

PROTOLYTIC EQUILIBRIA IN HOMOGENEOUS AND  
HETEROGENEOUS AQUEOUS SYSTEMS  
OF METHYL- AND PROPYLPARABEN AND  
INFLUENCE OF  $\beta$ -CYCLODEXTRIN ON  $pK_a$  VALUES

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**Abstract**

Acidity constants ( $K_a$ ) and intrinsic hydrosolubility ( $K_{s0}$ ) of two parabens, methylparaben and propylparaben, were spectrophotometrically determined at 25°C and a constant ionic strength of  $I=0.1$  M (NaCl). Based on the determined equilibrium constants  $K_{s0}$  and  $K_a$ , equilibrium constant  $K_{s1}$  in heterogeneous system of methyl- and propylparaben was calculated. Solubility of the two parabens as a function of solution acidity was calculated applying the equation:

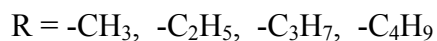
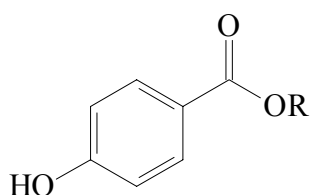
$$S = K_{s0} + \frac{[K_{s1}]}{[H_3O^+]}$$

In addition, the effects of varying  $\beta$ -cyclodextrin concentrations on  $pK_a$  values of the two parabens were examined. The results showed that  $\beta$ -CD influenced absorption spectra of the parabens (a decreased absorptivity and a bathochromic shift of the absorption maxima), but their  $pK_a$  values remained almost unchanged.

**Key words:** Methylparaben, propylparaben, solubility, acidity constant.

## Introduction

Due to their antimicrobial and antifungal action, alkyl esters of *p*-hydroxybenzoic acid (parabens) are widely applied as preservatives in numerous pharmaceutical preparations (oral solutions, creams and lotions), cosmetics and food industry. Pharmaceutical preparations contain parabens in the concentrations up to 0.25%, while cosmetic preparations and alimentary products usually contain up to 0.1% parabens (1).



Parabens are known to express their activity within a broad range of pH values (4 - 8) and this is advantageous for their use as preservatives. Generally, the efficiency of parabens is higher in acid media. Parallel to the increase of the chain length of alkyl substituent, paraben activity is also increasing, accompanied by a decrease of their hydrosolubility. Since hydroxyl group in the molecule of parabens is a subject to protolysis ( $pK_a$  8.4) (2), acidity of the medium also affects the solubility of parabens, but there is no data on this phenomenon in the available literature. Connected to that, the aim of the present study was to examine the effect of pH on hydrosolubility of two most frequently applied parabens, methyl-*p*-hydroxybenzoate (methylparaben, MP) and propyl-*p*-hydroxybenzoate (propylparaben, PP). Matsuda et al. (3) demonstrated an increased paraben solubility in the presence of  $\beta$ -cyclodextrin ( $\beta$ -CD) and a part of this work was focused on the examinations of the influence of  $\beta$ -CD on  $pK_a$  values of methyl - and propylparaben.

## Experimental

### *Reagents and apparatus*

Working standards of methyl- and propylparaben were obtained from Srbolek a.d. Pharmaceutical Works (Belgrade, Serbia & Montenegro). All chemicals used throughout this study (HCl, NaOH, ethanol) were Merck (Germany) products of analytical grade of purity.

For spectrophotometric measurements, a GBC Cintra 20 spectrophotometer (GBC Scientific Equipment Pty Ltd., Australia) with 1.0 cm quartz cuvettes was used. pH values were determined with a PHM-84 pH meter with a combined GK 2401B electrode (Radiometer). For the calculation of the measured pH values ( $25 \pm 0.1^\circ\text{C}$ ;  $I=0.1$  M (NaCl)) in  $[\text{H}_3\text{O}^+]$ , the following relation was used (4):  $p\text{c}_\text{H} = -\log[\text{H}_3\text{O}^+] = \text{pH} - 0.12$ .

### *Determination of equilibrium constant $K_{s0}$*

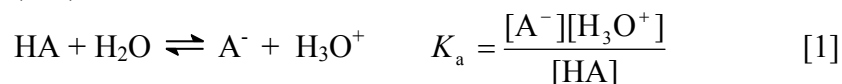
An excess of solid paraben was treated with 0.1 M NaCl solution and 0.1 M HCl was added to obtain pH values within the range of 2.5 - 4. The solutions were thermostated at  $25^\circ\text{C}$  with a strong stirring. After the equilibrium was established (4 h), undissolved part of paraben was removed by filtration. Aliquots of the filtrate were diluted with 0.01 M HCl and the concentration of paraben was determined spectrophotometrically at 255 nm, previously checking whether it obeys the Beer's law.

### *The effect of $\beta$ -CD on $pK_a$ values*

Four series of paraben  $5 \times 10^{-5}$  M solutions within the pH range from 4-12, without  $\beta$ -CD or supplemented with it ( $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  M) were prepared. Ionic strength of the solutions was constant 0.1 M (NaCl). The solutions were thermostated at  $25^\circ\text{C}$ . For  $pK_a$  determinations, the absorbancies of the solutions were measured at 295 nm (in the absence of  $\beta$ -CD and in the presence of  $10^{-4}$  M  $\beta$ -CD) and at 297 nm (in the presence of  $10^{-3}$  M and  $10^{-2}$  M  $\beta$ -CD), against the corresponding blanks.

## Results and Discussion

Within the pH range from 0 - 14, both methyl- and propylparaben are the subjects to protolysis of the hydroxyl group, i.e. they behave as acids (HA):



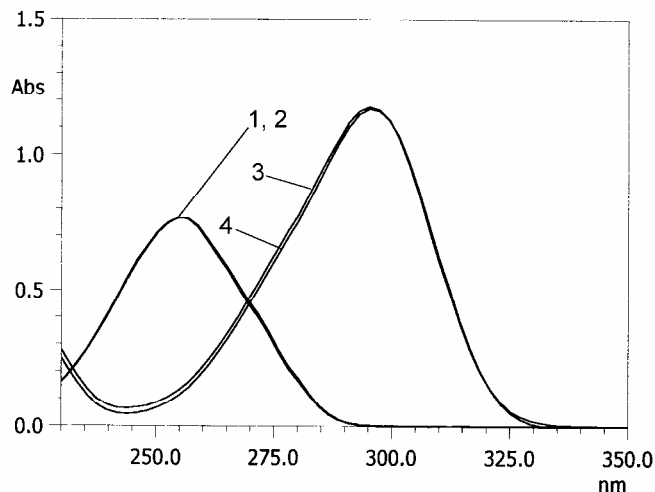
Because both parabens in molecular form are slightly soluble in water, the following equilibria between the solid phase ( $\text{HA}_s$ ) and saturated aqueous solution of these compounds get established:



There is the following relationship between the constant of acidity equilibrium and the equilibrium constants in the heterogeneous system :

$$K_a = \frac{K_{s1}}{K_{s0}} \quad [4]$$

Acidity constants were spectrophotometrically determined. The choice of optimal wavelength was based on the absorption spectra of paraben solutions pH 2.0 (molecular form) and pH 11.0 (anionic form) (Fig. 1). The data obtained by comparison of the corresponding absorption spectra of methyl- and propylparaben enabled us to conclude that both methyl- and propyl- radical influenced spectral characteristics of the examined parabens to almost the same extent. Namely, the two parabens had about the same absorptivity with the absorption maxima at the same wavelengths (molecular and anionic form at 255 nm and 295 nm, respectively). Acidity constants ( $\text{p}K_a$ ) determined by classical spectrophotometric method (5) at 295 nm were 8.03 and 8.00 for methyl- and propylparaben, respectively.



**Figure 1. Absorption spectra of molecular (pH 2.0) and anionic (pH 11.0) form of parabens ( $5 \times 10^{-5}$  M).**

**Molecular form: 1) methylparaben, 2) propylparaben ;**

**Anionic form: 3) methylparaben, 4) propyl paraben.**

**Slika 1. Apsorpcioni spektri molekuskog (pH 2.0) i anjonskog (pH 11.0) oblika metilparabena i propilparabena ( $c=5 \times 10^{-5}$  M).**

The constant  $K_{s0}$  was determined in a heterogeneous system within the pH interval from 2.5 - 4, in which the molecular form of the parabens is dominant. Under these conditions, the solubility (S) is independent on pH value and represents the intrinsic solubility:

$$S = [HA] = K_{s0} \quad [5]$$

The experimental determined values of intrinsic solubility of parabens ( $K_{s0}$ ) are presented in Table I.

**Table I** Experimental determined intrinsic solubility in water of parabens ( $K_{s0}$ )

**Tabela I** Osnovna rastvorljivost parabena u vodi ( $K_{s0}$ ) eksperimentalno određena.

Parabens	pH	$K_{s0}$ (M)	$K_{s0} \pm sd$
Methylparaben	2.5	$1.39 \times 10^{-2}$	$1.42 \times 10^{-2} \pm 3.11 \times 10^{-4}$
	2.8	$1.46 \times 10^{-2}$	
	3.1	$1.41 \times 10^{-2}$	
	3.5	$1.40 \times 10^{-2}$	
	3.9	$1.45 \times 10^{-2}$	
Propylparaben	2.6	$1.80 \times 10^{-3}$	$1.80 \times 10^{-3} \pm 3.36 \times 10^{-5}$
	2.8	$1.79 \times 10^{-3}$	
	3.0	$1.82 \times 10^{-3}$	
	3.4	$1.85 \times 10^{-3}$	
	3.9	$1.76 \times 10^{-3}$	

Based on the obtained  $K_a$  and  $K_{s0}$  values, the constant  $K_{s1}$  was calculated applying the equation [4]. The values of equilibrium constants are listed in Table II. By comparing the  $pK_a$  values, it can be concluded that the alkyl radical in alkoxy carbonyl group does not affect the acidity of the examined parabens. However, the intrinsic solubility of propylparaben ( $1.80 \times 10^{-3}$  M) is approximately 8-fold lower than that of methylparaben ( $1.42 \times 10^{-2}$  M).

**Table II** Equilibrium constants in homogeneous and heterogeneous system of methyl- and propylparaben.  $t=25$  °C;  $I=0.1$  M (NaCl).

**Tabela II** Ravnotežne konstante u homogenom i heterogenom sistemu metilparabena i propilparabena.  $t=25$  °C;  $I=0.1$  M (NaCl).

Constant	Methylparaben	Propylparaben
$K_a \pm sd$	$9.33 \times 10^{-9} \pm 1.15 \times 10^{-10}$	$1.00 \times 10^{-8} \pm 6.1 \times 10^{-10}$
$pK_a \pm sd$	$8.03 \pm 0.01$	$8.00 \pm 0.03$
$K_{s0} \pm sd$	$1.42 \times 10^{-2} \pm 3.11 \times 10^{-4}$	$1.80 \times 10^{-3} \pm 3.36 \times 10^{-5}$
$pK_{s0} \pm sd$	$1.85 \pm 0.01$	$2.74 \pm 0.01$
$K_{s1} \pm sd$	$1.32 \times 10^{-10} \pm 3.04 \times 10^{-12}$	$1.82 \times 10^{-11} \pm 1.26 \times 10^{-12}$
$pK_{s1} \pm sd$	$9.88 \pm 0.01$	$10.74 \pm 0.03$

Knowledge on equilibrium constants in a heterogeneous system enables the calculations of paraben solubility as a function of acidity of a solution:

$$S = [\text{HA}] + [\text{A}^-] = K_{s0} + \frac{K_{s1}}{[\text{H}_3\text{O}^+]} \quad [6]$$

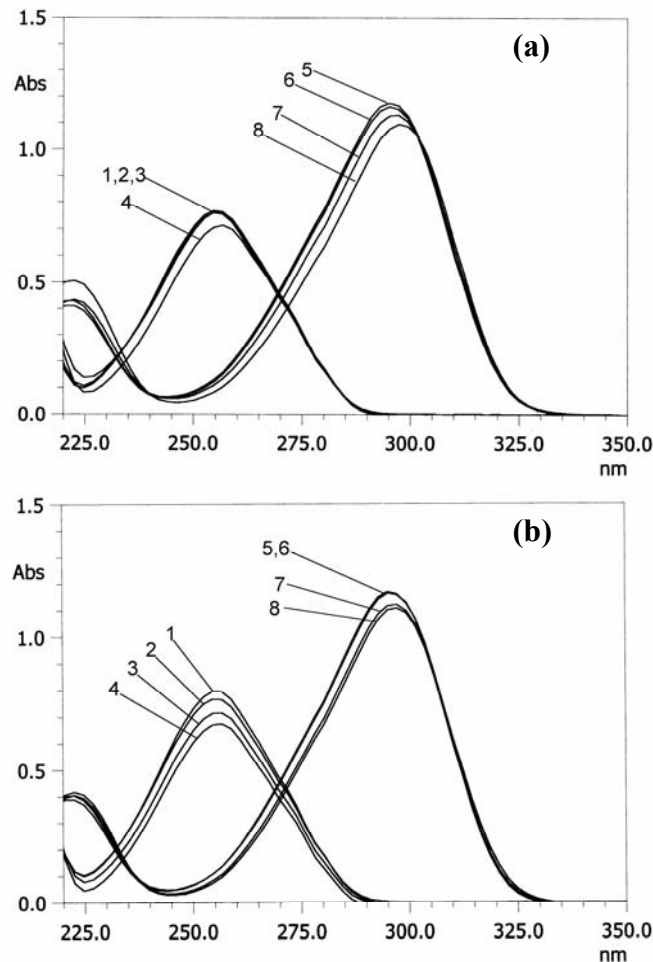
The equation [6] can be applied within the pH range from 6 - 10, in which molecular and anionic paraben forms are in equilibrium. At pH < 6, paraben solubility is constant and equal to the  $K_{s0}$  value.

Matsuda et al. (2) confirmed the formation of inclusion complexes of parabens with  $\beta$ -cyclodextrin. This prompted us to study the effect of  $\beta$ -cyclodextrin on  $pK_a$  of the two parabens. Absorption spectra of molecular (pH 2.0) and anionic (pH 11.0) forms of methyl- and propylparaben in the absence and in the presence of different  $\beta$ -CD concentrations ( $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  M) are depicted in Fig. 2. Although it is evident that  $\beta$ -CD influenced absorption spectra of the parabens, their  $pK_a$  values remained almost unchanged (Table III).

**Table III** The effect of varying  $\beta$ -cyclodextrin concentrations of  $pK_a$  values of methyl- and propylparaben.  
 $t=25$  °C;  $I=0.1$  M (NaCl).

**Tabela III** Uticaj različitih koncentracija  $\beta$ -ciklodekstrina na  $pK_a$  vrednosti metilparabena i propil parabena.  
 $t=25$  °C;  $I=0.1$  M (NaCl).

Paraben	$pK_a \pm sd$		
	$10^{-4}$ M $\beta$ -CD	$10^{-3}$ M $\beta$ -CD	$10^{-2}$ M $\beta$ -CD
Methylparaben	$8.00 \pm 0.01$	$8.01 \pm 0.01$	$8.02 \pm 0.01$
Propylparaben	$8.01 \pm 0.02$	$8.00 \pm 0.02$	$8.02 \pm 0.01$



**Figure 2. Absorption spectra of molecular (pH 2.0) and anionic (pH 11.0) form of parabens ( $5 \times 10^{-5}$  M) in the absence and in the presence of  $\beta$ -cyclodextrin.**

- (a) 1 - methylparaben (molecular form); 2, 3 and 4 - methylparaben (molecular form) in the presence of  $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  M  $\beta$ -CD, respectively; 5-methylparaben (anionic form); 6, 7 and 8 - methylparaben (anionic form) in the presence of  $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  M  $\beta$ -CD, respectively;
- (b) 1 - propylparaben (molecular form); 2, 3 and 4 - propylparaben (molecular form) in the presence of  $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  M  $\beta$ -CD, respectively; 5 - propylparaben (anionic form); 6, 7 and 8 - propylparaben (anionic form) in the presence of  $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  M  $\beta$ -CD, respectively;

**Slika 2. Apsorpcioni spektri molekuskog (pH 2.0) i anjonskog (pH 11.0) oblika metilparabena i propilparabena ( $c=5 \times 10^{-5}$  M), bez i u prisustvu  $\beta$ -ciklodekstrina.**



As shown in Fig. 2, the increase of the  $\beta$ -CD concentration resulted in a parallel shifting of the absorption maxima towards higher wavelengths and at the same time in a decrease of paraben absorptivity. At the highest  $\beta$ -CD concentration applied in the present study, the absorption maxima of molecular and anionic form of either of the parabens studied were shifted by 2 nm. The presence of  $10^{-2}$  M  $\beta$ -CD led to about 7% decrease of the absorption maxima of both methylparaben forms. Under the same conditions, this decrease of propylparaben made 5% and 15% for molecular and anionic form, respectively. These changes in the paraben absorption spectra occurring in the presence of  $\beta$ -CD point to their mutual interactions resulting in the formation of bonds which actually did not influence protolysis of the parabens, themselves.

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# PROTOLITIČKE RAVNOTEŽE U HOMOGENIM I HETEROGENIM VODENIM SISTEMIMA METIL- I PROPILPARABENA I UTICAJ $\beta$ -CIKLODEKSTRINA NA $pK_a$ VREDNOSTI

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## Izvod

Spektrofotometrijski su određene kiselinske konstante ( $K_a$ ) i osnovna rastvorljivost ( $K_{s0}$ ) u vodi metilparabena i propilparabena. Određivanja su vršena na temperaturi od 25 °C i pri konstantnoj jonskoj sili  $I=0.1$  M (NaCl). Na osnovu određenih ravnotežnih konstanti  $K_{s0}$  i  $K_a$  izračunata je ravnotežna konstanta  $K_{s1}$  u heterogenom sistemu parabena. Rastvorljivost parabena u funkciji kiselosti rastvora izračunata je primenom jednačine

$$S = K_{s0} + \frac{[K_{s1}]}{[H_3O^+]}$$

Pored toga, ispitan je uticaj različitih koncentracija  $\beta$ -ciklodekstrina na  $pK_a$  vrednosti datih parabena.

**Ključne reči:** metilparaben, propilparaben, rastvorljivost, kiselinska konstanta.

## References

1. Martindale. The Extra Pharmacopoeia, 33rd ed. London: The Pharmaceutical Press, 2002: 1149.
2. Martindale. The Extra Pharmacopoeia, 29th ed. London: The Pharmaceutical Press, 1989.
3. Matsuda H, Ito K, Sato Y, Yoshizawa D, Tanaka M, Taki A, et al. Inclusion complexation of p-hydroxybenzoic acid esters with 2-hydroxypropyl- $\beta$ -cyclodextrins. On changes in solubility and antimicrobial activity. *Chemical & Pharmaceutical Bulletin* 1993; 41: 1448-52.
4. Pfendt L, Sladić D, Janjić T, Popović G. Study of heterogeneous equilibria in saturated aqueous solutions of some 7-chloro-1,4-benzodiazepines. *Analyst* 1990; 115: 383-7.
5. Albert A, Serjeant EP. The determination of ionization constants. 2nd ed. London: Chapman and Hall, 1971: 44.