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THE STUDY OF INTERACTIONS BETWEEN IBUPROFEN AND BOVINE SERUM ALBUMIN

Review: Professor Stanisław Wysocki, Ph.D., D.Sc.

Investigation of the interactions between ibuprofen and Bovine Serum Albumin was carried using UV-Vis absorption and fluorescence methods. There was a quenching effect found between these two molecules in the fluorescence methods.

1. Introduction

Drugs' effectiveness in the human body is measured by their pharmacokinetics and pharmacodynamics. Pharmacokinetics is the study of the processing of drug in the body, which includes, assessing the absorption, distribution, metabolism, and excretion of the drug. Pharmacodynamics, on the other hand, studies the mechanism of action of the drug in the human body. Drugs become more efficacious as their concentration increases at the site of action, up to a certain limit where they start becoming toxic. Hence, determining the correct dosing regimen is critical in achieving the desired effect. Dosing regimens for drugs are determined based on several pharmacokinetic parameters, such as volume of distribution and total clearance from the body. Both of these parameters are influenced by the binding of the drug to plasma proteins, such as Human Serum Albumin (HSA) and α_1-acidic glycoprotein (AGP). Only free drug that is

unbound by plasma proteins can react at the site of action, so it's essential to have low affinity between the drug and the plasma protein at the site of action.

Ibuprofen is a non-steroidal-anti-inflammatory-drug (NSAID) that is used as an active compound in analgesic, antipyretic and anti-rheumatic medicaments (Fig. 1.) It belongs to the group of the NSAID and its chemical name is (2-[4-(2-methylpropyl)phenyl]propanoic acid (pKa = 4.91) [1]. Its normal therapeutic concentration in blood is about 50 mg/L, however, it is toxic at the concentration of 250 mg/L or higher [2].

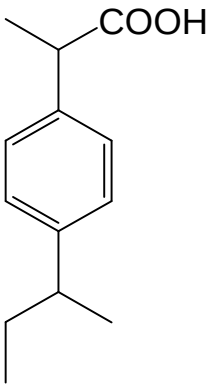

Fig. 1. The chemical structure of ibuprofen

The exact mechanism of action NSAID is not entirely known, it is very likely that limitation of prostaglandin synthesis due to COX inhibition is involved in it. This process is also the main cause of one of the side effects of NSAIDs – high susceptibility of stomach to corrosion by gastric acid. It is because inhibition of COX also decreases prostaglandins in the epithelium of the stomach. Moreover, NSAID are potential drugs in cancer therapy. It is likely that they may block tyrosine residue in topoisomerase enzymes. DNA topoisomerases are essential enzymes for facilitating such important dynamic processes in the cell as replication, transcription, recombination and repair. They work by breaking (transiently) the DNA backbone bonds utilizing a cycle of transesterification reactions mediated by a specific tyrosine residue in the enzyme [3]. The vital role that they serve in DNA - replication and thus cellular mitosis serves as an excellent weak point with which to attack. Death of tumor cell is expected to be inevitable consequence of blocking tyrosine residue in topoisomerases by NSAID.

Human Serum Albumin (HSA) is a carrier for several drugs inter alia ibuprofen and by this it plays an important role in the regulation of plasma concentrations of these, including both endogenous and exogenous compounds. HSA shares 76 % homology with Bovine Serum Albumin (BSA) and in many cases can be used as its substitute. The BSA molecule is formed with 582 amino acid residues with tryptophan at a position 134 and 212 [4]. Since it is easily

accessible, at the low cost, most of our studies have been performed using BSA [5]. The aim of the experiment is to explore and verify the molecular interactions between ibuprofen and BSA. Researches were carried out using spectrophotometric techniques [6].

2. Experimental

2.1. Chemicals

Ibuprofen and BSA used in this project were commercial products purchased at Sigma-Aldrich and were used as received. All chemicals were dissolved in physiological solution with 0.17 M NaCl (to maintain a physiological ionic strength), and 0.01 M phosphate buffer (pH = 7.0).

2.2. Experimental equipment

Absorption measurements were made using a Nicolet Evolution 300 UV-Vis spectrophotometer from Thermo Electron Corporation with resolution 0.5 nm and range 0-6. Steady-state fluorescence measurements were made using spectrofluorometer Generic Fluoromax-2 from Jobin Yvon-Spex with excitation resolution of 13 nm. Solutions were placed into a 10 mm quartz cuvette and temperature was kept constant at 26°C.

2.3. Software-analysis

The data collected in the experiment was analyzed using Microsoft Excel and Origin software. This program was applied to obtain calculations and display the results in a graphic form.

3. Results and discussion

3.1. Experimental results

3.1.1. UV-Vis spectra of pure ibuprofen and BSA

In Fig. 3.1 the spectrum for ibuprofen shows maximum at 264 nm and 272 nm.

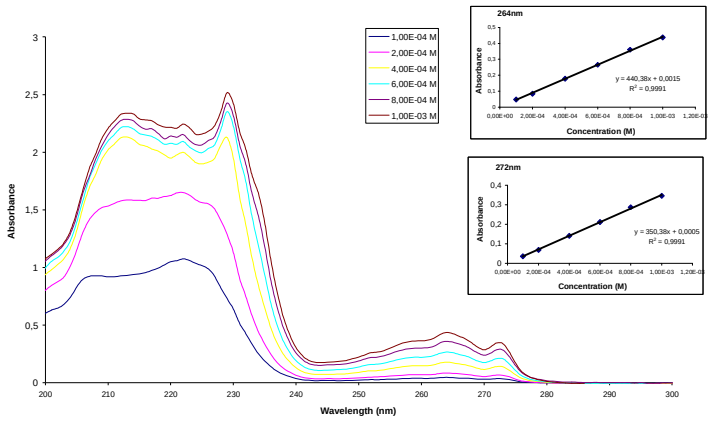

Fig. 3.1. UV-Vis spectrum of ibuprofen ($1x10^{-4}$ - $1x10^{-3}$ M) and its dependence on concentration at 264 nm and 272 nm

The molar extinction coefficient was determined using the Beer-Lambert law:

$$A = \varepsilon \cdot l \cdot c \qquad (1)$$

where: A – absorbance
ε – molar extinction coefficient with units of L $mol^{-1}cm^{-1}$ or $M^{-1}cm^{-1}$
l – path length of the sample - that is the path length of the cuvette in which the sample is contained (in our case 1 cm)
c – concentration of the compound in solution, expressed in mol L^{-1}

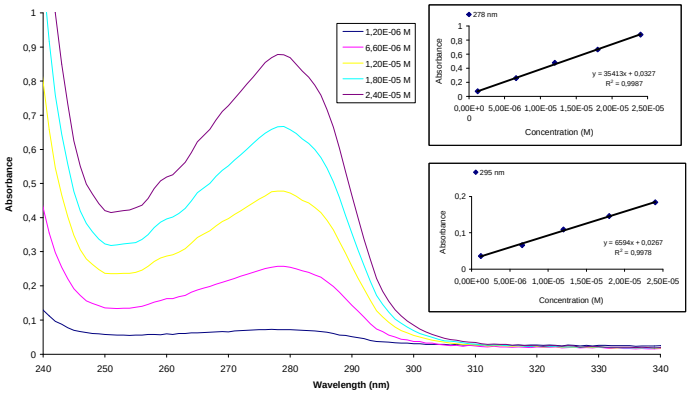

Fig. 3.2. UV-Vis spectrum of BSA ($1,2x10^{-6}$ – $2,4x10^{-5}$ M) and its dependence on concentration at 278 nm and 295 nm

BSA UV-Vis spectrum in Fig 3.2 shows a maximum at 278 nm. The experimental extinction coefficient is 36600 $M^{-1}cm^{-1}$ at 278 nm and 6600 $M^{-1}cm^{-1}$ at 295 nm. Peak at 278 nm differs from literature values which is 279 nm [7]. There is a perfect linear dependence of absorbance on the concentration of the solution. Obtained data corresponds reasonably with literature [8].The wavelength 295 nm were chosen following J.R. Lakowicz [10] due to the overwhelming absorption of tryptophan over absorption of tyrosine residue of both molecules at this spectral region. Thus selective excitation of tryptophan residue can be archived using 295 nm.

Table 1

Molar extinction coefficients of BSA and ibuprofen

Molecule	ε_{max} (M^{-1} cm^{-1})	ε_{295nm} (M^{-1} cm^{-1})
BSA	36600 (± 700) (278 nm)	6600 (± 100)
Ibuprofen	346 (± 5) (272 nm) 437 (± 6) (264 nm)	0

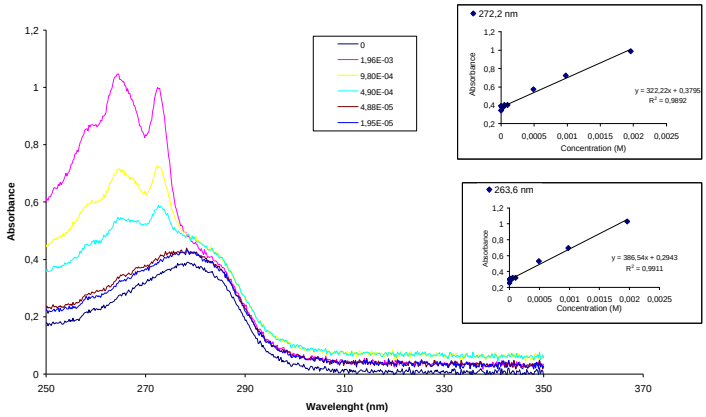

Fig. 3.3. UV-Vis spectrum of ibuprofen - BSA (0 – 1,95x10^{-5} M) and its dependence on concentration at 272 nm and 263 nm

Spectrum in Fig 3.3 shows two peaks, the first one at 263 nm, the second one at 272 nm. The calibration curves in Fig 3.1, 3.2 and 3.3 show a linear relationship between concentration and absorbance.

3.1.2. Fluorescence spectra

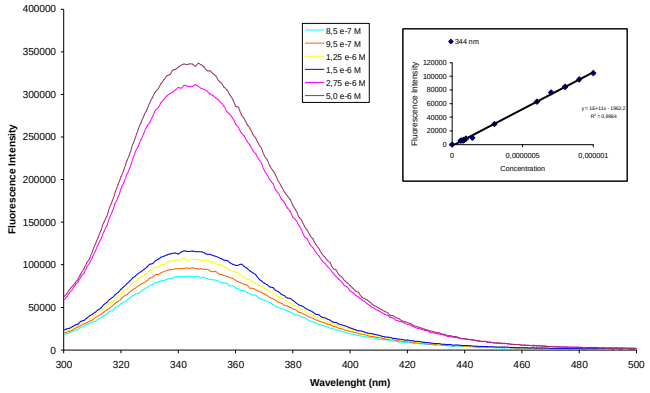

Fig. 3.4. Fluorescence spectrum of BSA ($8{,}5x10^{-7}$ – $5.0x10^{-6}$ M) and its dependence on concentration at 344 nm (λ_{ex}=295nm)

Fluorescence emission spectrum of BSA in Fig 3.4 shows a maximum at 344 nm when the exciting wavelength is 295 nm. This value obtained correlates with literature values [9]. This emission is attributed to the two tryptophans of BSA: trpyptophan-134 conserved in all albumins and trpyptohan-213 characteristic of bovine albumin.

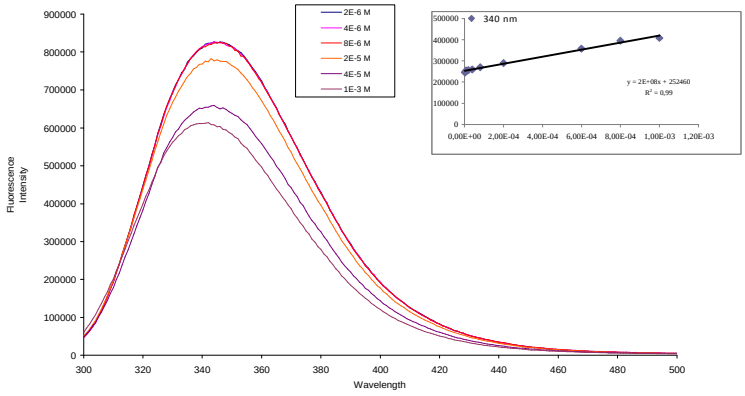

Fig. 3.5. Fluorescence spectrum of ibuprofen ($2{,}0x10^{-6}$ – $8{,}0x10^{-4}$ M). Fluorescence intensity dependence on the concentration at 340 nm (λ_{ex}=295nm)

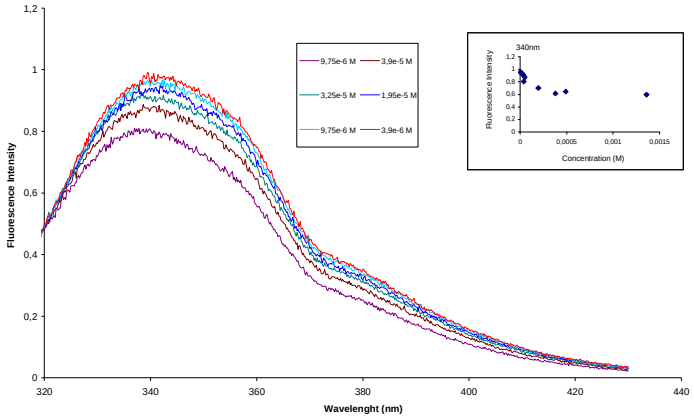

Fig. 3.6. Fluorescence spectrum of ibuprofen-BSA ($3{,}9\text{x}10^{-6}$ – $3{,}8\text{x}10^{-5}$ M). Fluorescence intensity dependence on the concentration at 340 nm (λ_{ex} = 295nm)

Quenching data were analyzed according to modified Stern-Volmer equation:

$$F' = F_0' + F_0\left(\frac{[M]_0 - [Q]_0 - \frac{1}{K} + \sqrt{\Delta'}}{2[M]_0} - 1\right) \qquad (2)$$

$$\Delta' = \left([M]_0 + [Q]_0 + \frac{1}{K}\right)^2 - 4[M]_0[Q]_0$$

where: F', F_0' – fluorescence intensity of BSA in the absence and in the presence of quencher

$F_0 = F_0' - F_{in}$

K – equilibrium constant of complex formation

$[M]_0$ – concentration of BSA

$[Q]_0$ – concentration of the free quencher

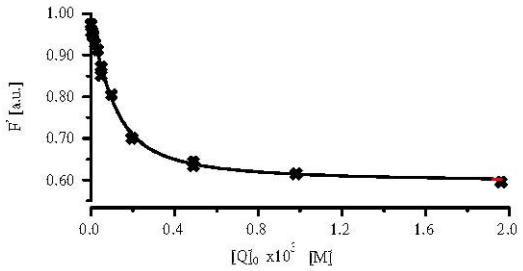

Fig. 3.7. The quenching of BSA fluorescence at 340nm by ibuprofen ($\lambda_{ex.}$ = 295nm) Solid line represents fitting of experimental data to the eq. 2. $[M]_0 = 1.1 \cdot 10^{-4}$ ($\pm 3 \cdot 10^{-5}$)M, K = 18000 (± 2000), F_0 = 0.38 (± 0.012), F_0' = 0.971 (± 0.005)

The following formula was used to show quenching of BSA fluorescence with increasing ibuprofen concentrations [10]:

$$\frac{F_0}{\Delta F} = \frac{1}{f_a \cdot K \cdot [Q]_0} + \frac{1}{f_a} \tag{3}$$

where: F_0 – fluorescence intensity of BSA

$\Delta F = F_0 - F$ (F: intensity of fluorescence in the presence of quencher)

f_a – constant

K – equilibrium constant of complex formation

$[Q]_0$ – concentration of quencher

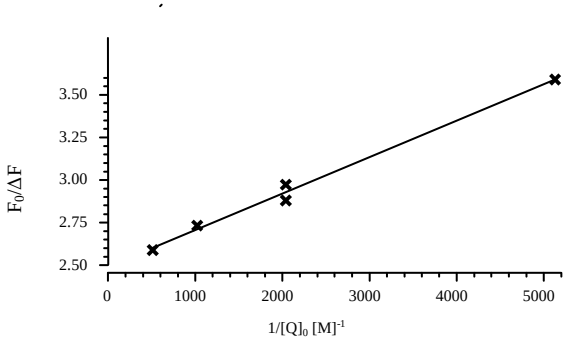

Fig. 3.8. Modified Stern – Volmer plot. Solid line represents fitting to the eq 3

Fluorescence of BSA is quenched by ibuprofen as show in Fig. 3.7. and 3.8. Fluorescence intensity is proportional to the concentration.

Number of binding sites of BSA (n) were confirmed base on Scatchard model (eq.4) [12]

$$\frac{\nu}{L} = nK - \nu K \tag{4}$$

where: ν – ration of bound quencher to total protein concentration

L – free ligand concentration

n – number of binding sites

K – equilibrium constant of complex formation

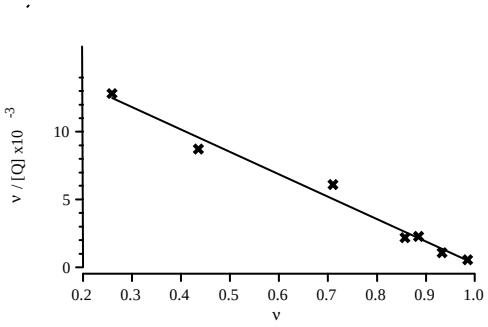

Fig. 3.9. Scatchard plot. Solid line represents experimental data fitting to the eq 4. K = 17000 (± 1000), n = 1.01 (± 0.05)

4. Conclusions

Our spectrophotometric researches were focused on interactions of BSA with ibuprofen. In the presence of ibuprofen a static quenching of BSA fluorescence due to ground state complex formation was observed. Base on Scatchard model it was proved that only one binding site of BSA was involved in interactions with ibuprofen. The equilibrium constant (K) of complex formation was found to be equal to 18000 M^{-1}.

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BADANIA ODDZIAŁYWAŃ POMIĘDZY IBUPROFENEM I SUROWICZĄ ALBUMINĄ WOŁOWĄ (BSA)

Streszczenie

W pracy zbadano oddziaływania pomiędzy ibuprofenem i surowiczą albuminą wołową przy użyciu metod spektroskopii absorpcyjnej UV-Vis oraz metod fluorescencyjnych. Wyniki wskazują na występowanie efektu wygaszania BSA przez ibuprofen.

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