DOI: 10.6060/ivkkt.20256810.7207

УДК: 547.466:543.2

ИССЛЕДОВАНИЕ ЭКСТРАКЦИИ ТРЕОНИНА СОПОЛИМЕРАМИ НА ОСНОВЕ N-ВИНИЛФОРМАМИДА

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Предложены новые экстракционные системы на основе N-винилформамида для извлечения треонина с целью его последующего определения в водных средах. Такие системы отвечают требованиям «зеленой» химии, являются нетоксичными, негорючими, и способны обеспечить практически полное извлечение треонина. В работе изучено межфазное распределение треонина в системах с поли-N-винилформамидом, сополимерами N-винилформамида с N-винилимидазолом, 1-винил-3,5-диметилпиразолом, 1-метакрилоил-3,5-диметилпиразолом. В статье приведены рассчитанные коэффициенты распределения и степень извлечения треонина в диапазоне его концентраций 1,0 – 3,0 мг/см^3. Изучено влияние концентрации экстрагентов, природы высаливателя, соотношения объемов водной и органической фаз, времени и температуры экстракции на количественные характеристики межфазного распределения. Установлены оптимальные условия экстракции, при которых степень извлечения треонина сополимерами N-винилформамида достигает 97%: экстрагент сополимер N-винилформамид с 1-метакрилоил-3,5-диметилпиразолом с концентрацией 0,25 моль/дм^3, соотношение фаз 10:3, концентрация треонина 2,5 - 3,0 мг/см^3. Показаны преимущества экстракции сополимерами N-винилформамида по сравнению с другими полимерами. Определение треонина в водной фазе после экстракции осуществлено методом капиллярного электрофореза при длине волны 254 нм. Обработка результатов электрофоретического определения аминокислоты проведена с помощью программного обеспечения "Эльфоран" для Windows. Предложены схемы межфазного взаимодействия в системе поли-N-винилформамид – треонин за счет образования водородных связей с учетом особенностей свойств и строения экстрагента и аналита. Разработанная методика экстракционно-электрофоретического определения треонина в водных средах может быть рекомендована для оценки качества пищевых продуктов и белковых смесей.

Ключевые слова: треонин, экстракция, N-винилформамид, сополимер, капиллярный электрофорез

Для цитирования:
Мокшина Н.Я., Пахомова О.А., Иванчура П.В. Исследование экстракции треонина сополимерами на основе N-винилформамида. *Изв. вузов. Химия и хим. технология*. 2025. Т. 68. Вып. 10. С. 58–63. DOI: 10.6060/ivkkt.20256810.7207.

For citation:
Mokshina N.Ya., Pakhomova O.A., Ivanchura P.V. Study of threonine extraction by N-vinylformamide based copolymers. *ChemChemTech [Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.]*. 2025. V. 68. N 10. P. 58–63. DOI: 10.6060/ivkkt.20256810.7207.

STUDY OF THREONINE EXTRACTION BY N-VINYLFORMAMIDE BASED COPOLYMERS

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New extraction systems based on N-vinylformamide for the extraction of threonine for its subsequent determination in aqueous media are proposed. Such systems meet the requirements of "green" chemistry, are non-toxic, non-flammable, and are capable of providing almost complete extraction of threonine. The interphase distribution of threonine in systems with poly-N-vinylformamide, copolymers of N-vinylformamide with N-vinylimidazole, 1-vinyl-3,5-dimethylpyrazole, 1-methacryloyl-3,5-dimethylpyrazole was studied. The article presents the calculated distribution coefficients and the degree of extraction of threonine in the concentration range of 1.0 - 3.0 mg/cm^3. The influence of the extractant concentration, the nature of the salting out agent, the ratio of the volumes of the aqueous and organic phases, the time and temperature of extraction on the quantitative characteristics of the interphase distribution was studied. Optimum extraction conditions have been established, at which the degree of threonine extraction by N-vinylformamide copolymers reaches 97%: extractant is a copolymer of N-vinylformamide with 1-methacryloyl-3,5-dimethylpyrazole with a concentration of 0.25 mol/dm^3, phase ratio is 10:3, threonine concentration is 2.5 - 3.0 mg/cm^3. The advantages of extraction by N-vinylformamide copolymers were shown in comparison with other polymers. Determination of threonine in the aqueous phase after extraction is carried out by capillary electrophoresis at a wavelength of 254 nm. The results of electrophoretic determination of the amino acid are processed using the Elforan software for Windows. Schemes of interphase interaction in the poly-N-vinylformamide – threonine system due to the formation of hydrogen bonds are proposed, taking into account the features of the properties and structure of the extractant and analyte. The developed method of extraction-electrophoretic determination of threonine in aqueous media can be recommended for assessing the quality of food products and protein mixtures.

Keywords: threonine, extraction, N-vinylformamide, copolymer, capillary electrophoresis

INTRODUCTION

Threonine is an essential amino acid that must be supplied to the body with food, and it is also involved in the synthesis of vitamin B_{12} [1, 2]. In recent years, the share of special-purpose products (sports nutrition, energy gels, chewing gum, bars and drinks) containing amino acids has increased sharply. Such products help to increase physical endurance, promote muscle mass growth and are used not only by athletes, but also by representatives of professions that require a lot of effort and energy [3, 4]. Amino acids, including threonine, are involved in fat metabolism and also perform vital functions for the human body: structural, digestive, antioxidant, neurotransmitter, immunomodulatory and others [5, 6]. Threonine is an amino acid, high doses of which are used for recovery after surgeries and injuries, and a deficiency leads to negative consequences for the central nervous system. Control of the threonine content in medicines and food products is very important and for this aim it is necessary to develop new methods for extraction and determination of this amino acid.

The need to control the content of amino acids in foods and drinks without destroying the multi-component matrix of the object is of particular relevance.

Liquid extraction is the most effective stage of sample preparation for isolating amino acids and subsequently determining them in aqueous media using physicochemical methods [7-9]. Water-soluble homo- and copolymers of N-vinylformamide, which meet the requirements of "green" chemistry and exclude the use of harmful organic substances, are successfully used as amino acid extractants [10, 11]. There have been no systematic research regarding threonine. However, there are only isolated results of polymer extraction [12].

The aim of the work is to study of the mechanism and calculation of extraction characteristics of the interphase distribution of threonine in water-salt systems based on N-vinylformamide. To solve the set tasks, polymers were synthesized, threonine was extracted from aqueous solutions using poly-N-vinylformamide and copolymers of N-vinylformamide with N-vinylimidazole, 1-vinyl-3,5-dimethylpyrazole, 1-methacryloyl-3,5-dimethylpyrazole, and amino acid determination was carried out using capillary electrophoresis.

EXPERIMENTAL PROCEDURE

N-vinylformamide (VF) was used to synthesize poly-N-vinylformamide (PVF). The process was carried out in the presence of an inhibitor (hydroquinone) at Tbp = 74 °C. Copolymers of N-vinylformamide – N-vinylimidazole (VF-VI), N-vinylformamide – 1-vinyl-3,5-dimethylpyrazole (VF-VDMP) and N-vinylformamide – 1-methacryloyl-3,5-dimethylpyrazole (VF-MDMP) were obtained by radical copolymerization in a dioxane solution at T = 65 °C in the presence of azo-bis-isobutyric acid dinitrile as an initiator and in the range of comonomer ratios of 0.1-0.9 mole fractions [12, 13]. The structures of threonine, the initial monomers and copolymers are shown in Fig. 1.

Liquid extraction was carried out in graduated test tubes with a capacity of 20 cm^3. A salting-out solution (ammonium sulfate) with a concentration of 15-20% by weight was prepared in advance, then 10 cm^3 of an aqueous-salt solution of threonine with a concentration of 1.0-3.0 mg/cm^3 and 1-3 cm^3 of an extractant solution with a concentration of 0.20-0.25 g/cm^3 were placed in the test tubes. After interphase equilibrium was established (10-15 min), the ratio of the volumes of the equilibrium phases r was measured, which changed due to partial dissolution of the copolymers in the aqueous-salt solution of the amino acid. The distribution coefficients D and the degree of extraction R (%) of threonine are calculated using known formulas [11, 12].

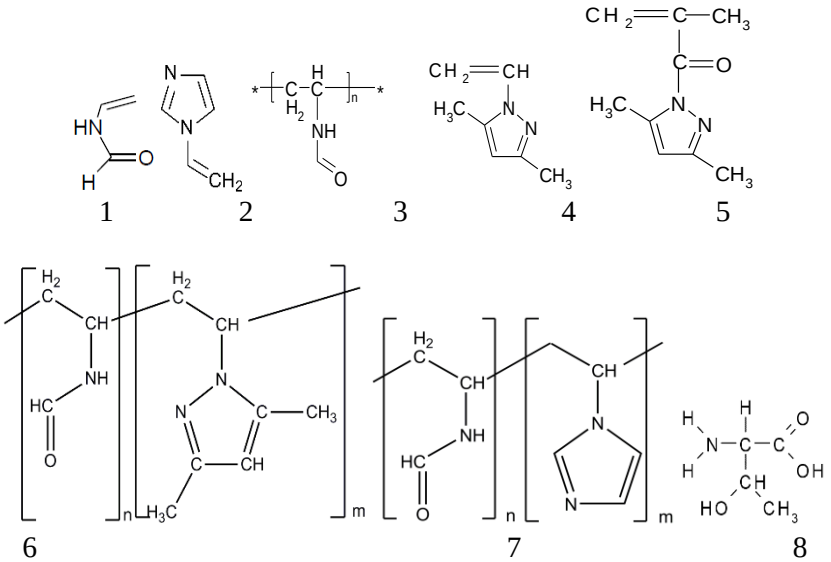

Fig. 1. Structures of VF (1), VI (2), PVF (3), VDMP (4), MDMP (5), VF-VDMP (6), VF-VI (7), threonine (8)
Рис. 1. Структуры ВФ (1), ВИ (2), ПВФ (3), ВДМП (4), МДМП (5), ВФ-ВДМП (6) и ВФ-ВИ (7), треонина (8)

0.5-1.0 cm^3 of the aqueous phase was placed in a dry Eppendorf tube, centrifuged for 5-10 min at 5000 rpm and analyzed by capillary electrophoresis under the following conditions: Kapel 105M system with spectrophotometric detection (Lumex, St. Petersburg), voltage +25 kV, temperature 30 ± 1 °C, water samples under a pressure of 300 mbar for 20 s [14, 15].

RESULTS AND ITS DISCUSSION

Previously, we studied the extraction of some amino acids with N-vinylformamide-based copolymers and established the optimal process conditions [11, 16]. Tables 1 and 2 show the distribution coefficients and the degree of extraction of threonine during its extraction with PVF and N-vinylformamide based

copolymers. The concentrations of PVF and VF-VI were 0.2 g/cm^3, VF-VDMP and VF-MDMP – 0.25 g/cm^3. For all systems, the extraction characteristics were calculated at different amino acid concentrations and ratios of the water-salt and organic phases. At r = 10:3 and a threonine concentration of 3.0 mg/cm^3, the maximum degrees of threonine extraction were obtained. However, it should be noted that the difference in the R values in systems with r = 10:2 and r = 10:3 is insignificant.

Table 1

Extraction characteristics of threonine when extracted by the copolymer VF-VI and PVF (n = 3, P = 0.95)

***Таблица 1.* Экстракционные характеристики треонина при извлечении сополимером ВФ-ВИ и ПВФ (n = 3, P = 0,95)**

Concentration of threonine, mg/cm^3	PVF		VF-VI	
	D	R, %	D	R, %
r = 10:1				
1.0	86±6	89.6	61±2	85.9
2.0	74±4	88.1	64±2	86.5
2.5	126±9	92.6	77±3	88.5
3.0	132±9	92.9	68±2	87.1
r = 10:2				
1.0	109±6	95.6	101±5	95.3
2.0	128±6	96.2	123±6	96.1
2.5	132±7	96.4	120±6	96.0
3.0	125±6	96.2	118±4	95.9
r = 10:3				
1.0	94±3	96.9	74±3	94.8
2.0	89±2	96.7	88±4	94.6
2.5	110±5	97.3	91±4	96.8
3.0	101±5	97.1	78±2	93.4

Table 2

Extraction characteristics of threonine when extracted by copolymers VF-MDMP and VF-VDMP (n = 3, P = 0.95)

***Таблица 2.* Экстракционные характеристики треонина при извлечении сополимерами ВФ-МДМП и ВФ-ВДМП (n = 3, P = 0,95)**

Concentration of threonine, mg/cm^3	VF-MDMP		VF-VDMP	
	D	R, %	D	R, %
r = 10:1			r = 10:2	
1.0	64±2	86.5	58±1	92.0
2.0	78±2	88.6	60±2	92.3
2.5	89±3	89.9	64±2	92.7
3.0	80±2	88.9	66±2	92.9
r = 10:2			r = 10:4	
1.0	115±7	95.8	40±1	94.1
2.0	128±7	96.2	36±1	93.5
2.5	125±6	96.2	42±1	94.3
3.0	136±6	96.5	44±2	94.6
r = 10:3			r = 10:5	
1.0	120±5	97.6	62±1	96.8
2.0	121±5	97.6	65±2	97.0
2.5	131±6	97.8	59±1	96.7
3.0	135±5	97.9	68±2	97.1

In the VF-VDMP extraction systems, no differences in the degrees of extraction were observed at different phase ratios either. At a phase ratio of 10:1, quantitative extraction of threonine was achieved as a result of double extraction. It should be noted that in the previously studied N-vinylcaprolactam – VDMP system, the degree of threonine extraction is 97% with a phase ratio of 10:4 [12]. In systems based on N-vinylformamide (PVF and VF-MDMP), it is also possible to achieve almost complete extraction of threonine (97% and higher) with the same concentration of amino acid in the solution, but with a phase ratio of 10:3, which allows reducing the consumption of the extractant. Homo- and copolymers of N-vinylformamide showed almost the same high extraction capacity with respect to threonine, which significantly exceeded the efficiency of amino acid extraction, for example, with poly-N-vinylpyrrolidone [17].

A further increase in the concentration of threonine did not lead to an increase in the degree of its extraction, which may be due to the limited ability of threonine to form intermolecular bonds with extractants (Fig. 2). Threonine is an amino acid with non-dissociating polar but uncharged side radicals that are capable of forming hydrogen bonds between themselves [18, 19]. Fig. 2 shows the interaction scheme in the PVF – threonine system, taking into account the structural features of the amino acid (Fig. 1). An analysis of quantum-chemical modeling of hydrogen bonds in threonine molecules and calculations of energy characteristics given in [18] showed that the stabilization energy of the O…H–O hydrogen bond in threonine is 13.65 kcal/mol, and the charge transfer value is 0.032. These data indicate the existence of a medium-strength H-bond between the –COO– and –OH groups in threonine. Obviously, the mechanisms of interphase interaction of threonine with other extractants based on N-vinylformamide will be similar.

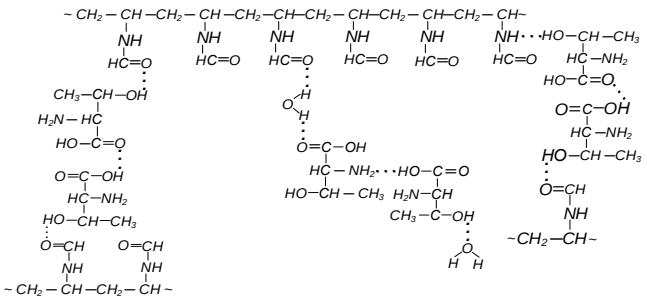

Fig. 2. Scheme of interaction in the PVF-threonine system

Рис. 2. Схема взаимодействия в системе ПВФ – треонин

Electrophoretic determination of threonine in the aqueous phase after extraction was carried out at a wavelength of 254 nm. The leading electrolyte was a

phosphate buffer solution (pH = 7.8 ± 0.2) with the addition of β-cyclodextrin (Fig. 3) [20, 21]. Two electropherograms of each portion of the prepared sample were recorded. Identification and determination of threonine in the sample were carried out, and the results were processed using the Elforan software for Windows.

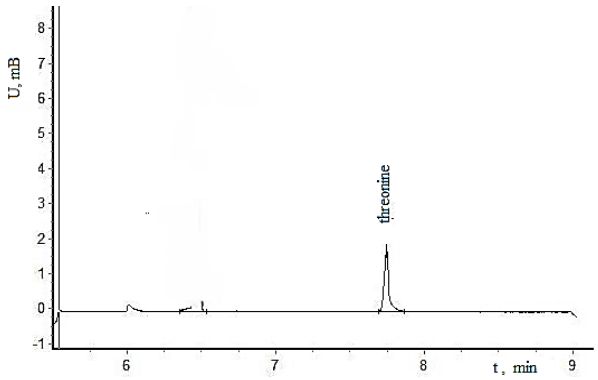

Fig. 3. Electropherogram of threonine
Рис. 3. Электрофореграмма треонина

CONCLUSIONS

Extraction systems based on water-soluble polymers represent a variant of "green" extraction, which are used harmless, environmentally friendly reagents. In this work, the extraction capacity of poly-N-vinylformamide and copolymers of N-vinylformamide with N-vinylmidazole, 1-vinyl-3,5-dimethylpyrazole and 1-methacryloyl-3,5-dimethylpyrazole with respect to threonine was studied. Distribution coefficients and the degree of threonine extraction were calculated for all the studied extraction systems. The concentrations of the amino acid and aqueous polymer solutions, the ratio of the volumes of the water-salt and organic phases, at which the maximum extraction characteristics are achieved, were determined. The amino acid was determined after extraction by capillary electrophoresis. Using the example of the PVF - threonine system, a mechanism for the formation of intermolecular hydrogen bonds of the amino acid with extractants was proposed.

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

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

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Поступила в редакцию 20.01.2025
Принята к опубликованию 23.03.2025

Received 20.01.2025
Accepted 23.03.2025