STUDY OF INTERACTION OF DYES WITH BIOPOLYMER CHITOSAN

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Abstract

The presented work was focused on the study of interactions of azo dyes (model diffusion probes) with cationic biopolymer chitosan. The interactions were realized via diffusion processes in hydrogel media based on thermoreversible agarose. The main aim was study of influence of solution pH on the diffusion process. Interactions of used dyes are based on electrostatic character. The amino group of chitosan interacts with the functional group of chosen anionic dyes (sulfonic group) and thus affects the process of diffusion. Organic anionic dyes Chicago Sky Blue 6B (C.I. 24 410), Sirius Red (C.I. 35 780) and Reactive Blue 49 (C.I. 621 526) were chosen for this experiment. Used diffusion technique was based on monitoring of the time progression of diffusion profile by UV-VIS spectrophotometry. The presented work follows the previous experiments and shows comprehensive view of the reactivity of chitosan and its behavior in different systems. Unsteady diffusion in cuvettes appears to be universal method for the study of reactivity of biopolymers and for the study of transport processes in hydrogel media.

Keywords: Diffusion, chitosan, hydrogel, agarose, organic dye, reactivity of biopolymers

1. INTRODUCTION

Each life organism is composed by a lot of complex macromolecules which are widely called biopolymers. There are compounds, such as proteins, nucleic acids or polysaccharides which are responsible for functioning of the organism. All these compounds are commonly used in industrial application, especially in pharmaceutical industry. One of the important biopolymers which is often used in pharmaceuticals applications is a linear polysaccharide chitosan. From a chemical point of view is chitosan partially deacetylated *N*-acetyl glucosamine and commercially is produced by deacetylation of chitin, which is the second most widespread polysaccharide. Different types of chitosan are distinguished especially by degree of deacetylation and length of chain, respectively molecular weight [1]. In the nature is chitosan most commonly occurs in the mixture with chitin. Due to its biodegradability and biocompatibility is chitosan significant material for use in many pharmaceutical applications. It also corresponds with high number of patents and articles focused on chitosan published in the last years [2].

Chitosan is a cationic biopolymer due to presence of amino groups which can be protonated in solutions. The presented experiment is based on reactivity of chitosan, especially its amino groups whose reactivity was studied by a simple diffusion technique called unsteady diffusion in cuvettes. The main aim of this work was study of influence of solution pH on diffusion process into hydrogel matrix. Used diffusion technique was based on periodic monitoring of the time progression of diffusion profile used model probes (azo dyes) by UV-VIS spectrophotometry. Interactions of azo dyes with chitosan were based on electrostatic character because amino group of chitosan interacted with sulfonic group of chosen azo dyes and thus affected the diffusion process. The observed parameter was effective diffusion coefficient.

2. EXPERIMENTAL PART

2.1. Materials

Agarose (≤ 10 % w/w humidity), chitosan (75-85 % degrees of deacetylation), 95 % w/w acetic acid, sodium hydroxide and used azo dyes Chicago Sky Blue 6B (C.I. 24 410), Sirius Red (C.I. 35 780) and Reactive Blue 49 (C.I. 621 526) were purchased from Sigma-Aldrich®, structures of dyes are shown in the figures below (**Figure 1-3**). Buffer solutions with different pH were prepared using: disodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate and citric acid which were purchased from Penta®. These chemicals were used for preparation of buffer solutions with pH = 3, pH = 7 and pH = 11. Each of used organic dyes was dissolved in buffers. Final concentrations of organic dyes in buffers were different for each type of dye (Chicago Sky Blue 6B c = 0.01 g/dm^3, Sirius Red c = 0.04 g/dm^3 and Reactive Blue 49 c = 0.10 g/dm^3). Solution of chitosan was prepared by dissolving 0.001 g of chitosan in 50 ml 5 % w/w acetic acid and stirred for 24 hours. Then pH of solution was adjusted using sodium hydroxide to pH value pH = 7 and diluted by distilled water to volume 100 ml. Prior to diffusion experiments calibration curves of all dyes in agarose gel for each value of pH were measured. Hydrogels for diffusion experiments contained the same concentrations of agarose (1.0 % w/w) but different concentrations of chitosan in hydrogel matrix (0 % w/w and 0.001 % w/w). Diffusion experiments were realized in plastics UV-VIS cuvettes (PMMA, 10 x 10 x 45 mm) so this PMMA cuvettes were filled by hydrogel solutions prepared by heating. Cooling of the agarose solutions at room temperature led to the hydrogel formation and then were cuvettes placed for 72 hours at room temperature into glass diffusion container including 250 ml of dye buffer solution.

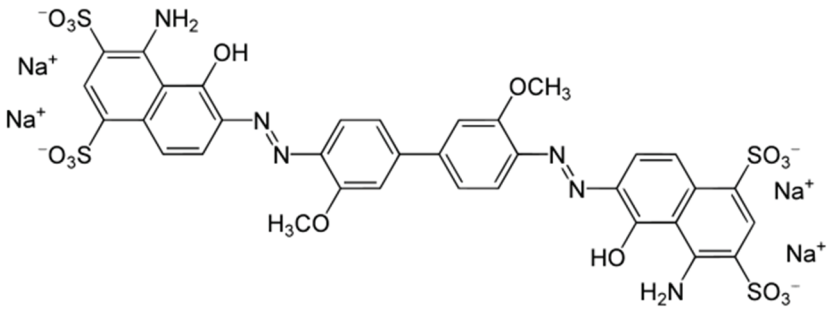

Figure 1 Structure of dye Chicago Sky Blue 6B C.I. 24 410 [3]

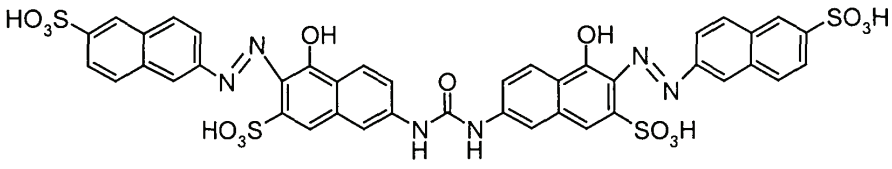

Figure 2 Structure of dye Sirius Red C.I. 35 780 [4]

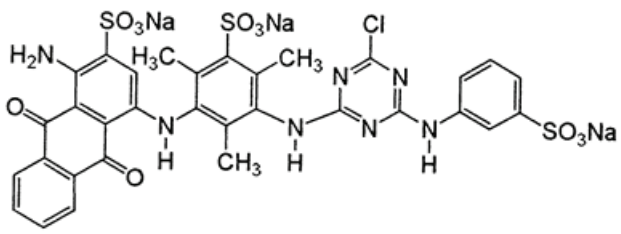

Figure 3 Structure of dye Reactive Blue 49 C.I. 621 526 [5]

2.2. Methods

PMMA cuvettes with hydrogel were measured by UV-VIS spectrophotometer at time interval 24, 48 and 72 hours. Vertically adjustable holder for cuvettes was made for this purpose which allowed to measure cuvettes at different distances from the interface hydrogel-solution. This procedure allowed to obtain complex UV-VIS spectra in different distances of cuvettes. All cuvettes placed in dye buffer solution (pH = 3, pH = 7 and pH = 11) were measured by this UV-VIS technique (**Figure 4**, **Figure 5**) and concentration profiles in each cuvette were obtained by using calibration curves.

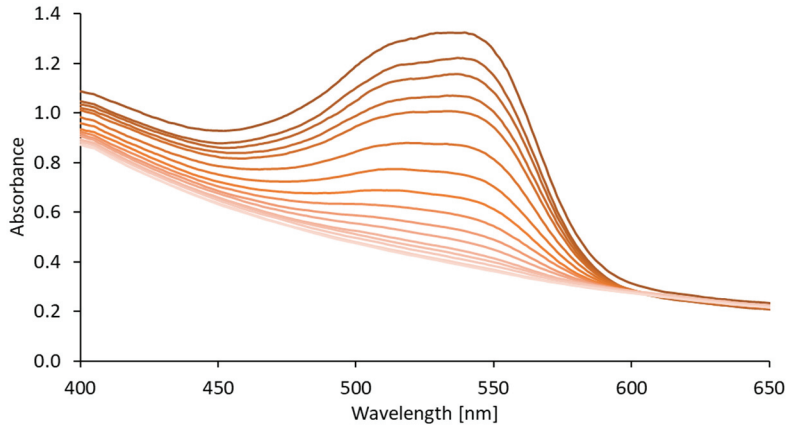

Figure 4 UV-VIS spectra correspond to different distances from interface hydrogel-solution in cuvette placed in dye Sirius Red after 72 hours

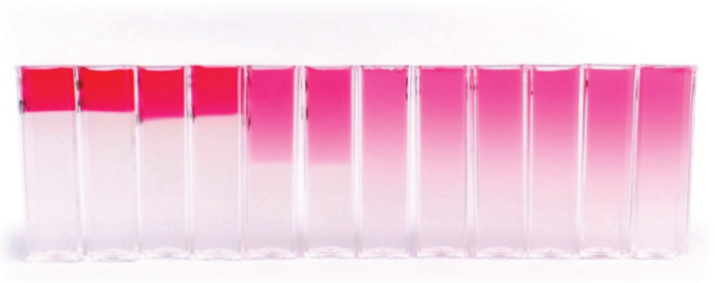

Figure 5 Cuvettes with hydrogel placed in dye buffers of Sirius Red after 72 hours at different pH (from left 6 cuvettes contained hydrogel with chitosan concentration 0.001 % w/w, the rest of hydrogel cuvettes without any addition of chitosan)

2.3. Results and discussion

The most important parameter which corresponds to diffusion process and its speed into hydrogel matrix is effective diffusion coefficient. This parameter was calculated for each cuvette. By evaluation of experimental data was found that pH of solution significantly affects speed of diffusion process in cuvettes with addition of chitosan.

As was already mention in the previous chapter chitosan is a cationic polymer due to presence of amino groups. Amino group of chitosan is protonated in solution and thus interacts with anionic sulfonic group of used dyes. These electrostatic interactions between opposite charged functional groups affect speed of penetration of dyes to the hydrogel matrix. Amino group of chitosan has a pK_a = 6.5. In alkaline solutions is small number of amino groups protonated so only few groups interacts with sulfonic groups of azo dyes. Otherwise acidic solutions lead to formation of $-NH_2^+SO_3^-$ respective $=NH^+SO_3^-$ complexes which affect diffusion process via electrostatic interactions of used dyes [6]. **Figure 6** below shows influence of decreasing pH value of solutions on effective diffusion coefficient for cuvettes with chitosan addition. Hydrogel cuvettes containing just 1 % w/w

agarose gel did not show any influence of diffusion on different pH of solution. Organic azo dye Sirius red showed the most intensive interactions with chitosan in comparison with other used dyes (Chicago Sky Blue 6B and Reactive Blue 49).

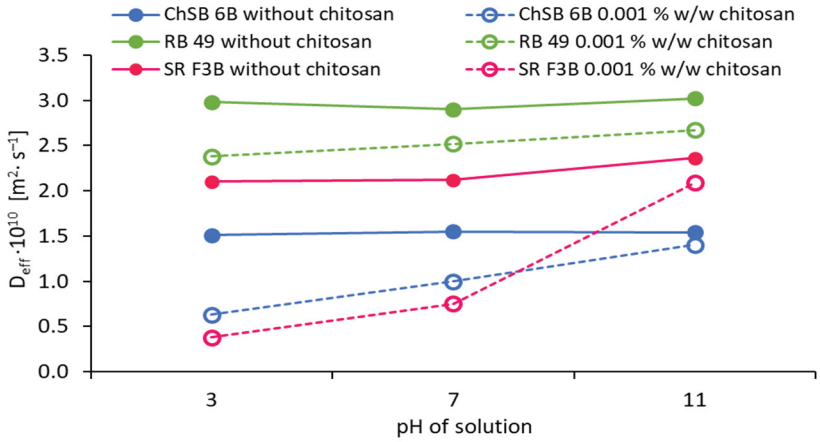

Figure 6 The influence of pH value of solution on effective diffusion coefficient

The obtained spectrophotometric data were evaluated using Origin 8 software. The baseline of each sample was corrected using preset function. Effective diffusion coefficients were obtained by fitting of experimental data to theoretic model based on Fick's laws. Mathematical model corresponds to:

$$c = c_0 \cdot \mathrm{erfc} \frac{x}{\sqrt{4 D_{eff} t}},$$

where c is concentration of diffuse compound, c_0 is a concentration of compound in the phase interface, x is diffusion length, D diffusion coefficient of used compound, t is time of diffusion and erfc is an error function [7]. Fitting of theoretical model was performed by the smallest square method using Solver function in MS Excel. The table below (**Table 1**) shows summary of results for effective diffusion coefficients and concentrations of dyes in solution-hydrogel interface for all tested dyes.

Table 1 Effective diffusion coefficients for hydrogels with chitosan (0.001 % w/w) and without chitosan depending pH of solution

pH of solution	$D_{eff}\cdot10^{10}$ [m^2 s^{-1}]	
Chicago Sky Blue 6B 0.01 g/dm^3		
	0.000 % w/w chitosan	0.001 % w/w chitosan
3	1.51 ± 0.05	0.63 ± 0.08
7	1.55 ± 0.07	1.00 ± 0.05
11	1.54 ± 0.10	1.40 ± 0.13
Sirius Red 0.04 g/dm^3		
	0.000 % w/w chitosan	0.001 % w/w chitosan
3	2.05 ± 0.12	0.38 ± 0.03
7	2.04 ± 0.10	0.74 ± 0.04
11	2.00 ± 0.12	2.01 ± 0.20
Reactive Blue 49 0.10 g/dm^3		
	0.000 % w/w chitosan	0.001 % w/w chitosan
3	2.98 ± 0.09	2.38 ± 0.13
7	2.90 ± 0.13	2.52 ± 0.05
11	3.02 ± 0.07	2.67 ± 0.08

3. CONCLUSION

Reactivity of biopolymers can be tested and measured by many experimental techniques. This study is focused on diffusion method Unsteady diffusion in cuvettes which seems to be a simple tool for reactivity testing of many compounds. This experiment follows the previous experiments which were focused on testing of different conditions, such as temperature or ionic strength and their influence on diffusion speed in hydrogel matrix with biopolymers. It helps to better understand to chitosan and other biopolymers and their behavior in different systems. The study of biopolymers using diffusion techniques has a lot of advantages, such as cheap or simplicity. But this technique is still relatively "new" and should be further tested and optimized. High amount of water in hydrogel matrix can simulate conditions suitable for use in e.g. pharmacy. The obtained results will be compared with other diffusion techniques (sorption experiments, diffusion in diaphragm cell…) in the future [8].

ACKNOWLEDGEMENTS

This research was supported by Materials Research Centre at FCH BUT-Sustainability and Development, REG LO1211, with financial support from National Programme for Sustainability (Ministry of Education, Youth and Sports).

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