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QUANTITATIVE ASSAY OF CHROMIUM(III) IN YEAST CULTURES USING CHROMAZUROL S AND SURFACTANTS FOR MONITORING CHROMATE REMEDIATION PROCESSES

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Because microorganisms are regarded as a potential means for bioremediation of chromium compounds and the yeasts are considered to be the source of chromium pharmaceutical biocomplexes, the development of the methods for chromium assay in real biological samples is of a significant biotechnological importance. A new sensitive method for the photometric assay of total chromium in the form of Cr(III) in microbial cultures after their mineralization was developed using the reaction with chromazurol S in the presence of the surfactants, sodium dodecyl sulfate (SDS) or cetyltrimethylammonium bromide (CTMAB). The optimal values of chromazurol S and surfactants concentrations were determined to achieve the maximal sensitivity of the analysis. Molar extinction coefficients of the complexes in 0.2 M sulfuric acid were shown to equal 27 and 98.9 $мМ^{-1} \cdot см^{-1}$ in cases of using SDS and CTMAB, respectively. The latter variant of chromium (III) assay is 3 times more sensitive than diphenylcarbazide method. The threshold sensitivity of chromazurol method is approximately 100 and 25 ng of chromium per 1 ml of the final reaction mixture for two variants of the assay in cases of using SDS and CTMAB, respectively. A convenient and relatively fast method for mineralization of the biological samples was optimized using perhydrol and sulfuric acid. It was shown that the developed methods can be used for the monitoring of chromate-reduction processes in yeast cultures by the final product Cr(III), as well as for determination of the total chromium content in microbial cultures or in the cells after their mineralization.

Key words: chromium(III), chromazurol S, sodium dodecylsulfate, cetyltrimethylammonium bromide, photometric assay, yeast culture.

In different biological systems, chromium can be represented both as a necessary microelement (Cr^{+3}) and a dangerous environmental pollutant (especially in the forms of chromates/bichromates — Cr^{+6}). Trivalent chromium (in the amounts of 50–200 μg per day) is necessary for normal vital activity of animals and people due to the role it plays in metabolism of glucose, cholesterol, and fats [1–3]. This microelement is a significant component of glucose tolerance factor, which, synergically with insulin, activates glucose consumption [4]. Chromium can also take part in regulation of gene expression as zinc mimetic. Cr (III) complexes have already become an integral part of pharmaceutical practice as the factors preventing diabetes and stimulating weight loss. On the other hand, the wide industrial use of chromium compounds results in accumulation of chromium in waste waters, soil, and plants, which leads to chronic or sometimes acute intoxication of human organism. The facts mentioned above testify to a great need for selective, sensitive, and, at the same time, non-expensive methods of chromium analysis in biological samples, including those for control of diabetes type II treatment [5, 6]. Applied microbiology is considered to be the important area in chromium analysis, as microorganisms are regarded as potential means for bioremediation of chromium compounds [7], and the yeasts are considered to be the source of chromium biocomplexes, which may be of pharmaceutical significance [8, 9].

Nowadays there are various chemical and physico-chemical methods of chromium content determination, namely, gravimetric methods, titrimetric methods (redox, chelatometric, potentiometric, amperometric, coulometric titration), photometric methods (based on light-absorption of Cr(VI) and Cr(III) ions as well as of their compounds with inorganic and organic reagents), luminescent, polarographic, kinetic (oxidative reaction by hydrogen peroxide, potassium bromate), methods of isotopic

процедуру (схему) одержання морфогенних калюсних тканин наведено на рис. 2.

За цією схемою одержали велику кількість регенерантів, які можна мультиплікувати так само, як і описані вище рослини, отримані прямою регенерацією. Одержані регенеранти досліджуються методами ПЛР-аналізу для вивчення можливих генетичних відмінностей їх від вихідних цибулин.

Таким чином, для рідкісної лікарської рослини унгернії Віктора уперше розроблено умови мікроклонального розмноження як прямою регенерацією із фрагментів лусок різних цибулин, так й індукуванням регенерації із пасивованих тривалий час калюсних тканин у культурі *in vitro*. Встановлено, що оптимальним середовищем як для індукування регенерації, так і для отримання та вирощування у пересадній культурі калюсних культур є живильне середовище з мінеральною основою за Воллосовичем та ін. Підібрано оптимальні співвідношення стимуляторів росту, зокрема ауксинів і цитокінінів, для кожного з вивчених процесів — прямої регенерації, індукції калюсоутворення, тривалого вирощування калюсних тканин зі збереженням морфогенного потенціалу, індукції редиференціації, мультиплікації і вирощування регенерантів *in vitro*. Виявлено, що освітлення пригнічує утворення регенерантів і калюсоутворення на первинних експлантах, а також гальмує процеси регенерації мікроцибулинок пасивованими калюсними тканинами та ріст калюсних тканин. Встановлено відмінності реакції різних цибулин унгернії Віктора на ті самі умови введення в культуру *in vitro*, що, очевидно, зумовлено відмінностями генотипів використаних цибулин, виявленими раніше методом RAPD ПЛР-аналізу.

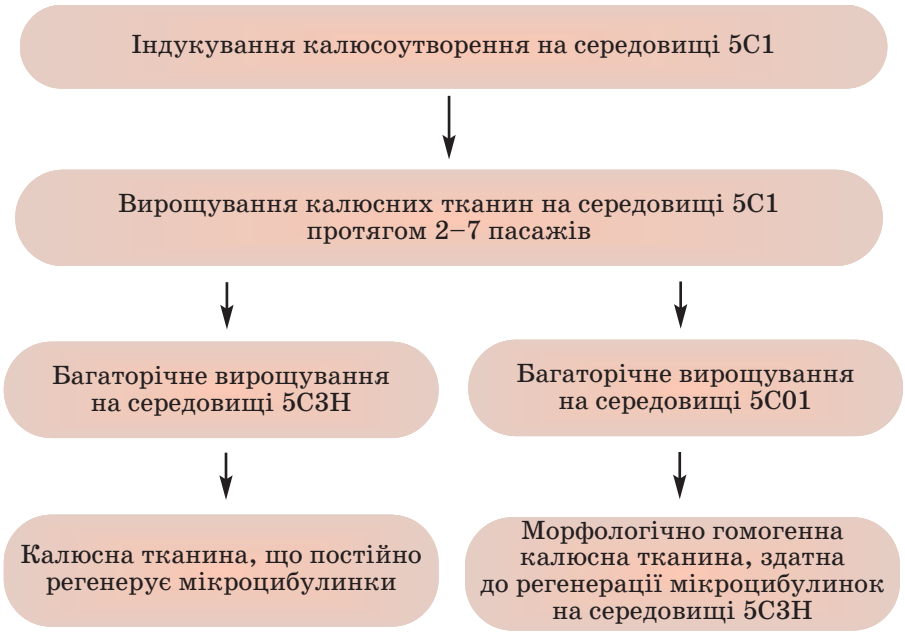

Рис. 2. **Схема одержання морфогенних калюсних тканин *U. victoris*, здатних до регенерації протягом не менше 6 років** (склад живильних середовищ — табл. 1)

dilution, gas-chromatographic methods, magnetic relaxation analysis, emission flame photometry, atomic absorption spectrometry, atomic fluorescence flame spectrometry, X-ray-fluorescence analysis, mass spectrometric analysis, radioactive and neutron activation analysis, *etc*. [10, 11]. Recently, potentiometric chemical sensors based on ion-selective electrodes for Cr (III) [12–14] and chromate [15, 16] analysis as well as amperometric chromate-selective biosensor based on bacterial cytochrome c_3 [17] have been developed. Photometric method of Cr (III) content determination through the reaction with chromazurol S has been described [18, 19].

The vast majority of methods of chromium analysis are designed for inorganic objects (industrial waters, alloys, minerals) and only a few of them are designed for biological samples. Spectrophotometry and atomic absorption spectrophotometry [10, 20, 21] can be singled out among the most commonly used methods of Cr analysis in biological material, however, the latter does not allow determining different Cr valency forms. Cr(VI) (chromate) is determined photometrically using diphenylcarbazide [22, 23], which may also be used for determination of total Cr content after Cr(III) oxidation to chromate by permanganate or other oxidants [10]. Regardless of a wide use of diphenylcarbazide method, it is worth mentioning that this method is sensitive to negative influence of various oxidants, *e.g.* Fe(III), Hg(II), molybdate, and vanadate ions.

The photometric method of determining Cr(III) using the reaction with chromazurol S is described [18, 19]. The development of a new and more sensitive variant of chromazurol method for Cr(III) determination using different surfactants, as well as the approbation of the optimized method for the analysis of biological samples, in particular determination of Cr(III) content in microbial yeast cultures in the process of chromate reduction were set to be the aims of our work. The research resulted in development of the sensitive photometric method for total Cr(III) content determination in mineralized biological samples, which can be of wide practical application.

Materials and Methods

Chemicals

All chemicals used, *i.e.* chromazurol S (China), cetyltrimethylammonium bromide (*Chemapol*, the Czech Republic), sodium dodecyl sulfate (*Sigma*), diphenylcarbazide, perhydrol (33%) as well as all other chemicals were analytical reagent grade or better. The water used was subdued to three-stage purification using DEMIWA 10 roi equipment.

Yeast strains

Histidine-dependent strain of *P. guilliermondii* ATCC90191 (L2) was used.

Cultivation

The yeast cells were cultivated at 30 °C in Erlenmeyer's flasks on a circular shaker (200 rpm) in Burkholder's medium [24] of the following composition: saccharose (2%), yeast extract (0.1%), and histidine (40 mg/l). Sterile chromate solution (K_2CrO_4) in the concentration of 1.5 μmoles/ml (1.5 mM) was added at the log phase of growth at the cell concentration of 0.3 mg/ml. Yeast biomass was determined turbodimetrically using photocolorimeter FEC-56M (λ_{max} = 540 nm). Dry mass (mg/ml) was calculated by gravimetrically obtained calibration curve.

Mineralization of yeast culture samples

Using the mixture of nitric and sulfuric acids

The samples of 0.5-1 ml in thermostable test tubes were placed in an aluminum block, evaporated, treated with 0.25 ml mixture of H_2SO_4 and HNO_3 (1:2.5) and heated at 240 °C till complete burning and nitroso-sulfuric acid removal [25]. The residues were neutralized by adding 0.1–0.12 ml 10 M NaOH, 0.2 ml 1 M acetate buffer (pH 5.0), and subacid pH level was achieved by adding 1 M NaOH, when needed.

Using nitric acid and hydrobromic acids, HBr and $KBrO_3$ mixture (performed in accordance with [26])

Using perhydrol in acid medium (optimal variant)

The samples of 0.02–0.5 ml in thermostable test tubes were heated in aluminum block at 120 °C and evaporated until they were almost dried. After adding 0.2 ml of sulfuric acid (1:3), the samples were heated at 120 °C till slight carbonization of the biomass. After water evaporation, 0.4 ml of perhydrol was added, and the samples were heated again to complete removal of vapors and clearing. The treatment of samples by perhydrol was repeated, if needed (in the case when cell biomass exceeds 3 mg, brown coloring of under-burned products may appear after one time perhydrol treatment), gradually increasing the temperature to 170 °C. Finally, 0.2 ml of water were added and the mixture was evaporated until only sulfuric acid remained and blue coloring of Cr(III) appeared. After cooling, 1 ml of water was added to the mixture, neutralized to subacid reaction by adding 0.5 ml 1 M NaOH and 0.5 ml 1 M acetate buffer (pH 3.5) (avoiding alkalizing of the samples). Volume of the mixture was adjusted to 5 ml by adding water.

Cr(III) model biocomplex formation

The mixture was prepared in the molar ratio of 1:2 [Cr(III):reduced glutathione], sustained at room temperature for 24 hours. Quantitative formation of the complex was approved by spectrophotometer at extinction maxima of 410 and 565 nm.

Development of photometric method of Cr(III) determination was performed as described below in **Results and Discussion**. Herein, the description of final (optimized) analysis procedures using two surfactants [sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTMAB)] is presented. 10 mM Cr(III) solution, obtained by dissolving of a definite aliquot of metallic chromium in 1 M sulfuric acid, was used as the standard. The analyte solutions were prepared by dissolving the initial chromium solution with 10 mM sulfuric acid or 10 mM acetate buffer, pH 3.5, to the final concentration of 0.05 mM.

The method with the use of SDS

Using 5 ml graduated test tubes, 1 ml 1% SDS, 0.5 ml 1M acetate buffer (pH 3.5), 0.5 ml 0.06% chromazurol S, 0.1–0.5 ml of the analyzed sample were mixed, and the volume was adjusted to 4 ml by distilled water. The samples were placed in boiling water bath for 30 min. After cooling, 1 ml 1 M sulfuric acid was added, and the final volume was adjusted to 5 ml by water, if needed. The mixtures were photometrically tested in 1 cm cuvettes using photocolorimeter KFK-2MP (with λ_{max} = 590 nm) or spectrophotometer (λ_{max} = 610 nm). The «blank» sample (all components but chromium) and standard Cr(III) solutions in two concentrations were treated by the same way. Cr(III) concentration was calculated on the base of optic density for the standard or using calibration curve.

In model investigations (see Fig. 1), the solutions without sulfuric acid (final component concentrations were 1.25 times higher than those in the finally selected variant of analysis with acid) were also photometrically analyzed.

The method with the use of CTMAB

Using 5 ml graduated test tubes, there were mixed 0.2 ml 0.4% CTMAB, 1 ml 0.5 M acetate buffer (pH 3.5), 1 ml 0.03% chromazurol S, 0.1–0.5 ml of the analyzed sample, and the volume was adjusted to 4 ml by distilled water. The samples were placed in boiling water bath for 40 min. After cooling, 1 ml 1 M sulfuric acid was added, and then water to the final volume of 5 ml, if needed. The solutions were photometrically tested in 1 cm cuvettes using KFK-2MP (with λ_{max} = 590 nm) or spectrophotometer (λ_{max} = 610 nm). The «blank» sample (all components but chromium) and standard Cr(III) solutions in two concentrations were treated by the same way. Cr(III) concentration was calculated on the base of optic density for standard or using calibration curve.

Statistical and regression analyses of calibration curves were performed using Microcal Origin 6.0 software.

Results and Discussion

The formation of colored Cr(III) complexes with chromazurol S [18] and photometric method for the metal assay using different activation means for Cr(III) are described in literature [19]. Molar extinction coefficients of Cr(III) complexes with chromazurol S in subacid medium (pH 3.0–5.0) are 59 and 71 $mM^{-1}\cdot cm^{-1}$ (the former is for reagent surplus and the latter — for metal ions surplus) [18], which is almost two times higher than the similar coefficient for chromate assay by the diphenylcarbazide method (33 $mM^{-1}\cdot cm^{-1}$) [27]. On the other hand, a number of transition metal ions are known to form triple complexes with organic dyes and surfactants with high values of molar extinction, for example, in the case of Cu(II)-chromazurol S-surfactant complexes [28]. Our goal was to develop selective and highly sensitive variant of photometric determination of Cr(III) using chromazurol S and various surfactants of anionic (sodium dodecyl sulfate, SDS) and cationic (cetyltrimethylammonium bromide, CTMAB) nature.

50 mM acetate buffer (pH 3.5) was selected as the working buffer. At higher pH values (4.5–5.5) chromazurol S complex with Fe(III), which is often present in biological samples, can be formed. On the other hand, at pH < 3.0 the rate of Cr(III) interaction with the reagent decreases significantly.

The improvement of the assay method consisted in the study of optimal concentrations of chromazurol S and surfactant, as well as of optimizing conditions for photometry of the final reaction mixture – without changing pH or after its preliminary acidification by sulfuric acid. Extinction spectra were obtained using Shimadzu UV-1650 PC spectrophotometer. Optical density of the solutions was measured using photocolorimeter KFK-2MP or spectrophotometer SF-46.

Optimization of SDS-chromazurol method

Experiments revealed that for Cr(III) assay by SDS-method, the optimal concentration of

chromazurol S (recalculated for final photometric solution after sulfuric acid addition) equals 0.006% and SDS — 0.2%. Acidification of the reaction mixture with sulfuric acid (to 0.2 M) before the photometry is reasonable due to the three following reasons: 1) the background extinction (optical density for the «blank» sample) in supraacid medium is three times lower when compared to subacid medium (pH 3.5); 2) higher stability of optical densities of the solutions; 3) higher assay selectivity as a result of Fe(III)-chromazurol S complex destruction in supraacid medium, which is significant for avoiding its interfering influence on chromium assay. Maximal extinction of the complex is observed in 0.2 mM sulfuric acid at 590–610 nm. Sensitivity of the method is enhanced significantly with increasing chromazurol S concentration in the range from 0.003 to 0.006% (2.4 times), and in a lesser extent — when chromazurol S concentration is increased to 0.01% (only by 5.7% compared to the concentration of 0.006%). The optimal concentration of SDS, in terms of the best sensitivity, is in the range of 0.1–0.4% (Fig. 1). The molar extinction of the formed complex equals to 27.0±0.75 $mM^{-1} \cdot cm^{-1}$ at optimal reaction conditions (supraacid medium), which is close to the similar coefficient for the diphenylcarbazide method. Although the proposed variant of analysis is inferior in sensitivity, compared to photometry in subacid medium, it is not sensitive to interfering influence of Fe(III) ions, as Fe(III) complex with chromazurol S is dissociated in supraacid medium. Our experiments confirmed the absence of reaction between chromazurol S and model solutions of Fe ions in two valence states at the final concentrations in photometric solution from 0.001 to 0.1 mM (Fe^{3+}) and 0.001 to 0.1 mM (Fe^{2+}).

Figure 2 presents the calibration curve for determination of Cr(III) content using SDS-chromazurol method at the optimized conditions. Linear dependency between optical density and Cr concentration is observed: linear regression coefficient $R = 0.999$; $p < 0.0001$. The threshold of Cr(III) assay by this method is around 20 μM or 1.0 μg/ml (recalculation was made for initial (analyzed) sample at the relation of 0.5 ml sample per 5 ml of photometric solution).

Optimization of CTMAB-chromazurol method

To develop the other variant of Cr(III) determination using CTMAB, optimal reaction conditions have been defined, namely, concentrations of components, optical wavelength, and the influence of acidification of the reaction mixture. Spectral measurements (spectra are not presented) showed that maximal extinction for reagent (without Cr adding) was observed at 501 nm (pH 3.5) and 464 nm (0.2 M H_2SO_4). Extinction peak for Cr(III)-chromazurol S complex in the presence of CTMAB is shifted to the area of 580–610 nm at two mentioned above acidity parameters. As in supraacid medium (0.2 M H_2SO_4) extinction maxima for reagent and colored product are more distant on the scale of wavelengths (better reaction contrast), supraacid conditions (adding sulfuric acid up to 0.2 M), and the wavelength for photometry of 610 nm (spectrophotometer) and 590 nm (photoco-

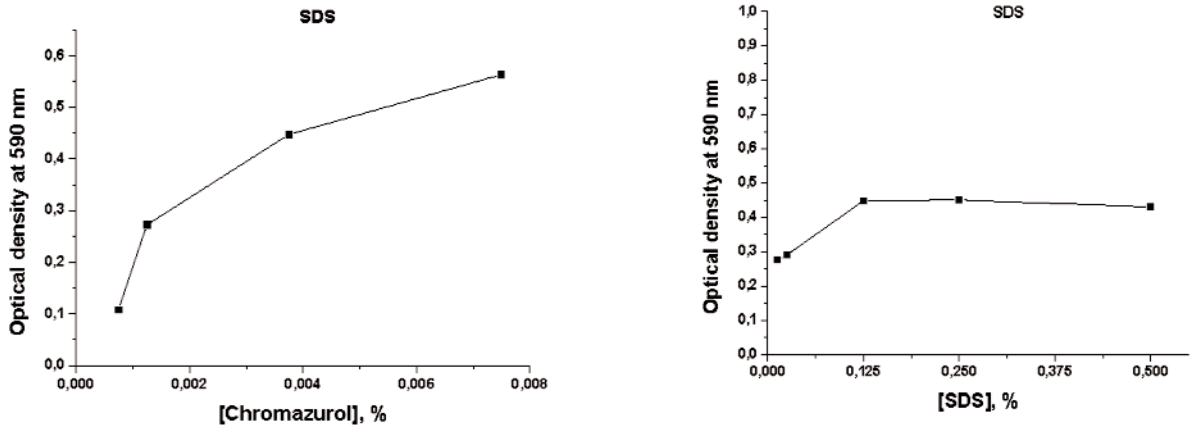

Fig. 1. **Optimization of chromazurol S (left) and SDS (right) concentrations in the reaction mixture for the assay of Cr(III) using SDS-chromazurol method (the data concern the reaction with no acidification of the reaction mixture using sulfuric acid).**

Reaction conditions: left chart: [SDS] = 0.25%; right chart — [chromazurol S] = 0.00375%. Optical density values of the samples (with 0.01 мМ Cr(III)) were measured in 1 cm cuvettes at 590 nm using KFK-2M photocolorimeter vs «blank» sample (with no Cr)

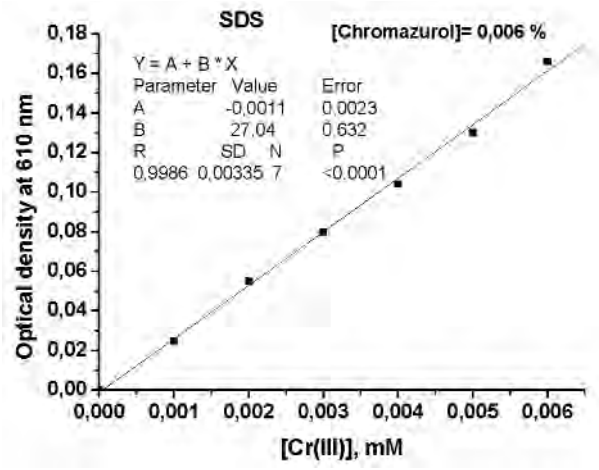

Fig. 2. **Calibration curve diagram for Cr(III) determination using SDS-chromazurol method at chromazurol S concentration in the reaction mixture 0.006%, and SDS — 0.2%.** Y-axis — optical density, after acidification with sulfuric acid (0.2 M), X-axis — final Cr concentration in photometric mixture

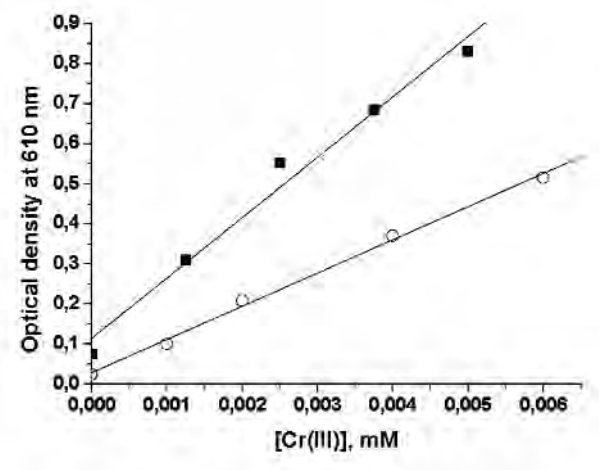

Fig. 3. **The dependence of optical density of the reaction mixture at 610 nm on final Cr(III) concentration: without acid (top curve, ■) and with acid (bottom curve, O).** Chromazurol S concentration — 0.003 and 0.00375 %, and CTMAB — 0.016 and 0.02 % for variants with and without acid, respectively

lorimeter KFK-2MP) were selected for the assay. Though molar extinction of Cr complex is 1.5 times lower at supraacid conditions (Fig. 3), this variant was selected as the reasonable one due to a lower background signal as well (optical density for the «blank» sample).

Experiments for optimization of CTMAB-chromazurol assay method showed that optimal concentration of chromazurol S (recalculated for the final photometric solution in the presence of sulfuric acid) was 0.006%, and CTMAB — 0.016%. The sensitivity of the method is linearly dependent on chromazurol S concentration in the range from 0.0005 to 0.003% (Fig. 4). Further increase of chromazurol S concentration up to 0.006% results in enhanced assay sensitivity, however, this positive effect is accompanied by undesirable increase of optical density for the «blank» sample. The influence of CTMAB surfactant concentration on the formation of the complex was shown to be more complicated, than in case with SDS: initially, the positive influence of CTMAB is observed (to the concentration of 0.01–0.015%), which was changed by gradual decrease of optical density (higher than 0.2% CTMAB). It is noteworthy that optimal CTMAB concentration in molar presentation (about 0.4 mM) is more than 16 times lower than the optimal SDS concentration (about 7 mM) at the same molar concentration of chromazurol S being around 0.1 mM (Fig. 5). These results allow to suggest trivalent complex formation in the system of Cr(III)-chromazurol S-CTMAB, similar to the described complex for Cu(II) ions [28].

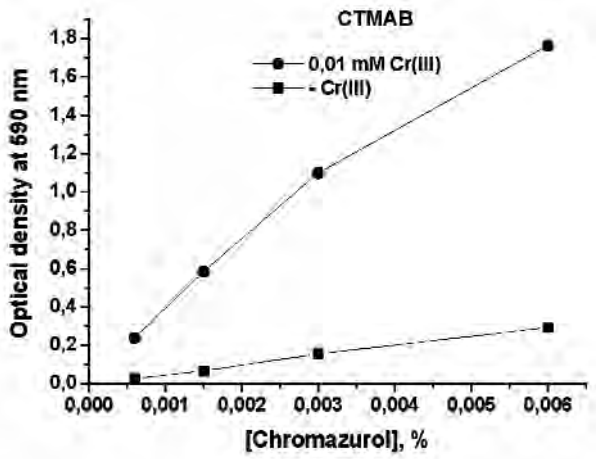

Fig. 4. **Influence of chromazurol S concentration in the reaction mixture on sensitivity of Cr(III) determination using CTMAB-chromazurol method.** Surfactant concentration — 0.016 %. Top curve (•) corresponds to optical density of samples at Cr concentration 0.01 мМ, and the bottom one (■) — optical density of «blank» samples (with no Cr). Photometry was performed with no sulfuric acid addition

Molar extinction coefficient for Cr(III)-CTMAB-chromazurol S complex in 0.2 M H_2SO_4 at the optimal reaction conditions (0.006% chromazurol S and 0.016% CTMAB) at λ = 610 nm is 98.9 $mM^{-1} \cdot cm^{-1}$, which exceeds the corresponding coefficient for the colored product for SDS-chromazurol method more than 4 times, and it exceeds the coefficient for diphenylcarbazide method more than 3 times. The threshold for Cr(III) determination using CTMAB-chromazurol method was around 5 μM or 0.25 μg/ml (recalculating for the initial

(analyzed) sample in the ratio of 0.5 ml of the sample per 5 ml of photometric solution). In subacid solution (pH 3.5) this complex has a higher molar extinction (up to 148 $mM^{-1}\cdot cm^{-1}$), which allows achieving the maximal sensitivity of the photometric method, however, from the standpoint of complex stability and selectivity, the determination is more reasonable to be performed by photometric measuring in 0.2 M H_2SO_4. Absorbance of supraacid solutions remains stable for at least 2 hours.

The study on the influence of boiling duration on colored complex formation revealed the optimal time for quantitative reaction to be 30 min for SDS-variant and 40 min — for CTMAB-method (Table 1).

Table 1. **Influence of boiling time on apparent values of molar extinction ($mM^{-1}\cdot cm^{-1}$) for Cr(III) assay by chromazurol method in the presence of SDS and CTMAB in acid solution (0.2 M H_2SO_4).**

Variant	Boiling time, min					
	10	20	30	40	50	60
SDS	18.0± 1.5	22.9± 1.3	26.2± 1.9	27.9± 0.95	28.0± 0.81	–
CTMAB	–	65.9± 2.5	83.6± 6.0	95.5± 8.2	–	90.9± 2.6

Both SDS-variant and CTMAB-method are insensitive to negative influence of Fe ions, as the formation of colored complex of chromazurol S with model solutions of Fe ions at the final concentration of 0.001 to 0.1 mM (Fe^{3+}) and from 0.01 to 1 mM (Fe^{2+}) in the mixture was not observed (data not shown).

As the developed methods of photometric determination of Cr(III) belong to ultramicromethods (esp. CTMAB-variant), in order to avoid possible errors due to different quality of water, chemicals, *etc*. it is recommended to set Cr(III) standard in each series of analytical assays for its correlation with the calibration curve (Fig. 6). It is also recommended to apply the test «added-determined» occasionally for the biological sample mineralization to control a completeness of this process (see next chapter).

Validation of the developed methods for Cr(III) determination on model samples and real biological objects

The reliability of both variants of Cr(III) analyses was tested using Cr(III) model biocomplex with reduced glutathione and real yeast culture samples cultivated in the presence of chromate, which were shown to reduce Cr(VI) to Cr(III)-containing complexes [8]. Besides, there were used the culture samples with additionally added chromate, Cr(III) or their mixtures after cultivation completion. The samples were exposed to acid oxidative mineralization before determining Cr(III) content. Since the search for mineralization methods in the literature did not reveal unambiguous results on which variant is the best for Cr content determination, we validated 4 most commonly used approaches of burning and mineralization of biological samples: 1) thermal treatment by the mixture of sulfuric and nitric acids; 2) burning in nitric acid with further treatment of samples with hydrobromic acid; 3) oxidation using HBr + $KBrO_3$ mixture; 4) oxidation by perhydrol in acidic medium. The following criteria were used to select the optimal mineralization method, namely, the completeness of biological material oxidation, the transfer of all Cr forms into Cr(III) mineral salts, operational processability (temperature and time mode, convenience of the subsequent operation of achieving the needed pH level of samples), and, of course, the reliability of discoveries of all Cr forms as Cr(III). It was shown that the mineralization conditions significantly influence on the reliability of Cr determination in biological samples. The experiments performed resulted in development of the optimal mineralization procedure with the use of perhydrol in the presence of middle concentration of sulfuric acid. The commonly used mineralization method by using the mixture of sulfuric and nitric acids was revealed to be inconvenient to perform due to the use of concentrated acids and high burning temperatures (200–220 ^{0}C for oxidation of organic material, and ~300 ^{0}C for nitrosulfuric acid destruction, which prevents determining Cr), time of the operation (more than 2 hrs), the necessity for individual neutralization mode of certain samples after burning, due to different pH levels. Other less commonly used mineralization methods: i) using nitric and hydrobromic acid (the former for burning the sample and the latter for removing HNO_3 surplus); the mixtures of HBr + $KBrO_3$, which generates free Br_2 as the oxidant [26], require less strict conditions, however, the complete oxidation is achieved at significant reagent amounts, which makes it impossible to perform serial burning of multiple samples in regular test tubes placed in aluminum block. The use of perchloric acid as an oxidizing agent component is inconvenient due to the risk of explosion [26]. Our experience showed that the most suitable method of oxidative mineralization of biological material for Cr determination is the method which involves perhydrol (concentrated hydrogen

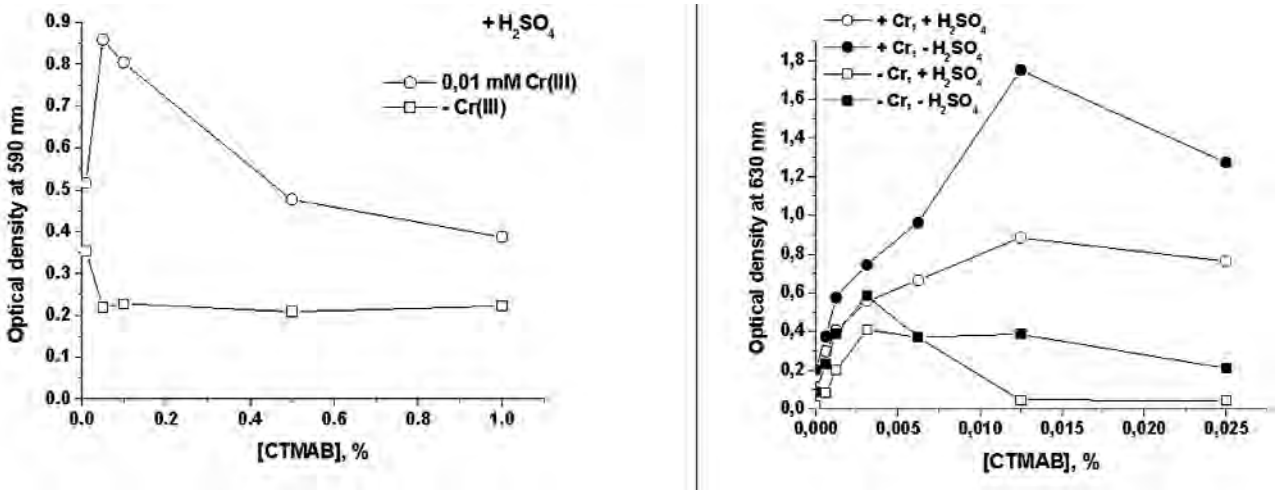

Fig. 5. **CTMAB influence in different concentration ranges on colored product formation for Cr(III) assay using chromazurol method. The data are presented for both variants of the reaction mixture: acidified («+H_2SO_4»: ○, □) and without sulfuric acid addition («−H_2SO_4»: ●, ■).**
Reaction conditions: chromazurol S concentration — 0.00375 % (without acid) and 0.003% (with acid). Cr(III) concentration in samples (●, ○) — 0.01 мМ. «Blank» samples (■, □) did not contain Cr

peroxide solution) used in the presence of sulfuric acid. The procedure lasts about 2 hours, at relatively high temperature (120–170 °C), the oxidizing reagent does not create any by-products, which would interfere with Cr determination, and the concentration of residual acid does not vary significantly after burning (thermal decomposition does not take place, which makes more easy to perform the neutralization procedure to the necessary pH level).

Total Cr analysis in model mineralized samples using the chromazurol method in two variants showed a good correlation of Cr content values with the expected ones for different types of samples: chromate water solution, Cr(III), and their mixtures; cultures cultivated in the presence of chromate, and those with additional introduction of Cr in the two valence states directly before culture mineralization; Cr(III) complex with glutathione (Table 2). In the majority of the samples the level of Cr detecting was higher than 90–95%.

Thus, a novel sensitive method for photometric determination of total chromium

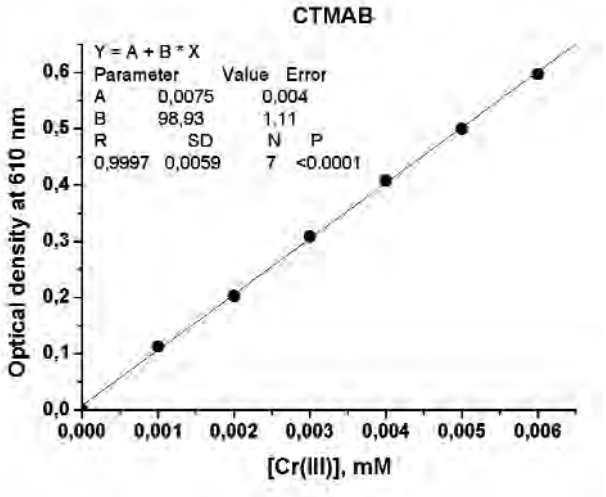

Fig. 6. **Calibration curve for Cr(III) determination using CTMAB-chromazurol method at chromazurol concentration of 0.006%, and CTMAB — 0.016% in the reaction mixture.**
Y-axis — optical density of mixtures, acidified by sulfuric acid (0.2 M),
X-axis — final Cr concentration in photometric mixture. Optical densities of the samples were measured vs. «blank» sample

Table 2. **Validity of determination of total Cr(III) in the mineralized samples by SDS-chromazurol method (results for CTMAB-method are presented in brackets) using a test «added-determined»**

№	Sample	Cr added, μmoles				Cr determined, μmoles M±m	Deviation from expected, %
		In a medium	Additionally before mineralization				
			Cr(III)	Cr(VI)	Total		
1	Yeast culture (0.5 ml; 2.8 mg of cells) after reduction of 1.5 mM of chromate	0.75			0.75	0.615±0.031 (0.589±0.040)	-18.0 (-21.5)
2	The same; Cr(III) added	0.75	0.5	–	1.25	1.15±0.051 (1.057±0.028)	–8.0 (-15.4)
3	The same; Cr(VI) added	0.75	–	0.5	1.25	1.11±0.055 (1.177±0.030)	–11.2 (-5.8)
4	The same; Cr(III) and Cr(VI) added	0.75	0.25	0.25	1.25	1.22±0.108 (1.014±0.044)	–2.4 (-18.9)
5	Water, Cr added	–	1.25	–	1.25	1.22±0.044 (1.27±0.050)	–2.4 (+1.6)
6	Water, Cr added	–	–	1.25	1.25	1.20±0.102 (1.254±0.062)	–4.0 (+0.3)
7	Water, Cr added	–	0.625	0.625	1.25	1.33±0.062 (1.39±0.075)	+6.4 (+11.2)
8	Cr(III)-Glut complex	1.0	–	–	1.0	0.97±0.070 (1.04±0.043)	–3.0 (+4.0)

Abbreviation: Glut — reduced glutathione.

content in microbial cultures after acidative-oxidative mineralization using reaction with chromazurol S in the presence of two surfactants – sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTMAB) has been developed. The latter variant of the method developed is 3 times more sensitive compared to diphenylcarbazide method. Both variants of chromazurol method are simpler in terms of performance and do not require any tedious procedure of transforming all Cr forms to chromate after mineralization of the sample. Threshold sensitivity of chromazurol method is around 100 and 25 ng of chromium in 1 ml of photometric samples for two variants of analysis using SDS and CTMAB, respectively. SDS variant is less sensitive, but this variant is more reproducible. A convenient and relatively fast method of mineralization of biological samples using perhydrol in the presence of sulfuric acid is optimized. The reliability of chromazurol method for total chromium content determination in real biological samples after mineralization is presented.

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ВИЗНАЧЕННЯ ВМІСТУ ХРОМУ(III) У ДРІЖДЖОВИХ КУЛЬТУРАХ ЗА ДОПОМОГОЮ ХРОМАЗУРОЛУ S ТА ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН ДЛЯ СКРИНІНГУ ПРОЦЕСІВ БІОРЕМЕДІАЦІЇ ХРОМАТУ

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Оскільки мікроорганізми розглядаються як потенційний засіб для біоремедіації сполук хрому, а дріжджі — як джерело біокомплексів хрому фармацевтичного значення, актуальною біотехнологічною проблемою є розробка методів визначення вмісту хрому в реальних зразках біологічного походження. Розроблено новий чутливий метод фотометричного визначення сумарного хрому у формі Cr(III) в мікробних культурах після їх мінералізації з використанням реакції з хромазуролом S у присутності поверхнево-активних речовин (ПАР) — додецилсульфату натрію (SDS) або цетилтриметиламонійброміду (CTMAB). Визначено оптимальні величини концентрації хромазуролу S і ПАР для досягнення максимальної чутливості аналізу. Показано, що молярні коефіцієнти екстинкції комплексів в 0,2 М сірчаній кислоті дорівнюють 27,0 і 98,9 $мМ^{-1}{\cdot}см^{-1}$ — у випадку застосування SDS і CTMAB, відповідно. Останній варіант визначення хрому(III) чутливіший порівняно із дифенілкарбазидним методом у 3 рази. Нижня межа чутливості хромазурольного методу становить близько 100 і 25 нг хрому в 1 мл фотометрованої проби для двох варіантів аналізу із застосуванням SDS і CTMAB, відповідно. Оптимізовано зручний і порівняно швидкий метод мінералізації біологічних зразків з використанням пергідролю у присутності сірчаної кислоти. Розроблені методи аналізу можна застосовувати для моніторингу процесів відновлення хромату культурами дріжджів з утворення кінцевого продукту Cr(III), а також для визначення загального вмісту хрому в мікробних культурах чи клітинах після їх мінералізації.

Ключові слова: хром(III), хромазурол S, додецилсульфат натрію, цетилтриметиламонійбромід, фотометричний аналіз, культура дріжджів.

ОПРЕДЕЛЕНИЕ СОДЕРЖАНИЯ ХРОМА(III) В ДРОЖЖЕВЫХ КУЛЬТУРАХ С ПОМОЩЬЮ ХРОМАЗУРОЛА S И ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ ДЛЯ СКРИНИНГА ПРОЦЕССОВ БИОРЕМЕДИАЦИИ ХРОМАТА

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Поскольку микроорганизмы рассматриваются как перспективное средство для биоремедиации соединений хрома, а дрожжи — как источник биокомплексов хрома фармацевтического значения, актуальной биотехнологической проблемой является разработка методов определения содержания хрома в реальных образцах биологического происхождения. Разработан новый чувствительный метод фотометрического определения суммарного хрома в форме Cr(III) в микробных культурах после их минерализации с использованием реакции с хромазуролом S в присутствии поверхностно-активных веществ (ПАВ) — додецилсульфата натрия (SDS) или цетилтриметиламмонийбромида (CTMAB). Найдены оптимальные величины концентрации хромазурола S и ПАВ для достижения максимальной чувствительности анализа. Показано, что молярные коэффициенты экстинкции комплексов в 0,2 М серной кислоте равны 27,0 и 98,9 $мМ^{-1}{\cdot}см^{-1}$ – в случае применения SDS и CTMAB, соответственно. Последний вариант определения хрома(III) превышает по чувствительности дифенилкарбазидный метод определения хромата в 3 раза. Пороговая чувствительность хромазурольного метода составляет 100 и 25 нг хрома в 1 мл фотометрируемого раствора для двух вариантов анализа с применением SDS и CTMAB, соответственно. Оптимизирован удобный и сравнительно быстрый метод минерализации биологических образцов с использованием пергидроля в присутствии серной кислоты. Разработанные методы анализа применимы для мониторинга процессов восстановления хромата культурами дрожжей по образованию конечного продукта — Cr(III), а также определения общего содержания хрома в микробных культурах или клетках после их минерализации.

Ключевые слова: хром(III), хромазурол S, додецилсульфат натрия, цетилтриметиламмонийбромид, фотометрический анализ, культура дрожжей.