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CHOICE OF A METHOD OF PREPARING SAMPLES FOR RECORDING THE RADIOACTIVITY OF COMPLEXES OF ^{14}C-MACROTETROLIDE ANTIBIOTICS WITH ALKALI-METAL PICRATES

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UDC 577.181.6:57.088.6

A comparison of the method of preparing samples for recording the radioactivity of complexes of ^{14}C-macrotetrolide antibiotics with the picrates of univalent cations using a correction for color quenching and the method with the washing out of the water-soluble (including colored) components has shown the possibility of obtaining adequate reproducible results by both methods.

Macrotetrolide antibiotics produced by various strains of streptomycetes are ionophoric compounds that, in nonaqueous media, form strong complexes with the ions of univalent cations, especially ammonium, and this has been made the basis of the quantitative determination of these compounds by an extraction-spectrophotometric method in the presence of an excess of alkali-metal picrates [1, 2]. In this connection, it is important to study the distribution of the macrotetrolide antibiotics themselves between the organic and aqueous phases. The most suitable method for estimating these compounds in the different phases is the radioindicator method. However, the measurement of the radioactivity of complex multicomponent systems requires the conditions for obtaining a reproducible and comparable result to be clarified beforehand. With this aim, in samples containing ^{14}C-labeled macrotetrolides, picric acid, and alkali-metal picrates, we first determined the true activity of the samples of ^{14}C-macrotetrolides in ZhS-106 scintillation fluid using the method of internal standards to introduce corrections for quenching (Table 1, variant 1). Samples of ^{14}C-macrotetrolides with the addition of picric acid and ammonium and sodium picrates were estimated by the same program (Table 1, variants 2, 3, and 4).

TABLE 1. Activities of Preparations of ^{14}C-Macrotetrolides in the Presence of Picric Acid and of Ammonium and Sodium Picrates

Experimental conditions	Activity of the sample		Counting efficiency	Error, %
	disint./min	error,%		
1. ^{14}C-Macrotetrolides	17391±460	2,64	0.9263±0.0010	0,11
2. ^{14}C-Macrotetrolides+ picric acid	3125±149	4.79	0,7645±0,0332	4,34
3. ^{14}C-Macrotetrolides+ ammonium picrate	2817±67	2,97	0,7137±0,0161	2,26
4. ^{14}C-Macrotetrolides+ sodium picrate	2010±81	4,03	0.8694±0,0191	2,20

N. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 266-269, March-April, 1987. Original article submitted June 5, 1986; revision submitted December 15, 1986.

0009/3130/87/2302-0223$12.50

TABLE 2. Activities of ^{14}C-Macrotetrolide Preparations in the Presence of Picrates and of Absolute Ethanol

Experimental conditions	Activity of the sample		Counting efficiency	Error, %
	disint./min	error, %		
1. ^{14}C-Macrotetrolides + absolute ethanol	17159±471	2,75	0,8971±0,0002	0,03
2. ^{14}C-Macrotetrolides + absolute ethanol + picric acid	3339±137	4,10	0,5310±0,0548	10,32
3. ^{14}C-Macrotetrolides + absolute ethanol + ammonium picrate	3072±90	2,94	0,5083±0,0651	12,80

Analysis of the results obtained showed that it was impossible to compare the results of a control sample and samples with the addition of picric acid and ammonium and sodium picrates. The macrotetrolide antibiotics of the control variants dissolved completely in the scintillation liquid, giving homogeneous samples and their counting was performed with a high efficiency (above 92%). The activity values of samples of ^{14}C-macrotetrolides with picric acid and picrates were greatly lowered, since these samples proved to be heterogeneous because of their poor solubility in the scintillation liquid and they were colored. In order to have the possibility of accurately determining the true activity of such samples, it was necessary to perform further work in two directions: to obtain homogeneous samples and to eliminate the conditions lowering activity through color quenching.

To obtain homogeneous samples, the picric acid and picrates were dissolved in absolute ethanol, and the scintillation liquid with the previously produced labeled antibiotic was added to the solution so obtained. In order to maintain constant conditions, absolute ethanol was also added to the control sample (Table 2). As a result, the samples were obtained in which there were no precipitates whatever. This expedient for obtaining homogeneous samples was used in all the subsequent experiments. However, because of the complete dissolution of the colored compound and, consequently, an increase in the intensity of coloration, color quenching increased [3]. A determination of activity showed that the efficiency of counting decreased insignificantly as a result of the addition of the absolute ethanol (variants 1 in Tables 1 and 2), but fell greatly in the presence of picric acid and ammonium picrate (the other variants of Tables 1 and 2).

In order to answer the question of the introduction of a correction for color quenching, we investigated the dependence of the activity of the preparation on the concentrations of picrates (Table 3). The results obtained indicate that the true activity is practically independent of the additives used and of concentration variations.

In addition, a satisfactory agreement of the results was observed (the maximum error amounted to 7.94%). However, a marked decrease in counting efficiency and a large scatter of this magnitude in parallel samples, were observed when the relative concentration of the picrates was increased (to 0.1018), an error of more than 11% being possible.

Thus, work with samples colored by picric acid is connected with the fact that the main advantage of the liquid scintillation method — its high efficiency — is lost. A marked decrease in counting efficiency may apparently indicate the existence in the experimental samples of, in addition to color quenching, factors appearing through an interaction of the macrotetrolides, the alcohol, the alkali-metal picrates, and the scintillation liquid, which is extremely difficult to allow for completely in drawing up a program for absolute counting. Consequently, in seeking an answer to the question of the choice of a method of preparing samples for recording the radioactivities of macrotetrolides under these conditions we investigated an alternative variant — the elimination of ethanol, alkalis, and colored compounds, from the counting bottles in the final stages. For this purpose, samples that had already been counted under the conditions of Table 3 were washed with distilled water until they were completely decolorized and were then dried with sodium sulfate and recounted (Table 4).

As a result of these operations, as was to be expected, the true radioactivity fell somewhat. The predominating conclusion was that the mean error of the determination had decreased to 4.29% (Tables 3 and 4) and, which is the main thing, a stable and fairly high counting efficiency of approximately 0.9 had again been achieved.

TABLE 3. Activity of a Preparation of ^{14}C-Macrotetrolides in the Presence of Absolute Ethanol and Picrates at Various Concentrations

Experimental conditions		Activity of the sample		Counting efficiency	Error, %
additives	molar ratios of the concentrations, [C]picrates/[C]macrolides	disint. /min	error, %		
1. ^{14}C-Macrotetrolides + absolute ethanol + potassium picrate	100	1460±110	7,53	0,1058	3,01
	75	1325±43	3,24	0,2987	11,28
	25	1363±90	6,60	0,6228	1,23
2. ^{14}C-Macrotetrolides + absolute ethanol + ammonium picrate	75	1318±37	2,85	0,3336	10,06
	50	1365±39	2,89	0,4425	8,39
	25	1404±111	7,94	0,6230	3,80
Mean error, %			5,17		

TABLE 4. Activity of a Preparation of ^{14}C-Macrotetrolide after the Scintillation Liquid had been Washed Free from Absolute Ethanol and Alkali-metal Picrates

Experimental conditions		Activity of the sample		Counting efficiency	Error, %
additives	molar ratio of the concentrations [C]picrates/[C]macrotetrolides	disint / min	error, %		
1. ^{14}C-Macrotetrolides + absolute ethanol + potassium picrate	100	1239±100	8,07	0,8814	0,65
	75	1205±44	3,66	0,8848	1,50
	25	1204±68	5,64	0,9110	0,22
2. ^{14}C-Macrotetrolides + absolute ethanol + ammonium picrate	50	1270±21	1,65	0,9071	0,40
	25	1252±39	2,44	0,9078	0,67
Mean error, %			4,29		

Consequently, where necessary in complex cases it is possible to use similar expedients to obtain comparable results.

EXPERIMENTAL

A preparation of ^{14}C-labeled macrotetrolides was used. To obtain it, the streptomycete *Str. chrysomallus* var. *carotenoides* was grown in a 100-liter fermenter at 28°C with the gentlest possible working of the stirrer for 60 h. The mycelial mass obtained was separated from the culture liquid, washed with Tris-HCl buffer at pH 7.8-8.0 and placed in a solution of [^{14}C]glucose with a specific activity of 2.18 μCi/ml, and it was then cultivated at 28°C for 30 min. After this, the macrotetrolides were extracted from the mycelium with acetone, ethyl acetate was added to the acetone extract and it was washed three times with water, and was evaporated and the residue was recrystallized from n-hexane. This gave a white acicular crystalline preparation of ^{14}C-labeled macrotetrolides with a specific activity of 0.00484 μCi/100 mg.

The specific activity of the preparation obtained and the recording of radioactivities of the experimental samples were carried out in a Rackbeta 1217 (LKB) liquid scintillation counter using ZhS-106 scintillation mixture prepared in a toluene base.

The quantitative determination of macrotetrolides was carried out spectrophotometrically on a Spekol 10 instrument (Zeiss) by Suzuki's method [4].

SUMMARY

A comparison of two methods of preparing samples for recording radioactivities of complexes of ^{14}C-macrotetrolide antibiotics with picrates of univalent cations, namely, a method using a program for color quenching and a method in which the water-soluble components, including the colored ones, are washed out, has shown that they give adequate and reproducible results. However, some limitations must be mentioned. The first method is simpler in use

but on working with low levels of activity the reliability of the results falls. The second method, although it makes the process more laborious, nevertheless provides the possibility of counting samples with higher efficiency at any concentrations of quenchants and with any levels of activity.

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PHOTOPHYSICAL AND PHOTOCHEMICAL PROPERTIES OF LIGNIN CHROMOPHORES IN AQUEOUS SYSTEMS

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UDC 546.14:547.922.3:628.31

The results are given of an investigation of the changes in the physicochemical properties of the chromophores of lignin substances of effluents by photopotential, luminescence, and ESR spectroscopy and the polarographic determination of oxygen as a function of the number of quanta of incident energy in the interval from 300 to 600 nm. It has been established that under the action of light a change in the redox properties, an increase in the rate of consumption of oxygen, the formation of radical intermediate products, and the appearance of excited triplet states of the lignin chromophores take place in the lignin substances. Action spectra of the photopotential, of the yield of EPCs of free radicals, and of the consumption of oxygen by the lignin substances in the interval from 300 to 600 nm have been obtained.

In nature few polymers are found in which there is such a diverse set of extremely dynamic and labile chromophoric systems as lignin preparations. Depending on the type of treatment to which the lignin is subjected in industrial processes during the production of cellulose, preparations of it differ considerably in their physicochemical properties.

An analysis of the products of the far-reaching breakdown of lignin under the action of lights and atmospheric oxygen has been the subject of a number of investigations. In the opinion of the authors concerned [1-5], an important role in the photooxidation of lignin under the action of light in the presence of oxygen is played by phenoxyl radicals, photosensitizers, singlet oxygen, and carbonyl groups in an excited state.

There have been far fewer investigations on the analysis of the photophysical properties of lignin chromophores and the chemical transformations preceding far-reaching oxidative breakdown. Results due to Sergeev and Chupka [6] show that the initial stages of the oxidation of lignin are accompanied by chemiluminescence in the spectra of which there are maxima at wavelengths of 490, 560, and 640 nm which are due to the de-activation of the triplet states of carbonyl groups and of various forms of singlet oxygen. It has been established that in lignin macromolecules, which have a globular form, there is an intensive migration of the energy of electronic excitation, as a result of which the lignin behaves, according to an expression of Swedish workers [7], as a "single chromophore." The most active accepters of energy are aldehydes, ketones, quinones, quinone methides, and stilbenes.

The fact that the lignin chromophores possess photoactivity and are labile under the action of light and atmospheric oxygen acquires particular importance in connection with the setting up of investigations to establish standards for the maximum acceptable concentrations

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Siberian Scientific-Research Institute of Cellulose and Board Bratsk. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 269-274, March-April, 1987. Original article submitted April 25, 1986; revision submitted December 10, 1986.

0009/3130-87/2302-0226$12.50