsorption temperature. The values of calculated adsorption enthalpies decreased (from 13.09 to 10.51 J·mol$^{-1}$) with an increase of the temperature. Moreover, determined adsorption enthalpies assumed values lower than 100 kJ/mol and thus we can presume that it is physical adsorption.

CONCLUSIONS

The above considerations lead to a conclusion that between the investigated drugs: diclofenac and sucralfate occurs an antagonistic interaction consisting in adsorption of the drug on sucralfate.

The level of adsorption largely depends on pH. The highest adsorption capability was found in samples at pH 5.0, i.e. at pH corresponding to that in the gastric juice after a meal. Adsorption capability of the investigated drugs also depends on their concentration and on the form of sucralfate.

Mathematical interpretation of the findings made possible to plot Langmuir and Freundlich adsorption isotherms. Freundlich linear relation is correlated and this fact speaks in favor of multilayer adsorption. Adsorption decreases with increased temperature as does the temperature of adsorption and the values of these parameters point to a physical character of adsorption.

The above mentioned factors affecting the level of adsorption should be considered under clinical conditions with simultaneous administration of the drugs, in order to obtain the optimum therapeutic effects.

REFERENCES


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