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ИММОБИЛИЗАЦИЯ РЕАКТИВНЫХ КРАСИТЕЛЕЙ НА ПОВЕРХНОСТИ УЛЬТРАФИЛЬТРАЦИОННЫХ МЕМБРАН НА ОСНОВЕ ПОЛИ-М-ФЕНИЛЕНИЗОФТАЛАМИДА

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Получены ультрафильтрационные мембраны на основе поли-м-фениленизофталамида (ПА) и его смесей, содержащих 5 мас.% (ПА-5) и 10 мас.% (ПА-10) сополимера акрилонитрила с N,N-диметил-N,N-диаллиламмоний хлоридом. Показана возможность их использования для иммобилизации применяемых в аффинной хроматографии в качестве лигандов реактивных красителей: Cibacron Blue 3GA, Cibacron Brilliant Yellow 3GP, Reactive Red 120. Установлено, что иммобилизация в основном происходит за счет электростатического взаимодействия анионных групп красителей и катионных групп полимерной матрицы. Вклад гидрофобных взаимодействий краситель/мембрана является существенным и возрастает с уменьшением концентрации ионных групп на поверхности материала. В зависимости от вида мембраны и природы красителя максимальная экспериментальная адсорбционная емкость составляет от 0,05 до 0,29 мкмоль·см$^{-2}$. Показана целесообразность применения для описания адсорбции красителей двухпараметрической модели Ленгмюра. Хорошее соответствие экспериментальных изотерм адсорбции модели Фрейндлиха наблюдается при невысоких концентрациях красителей в растворе. Установлено, что увеличение содержания ионных групп в полимерной матрице приводит к росту устойчивости иммобилизации красителя. Для ПА-10 в зависимости от природы красителя десорбция в воде составляет от 2,0 до 3,9% и возрастает в водно-этанольном растворе до 7,1 – 18,0%. Для ПА значения десорбции изменяются в диапазонах 15,7 – 24,6 и 47,8 – 62,9% соответственно. Адсорбция мембранами бычьего сывороточного альбумина, лизоцима и миоглобина исследована в статическом режиме. Установлено, что для всех образцов иммобилизация красителя приводит к возрастанию их адсорбционной емкости. Выявлено влияние природы белка на адсорбцию его макромолекул на поверхности модифицированных мембран, что определяет возможность использования полученных материалов в афинной фильтрации.

Ключевые слова: ультрафильтрационные мембраны, реактивные красители, иммобилизация

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IMMOBILIZATION OF REACTIVE DYES ON THE SURFACE OF ULTRAFILTRATION MEMBRANES BASED ON POLY-M-PHENYLENISOPHTHALAMIDE

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Ultrafiltration membranes based on poly-m-phenylenisophthalamide (PA) and its mixtures containing 5 wt.% (PA-5) and 10 wt.% (PA-10) acrylonitrile copolymer with N, N-dimethyl-N, N-diallylammonium chloride were produced. Their possible application for reactive dyes immobilization used in affinity chromatography as ligands: Cibacron Blue 3GA, Cibacron Brilliant Yellow 3GP, Reactive Red 120 has been demonstrated. Immobilization has been stated to occur basically due to the electrostatic interaction of dyes anionic groups and polymer matrix cationic groups. The contribution of hydrophobic dye/membrane interactions is significant and increases alongside the decrease in ionic groups concentrations on the material surface. Depending on the membrane type and the dye nature, the maximum experimental adsorption capacity ranges from 0.05 to 0.29 μmol/cm^2. The possibility to apply the two-parameter Langmuir model for describing the adsorption of the dyes was shown. Good correspondence of the experimental adsorption isotherms of the Freundlich model was observed at low concentrations of dyes in the solution. It was also found that the increase in the ionic group amount in the polymer matrix causes an increase in the dye immobilization stability. Depending on the dye nature, desorption in water for PA-10 varied from 2.0 to 3.9%, it increases in water-ethanol solution up to 7.1 – 18.0%. The desorption values for PA varied in the range of 15.7 – 24.6 and 47.8 – 62.9%, respectively. The adsorption of bovine serum albumin, lysozyme, and myoglobin by membranes has been studied in a static mode. It is revealed that the dye immobilization for all samples causes an increase in their adsorption capacity. The influence of the protein nature on its macromolecules adsorption on the modified membrane's surface was revealed. Therefore, it is possible to use the developed materials in affine filtration.

Key words: ultrafiltration membranes, reactive dyes, immobilization

INTRODUCTION

Membrane technologies demand further advanced development for several reasons. Primarily, because traditional methods of substance separation, neutralization, and purification can't ensure their compliance with the ever-increasing environmental and economic requirements. According to expert assessment, in the future, global demand for membranes will be expanding by 8.5% per year on average [1]. Currently, the leaders of industrial application of membranes are water pretreatment and water treatment enterprises (about 50% of the market), food and beverage production (about 21%), pharmaceuticals, and medicine (about 9%) [2, 3]. Considering the current trends in science and technology development, probably, the latter component will show the most active growth in the near future. One of the key processes ensuring membranes and membrane technologies application in pharmaceuticals is ultrafiltration. Its application is largely stimulated by the possibility, in accordance with the tasks to be solved, of adapting, «adjusting» the properties of the resulting membranes. The membrane surface modification can be considered as the instrument of such «adjustment» to provide them with new qualities, such as separation activity and selectivity that are not initially inherent in the original polymer composition, as well as several additional properties providing the material with mechanical strength and resistance to contamination by organic components [4-8]. Affine filtration is a separate area where the various approaches to development that allows modifying the surface are in great demand. The basic theoretical considerations and their experimental implementation in

the membrane production were introduced to membrane technology from affinity chromatography, which proved its effectiveness in the processes of purification and separation of target protein components from biological systems [9-10]. Specific interaction in affine membranes is primarily provided by macro- and microligands immobilized due to covalent or non-covalent bonds [11-16], the latter is usually an electrostatic interaction of active component/membrane. Membranes based on cellulose and its derivatives, polyethersulfone, and other «traditional» polymers for membrane technology are used as matrix [17-21].

One of the most popular ligand groups in affine processes is reactive dyes. Their advantage is determined by low cost, availability, easy immobilization on the matrix surface, biological and chemical stability, and selectivity. However, despite common approaches, the choice of a certain dye as an affinity ligand for each specific protein system is an empirical and time-consuming process, including screening of several different dyes, immobilizing each of them on the matrix, and assessing their selectivity concerning the target component.

The research objective was to obtain affine materials by non-covalent immobilization of reactive dyes on ultrafiltration membranes surface, based on poly-m-phenylenisophthalamide.

EXPERIMENTAL TECHNIQUE

Samples of wet-spun ultrafiltration membranes made by the phase inversion method based on poly-m-phenyleneisophthalamide (OAO «Polymersintez», Russia) (PA) and its mixtures containing 5 wt.% (PA-5) and 10 wt.% (PA-10) of the acrylonitrile/N,N-dimethyl-N,N-diallylammonium chloride copolymer (75:25 mol.%) (OAO «Kaustik», Russia) were used in the work. Researched membranes were obtained on a nonwoven substrate. The thickness of the investigated membrane samples was □ 190 μm and the thickness of the substrate was □ 90 μm. The rated cut-off molecular weight of the membranes was □ 30 kDa and there ξ-potential value of +3.5 mV (PA), +6.7 mV (PA-5), and +9.5 mV (PA-10).

As components of immobilization were used dyes (Sigma-Aldrich) of the following structure:

The processing of membrane samples was carried out in static mode.

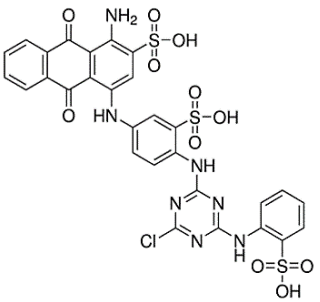
To measure the concentration of dyes, an SF-2000 spectrophotometer was used; the optical density of the solutions was monitored at a wavelength λ 620 (CB), 420 (CY), 540 (CR) nm.

To mathematically processing the experimental data, the two-parameter models by Langmuir and Freundlich were used [22-24]:

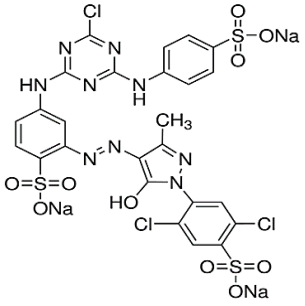
$$Q = \frac{Q_m \cdot b \cdot C}{1 + b \cdot C}$$

$$Q = K_F \cdot C^{1/n}$$

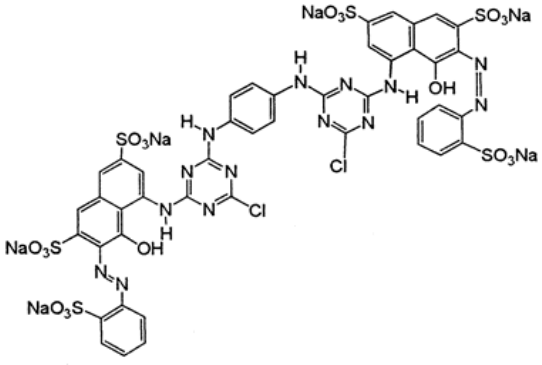
where Q – equilibrium adsorption capacity of the membrane, mg·cm^{-2}; C – concentration of the dye in solution at the moment of equilibrium, mg·dm^{-3}; Q_m – maximum adsorption calculated value, mg·cm^{-2}; b – constant, describing the affinity in adsorbate/adsorbent system, dm^3·mg^{-1}, K_F – constant, representing adsorption capacity, (dm^3)$^{1/n}$·mg$^{(1-1/n)}$·cm^{-2}, n – a parameter that indicates adsorption intensity.



Cibacron Blue 3GA (CB)



Cibacron Brilliant Yellow 3GP (CY)



Reactive Red 120 (CR)

To assess the compliance degree of the experimental data to the selected mathematical models, the values of the coefficient of determination (R^2) [23] were used.

The stability of dye immobilization was studied in the static mode. The duration of the experiment was 14 days. The degree of desorption was calculated by the formula:

$$W = \frac{Q_0 - Q_f}{Q_0} \cdot 100\%$$

where Q_0 and Q_f – initial and final adsorption capacity of the membrane for the dye, respectively, mg·cm^{-2}.

Adsorption of proteins (Sigma-Aldrich) (Table 1) by the membranes was investigated in the static mode. To measure the concentration of proteins, an SF-2000 spectrophotometer was used; the optical density of the solutions was monitored at a wavelength λ = 278 nm [25].

Table 1

Characteristics of proteins [26, 27]
***Таблица 1.* Характеристики белков [26, 27]**

Protein	Molecular weight, kDa	pI	Stokes radius of the molecule, r_s, Å	Structure
Bovine serum albumin (BSA)	66.4	4.9	34.0	Ala – 46; Phe – 27; Lys – 59; Pro – 28; Thr – 34; Cys – 35; Gly – 16; Leu – 61; Gln – 20; Val – 36; Asp – 40; His – 17; Met – 4; Arg – 23; Trp – 2; Glu – 59; Ile – 14; Asn – 14; Ser – 28; Tyr – 20
Lysozyme (Lys)	14.3	11.0	20.6	Asp – 7; Glu – 2; His – 1; Lys – 6; Tyr – 3; Arg – 11; α-amino – 1; α -carboxyl – 1
Myoglobin (Myo)	16.8	7.0	16.0	Asp – 8; Glu – 13; His – 7; Lys – 19; Tyr – 2; Arg – 2; α-amino – 1; α -carboxyl – 1; heme-carboxyl – 2

Table 2

Isotherms parameters for CB, CY, CR dyes adsorption by PA, PA-5 and PA-10 membranes
***Таблица 2.* Параметры изотерм адсорбции красителей CB, CY, CR мембранами ПА, ПА-5 и ПА-10**

Membrane	Dye	$Q_{m\ exp}$, mg·cm^{-2}	Langmuir isotherm			Freundlich isotherm		
			Q_m	b	R^2	K_{LF}	n	R^2
PA	CB	0.0552	0.0603	0.0376	0.987	0.0041	1.9589	0.769
	CY	0.0590	0.0638	0.0420	0.988	0.0050	2.0313	0.761
	CR	0.0758	0.0845	0.0315	0.986	0.0046	1.7864	0.829
PA-5	CB	0.0987	0.1543	0.0113	0.959	0.0027	1.3236	0.816
	CY	0.1191	0.1558	0.0065	0.948	0.0022	1.1718	0.761
	CR	0.1373	0.1890	0.0176	0.892	0.0060	1.4925	0.724
PA-10	CB	0.2243	0.4221	0.0055	0.952	0.0038	1.1321	0.756
	CY	0.2342	0.4409	0.0081	0.943	0.0054	1.2238	0.727
	CR	0.2951	0.4871	0.0068	0.938	0.0059	1.1685	0.723

Experiments were performed with samples with the area of 2 cm^2. The volume of the protein solution was 5 cm^3. To prepare the solutions, a universal buffer (the ionic strength of the solution is 0.02 mol·dm^{-3}) was used. The protein concentration in the solution was 0.25 g·dm^{-3}. Adsorptive properties of the membranes were assessed by the change of protein content in the solutions after maintaining samples in them until the reach of equilibrium. The adsorption capacity of the membranes Q was calculated as the ratio of the adsorbed substance mass to the sample area.

The number of parallel tests in series of experiments was no less than five. The results describing the experimental data were processed using the theory of errors. The confidence interval was calculated to the significance level of 0.05.

RESULTS AND DISCUSSION

The basic forces ensuring the intermolecular polymer – dye interaction are: Coulomb forces (when both the dye and polymer composition contains ionic groups); hydrophobic interactions between polymer and dye macromolecules and between the bound dye molecules; interaction of π-electrons between neighboring bound dye molecules [28]. The dominance of the polymer – dye ionic interactions determines the possibility of applying dyes as test systems allowing to determine the availability and nature of ionic groups on the membrane surface and demonstrate a proper correlation with the value and ξ-potential sign of the material surface [29]. All the membranes considered in this paper have positive ξ-potential of surface, its value rises when polymer, based on poly-m-phenylenisophthalamide and containing quaternary ammonium groups is introduced into the composition. The dyes selected as immobilization components include the sulfonate groups in acid (CB) and salt forms (CY, CR). Thus, Coulomb forces can be assumed to ensure the CB, CY, and CR dyes immobilization on the membranes' surface.

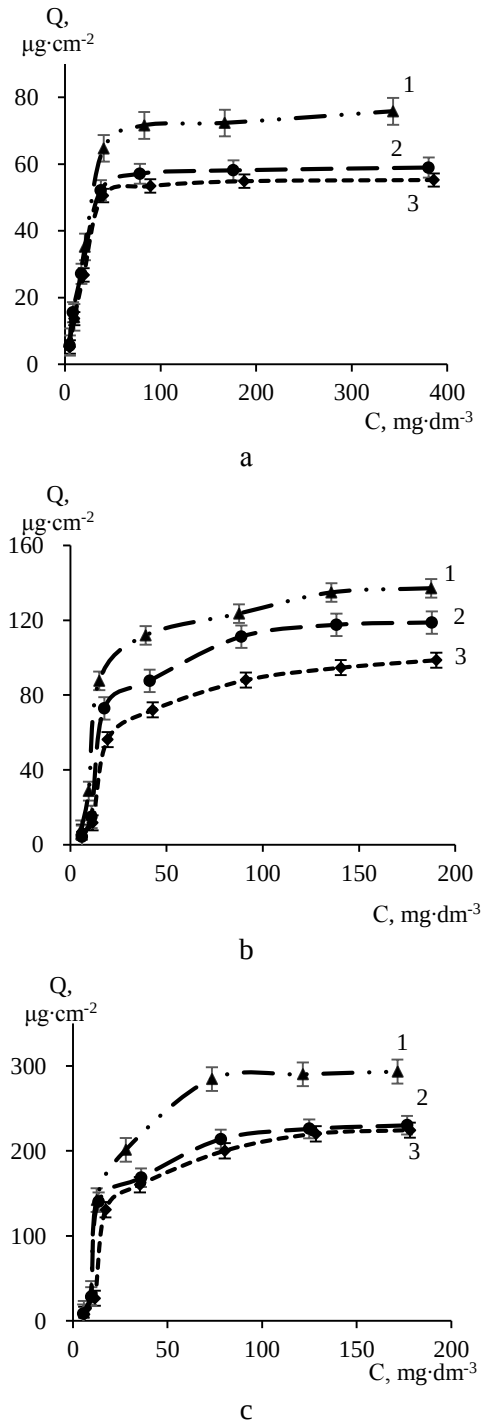


Fig. Experimental isotherms of adsorption CR (1), CY (2), CB (3) by membranes PA (a), PA-5 (b), PA-10 (c)
Рис. Экспериментальные изотермы адсорбции CR (1), CY (2), CB (3) мембранами ПА (a), ПА-5 (b), ПА-10 (c)

The experimental isotherms of dye adsorption by PA (a), PA-5 (b), and PA-10 (c) membranes are shown in Figure. The maximum experimental adsorption capacity of PA; PA-5 and PA-10 samples for CB, CY, and CR equals 0.07, 0.07, 0.05; 0.13, 0.14, 0.09 and 0.29, 0.28, 0.20 mmol·cm^{-2} respectively. The comparability of the obtained different dyes adsorption values for the similar membrane type and the nature of their changes for membranes PA, PA-5 and PA-10 proves that the ion binding of dye/membrane determines the immobilization mechanism of dye molecules on the surface. However, non-electrostatic forces (primarily hydrophobic interactions and hydrogen bonds) can significantly contribute to organic components immobilization [30], causing the difference in the adsorption capacity of similar membrane samples with CB, CY, and CR dyes.

The results of mathematical processing demonstrated the application preference of the two-parameter Langmuir model to characterize the adsorption in the studied systems (Table 2). Good consistency of the experimental adsorption isotherms of the Freundlich model was observed at low concentrations of dyes in the solution.

The static mode studies results of dyes' desorption from the membrane surface are presented in Table 3. The dyes' desorption rate in the discussed systems is basically determined by two main factors: the ionic groups amount in the studied material and the desorption medium nature. The received data demonstrated the increased contribution of Coulomb forces and decreased role of hydrophobic interactions when membranes containing a significant amount of the ionic groups are used as a matrix for immobilization. The desorption for PA-10, depending on the dye nature, varied from 2.0 to 3.9% in water solution and increased in water-ethanol solution to up to 7.1-18.0%. This fact demonstrates rather good stability of the developed systems in aqueous media.

The data presented in Table 4 proves the effect nature of the CB dye immobilization on the membranes' adsorption capacity in relation to the model proteins.

Table 3

Desorption of CB, CY, CR dyes immobilized on the surface of the membranes PA, PA-5 and PA-10

Таблица 3. **Десорбция красителей CB, CY, CR, иммобилизованных на поверхности мембран ПА, ПА-5 и ПА-10**

Membrane	Desorption degree, %	
	Water	Ethanol – water (60:40 vol.%)
PA(CB)	16.81±1.08	49.54±1.71
PA(CY)	22.34±1.11	61.75±1.13
PA(CR)	23.72±0.89	55.82±1.45
PA-5(CB)	6.75±0.28	18.52±1.54
PA-5(CY)	7.36±1.14	21.36±2.02
PA-5(CR)	9.31±1.06	20.12±1.87
PA-10(CB)	2.32±0.29	9.43±2.31
PA-10(CY)	3.21±0.34	16.11±1.84
PA-10(CR)	3.40±0.45	12.12±1.25

Table 4

Proteins adsorption by membranes based on poly-m-phenylenisophthalamide (pH=7.0, T=25°C)

Таблица 4. **Адсорбция белков мембранами на основе поли-м-фениленизофталамида (pH=7.0, T=25°C)**

Membrane	Adsorption capacity, mg·cm^{-2}		
	BSA	Lys	Myo
PA	0.012	0.035	0.027
PA(CB)	0.104	0.140	0.116
PA-5	0.019	0.039	0.028
PA-5(CB)	0.083	0.174	0.121
PA-10	0.034	0.008	0.025
PA-10(CB)	0.098	0.245	0.138

In all samples, the dyes' immobilization causes a significant increase of their adsorption capacity, regardless of the protein macromolecules charge (at pH = 7.0, macromolecules of BSA are negatively charged, Lys – positively, and Myo – not charged). It can be assumed that it is reasoned by the increase of hydrophobic interactions of protein/immobilized dye, which largely compensates the contribution of the electrostatic force to the protein adsorption on the membrane surface. Nevertheless, the difference in the received values for the same PA-10(CB) membrane indicates the interactions uniqueness implemented in the protein/dye/membrane system, which determines the possibility to use modified membranes in the affine filtration processes.

CONCLUSION

The research has proved the possibility of using porous membranes based on poly-m-phenylenisophthalamide as matrices for the immobilization of Cibacron Blue 3GA, Cibacron Brilliant Yellow 3GP and Reactive Red 120 dyes by adsorption mechanism. Primarily the immobilization is caused by electrostatic interaction of dye anionic groups and polymer matrix cationic groups. The contribution of hydrophobic interactions of dye/membrane system increases alongside the concentration decrease of ionic groups on the material surface. Depending on the membrane type and the dye nature, the maximum experimental adsorption capacity varies from 0.05 to 0.29 μmol·cm^{-2}. The resulting dye/membrane systems demonstrate good stability in aqueous environments. The main factor determining the dye fixation stability on the material surface is the presence and number of the corresponding ionic groups on it. For PA-10, depending on the dye nature, desorption varies from 2.0 to 3.9% in water solution and increases up to 7.1-18.0% in an aqueous-ethanol solution. The dye immobilization causes a significant increase in the membrane adsorption capacity, regardless of the adsorbed protein macromolecules charge. For PA-10 (CB) at pH = 7.0, the adsorption capacity for bovine serum albumin, lysozyme, and myoglobin is 98, 245, and 138 μg·cm^{-2} respectively. The protein nature influence on its macromolecules adsorption on the surface of the modified membrane opens up the prospects for the application of the developed materials in affine filtration.

The authors declare the absence a conflict of interest warranting disclosure in this article.

Авторы заявляют об отсутствии конфликта интересов, требующего раскрытия в данной статье.

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