

# **Austrian Journal of Technical and Natural Sciences**

**Nº 5–6 2020**

**May – June**

# Austrian Journal of Technical and Natural Sciences

Scientific journal  
№ 5–6 2020 (May – June)

ISSN 2310-5607

**Editor-in-chief** Hong Han, China, Doctor of Engineering Sciences

## International editorial board

Andronov Vladimir Anatolyevitch, Ukraine, Doctor of Engineering Sciences  
Bestugin Alexander Roaldovich, Russia, Doctor of Engineering Sciences  
S.R. Boselin Prabhu, India, Doctor of Engineering Sciences  
Frolova Tatiana Vladimirovna, Ukraine, Doctor of Medicine  
Inoyatova Flora Ilyasovna, Uzbekistan, Doctor of Medicine  
Kambur Maria Dmitrievna, Ukraine, Doctor of Veterinary Medicine  
Kurdzeka Aliaksandr, Russia, Doctor of Veterinary Medicine  
Khentov Viktor Yakovlevich, Russia, Doctor of Chemistry  
Kushaliyev Kaisar Zhalitovich, Kazakhstan, Doctor of Veterinary Medicine  
Mambetullaeva Svetlana Mirzamuratovna, Uzbekistan, Doctor of Biological Sciences  
Manasaryan Grigoriy Genrihovich, Armenia, Doctor of Engineering Sciences  
Martirosyan Vilena Akopovna, Armenia, Doctor of Engineering Sciences  
Miryuk Olga Alexandrovna, Kazakhstan, Doctor of Engineering Sciences  
Nagiyev Polad Yusif, Azerbaijan, Ph.D. of Agricultural Sciences  
Nemikin Alexey Andreevich, Russia, Ph.D. of Agricultural Sciences  
Nenko Nataliya Ivanovna, Russia, Doctor of Agricultural Sciences

Ogirko Igor Vasilievich, Ukraine, Doctor of Engineering Sciences  
Platov Sergey Iosifovich, Russia, Doctor of Engineering Sciences  
Rayiha Amenzade, Azerbaijan, Doctor of architecture  
Shakhova Irina Aleksandrovna, Uzbekistan, Doctor of Medicine  
Skopin Pavel Igorevich, Russia, Doctor of Medicine  
Suleymanov Suleyman Fayzullaevich, Uzbekistan, Ph.D. of Medicine  
Tegza Alexandra Alexeevna, Kazakhstan, Doctor of Veterinary Medicine  
Zamazay Andrey Anatolievich, Ukraine, Doctor of Veterinary Medicine  
Zhanadilov Shaizinda, Uzbekistan, Doctor of Medicine

**Proofreading** Kristin Theissen  
**Cover design** Andreas Vogel  
**Additional design** Stephan Friedman  
**Editorial office** Premier Publishing s.r.o.  
Praha 8 – Karlín, Lyčkovo nám. 508/7, PSČ 18600  
**E-mail:** pub@ppublishing.org  
**Homepage:** ppublishing.org

**Austrian Journal of Technical and Natural Sciences** is an international, German/English/Russian language, peer-reviewed journal. It is published bi-monthly with circulation of 1000 copies.

The decisive criterion for accepting a manuscript for publication is scientific quality. All research articles published in this journal have undergone a rigorous peer review. Based on initial screening by the editors, each paper is anonymized and reviewed by at least two anonymous referees. Recommending the articles for publishing, the reviewers confirm that in their opinion the submitted article contains important or new scientific results.

Premier Publishing s.r.o. is not responsible for the stylistic content of the article. The responsibility for the stylistic content lies on an author of an article.

## Instructions for authors

Full instructions for manuscript preparation and submission can be found through the Premier Publishing s.r.o. home page at:  
<http://ppublishing.org>.

## Material disclaimer

The opinions expressed in the conference proceedings do not necessarily reflect those of the Premier Publishing s.r.o., the editor, the editorial board, or the organization to which the authors are affiliated.

Premier Publishing s.r.o. is not responsible for the stylistic content of the article. The responsibility for the stylistic content lies on an author of an article.

Included to the open access repositories:



© Premier Publishing s.r.o.

All rights reserved; no part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without prior written permission of the Publisher.

Typeset in Berling by Ziegler Buchdruckerei, Linz, Austria.

Printed by Premier Publishing s.r.o., Vienna, Austria on acid-free paper.

## Section 1. Biology

<https://doi.org/10.29013/AJT-20-5.6-3-7>

Bagdasaryan Samvel,  
senior laboratory assistant,  
“Armbiotechnology” SPC, NAS RA  
E-mail: sambagd55@gmail.com

Babayan Bella,  
PhD student, researcher, “Armbiotechnology” SPC, NAS RA,  
National Polytechnic University of Armenia (NPUA)  
E-mail: bbg.15.04@mail.ru

Melkumyan Marina,  
PhD in Biology, senior researcher,  
“Armbiotechnology” SPC, NAS RA  
E-mail: marmelk2109@gmail.com

Kinosyan Marina,  
Researcher, “Armbiotechnology” SPC, NAS RA  
E-mail: marina.kinosyan@mail.ru

### POLYSORBATES BIODEGRADATION POTENTIAL AND PLASMID STABILITY OF SOIL *PSEUDOMONAS*

**Abstract.** The main aim of current research was a consideration of xenobiotics biodegradation potential of more than 50 strains of 7 species and 3 subspecies of mentioned bacteria and their antibiotic resistance as interacting parts of one system of adaptation of microorganisms to changing environment. And it has not only theoretical scientific importance, for understanding of bases of *Pseudomonas* and *Stenotrophomonas* resistance plasmids stability, but also has a practical significance for solving of modern ecological problems of new multidrug resistant pathogen forming and xenobiotic biodegradation processes in soil.

**Keywords:** *Pseudomonas*, biodegradation, antibiotic-resistance, plasmids, lipases.

#### Introduction

The problem of multi-drug resistance of microbes, according to WHO reports of recent years, is one of key importance research directions for upcoming century [1]. The multiple resistance of microorganisms to antimicrobial preparations is one of

the well-known properties of *Pseudomonas*, which are being isolated from clinics [2]. But the native strains of these bacteria, which were isolated from soil and other wet surfaces of natural and artificial origin, have the wide spectrum of resistance to antibiotics of II and III generations and they are able to

stable transfer this property to other Gram-negative bacteria too. Thus it has not only medical but also ecological and archeological significance, because of very broad life areal of these microorganisms.

According to the research of recent years, *Pseudomonas* and other cave microorganisms are endangering not only the health visitors and cave researchers, but also are becoming the new object for resistance research in aspects of both resistance new types discovery and the discovery of new antibiotics [3; 4]. Due to literature data, *Pseudomonas* are highly adaptive microorganisms. They have a wide diversity of enzymes and biochemical pathways of biodegradation of various native substances and xenobiotics [5; 6]. That is why the study of relation of stable antibiotic resistance persistence and the transfer of it with xenobiotic biodegradation genes localization in bacterial genome is very significant for understand the mechanisms of this problem.

#### Materials and Methods

During this research there were used strains from The National Culture Collection of Microorganisms of the MDC of "Armbiotechnology" SPC NAS RA. They were cultivated according to the standard protocols. For the resistance tests here were used 50mg/kg/

ml concentrations of 13 antibiotics of different classes and generations: penicillin/Pen, ampicillin/Amp, amoxicillin/Amx, augmentin/Amc, cefixime/Cfm, ceftriaxone/Cro, kanamycin/Kan, streptomycin/Str, gentamycin/Gen, ciprofloxacin/Cip; tetracycline/Tet; Chloramphenicol Chl) of amphenicoles, azithromycin (Azm) – of azalides of macrolides [7; 8].

Biodegradation potential was evaluated due to extracellular enzyme activity precipitation. Lipases activities tests were carried out according to standard microbiology and biochemistry protocols on solid cultural media, using substitution of carbon source to appropriate polysorbates –20,–40,–60,–65,–80,–85 with different length of fatty acids [9]. The genetical analyses was done by DNA electrophoresis, transformation and PCR. DNA isolation was done by alkaline extraction for plasmids and with benzyl-chloride method for total DNA. PCR analysis of antibiotic modification enzyme genes was done with the following primers: *aph(3')IV*, *aac(6')II*, *pCAT639*, *bla-OXA-10* [10]. The statistical verification of experiments was done by standard methods [11; 12].

#### Results

The results of polysorbate degradation tests are presented on (table 1).

Table 1. – Polysorbates biodegradation by *Pseudomonas*

Strain of micro-organism	Polysorbates												C+	C–	
	20		40		60		65		80		85				
<i>I</i>	G	A	G	A	G	A	G	A	G	A	G	A			
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>P. geniculata</i>	9335	3+	–	3+	–	3+	–	+	–	3+	–	3+	–	5+	–
	9336	3+	–	3+	–	3+	–	+	–	3+	–	3+	–	5+	–
	9337	3+	–	3+	3+	3+	–	2+	–	3+	–	3+	3+	5+	–
	9338	2+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–
	9339	3+	–	2+	–	3+	–	+	–	3+	–	3+	–	5+	–
	9340	2+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–
	9205	3+	–	+	2+	3+	–	3+	–	3+	–	2+	–	5+	–
	9203	3+	2+	+	–	2+	+	+	+	3+	3+	3+	–	5+	–
	9212	2+	+	2+	–	2+	+	+	+	2+	–	++	+	5+	–
<i>P. aeruginosa</i>	9059	3+	–	+	–	3+	–	3+	–	3+	+	3+	3+	5+	–
	9058	3+	–	3+	–	3+	–	3+	–	3+	–	3+	–	5+	–
	9056	–	–	–	–	–	–	–	–	–	–	–	–	5+	–

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>P. fluorescens</i>	9075	3+	+	3+	+	3+	-	2+	-	3+	-	3+	3+	5+	-
	9077	3+	-	3+	-	3+	-	2+	-	3+	-	3+	-	5+	-
	9252	3+	-	-	-	3+	-	+	-	3+	5+	3+	+	5+	-
	9089	3+	-	3+	-	3+	-	2+	-	3+	-	3+	-	5+	-
	9092	3+	-	3+	-	3+	-	2+	-	3+	-	3+	-	5+	-
	9095	3+	5+	2+	-	2+	-	2+	-	3+	-	3+	-	5+	-
	9096	3+	-	3+	-	3+	-	2+	-	3+	-	3+	-	5+	-

Polysorbates – 20, 40, 65, 80, 85; G – growth (after 5 days), A – activity; “+” – growth/lipase activity presence different intensity; “-” – growth/lipase activity absence; “C+” – positive control on nutrient agar media; “C-” – negative control on mineral cultural media

Table 2. – Resistance transfer in soil *Pseudomonas*

Rec	Donor	R/S of Rec	R/S of Donor	1	2	3	4	5	6	7	8	9	10	11	12	13	C2
I	<i>pUC18</i> (C1)	S	Amp	-	+	-	-	-	-	-	-	-	-	-	-	-	+
II	<i>S. maltophilia</i> 9302	S	Str, Pcn, Amx Amp	+	+	+	-	-	-	-	-	-	-	-	-	-	+
III	<i>pUC18</i>	Cip, Cro,	Amp	-	-	-	-	-	+	-	-	-	-	-	+	-	+
IV	<i>pUC18</i>	Cip, S	Amp	+	-	-	-	-	-	-	-	-	-	+	-	-	+
V	VOG16	Chl, Amc, Amx, Amp Pcn, Cfm	Kan	+	+	+	+	+	+	+	+	-	-	-	-	-	+
VI	<i>pUC18</i>	Cfm	Amp	-	+	-	-	+	-	-	-	-	-	-	-	-	+
III	<i>S. maltophilia</i> 9285	Cip, Cfm	Kan Amc Amx Amp Cfm	-	-	-	-	+	-	-	-	-	-	-	+	-	+
VI	<i>S. maltophilia</i> 9288	Chl, Amc, Amx, Amp, Cfm,	R, Cro <sup>S</sup> , Tet <sup>S</sup> , Azm <sup>S</sup> , Cip <sup>S</sup>	-	+	+	+	+	-	+	+	-	-	-	-	-	+
IV	<i>S. maltophilia</i> 9277	Cip	Amc Amx Amp Cfm	+	+	+	+	-	-	-	-	-	-	-	+	-	+
VI	<i>S. maltophilia</i> 9304	Cfm	Cro	-	-	-	-	+	-	-	-	-	-	-	-	-	+

Antibiotics: 1 – penicillin/Pen, 2 – ampicillin/Amp, amoxicillin/Amx, 3 – augmentin/Amc, 4 – cefixime/Cfm, 5 – ceftriaxone/Cro, 6 – kanamycin/Kan, 7 – streptomycin/Str, 8 – gentamycin/Gen, 9 – ciprofloxacin/Cip, 10 – tetracycline/Tet, 11 – Chloramphenicol/Chl, 12 – azithromycin /Azm; Rec. (Recipients): I – *P. aeruginosa* 9056, II – *E. coli* DH5a, III – *S. maltophilia* 9326, IV – *P. chlororaphis*, subsp. *chlororaphis* 9329, V – *P. fluorescens* 9092, VI – *P. taetrolens* 9243; S – sensitivity, R – resistance, C1 – plasmid transmission control by *E. coli* DH5a/*pUC18* plasmid, C2 – control on nutrient agar, “+” – growth, “-” – the absence of growth

Then the same procedure of transformation was repeated with other recipient strains. The same results were obtained in 6 used recipients of different species of bacteria. While the tests of stability, the divergence of results was detected. The stability of replication was detected for all the researched donors in case of *P. aeruginosa* 9056 recipients, except the *P. taetrolens* recipients which didn't demonstrate the stability after the cultivation of transformants using non selective nutrient agar media. Thus it might

be concluded that the cause of plasmid resistance in general is defined by the genome structure of *P. aeruginosa* [13]. In case of *P. taetrolens*, probably there is the system which is blocking the possibility of stable replication of donor DNA in recipient cells. The antibiotic resistance tests of all the obtained transformed strains are presented on table 5. The results of antibiotic resistance test were the same for each several donor and all the used recipients, thus in table is represented only one of 6 experiments of each series.

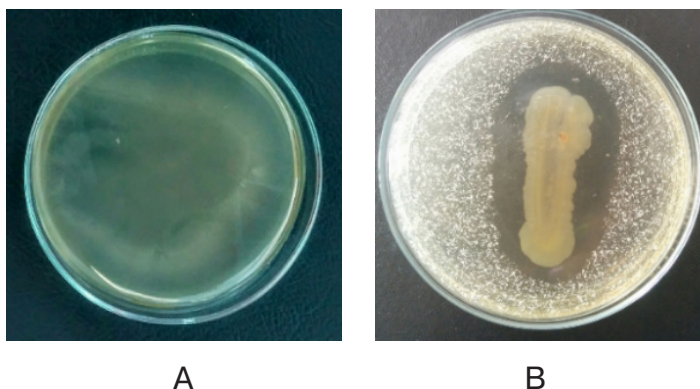


Figure 1. Lipase activity of different stains (A – *P. taetrolens* 9246 on polysorbate-85; B – *P. chlororaphis subsp. aurantiaca* 9061)

### Conclusions

During the experiments, it was found out that the genes of 5 types of polysorbate degrading lipases are different. They can have both plasmid and nucleoid localization in frames of one species and one subspecies. Probably, one part of these plasmid localized lipases can play a key role for antibiotic resistance plasmid stability in some strains of *P. taetrolens*, while the stability of plasmids from *P. fluorescens*, *P. chlororaphis* and *P. aeruginosa*, is predominantly caused

by genome of recipient strains. The maximal stability was detected on both selective and non-selective cultural media in recipient cells of *P. aeruginosa*.

### Acknowledgements

This research is supported by the research grant #microbio-5133, from the Armenian National Science and Education Fund (ANSEF) based in New York, USA and The Ministry of Education, Science, Culture and Sports of RA SCS, in frames of the research project № 18T-2I036.

### References:

1. Cotar A.-I., Chifiriuc M.-C., Dinu S., Bucur M., Iordache C., Banu O., Dracea O., Larion C., Lazar V. Screening of Molecular Virulence Markers in *St. aureus* and *P. aeruginosa* Strains Isolated from Clinical Infections, *Int. J. Mol Sci.*, 2010.– 11(12).– P. 5273–5291.
2. Critically Important Antimicrobials for Human Medicine, WHO AGISAR, V rev., 2017.– P. 20–37.
3. Babayan B. G., Mikaelyan A. R., Shahinyan S. M., Bagdasaryan S. A. Tartaric Acid New Derivatives Effect Against the Soil *Pseudomonas* and *Stenotrophomonas* As A Model For Research of Cave Infection Bacteria Antibiotic Resistance Combating, The Abstract book of Int. Conf. “Caves As Natural & Cultural Monuments”, Sep 11–13, 2019.– P. 22–23.

4. Suárez P., Gutiérrez A. V., Salazar V., Puche M. L. Virulence properties and antimicrobial resistance of *Pseudomonas aeruginosa* isolated from cave waters at Roraima Tepui, Guayana Highlands. *Lett Appl Microbiol.* 2020. 70(5).– P. 372–379.
5. Borkar P. S., Bodade R. G., Rao S. R., Khobragade C. N., Purification and characterization of extracellular lipase from a new strain: *P. aeruginosa* SRT 9, *Braz J Microbiol.*, 2009. – 40(2).– P. 358–366.
6. Pasumarthi R., Chandrasekaran S., Mutnuri S. Biodegradation of crude oil by *P. aeruginosa* and *E. fergusonii* isolated from the Goan coast., *Mar Pollut Bull.* 2013. – 15; 76(1–2).– P. 276–82.
7. Dolejska M., Literak I. Wildlife is Overlooked in the Epidemiology of Medically Important Antibiotic-Resistant Bacteria, *Antimicrob Agents Chemother*, 2019. – 25. 63(8) – pii: e01167–19.
8. Babayan B. G., Hovhannisyan N. A., Hovhannisyan A. M., Sargsyan A. S., Davidyan T. S., Resistance To  $\beta$ -Lactam Antibiotics In Some Soil *Stenotrophomonas* and *Pseudomonas*, *The Scientific-Heritage*, 2019. – 2(34).– P. 32–38.
9. Babayan B. G., Bagdasaryan S. A., Kinosyan M. H., Melkumyan M. A., Hovhannisyan N. A., Metabolic and Genetical Features of Biodegradation and Resistance Potential of Soil *Pseudomonas* sp. From The National Culture Collection of Microorganisms RA, *EJBL*, 2020. – 1. – P. 12–19.
10. Aksentijević K., Ašanin J., Milivojević D., Čolović S., Butorac A., Cindrić M., Mišić D. Differentiation between *Pseudomonas* and *Stenotrophomonas* Species Isolated from Fish Using Molecular & MALDI-TOF Method, *Acta Veterinaria-Beograd*, 2016. – 66(3).– P. 304–316.
11. Ashmarin I. P., Vorobyov A. A. The Statistical Methods in microbiological research [published in Russian], L. State pub. of Med Literature, 1962. – 182 p.
12. Chou D. K., Krishnamurthy R., Randolph T. W., Carpenter J. F., Manning M. C. Effects of Tween 20 and Tween 80 on the stability of Albutropin during agitation, *J Pharm Sci.*, 2005. – 94(6).– P. 1368–12381.
13. Molina L., Udaondo Z., Duque E., Fernández M., Molina-Santiago C., Roca A., Porcel M., de la Torre J., Segura A., Plesiat P., Jeannot K., Ramos J.-L. Antibiotic Resistance Determinants in a *P. putida* Strain Isolated from a Hospital, *PLoS One*. 2014. – 9(1): e81604.

## Section 2. Information technology

<https://doi.org/10.29013/AJT-20-5.6-8-13>

*Fesokha Vitalii Viktorovich,  
postgraduate student, the Faculty of Information Technology,  
Military Institute of Telecommunication and  
Information technologies named after the Heroes of Kruty,  
E-mail: vitaliifesokha@gmail.com*

*Subach Ihor Yuriiiovych,  
Doctor of Technical Sciences, Associate Professor,  
Email: igor\_subach@ukr.net*

*Kubrak Volodymyr Oleksandrovysh,  
postgraduate student,  
Email: volodymir.kubrak@ukr.net*

*Mykytiuk Artem Viacheslavovych,  
postgraduate student,  
Email: mukuta8888@gmail.com*

*Korotaiev Stanislav Oleksandrovysh,  
Engineer,  
Institute of special communications and information security  
National technical university of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"  
Email: meduha1998@ukr.net*

### **ZERO-DAY POLYMORPHIC CYBERATTACKS DETECTION USING FUZZY INFERENCE SYSTEM**

**Abstract.** The model of zero-day cyberattacks detection on information systems and networks of critical infrastructure objects is presented in the paper. The mechanism of detecting new intrusions is based on identification of polymorphic modification of classified cyberattacks presented as fuzzy condition-action rule.

**Keywords:** cyber security, information system, polymorphic cyberattack, fuzzy logic, intrusion detection system.

The ongoing development and active efforts of State agencies, centres and services to combat cyberterrorism in the world leading countries testifies

to the urgency of ensuring an appropriate level of protection for information systems and critical infrastructure networks.



One of the main focuses of their activities is to increase the effectiveness of the main security means of information systems and networks in order to protect them from destabilizing impacts (interferences) of cyberattacks, i.e. intrusion detection system (IDS), by promptly identifying signs of privacy compromise, availability and integrity of information resources and/or services.

Solving this issue in terms of compliance with IDS modern requirements to information security systems requires the use of approaches capable of detecting zero-day vulnerabilities (*0-day*), because it is their uncontrolled nature that can lead to unpredictable consequences at critical infrastructure facilities [1–5].

One of the promising approaches to solving the above issue is the implementation of cyberattack detection methods that simultaneously have the verification and adaptation capabilities, as well as the ability to analyze data with a sufficiently high degree of accuracy and reliability in conditions of uncertainty – methods based on the mathematical tool of the fuzzy sets and fuzzy logic [2–5].

However, supporting effective decisions to detect new cyberattacks according to this approach in the absence of the necessary linguistic variables included in the rules formed by experts is a rather complicated task. On the other hand, generation of a full possible set of rules with different combinations of linguistic variables is a rather complicated task and leads to a great computational complexity as well as an increase in the number of false alarms by the system.

This determines the relevance of further research, the purpose of which is to develop new models and methods of detection of cyberattacks, the fundamental foundation of which is the theory of fuzzy sets and fuzzy logic to detect zero-day cyberattacks.

**The purpose of the article** is to develop a model of cyberattacks detection on information systems and networks of critical infrastructure objects based on the application of a fuzzy logic system that will allow detecting zero-day cyberattacks.

**Analysis of recent studies and publications** [6–11] shows that the detection of this type of cyberattack is one of the most difficult issues of the cybersecurity and is usually solved by iteration, after identifying the consequences of their destructive activities and/or identifying them after a certain (fairly long) period of time. In order to detect the zero-day cyberattack, it is necessary to conduct a series of studies to establish the vector of impact, vulnerability of the software or the exploit that is using it to determine their characteristics for the subsequent creation of corresponding signatures (templates) or rules.

Today, there are two approaches to detecting such cyberattacks:

- Detection of malicious activity based on auditing software operation scenarios;
- detection based on existing experience in dealing with known disruptive forces.

The first approach is inappropriate for information networks of critical infrastructure facilities, as:

- it is practically impossible to verify the decision taken by the system;
- it is impossible to envisage a decision by the system in case of a software failure (hardware malfunction);
- too complicated process of creating scenarios of software functioning and level of detail (functioning at different levels);
- it is possible to envisage masking the cyberattack behavior at runtime as legitimate software.

For effective detection of zero-day cyberattacks it is reasonable to use the second approach based on the experience of dealing with already known intrusions, since the number of new attacks characterized by a fundamentally new set of features is quite small [6–9].

For example, the latest research by *Webroot Inc.* – a private American company that provides Internet security for consumers and businesses showed that in 2017 93% of malware and 95% of unwanted applications were *polymorphic* (occurring in several different forms of the same object), and this trend

continued during 2018–2019 [6,8,9]. However, this fact remained unnoticed by the major manufacturers of IDS. The security laboratory of *Avira* – a German company, developer of antivirus software, declares [6] that polymorphic cyberattacks are an increasingly common threat in recent years. Moreover, it is easier for malware developers to change the object shell using polymorphism and create new versions of malware based on existing malware with less effort. In addition, the architecture and principles of software that form the basis of the software stack (operating system kernel level, command system, memory management principles, runtime level) have not changed significantly over the past few decades.

On the basis of the above, most recent cyberattacks tend to have a certain, though not substantial, set of features inherent in the already classified incursions on which they were based. Cyberattacks that implement this approach are polymorphic forms of previously classified intrusions.

*Polymorphic cyberattack* is a cyberattack that is capable of changing its form (structure) with each instance. According to the time of modification, this approach is divided by:

- modification during initialization – once, before launching;
- modification *on the fly* – during its execution.

Below is a brief list of polymorphic cyberattacks, which have gained wide enough publicity [6]:

*CryptoLocker* encrypts files on users' devices in order to get a ransom money. The feature is access to information network resources through various scenarios.

*CryptXXX* is a ransomware created on the basis of *Angler Exploit Kit (EK)*, which used to encrypt files and steal passwords.

*VirLock* is the first polymorphic ransomware that locked victim's screen.

*Wanna Cry* is a ransomware cyberattack based on the *CryptXX* trojan. It used a vulnerability in older Windows operating systems.

In case of polymorphic cyberattack self-modification, a part of its code changes [6–11] (encryption, generation of program garbage), keeping the original algorithm intact (vector and body of the cyberattack), which contains the main program scenario of destructive activity implementation on resources (services) of the information system or network. Consequently, detection of such cyberattack in real time is practically impossible, which leads to signature development in the future for each of its forms, while the fact of invariability of functional purpose of the cyberattack body, which implements its vector, gives grounds for detection of zero-day cyberattacks based on identification of polymorphic modifications of already classified intrusions.

Therefore, in order to effectively detect polymorphic cyberattacks based on existing and rule-driven intrusions under conditions of uncertainty and incompleteness of the data to be processed, it is reasonable to determine the minimum required power of a set of linguistic variables of the fuzzy rule, which will ensure the functional belonging of a particular cyberattack to a certain class.

For the purpose of the analytical justification of the above, let us present a formal description of the model for detecting cyberattacks based on the proposed approach.

Definition of a state index (normal, detection of cyberattack) of an information system or network based on the application of the mathematical apparatus of the theory of fuzzy sets and fuzzy logical conclusion in the context of the above is considered as a process of decision-making on the state of the network based on the analysis of telemetry values of network traffic, *log (pcap)*-files of IDS, indicators of the state of the hardware component of hosts or their combination.

Hence, the problem of detection of cyberattacks is considered as a decision-making process in a system with a single output parameter  $y \in Y = [\underline{y}, \bar{y}]$  – an expert conclusion about its presence in the network based on the analysis of the input parameter

vector  $X_i = \left[ \underline{x}_i, \overline{x}_i \right], i = \overline{1, n}$  according to the cause-effect dependence:

$$y = f_y \{x_1, \dots, x_i, \dots, x_n\}, i = \overline{1, n} \quad (1)$$

and it comes down to finding a solution to an analytical formula:

$$X = (x_1, x_n) \rightarrow y = d_j (a_i^1, a_i^l, \dots, w_1, w_n) \in D = (d_1, d_m), \quad (2)$$

$$i = \overline{1, n}, \overline{1, m}$$

where  $y$  is linguistic description of the expert solution  $d \in D$  for the vector of values  $X = \{x_1, x_2, \dots, x_n\}$ ,  $a_i$  are numbers of combination of values of description parameters (linguistic terms)  $X$ , corresponding to the value  $d_j$ ,  $w_i$  is the weight of fuzzy rule,  $m$  – is the number of possible values of  $y$  variable.

Therefore, if the analytical description of some known (classified cyberattack) can be presented as:

$$X = \{x_1, \dots, x_i, \dots, x_n\}, i = \overline{1, n}, \quad (3)$$

then let us describe the polymorphic cyberattack on the basis of the principle of similarity of many of their features characterizing the body and vector of cyberattack [6–11] as:

$$X' = \{x'_1, \dots, x'_i, \dots, x'_m\}, i = \overline{1, m} \quad (4)$$

where  $n$  is the power of a known cyberattack rule set,  $m$  is the minimum required power of a polymorphic cyberattack rule.

To estimate the linguistic variables  $x_i, i = \overline{1, n}$  and  $y$  qualitative terms from the following term sets are used:

$$A_i = \{a_i^1, a_i^2, \dots, a_i^l\} - \text{term set } x_i, i = \overline{1, n},$$

$$D = \{d_1, d_2, \dots, d_m\} - \text{term set } y, \text{ where } a_i^p -$$

$p$ -th linguistic term  $x_i, p = \overline{1, l}, i = \overline{1, n}$ ;  $d_j - j$ -th linguistic term  $y$ .

Quantitative linguistic variables  $x_i, i = \overline{1, n}$  and  $y$  are classically determined by relations:

$$a_i^p = \int_{\underline{x}_i}^{\overline{x}_i} \mu^{a_i^p}(x_i) / x_i \quad (5)$$

$$d_j = \int_{\underline{y}_i}^{\overline{y}_i} \mu^{d_j}(y) / y \quad (6)$$

where  $\mu^{a_i^p}(x_i)$  is a function of the value of the input linguistic variable  $x_i \in \left[ \underline{x}_i, \overline{x}_i \right]$  of the term  $a_i^p \in A_i$ ,  $p = \overline{1, l}, i = \overline{1, n}$ ;  $\mu^{d_j}(y)$  is a function of the value of the variable  $y \in \left[ \underline{y}, \overline{y} \right]$  of the term  $d_j \in D, j = \overline{1, m}$ .

The qualitative linguistic variables  $x_i, i = \overline{1, n}$  and  $y$  are in turn determined by the following relations:

$$a_i^p = \sum_{k=1}^{q_i} \mu^{a_i^p}(v_i^k) / v_i^k \quad (7)$$

$$d_j = \sum_{r=1}^{q_m} \mu^{d_j}(y^r) / y^r \quad (8)$$

where  $\mu^{a_i^p}(v_i^k)$  is the membership degree of the element  $v_i^k \in U_i$  of the term  $a_i^p \in A_i, p = \overline{1, l}, i = \overline{1, n}, k = \overline{1, q_i}$ ;  $\mu^{d_j}(y^r)$  is the membership degree of the element  $y^r \in Y$  of the term  $d_j \in D, j = \overline{1, m}$ .

To establish the functional dependence (1) between the set of investigated parameters and the corresponding decision on the state of the network (system), after their linguistic evaluation by the experts, a system of fuzzy logical rules of the “IF-THEN, ELSE” type is formed in a knowledge base (KB):

$$\text{IF } (x_1 = a_1^{l1}) \text{ AND } (x_n = a_n^{l1}) \text{ (weight } w_{11}) \text{ OR } (x_1 = a_1^{lk_1}) \text{ AND } (x_n = a_n^{lk_1}) \text{ (weight } w_{1k_1})$$

THEN

$$y = d_1, \text{ ELSE}$$

$$\text{IF } (x_1 = a_1^{m1}) \text{ AND } (x_n = a_n^{m1}) \text{ (weight } w_{11}) \text{ OR } (x_1 = a_1^{mk_m}) \text{ AND } (x_n = a_n^{mk_m}) \text{ (weight } w_{1k_1})$$

THEN

$$y = d_m.$$

The next stage is the process of determining the minimum required number of the most important (informative) research parameters for each known class of cyberattacks, represented as fuzzy sets of linguistic variables that will describe them sufficiently. For example, for known generalized classes of cyberattacks as defined in *KDD Cup 1999 Data: User to Root (u2)* – unauthorized access to the privileged user (root), *Remote to Local (r2l)* – unauthorized remote access in order to gain access to the account, *Denial of Service (dos)* – disruption of the system ser-

vices in order to bring the service (server) to the denial of service to users, *Probe* – obtaining information about the target host based on scanning and probing will need only 4 fuzzy sets of the most significant linguistic variables.

For example, for the existing in KB fuzzy rules of network traffic telemetry analysis with division into classes of cyberattacks according to *KDD Cup 1999 Data* [12]:

$$X_{u2r} = \{x_1^1, x_1^2, \dots, x_1^{41}\},$$

$$X_{r2l} = \{x_2^1, x_2^2, \dots, x_2^{41}\},$$

$$X_{dos} = \{x_3^1, x_3^2, \dots, x_3^{41}\},$$

$$X_{probe} = \{x_4^1, x_4^2, \dots, x_4^{41}\}$$

allows to identify many of the most informative variables:

$$X'_{u2r} = \{x_1^1, x_1^2, \dots, x_1^m\},$$

$$X'_{r2l} = \{x_2^1, x_2^2, \dots, x_2^m\},$$

$$X'_{dos} = \{x_3^1, x_3^2, \dots, x_3^m\},$$

$$X'_{probe} = \{x_4^1, x_4^2, \dots, x_4^m\},$$

where  $m$  is the number of minimum required informative variables to determine the class of cyberattacks.

The next step is the formation of new additional rules in the KB by applying an intersection operation ( $\cap$ ) of fuzzy sets for previously existing rules  $X_i$  and  $X'_i$ :

$$\forall x \in X \mu_{X \cap X'}(x) = \min\{\mu_X(x); \mu_{X'}(x)\} \quad (9)$$

Therefore, for the model of detection of cyberattacks on the basis of the theory of fuzzy sets and fuzzy logic in conditions of uncertainty of the data to be processed, the possibility of their analysis with the minimum required completeness of the set of fuzzy rules is added, which allows to detect polymorphic cyberattacks created on the basis of known and described, because the most significant signs of intrusions characterize the body and their vector of influence.

By using  $\cup$  (OR)  $\cap$  (I) operations, the above system of logical statements is given as follows:

$$\bigcup_{p=1}^{k_j} \left\{ w_{jp} \left[ \bigcap_{i=1}^n (x_i = a_i^{jp}) \right] \right\} \rightarrow y = d_j, j = \overline{1, m} \quad (10)$$

Calculation of values of multidimensional membership functions for expert solutions, which may arise in an information system or network at fixed values of the input parameters specified above, is presented in the form of the following system of fuzzy logic equations with the replacement of their linguistic terms with the corresponding membership functions, and operations  $\cap$  and  $\cup$  on  $\wedge$  and  $\vee$ :

$$\mu^{d_j}(x_1, x_2, \dots, x_n) = \bigvee_{p=1}^{k_j} \left\{ w_{jp} \left[ \bigwedge_{i=1}^n \mu^{jp}(x_i) \right] \right\}, j = \overline{1, m} \quad (11)$$

where  $\wedge$  is the fuzzy logic AND,  $\vee$  is the fuzzy logic OR.

Since operations  $\wedge$  and  $\vee$  in the theory of fuzzy sets meet operations *max* and *min*, then:

$$\mu^{d_j}(x_1, x_2, \dots, x_n) = \max_{p=1, k_j} \left\{ w_{jp} \min_{i=1, n} \left[ \mu^{jp}(x_i) \right] \right\}, \quad (12)$$

$$j = \overline{1, m}$$

As a decision on the state of a system or network based on a fixed vector of input values (network traffic telemetry, *log (pcap)*-files of IDS, parameters of the hardware of hosts or their combination) is determined the result with the maximum value obtained as a result of convolution of functions of belonging of the terms of fuzzy rules of description of cyberattacks and corresponding weight coefficients.

Therefore, the model of detection of cyberattacks on information systems or networks of objects of critical infrastructure, which, unlike the existing ones, allows to detect zero-day intrusions based on the identification of polymorphic cyberattacks, as well as solve the problem of detecting cyberattacks in real time with the necessary completeness by forming additional fuzzy rules based on the definition of the most significant features for each class of cyberattacks.

---

### References:

1. Shanmugavadivu R., Nagarajan N. Network intrusion detection system using fuzzy logic. *Indian Journal of Computer Science and Engineering (IJCSE)*. 2011.– Vol. 2. – No. 1.– C. 101–111. ISSN: 0976–5166.
2. Mityushkin I., Mokin B., Rothstein O. *Soft Computing: identification of laws with fuzzy knowledge bases*. Monograph.– Vinnytsya: UNIVERSUM – Vinnitsa. 2002–145p.
3. Subach I., Fesokha V., Fesokha N. An analysis of existing decisions to prevent intrusion in information and telecommunication networks open on the basis of public licenses. *Information Technology and Security*, 2017. – Vol. 5. – No. 1. E-ISSN: 2518–1033.
4. Subach I., Fesokha V. Model of detection of anomalies in information and telecommunication networks of military management bodies on the basis of fuzzy sets and fuzzy logic, *Collection of scientific works of MITI – No. 3*. 2017. – P. 158–164.
5. Subach I., Fesokha V. Model of detecting cybernetic attacks on information-telecommunication systems based on description of anomalies in their work by weighed fuzzy rules. *Information Technology and Security*, 2017, – Vol. 5. – No. 2. E-ISSN: 2518–1033.
6. Casey Crane, *Polymorphic Malware and Metamorphic Malware: What You Need to Know*. May 21, 2019. URL: <https://www.thesslstore.com/blog/polymorphic-malware-and-metamorphic-malware-what-you-need-to-know>.
7. Nate Lord. *What is Polymorphic Malware? A Definition and Best Practices for Defending Against Polymorphic Malware*. September, 2018. URL: <https://digitalguardian.com/blog/what-polymorphic-malware-definition-and-best-practices-defending-against-polymorphic-malware>.
8. 2018 Webroot Threat Report. URL: [https://www-cdn.webroot.com/9315/2354/6488/2018-Webroot-Threat-Report\\_US-ONLINE.pdf](https://www-cdn.webroot.com/9315/2354/6488/2018-Webroot-Threat-Report_US-ONLINE.pdf)
9. 2019 Webroot Threat Report. URL: [https://www-cdn.webroot.com/9315/2354/6488/2018-Webroot-Threat-Report\\_US-ONLINE.pdf](https://www-cdn.webroot.com/9315/2354/6488/2018-Webroot-Threat-Report_US-ONLINE.pdf)
10. William Donald Hamilton, Jonathan Howard, Royal Society (Great Britain) Chapman & Hall. *Infection, Polymorphism and Evolution*. 1997.– P. 120. ISBN-13: 978–94–010–6523–8 e-ISBN-13: 978–94–009–0077–6 DOI: 10.1007/978–94–009–0077–6.
11. Mohssen Mohammed, Al-Sakib Khan Pathan. *Automatic Defense Against Zero-day Polymorphic Worms in Communication Networks*. ISBN9780367380038. Published September 19, 2019. by Auerbach Publications, – 337 P.
12. UCI Knowledge Discovery in Databases Archive. University of California, Irvine, CA 92697–3425. URL: <http://kdd.ics.uci.edu/databases/kddcup99/task.html>.

## Section 3. Medical science

<https://doi.org/10.29013/AJT-20-5.6-14-21>

*Hruzevskiy Olexander,  
docent, department of microbiology, virology and immunology  
Odessa National Medical University  
E-mail: gruzevskiy@ua.fm*

### **A STATUS OF CELLULAR IMMUNITY IN BACTERIAL DYSBIOSIS AND BACTERIAL VAGINOSIS**

**Abstract.** Systemic immunodeficiency contributes to the development of bacterial dysbiosis and bacterial vaginosis (BV), therefore, it is relevant to study the systemic response of the immune system, particularly its cellular component, and to search for informative criteria of the pathological process development.

Purpose of the study was to determine the status of cellular immunity by populations of T-lymphocytes in normocenosis, bacterial dysbiosis and BV.

As result in dysbiosis and BV, CD3 and CD4 T-cell immunodeficiency was identified. IRI is a diagnostic factor both for the formation of dysbiosis and for the diagnostics of BV. The model proposed allows us to predict the probability of development of BV with a high level of confidence.

**Keywords:** bacterial vaginosis, T-cell immunodeficiency.

Bacterial vaginosis (BV) is a common but poorly studied disease of the vagina, which promotes the development of local and systemic immunodeficiency and facilitates the transmission of human immunodeficiency virus (HIV) [1, 219–228], papillomavirus and cervical cancer [2, 9–18; 3, 553–558]. The problem of BV is very important for global health, because vaginal microbiota with high levels of streptococcus or enterobacteria, vaginal candidiasis and trichomoniasis causes an increased risk of pelvic inflammatory disease, pre-term birth and infections for mother and newly born child [4, 859–864]. Over the last decade, an incidence of BV has doubled and ranges from 26% to 40–45% [5, 472; 6]. For example, in the United States BV affects 29% of women, and in sub-Saharan Africa, where HIV is wide spread – 52% of women [7, 505–23].

BV is not usually associated with the redness, swelling, or pain observed in classical inflammation, so it is called “vaginosis”, not “vaginitis” [8, 555–63]. However, BV is related to “subclinical” inflammation of the genitals, which is determined by the hyperreactive response of the immune system [9, 965–76]. According to [10, 156–62], dysbiosis and BV are the main factors contributing to the persistent inflammation of the genital organs and facilitating HIV infection among such women.

It is the lack of local immunity that is an important factor for the occurrence of BV [11, 1399–1405; 12, 103–7]. The reason may be the ability of bacteria forming symbiote in BV to form an active biofilm, which causes suppression of the immune system, chronicity of the process, and resistance to antibi-

otic therapy [13]. In general, dysbiosis and BV are characterized by a decrease of systemic and local inflammatory response [14, 1–5], which, as found in the study [15, 481–7], correlates with an increase of *Gardnerella vaginalis* and *Mycoplasma hominis*.

Therefore, according to the foregoing, it is relevant to study the systemic response of the immune system, in particular of its cellular component, and to search for informative indicators of the pathological process.

Purpose of the study was to determine the status of cellular immunity by populations of T-lymphocytes in normocenosis, bacterial dysbiosis and BV.

### Material and methods

This study examined 298 women aged from 16 to 64 who saw gynecologist for a preventive examination or with complaints of genital discomfort of various degrees of manifestation. Subsequent observation excluded patients who had at least one of the definitely pathogenic microorganisms (*Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Herpes Simplex Virus 1, 2*) in the material taken. Presence in the smear of more than 15–20 leukocytes, which indicated of an inflammatory reaction, was also the reason for exclusion from the number of patients.

During the examination, scraping of epithelium from the posterolateral vaginal paries was made using a urogenital probe. Molecular-genetic studies were performed using polymerase chain reaction (PCR) method. DNA was extracted using a kit of reagents “Proba-GS” (“DNK-Technologiia” LLC, RF). Amplification of tubes with the reaction mixture was performed in the amplifier “DTLite” (“DNK-Technologiia” LLC, RF) using the amplification programs recommended by the manufacturer of the reagent kit. Investigation of vaginal biocenosis status was performed using the real-time PCR test system “Femoflor 16”, which allowed to quantify the biota [16, 30] by the following indicators: total bacterial mass (TBM), normobiota (*Lactobacillus spp.*; NB), obligate anaerobes (*Atopobium vaginalis*, *Eubacte-*

*rium spp.*, *Gardnerella vaginalis*, *Prevotella bivia*, *Porphyromonas spp.*, *Lachnobacterium spp.*, *Clostridium spp.*, *Megasphaera spp.*, *Veilonella spp.*, *Dialister spp.*, *Mobilunory spp.*, *Mobiluncus spp.*, *Peptostreptococ spp.*, *Sneathia spp.*, *Leptotrihia spp.*, *Fusobacterium spp.*), Facultative anaerobes (*Enterobacteriaceae spp.*, *Staphylococcus spp.*, *Streptococcus spp.*), mycoplasmas (*Ureaplasma urealyticum + parvum*, *Mycoplasma hominis + genitalium*) and yeast-like fungi (*Candida spp.*).

Criterion for the distribution of patients into groups was an index of conditionally pathogenic microflora (ICPM), which was calculated as the difference between the sum of all conditionally pathogenic microorganisms and the number of lactobacilli in lg GE/sample. In normocenosis ICPM was lower than –3 lg GE/sample (first group, n = 53); in grade I dysbiosis it was from –3 to –1 lg GE/sample (second group, n = 128); and in grade II dysbiosis (BV) it was more than –1 lg GE/sample (third group, n = 117) [17, 54–7; 18, 36–41]. In addition, groups with dysbiosis were subdivided into subgroups by normobiota indicator (NBI), which was calculated as the difference between TBM and lactobacilli (in lg GE/sample). In the 2<sup>nd</sup> group there are three subgroups: 1<sup>st</sup> subgroup – with NBI ≤ 0.3 lg GE/sample (n = 23), 2<sup>nd</sup> subgroup – with NBI from 0.3 to 1.0 lg GE/sample (n = 83), and 3<sup>rd</sup> subgroup – with NBI > 1 lg GE/sample (n = 22). In the 3<sup>rd</sup> group there are two subgroups: 1<sup>st</sup> subgroup – with NBI ≤ 1 lg GE/sample (n = 34), and 2<sup>nd</sup> subgroup – with NBI > 1 lg GE/sample (n = 83). The maximum degree of dysbiosis was recognized in the 2<sup>nd</sup> subgroup of the 3<sup>rd</sup> group, which corresponded to the condition of BV [16].

Subpopulations of T-lymphocytes in the blood were quantified using the method of their visualization in the rosette formation reaction with monoclonal antibodies to T-lymphocytes (CD3), T-lymphocytes helper (CD4), and T-lymphocytes killers/suppressor (CD8) using erythrocyte diagnostic tubes manufactured by “Granum” NPP (Ukraine). Lymphocyte suspension was separated on a density gradient d = 1.077 (“Granum” NPP, Ukraine). The

ratio CD4/CD8 (immunoreactivity index – IRI) was calculated.

For descriptive statistics, arithmetic mean value (M) and standard error of mean (m), median (Me), 1<sup>st</sup> and 3<sup>rd</sup> quartiles (Q1; Q3) were used. Variation series were compared using the Student's test (t), single- and multi-factor analysis of variance (criterion F). Paired independent data samples were compared using Mann-Whitney (U) test. Influence of factor variables on the dependent indicators was investigated using linear regression analysis. Regres-

sion coefficients ( $\beta$ ), probability of their contrast to the main hypothesis, Wald-statistics value and the maximum probability coefficient for nonlinear models were calculated. Operational characteristics were evaluated using ROC-diagrams. Significance of all the variations was taken at  $p < 0.05$ . Statistica 10 software package (StatSoft, Inc., USA) was used for statistical processing of the obtained data.

Results and Discussion. Indicators of cellular immunity in patients with normo- and dysbiosis are given in (Table 1).

Table 1. – Indicators of cellular immunity (M  $\pm$  m)

Group, subgroup	Leucocytes, G/l	CD3, G/l	CD4, G/l	CD8, G/l	IPI, rel. units	
1 <sup>st</sup> (normocenosis). n=53	6.104 $\pm$ 0.162	1.103 $\pm$ 0.042	0.629 $\pm$ 0.018	0.408 $\pm$ 0.009	1.592 $\pm$ 0.060	
2 <sup>nd</sup> (grade I dysbiosis). n=128	1 <sup>st</sup> , n=23	6.478 $\pm$ 0.296	1.147 $\pm$ 0.063	0.681 $\pm$ 0.037	0.428 $\pm$ 0.015	1.631 $\pm$ 0.104
	2 <sup>nd</sup> , n=83	7.142 $\pm$ 0.180	1.165 $\pm$ 0.031	0.690 $\pm$ 0.019	0.414 $\pm$ 0.006	1.695 $\pm$ 0.054
	3 <sup>rd</sup> , n=22	7.476 $\pm$ 0.337	1.159 $\pm$ 0.058	0.469 $\pm$ 0.020	0.422 $\pm$ 0.015	1.140 $\pm$ 0.061
3-я (grade II dysbiosis). n=117	1 <sup>st</sup> , n=34	8.658 $\pm$ 0.258	0.595 $\pm$ 0.014	0.442 $\pm$ 0.011	0.414 $\pm$ 0.011	1.094 $\pm$ 0.041
	2 <sup>nd</sup> , n=83	10.122 $\pm$ 0.183	0.413 $\pm$ 0.007	0.353 $\pm$ 0.007	0.508 $\pm$ 0.009	0.709 $\pm$ 0.017
Statistical procedure of comparison of the results (p)						
p(MW) <sup>1</sup>	0.282	0.527	0.190	0.218	0.709	
p(MW) <sup>2</sup>	0.001	0.276	0.048	0.758	0.309	
p(MW) <sup>3</sup>	0.001	0.446	<0.001	0.496	<0.001	
p(MW) <sup>4</sup>	<0.001	<0.001	<0.001	0.811	<0.001	
p(MW) <sup>5</sup>	<0.001	<0.001	<0.001	<0.001	<0.001	
F	58.834	126.594	73.392	23.089	72.095	
p	<0.001	<0.001	<0.001	<0.001	<0.001	

Notes: probability of discrepancies between the corresponding indicators in 1<sup>st</sup> and 2<sup>nd</sup> groups using Mann-Whitney test:  $p(MW)^1$  – in the 1<sup>st</sup> subgroup of the 2<sup>nd</sup> group,  $p(MW)^2$  – in the 2<sup>nd</sup> subgroup of the 2<sup>nd</sup> group,  $p(MW)^3$  – in the 3<sup>rd</sup> subgroup of the 2<sup>nd</sup> group,  $p(MW)^4$  – in the 1<sup>st</sup> subgroup of the 3<sup>rd</sup> group,  $p(MW)^5$  – in the 2<sup>nd</sup> subgroup of the 3<sup>rd</sup> group; F – result, and p – probability of the dispersion analysis of variance of differences of the corresponding indicators among the subgroups

Number of leukocytes in the blood under condition of normocenosis was slightly higher than the normal one (4.4–5.5 G/l [19, 960]). As the patients in 1<sup>st</sup> group did not experience inflamma-

tory processes and/or infectious diseases, we considered these values to be the normal variant.

As dysbiosis progressed, progression of leukocytosis was also observed (Fig. 1). Thus, the content of leukocytes in comparison with the 1<sup>st</sup> group was in-



creased by 1.2 times in the 2<sup>nd</sup> group ( $p = 0.001$ ), by 1.4 times in the 1<sup>st</sup> subgroup of the 3<sup>rd</sup> group and by 1.7 times in 2<sup>nd</sup> subgroup of the 3<sup>rd</sup> group ( $p < 0.001$  in both cases). Therefore, under conditions of devel-

opment of BV (2<sup>nd</sup> subgroup of the 3<sup>rd</sup> group), leukocytosis acquired the maximum value and exceeded such values in grades I and II dysbiosis (by 1.4 times and 1.2 times respectively;  $p < 0.05$  in both cases).

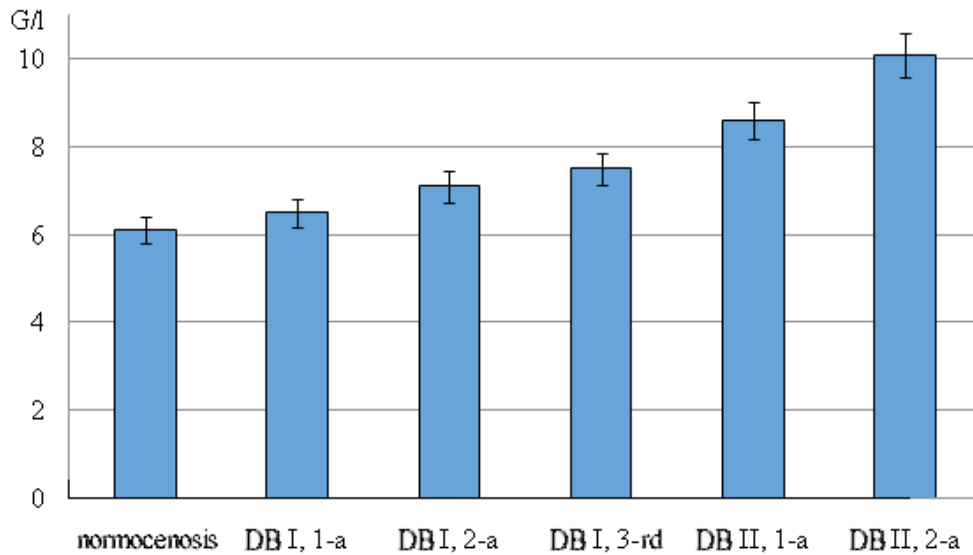


Figure 1. The absolute number of leukocytes in the blood (G/l) depending on the grade of dysbiosis; statistical significance of the differences is shown in Table 1

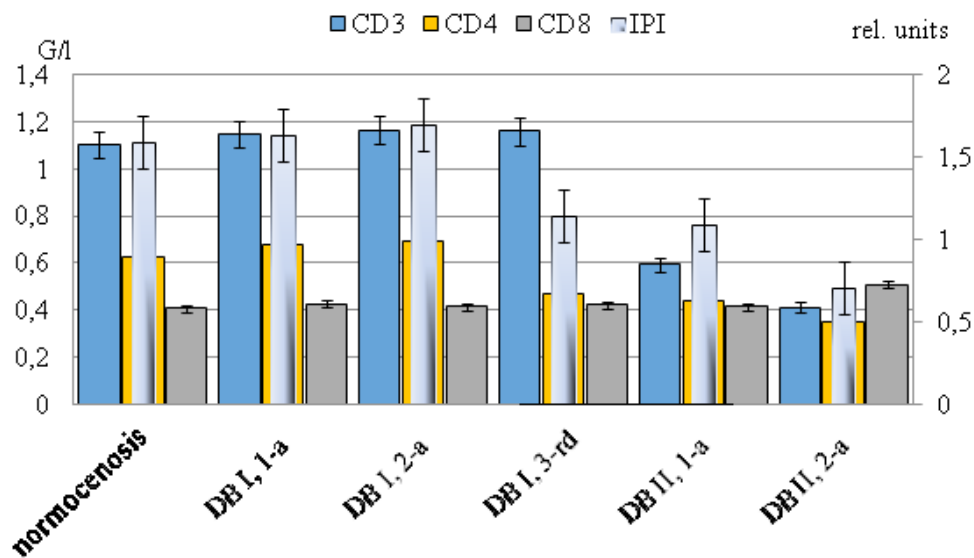


Figure 2. Content of T-lymphocytes in the blood by fractions (CD3, CD4 and CD8) (G/l; right axis) and IRI value (rel. units; left axis) depending on the grade of dysbiosis; statistical significance of the differences is shown in Table 1

CD3 is a multiprotein membrane complex that is present on the surface of T-lymphocytes and is the major coreceptor of T-cell receptor (TCR) [20, 576]. Due to this, CD3 is a major marker of T-

lymphocytes. As we can see from Table 1, in grade I dysbiosis (2<sup>nd</sup> group) in comparison with the 1<sup>st</sup> group (normocenosis) CD3 content in the blood did not actually change, whereas in grade II dysbiosis (3<sup>rd</sup>

group) it was reduced – by 1.8 times in the 1<sup>st</sup> subgroup and by 2.7 times in the 2<sup>nd</sup> subgroup ( $p < 0.001$  in both cases).

Such dynamics (Fig. 2) indicated the development of deep grade dysbiosis of CD3-lymphocytopenia. Accentuated decrease in the number of CD3-lymphocytes in the blood was a characteristic of BV, when it was statistically significantly lower than in the case of grade II dysbiosis (by 1.4 times;  $p < 0.001$ ). It indicated the progression of T-cell immunodeficiency with increasing severity of dysbiosis.

CD4 is a monomeric transmembrane glycoprotein that belongs to the immunoglobulin superfamily and is a marker of T-helper cells [20]. It is expressed on thymocytes (80–90%), mature T-lymphocytes (65% of T-helper cells), monocytes, macrophages, Langerhans cells, dendritic cells.

In our study, we observed a significant decrease in the number of CD4-lymphocytes in the blood in severe dysbiosis, namely in the 3<sup>rd</sup> subgroup of the 2<sup>nd</sup> group and in the 3<sup>rd</sup> group, by 1.3–1.8 times ( $p < 0.001$  for all these observations). To a maximum extent (by 1.8 times;  $p < 0.001$ ), CD4-lymphocyte content was reduced in BV, when it was significantly lower than in dysbiosis (by 1.3 times;  $p < 0.05$ ). Also, inhibition of T-lymphocytic component in BV was identified [21], which the authors associate with a decrease in predominantly gamma-delta-TcR T-lymphocytes, which recognize non-peptide microbial antigens, similar to NK-cells.

CD8 is transmembrane glycoprotein, which is a coreceptor of T-cell receptors (TCR). Like TCR, CD8 has the ability to bind to a class I major histocompatibility complex molecule [20]. Most of CD8 is represented by peripheral T-lymphocytes as a disulfide-linked alpha-chain homodimer, which is a marker of subpopulation of cytotoxic T-lymphocytes and T-suppressors. On the mature T-cells, either CD8 or CD4 is expressed.

In our study, in normocenosis and dysbiosis content of CD8-lymphocyte in the blood was almost the same (see Table 1 and Figure 2). 2<sup>nd</sup> subgroup

of the 3<sup>rd</sup> group was the exception – patients with BV showed a significant increase in content of CD8 lymphocytes in their blood (by 1.2 times compared to those in normocenosis;  $p < 0.001$ ).

Presence of pre-existing trends showing changes in CD3, CD4, CD8 lymphocyte content in the blood, and IRI values in the development of dysbiosis and BV, was confirmed by analysis of variance estimates among the subgroups (see Table 1): F value was from 23.1 to 126.6 ( $p < 0.001$  for all the indicators).

Thus, in dysbiosis, there was a gradual, associated with an increase of severity, increase of CD3- and CD4-lymphocytes in the blood, which was expressed to a maximum extent in case of BV. This allowed us to establish the progression of T-cell immunodeficiency in the development of dysbiosis. In contrast, the response of cytotoxic T-lymphocytes and T-suppressors (increase in CD8 content in the blood) was observed only in case of BV, which evidenced the development of compensatory activation of the cellular component of immune system.

The revealed pattern was confirmed and quantified by the dynamics of IRI (see Table 1 and Figure 2). In the 3<sup>rd</sup> subgroup of the 2<sup>nd</sup> group and in the 3<sup>rd</sup> group, IRI was significantly reduced in comparison with normocenosis (by 1.4–2.2 times;  $p < 0.001$ ). Moreover, in case of BV, IRI was reduced to the maximum extent – by 2.2 times in comparison with normocenosis, and in by 1.5–1.6 times in comparison with the indicators in dysbiosis ( $p < 0.001$  in all cases).

The analysis of variance (Table 2) confirmed the revealed differences of IRI among the groups of patients with normocenosis (1<sup>st</sup> group) in comparison with dysbiosis (2<sup>nd</sup> and 3<sup>rd</sup> groups). When analyzing the quantitative data, it was found that the value of IRI can be considered a diagnostic marker of development of dysbiosis. IRI is less than  $Q_1 = 1.340$  rel. units was the limit below which the dysbiosis could be statistically diagnosed ( $F = 20.47$ ;  $p < 0.001$ ).

Table 2.– Values of IRI in normocenosis and dysbiosis

Indicators	Groups		F	p
	1 <sup>st</sup> group (normocenosis), n=53	2 <sup>nd</sup> and 3 <sup>rd</sup> groups (grade I and II dysbiosis), n=245		
M±m	1.592±0.060	1.222±0.036	20.47	< 0.001
Me (Q1; Q3)	1.543 (1.340; 1.805)	1.081 (0.758; 1.335)		

Notes:  $M \pm m$  – mean value and standard error of the mean; Me (Q1; Q3) – median, 1<sup>st</sup> and 3<sup>rd</sup> quartiles; F – Fisher’s criterion for analysis of variance; p – statistical significance of differences among groups (accepted at  $p < 0,05$ )

Analysis of the effect of IRI on the development of BV compared to dysbiosis was performed when comparing the data of variance analysis of IRI values in all the subgroups with dysbiosis with the 2<sup>nd</sup> subgroup of the 3<sup>rd</sup> group (Table 3).

Table 3.– IRI values in dysbiosis and bacterial vaginosis

Indicators	Groups		F	p
	2 <sup>nd</sup> group and 1 <sup>st</sup> subgroup of the 2 <sup>nd</sup> group (dysbiosis), n=162	2 <sup>nd</sup> subgroup of the 3 <sup>rd</sup> group (BV), n=83		
M±m	1.484±0.040	0.709±0.017	186.032	< 0.001
Me (Q1; Q3)	1.401 (1.081; 1.805)	0.700 (0.595; 0.800)		

Notes:  $M \pm m$  – mean value and standard error of the mean; Me (Q1; Q3) – median, 1<sup>st</sup> and 3<sup>rd</sup> quartiles; F – Fisher’s criterion for analysis of variance; p – statistical significance of differences among groups (accepted at  $p < 0,05$ )

It is established that Q1-Q3 intervals for IRI in groups of patients with dysbiosis and BV do not intersect. In this regard, IRI less than 0.800 rel. units may be considered as a margin, below which there is a probability of BV development (F = 186.032; p < 0.001).

The fundamental possibility of predicting the development of dysbacteriosis and BV using math-

ematical models of immunity disorders is justified by [22]. According to [23], the need to find optimal and simple prognostic models is an important modern problem, especially in the context of the tendency of BV to become chronic.

Table 4.– Statistical significance of indicators, logistic regression coefficients and their probability for the dependent variable P<sub>BV</sub>

Indicators	Wald	β-coef.	±SE <sub>β</sub>	BI±95%	t	p
IPI	46.764	-10.546	1.542	-(13.568-7.523)	-6.826	< 0.001
Independent indicator	46.655	9.305	72.922	6.635-11.976	6.820	< 0.001

Notes: Wald – resulting value of Wald-statistics; β – regression coefficient; ± SEβ – regression coefficient error; BI ± 95% – 95% confidence interval; t – Student’s coefficient; p – significance of differences compared to the main hypothesis (accepted at  $p < 0,05$ )

Logistic regression analysis was performed to calculate the probability of BV development. IRI values were used as a factor trait. Presence or absence of BV

was the resulting feature (categorical values “YES” and “NO” were assigned indicator values: “1” and “0” respectively). When developing analysis model,

245 patients with dysbiosis were engaged, 83 patients among them had BV. To evaluate the contribution of IRI values to the probability of BV prediction ( $P_{BV}$ ), analysis of the resulting Wald-statistics value was conducted.  $\beta$ -coefficients of the regression equation, their standard errors, confidence intervals, and significance of differences compared to the main hypothesis were calculated (Table 4).

Calculated  $\beta$ -coefficients of the regression equation, their standard errors and confidence intervals indicate of the presence of adverse relation of IRI with the probability of BV development ( $t = -6,826$ ;  $p < 0.001$  compared with the main hypothesis). Operational characteristics of the developed model, calculated using ROC-analysis, showed the satisfactory quality:  $AUC = 0.961 \pm 0.011$  ( $CI \pm 95\% 0.940-0.983$ ;  $p < 0.001$ ). Assessment of the model from the point of view of conformity with the used regression construction method also had satisfactory parameters:  $-2\log = 115,426$  ( $\chi^2 = 198,281$ ;  $p < 0.001$ ;  $df = 1$ ). Accuracy of the error-free detection of BV was 91.0%.

Thus, it was determined that IRI is a diagnostic factor both for formation of dysbiosis and for diagnostics of BV. The model proposed meets the quality criteria and allows us to predict the probability of BV development with a high degree of certainty.

### Conclusions

1. In dysbiosis on the background of general leukocytosis, there was a gradual, related to the progression of severity of dysbiosis, decrease in the content of blood of CD3- and CD4-lymphocytes, which was expressed to a maximum extent in BV. This allowed us to establish the progression of T-cell immunodeficiency in the development of dysbiosis.

2. The response of cytotoxic T-lymphocytes and T-suppressors (CD8) was observed only in BV. IRI was a diagnostic factor both for the formation of dysbiosis ( $IRI < 1.340$  units) and for the diagnostics of BV ( $IRI < 0.800$  units).

3. Regression model developed meets the required criteria and allows us to predict the probability of development of BV with an accuracy of 91.0%.

### References:

1. McKinnon L. R., Achilles S. L., Bradshaw C. S., Burgener A., Crucitti T., Fredricks D. N., Jaspán H. B., Kaul R. et al. The evolving facets of bacterial vaginosis: implications for HIV transmission. *AIDS Res Hum Retroviruses*. 2019. Mar, – 35(3). – P. 219–228. Doi: 10.1089/AID.2018.0304.
2. Brusselaers N., Shrestha S., van de Wijgert J., Verstraelen H. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *Am J Obstet Gynecol*. 2019. Jul, – 221(1). – P. 9–18.e8. Doi: 10.1016/j.ajog.2018.12.011.
3. Reid G. Is bacterial vaginosis a disease? *Appl Microbiol Biotechnol*. 2018. Jan, – 102(2). – P. 553–558. Doi: 10.1007/s00253-017-8659-9.
4. Van de Wijgert JHHM, Jaspers V2. The global health impact of vaginal dysbiosis. *Res Microbiol*. 2017. Nov-Dec, – 168(9–10). – P. 859–864. Doi: 10.1016/j.resmic.2017.02.003.
5. Kira E. F. [Bacterial vaginosis]. – Moscow: Medical Information Agency, 2012. – 472 p. [in Russian].
6. Mark K. S., Tenorio B., Stennett C. A., Ghanem K. G., Brotman R. M. Bacterial vaginosis diagnosis and treatment in postmenopausal women: a survey of clinician practices. *Menopause*. 2020. Mar, 2. Doi: 10.1097/GME.0000000000001515.
7. Kenyon C., Colebunders R., Crucitti T. The global epidemiology of bacterial vaginosis: A systematic review. *Am J Obstet Gynecol* 2013. – 209. – P. 505–23. Doi: 10.1016/j.ajog.2013.05.006.
8. Mitchell C., Marrazzo J. Bacterial vaginosis and the cervicovaginal immune response. *Am J Reprod Immunol*. 2014. – 71. – P. 555–63. Doi: 10.1111/aji.12264

9. Anahtar M. N., Byrne E. H., Doherty K. E., Bowman B. A., Yamamoto H. S., Soumillon M., Padavattan N., Ismail N. et al. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity*. 2015. May, 19. – 42(5). – P. 965–76. Doi: 10.1016/j.immuni.2015.04.019.
10. Passmore J. A., Jaspán H. B., Masson L. Genital inflammation, immune activation and risk of sexual HIV acquisition. *Curr Opin HIV AIDS*. 2016. Mar, – 11(2). – P. 156–62. Doi: 10.1097/COH.0000000000000232.
11. Muzny C. A., Taylor C. M., Swords W. E., Tamhane A., Chattopadhyay D., Cerca N., Schwebke J. R. An updated conceptual model on the pathogenesis of bacterial vaginosis. *J Infect Dis*. 2019. Sep 26. – 220(9). – P. 1399–1405. Doi: 10.1093/infdis/jiz342.
12. Gruzevskyy O. A. [Colonization resistance in vaginal dysbiosis: the state of humoral and cellular links]. *Bul marine med*. 2017. – 4(77). – P. 103–7. [in Russian].
13. Vestby L. K., Grønseth T., Simm R., Nesse L. L. Bacterial biofilm and its role in the pathogenesis of disease. *Antibiotics (Basel)*. 2020. Feb, – 3; 9(2). pii: E59. doi: 10.3390/antibiotics9020059.
14. Muzny C. A., Schwebke J. R. Pathogenesis of bacterial vaginosis: discussion of current hypotheses. *J Infect Dis*. 2016. Aug, 15; 214 Suppl 1. – P. 1–5. Doi: 10.1093/infdis/jiw121.
15. Cox C., Watt A. P., Mc Kenna J. P., Coyle P. V. *Mycoplasma hominis* and *Gardnerella vaginalis* display a significant synergistic relationship in bacterial vaginosis. *Eur J Clin Microbiol Infect Dis*. 2016. Mapr. – 35(3). – P. 481–7. Doi: 10.1007 / s10096–015–2564-x.
16. Lipova E. V., Boldyreva M. N., Trofimov D. Yu., Vitvitskaya Yu.G. [Femoflor. Urogenital infections caused by conditionally biota in women of reproductive age (clinical and laboratory diagnostics). Manual for doctors]. – Moscow: DNA technology. 2015. – 30 p. [in Russian].
17. Hruzevskiy O. A., Vladymirova M. P. [Results of a complex bacteriological study of vaginal contents under the conditions of bacterial vaginosis]. *Ach biol and med*. 2014. – 2. – P. 54–7. [in Ukrainian].
18. Hruzevskiy O. A. [Normocenosis of the vagina: qualitative and quantitative characteristics] *Odessa Med J*. 2015. – 1(147). – P. 36–41. [in Ukrainian].
19. Tits N. U. *Encyclopedia of clinical laboratory tests*. – Moscow: Labinform. 1997. – 960 p. [in Russian].
20. Delves P. J., Martin S. J., Burton D. R., Roitt I. M. *Roitt's Essential Immunology*, 13<sup>th</sup> Edition. 2016. Wiley-Blackwell, – 576 p.
21. Alcaide M. L., Strbo N., Romero L., Jones D. L., Rodriguez V. J., Arheart K., Martinez O., Bolivar H., Podack E. R., Fischl M. A. Bacterial vaginosis is associated with loss of gamma delta T cells in the female reproductive tract in women in the Miami Women Interagency HIV study (WIHS): A cross sectional study. *PLoS One*. 2016. Apr 14. – 11(4): e0153045. Doi: 10.1371/journal.pone.0153045.
22. Fichorova R. N., Morrison C. S., Chen P. L., Yamamoto H. S., Govender Y., Junaid D., Ryan S., Kwok C., Chipato T., Salata R. A., Doncel G. F. Aberrant cervical innate immunity predicts onset of dysbiosis and sexually transmitted infections in women of reproductive age. *PLoS One*. 2020. Jan 8. – 15(1). – e0224359. Doi: 10.1371/journal.pone.0224359.
23. Sobel J. D., Kaur N., Woznicki N. A., Boikov D., Aguin T., Gill G., Akins R. A. Prognostic indicators of recurrence of bacterial vaginosis. *J Clin Microbiol*. 2019. Apr 26. – 57(5). – pii: e00227–19. Doi: 10.1128/JCM.00227–19.

<https://doi.org/10.29013/AJT-20-5.6-22-25>

Tabatadze Nino,  
Tsomaia Irma,  
Chikovani Anton,  
Gigoshvili Tamar,  
Sokhumi State University, Tbilisi, Georgia  
E-mail: nino\_tabatadze@yahoo.com

## BIOLOGICALLY ACTIVE SUBSTANCES OF *CEPHALARIA GIGANTEA* GROWING IN GEORGIA

**Abstract.** Among the biologically active substances, the roots of *Cephalaria gigantea* mainly contain alkaloids, phenolic compounds and triterpene glycosides. Studies have shown that the total amount of saponins is less than 14 glycosides. Eleven individual compounds have been isolated, named giganteosides A, B, C, D, E, F, G, H, I, J, J', K, L,

Antifungal and antiprotozoal activities of several giganteosides and enriched fractions have been evaluated. The alkaloid fraction has shown an antimalarial effect. Monodesmosides – giganteosides D and E were evaluated for their cytotoxicity against human non pigmented melanoma MEL-5 and human leukemia HL-60. Of exceptional importance is the anticonvulsive effect of the aqueous extracts of *C. gigantea* roots in pentylenetetrazol and audiogenic seizure models, with minimal toxic effects.

High-performance liquid chromatography method has been developed for the qualitative detection of the phenolic compounds. This method will be used for the standartization of the expected anticonvulsive drug form.

**Keywords:** Triterpene saponins, giganteoside, HPLC, antifungal, anticonvulsive activity.

*Cephalaria gigantea* (Ledeb.) Bobr. (fam. *Dipsacaceae*) is an endemic plant of Caucasus. Among the biologically active substances, the roots of the plant mainly contain alkaloids, phenolic compounds and triterpene glycosides [1–2]. In folk medicine, the aqueous extract of the roots of *Cephalaria* has been used successfully to treat epilepsy [3].

In order to identify biologically active substances and to determine their pharmacological activities, we have developed an effective method of separation and purification the sum of saponins from the roots of the plant, fractionation and division into individual compounds.

Studies have shown that the total amount of saponins is less than 14 glycosides. Eleven individual

compounds have been isolated, named giganteosides A, B, C, D, E, F, G, H, I, J, J', K, L, M, N. The chemical structures of individual molecules were established on the basis of physical-chemical methods, by 1D and 2D NMR experiments (<sup>1</sup>H, <sup>13</sup>C, gs-COSY, gs-HMBC, gs-HMQC and gs-HSQC-TOCSY) and mass spectrometry (MALDI-TOF, ESI-HR-MS) [4–9].

Giganteosides are the derivative of two aglycones – oleanolic acid and hederagenin.

**Giganteoside D** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\beta$ -D-Xylp(1 $\rightarrow$ 3)- oleanolic acid;

**Giganteoside E** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\beta$ -D-Xylp(1 $\rightarrow$ 2)- $\alpha$ -L-Arap(1 $\rightarrow$ 3)- hederagenin;

**Giganteoside G** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\beta$ -D-Xylp(1 $\rightarrow$ 3)-oleanolic acid – 28-O- $\beta$ -D-Glcp;

**Giganteoside H** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\beta$ -D-Xylp(1 $\rightarrow$ 2)- $\alpha$ -L-Arap(1 $\rightarrow$ 3)-hederagenin-28-O- $\beta$ -D-Glcp;

**Giganteoside I** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 4)- $\beta$ -D-Glcp(1 $\rightarrow$ 4)-[ $\alpha$ -L-Rhap(1 $\rightarrow$ 2)]- $\alpha$ -L-Arap(1 $\rightarrow$ 3)-oleanolic acid-28-O- $\beta$ -D-Glcp(1 $\rightarrow$ 6)- $\beta$ -D-Glcp;

**Giganteoside J** –  $\beta$ -D-Glcp(1 $\rightarrow$ 3)- $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\alpha$ -L-Arap(1 $\rightarrow$ 3)-hederagenin – 28-O- $\beta$ -D-Glcp(1 $\rightarrow$ 6)- $\beta$ -D-Glcp;

**Giganteoside J'** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 3)- $\beta$ -D-Glcp(1 $\rightarrow$ 3)- $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\alpha$ -L-Arap(1 $\rightarrow$ 3)-hederagenin – 28-O- $\beta$ -D-Glcp(1 $\rightarrow$ 6)- $\beta$ -D-Glcp;

**Giganteoside K** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 4)- $\beta$ -D-Glcp(1 $\rightarrow$ 4)-[ $\alpha$ -L-Rhap(1 $\rightarrow$ 2)]- $\alpha$ -L-Arap(1 $\rightarrow$ 3)-hederagenin – 28-O- $\beta$ -D-Glcp(1 $\rightarrow$ 6)- $\beta$ -D-Glcp;

**Giganteoside L** –  $\alpha$ -L-Rhap(1 $\rightarrow$ 2)- $\beta$ -D-GlcAp(1 $\rightarrow$ 3)-hederagenin-28-O- $\beta$ -D-Glcp(1 $\rightarrow$ 6)- $\beta$ -D-Glcp;

**Giganteoside M** –  $\beta$ -D-Galp(1 $\rightarrow$ 2)- $\beta$ -D-GlcAp(1 $\rightarrow$ 3)-oleanolic acid 28-O- $\beta$ -D-Glcp(1 $\rightarrow$ 6)- $\beta$ -D-Glcp;

**Giganteoside N** –  $\beta$ -D-Galp(1 $\rightarrow$ 2)- $\beta$ -D-GlcAp(1 $\rightarrow$ 3)-hederagenin – 28-O- $\beta$ -D-Glcp(1 $\rightarrow$ 6)- $\beta$ -D-Glcp.

Saponins containing glucuronic acid in the sugar chain were reported for the first time in *Cephalaria* species.

It has been established that the roots of *Cephalaria* from different regions of Georgia, do not differ from each other in the content of biological active components.

Biological studies have shown the antifungal, antiprotozoal, cytotoxic and anticonvulsant effects of *Cephalaria* root's saponins extract, alkaloid fraction and individual glycosides [10–13].

The fungicidal and antiprotozoal activities of individual substances and enriched fractions from the roots of *Cephalaria* were studied in the Laboratory of Botany and Parasitology of the faculty of Phar-

macy at the University of Mediterranean (Marseille, France).

The antifungal activity of saponins has been determined against yeasts and dermatophytes with an agar dilution method as previously described [14]. The tested concentration range was 1–2 mg/ml for crude extract, and 0.06  $\mu$ g/ml to 200  $\mu$ g/ml for the individual compounds. The reference antifungal agents were amphotericin B for yeasts and ketokonazole for dermatophytes. The minimal inhibitory concentration of saponins has been determined.

The highest antifungal activity was obtained with monodesmoside – giganteoside E (MIC 12.5  $\mu$ g/ml), which confirms the higher activity of hederagenin derivatives compared with glycosides of oleanolic acid. The most sensitive yeast species were *Candida glabrata* and *C. kefyr*.

The antiprotozoal activities against *Leishmania infantum* and *Trichomonas vaginalis* have been evaluated as described in [15]. The inhibitory concentration (IC<sub>50</sub>) and lethal dose (LD<sub>100</sub>) were determined with amphotericin B and metronidazole as references, respectively.

The most active product against *Trichomonas vaginalis* is a mixture (50/50) of monodesmosides – giganteosides D + E (IC<sub>50</sub> 1–2.5  $\mu$ g/ml). Giganteoside E with aglycone as hederagenin has shown more strong activity (IC<sub>2.5</sub>–5  $\mu$ g/ml) than giganteoside D (IC<sub>5</sub>  $\mu$ g/ml).

No significant activity was detected against *Leishmania infantum*. The synergism of activity has been found in the case of mixture of two monodesmosides (50:50).

Monodesmosides – giganteosides D and E were evaluated for their cytotoxicity against human non pigmented melanoma MEL-5 and human leukemia HL-60 cell line by using the metabolic MTT or WST1 cell viability assay, camptothecin was used as positive control [16]. The tested compounds exhibited interesting antiproliferative effect on human cell lines with IC<sub>50</sub> values in the range 3.15–7.5  $\mu$ M.

The leukemia HL-60 line was more sensitive to these monodesmosides and the most active product against HL-60 was giganteoside D (3.15  $\mu$ M) compared with giganteoside E (6.8  $\mu$ M). The cytotoxic activity may be related to the rhamnose residue of the sugar chain attached at C3 of the aglycone as it has been reported previously [17].

Preliminary phytochemical analysis showed the presence of 0,2% alkaloids in the plant's roots. These include 3 known alkaloids: gentianine, gentianidine, gentianaine. Alkaloid containing fractions of the roots of *C. gigantea* were active against *Plasmodium falciparum*, a parasite that causes malaria (IC50–27.4 $\mu$ l) [2].

The anticonvulsive effects of the aqueous extract of *C. gigantea* was studied using pentylenetetrazol (PTZ) and audiogenic seizure models (The Krushinsky-Molodkina and Wistar rat strains). In PTZ-model onset of tonic-clonic seizures, latencies to the beginning of the seizure activity and mortality and similarly in audiogenic seizures behavioral convulsive reactions, facial automatisms and latency to wild running were evaluated.

It was shown that the aqueous extract demonstrates anticonvulsant properties as in both model, as in both routes of administration -peroral and intraperitoneal. Extract appeared most effective in case of preliminary repetitive administration: in PTZ model seizures were eliminated in 9 of 10 animals and in audiogenic model convulsive response was no more observable during 7 days of treatment termination.

It was found also that sedative properties are characteristic of this antiseizure extract according to the sleeping prolongation in barbiturate treated mice. Safety of the extract was evaluated in toxicological acute and chronic experiments, and consequently it is considered a substance of moderate general toxicity.

Strong anticonvulsive effect and a very low toxicity of *Cephalaria* root's extract gives an opportunity for the creation of a new medicine of plant origin for prevention and treatment of psychosomatic forms of epilepsy.

Studies have shown higher concentration of phenolic compounds (both flavonoids and phenolic acids) in the roots extract of *Cephalaria*. Up to 9 compounds were identified.

High-performance liquid chromatography method has been developed for the qualitative detection of the phenolic compounds. The mixture of water (0,1% HCOOH) – acetonitrile (0,1% HCOOH) was used as a mobile phase with a gradient condition (acetonitrile 5%  $\rightarrow$  40%). All solutions were of HPLC purity, the concentrations of the sample and standards were 10 mg/mL and 1 mg/mL in MeOH, respectively. Solid phase – C18 reversed phase column Kinetex XB-Rp18, 250X4.6mm (Phenomenex), run time 25 min. UV detection was performed at 254, 280, 325 and 365 nm.

The following compounds were used as standards: rutin, caffeic acid, chlorogenic acid, cumaric acid, ferulic acid, hyperoside, luteolin and quercitrin. On the basis on the retention time, using corresponding standards and UV spectra, there have been identified 3 main compounds – chlorogenic acid, caffeic acid and flavonoid glycoside quercitrin. The obtained result allow us to performed standardization of biological and chemical markers of the anticonvulsive drug form [18].

Thus, the *Cephalaria gigantea* is a very perspective medicinal source due to its large botanical resources in Georgia, a rich chemical composition and a wide range of biological activities.

### References:

1. Flora of Georgia, XIII, edit. 2. Caprifoliaceae-Campanulaceae, – Tbilisi, “Mecniereba”. 2001. – P. 86–100. (in Georgian).
2. Tabatadze N. Avtoreferat PhD, – Tbilisi, 2004. – 42 p. (in Georgian).



3. Zviadadze L. D., Dekanosidze G. E., Kemertelidze E. P. Scientific works of Institute of Pharmacochemistry, – Tbilisi, – 13, 1976. – P. 143–146. (in Russian).
4. Zviadadze L. D., Dekanosidze G. E., Djikia O. D., Kemertelidze E. P., Shashkov A. S. Biologicheskaja chimia, – 7(5). 1981. – P. 736–740. (in Russian).
5. Zviadadze L. D., Dekanosidze G. E., Djikia O. D., Kemertelidze E. P. Chemistry of Natural Compounds, 1983. – P. 46–49. (in Russian).
6. Tabatadze N., Mshvildadze V., Dekanosidze G., Zviadadze L., Elias R., Ollivier E., Faure R., Balansard G. Bulletin of the Georgian Academy of Sciences, – 171(2). 2005. – P. 296–299.
7. Tabatadze N. A., Zviadadze L. D., Dekanosidze G. E., Kemertelidze E. P. Georgia Chemical Journal, – 3(2). 2003. – P. 156–157. (in Russian).
8. Tabatadze N., Elias R., Faure R., Gerken P., De Paul-Gillet M. C., Kemertelidze E., Chea A. and Ollivier E. Chem. Pharm. Bull. – 55(1). 2007. – P. 102–105.
9. Tabatadze N., Vachnadze N., Tabidze B., Getia M., Gogitidze N., Mshvildadze V., Dekanosidze G. Experimental and Clinical medicine, – 4, 2014. – P. 93–97. (in Georgian).
10. Tabatadze N., Zviadadze L., Favel A., Delmas F., Giorgio C. Di, Balansard G., Kemertelidze E. Bulletin of the Georgian academy of Sciences, – 165(2). 2002. – P. 318–320.
11. Gerken P., Dobson R., Tabatadze N., Mshvildadze V., Elias R., Peulen O. J., Jolois O. M. and De Paul-Gillet M. C. Anticancer Research, – 27. 2007. – P. 2529–2534.
12. Sixarulidze I., Gogitidze N., Mushkiashvili N., Gabelaia M., Vachnadze N., Dekanosidze G. Allergology and immunology, – 11(2). 2010. – P. 170–172. (in Russian).
13. Gogitidze N., Mushkiashvili N., Gedevanishvili M., Tabatadze N., Dekanosidze G. Georgian Medical News, – 3, 264. 2017. – P. 127–131. (in Russian).
14. Favel A., Steinmetz M. D., Regli P. et al. Planta Medica, – 60, 1, 1994. – P. 50–53.
15. Delmas F., Gasquet M., Timon-David P. et al. J. Europ. J. Med. Chem., – 28, 1. 1993. – 23 p.
16. Pisha E., Chai H., Lee I. S., Chagwedera T. E., et al. Nature Med., – 1, 1995. – P. 1046–1051.
17. Barthomeuf C., Debiton E., Mshvildadze V. D., Kemertelidze E. P., Balansard G. Planta med., – 68. 2002. – P. 672–675.
18. Tabatadze N., Tabidze B., Getia M., Mshvildadze V., Pichette A., Dekanosidze G., Kemertelidze E. Bulletin of the Georgian Academy of Sciences, – 11(2). 2017. – P. 118–122.

## Section 4. Technical sciences

<https://doi.org/10.29013/AJT-20-5.6-26-31>

*Abdukarimov Mirzohid Muratovich,  
the senior researcher (basic doctor),  
Tashkent state technical university*

*Yodgorov Normahmad,  
Doctor of Chemistry, professor,  
Tashkent state technical university*

*Jalilov Abdulahad Turobovich,  
director of Tashkent chemical-technological research institute  
Doctor of Chemistry, professor, academic of science  
E-mail: adler\_219@mail.ru*

### **SYNTHESIS OF NEW ANTICORROSION COATINGS BASED ON GOSSIPOL RESIN**

**Abstract.** The article discusses the results of the study of the competitive anticorrosive coatings synthesis based on gossypol resin and phosphoric acid. The resistant modified anticorrosive coatings are presented as a result of the gossypol resin, 3-chloroxypropane, monoethanolamine and diethanolamine interaction. Synthesized anticorrosive compositions based on gossypol resin and 3-chloroxypropane, which mechanism is characterized not only by the barrier type of protection, but also by the acquisition of rust modifying properties with improved physical mechanical and technological indicators of high adhesive properties and aging resistance, as well as a wide temperature range of plasticity, increased heat and frost resistance have been obtained.

**Keywords:** gossypol resin, anticorrosion coatings, 3-chlor 1,2-oxypropane (ChOP), monoethanolamine (MEA), diethanolamine (DEA), urotropin, adhesion, range of plasticity, type of protection.

In connection with the need to improve production efficiency, there is a necessity to develop new technologies that ensure the integrated use of raw materials and the disposal of industrial waste, which, in turn, lead to preservation of raw materials and improvement of the ecological situation.

At present, the need of the Republic of Uzbekistan for anticorrosion materials is provided by import. Creating a technological basis for waste chemi-

cal processing in order to obtain import-substituting, anticorrosive commodity products for the needs of the country is relevant.

This paper proposes development of physicochemical and technological bases for the production of anticorrosive materials from waste oil industry.

On the basis of the experimental studies carried out using modern chemical and physicochemical methods, the corrosion rate was determined by the

polarization resistance method on the corrosion rate meter R-5035.

It is established that as a result of the interaction of gossypol resin and 3-chloroxypropane (OCP), monoethanolamine (MEA) and diethanolamine (DEA) stable modified anti-corrosion coatings are formed.

Synthesized anticorrosive compositions based on gossypol resin and 3-chloroxypropane (OCP), which mechanism is characterized not only by the barrier type of protection, but also by acquiring the properties of corrosion modifiers, also have several advantages from the previous analogs:

- improved physical-mechanical and technological indicators;
- high adhesive properties and resistance to aging;
- wide temperature range of plasticity, high heat and cold resistance.

In the production of cottonseed oil and fatty acids, lots of secondary products and wastes such as gossypol resin and soap stock are generated. It is known that gossypol resin is an aromatic compound with phenolic, hydroxyl groups and a carbonyl group in ortho position to a hydroxyl group.

In the gossypol resin, 12% of nitrogen-containing compounds, 36% of gossypol conversion products, which retained naphthol hydroxyls and 52% of fatty and oxyfatty acids in the form of lactones, have been found [1].

The above mentioned resin exhibits acidic properties as well as properties of phenolic and aldehyde compounds. The presence of phenolic, carboxyl, carbonyl functional groups allows modification of gossypol resin and converts it to a water-soluble state [2; 3; 4; 5].

It is known that polyphenols, fatty acids, hydrocarbons, nitrogen- and phosphorus-containing compounds, as well as gossypol transformation products are present in the gossypol resin. The presence of naphthalene core compounds in its composition also makes the products of gossypol resin thermal, chemo- and radiation-resistant, and makes the pres-

ence of phenolic hydroxyls and aldehyde groups reactive with high complexing properties.

In many respects, it can successfully replace expensive anti-corrosion coatings, which deficit is felt every year. The preparation of anticorrosive materials on the basis of gossypol resin is associated with specific features and requires the search for certain conditions, as well as the use of non-traditional additives – modifiers [3].

In order to reduce costs and improve operational properties, an anti-corrosion coating technology based on gossypol resin has been developed and introduced into production. Based on the use of cheap and affordable raw materials, the production of anti-corrosion coatings has a high level of organization of technological processes and relatively high economic efficiency.

One of the advantages of the obtained bitumen anticorrosive composite materials is their versatility. In particular, by selection of appropriate modifiers and solvents improved anti-corrosion coatings and paints can be obtained from them [4].

Today in domestic practice there are over 100 different compositions for inhibiting the corrosion of steel. The disadvantages of the existing anti-corrosion materials are their high cost and low accessibility, as well as the impossibility of use their to combat multicomponent salt and acid corrosion.

The use of readily-available gossypol resin and its modifications as the basis of the anti-corrosion coating is due to the fact that it contains phenol, hydroxyl and carboxyl groups that interact with corrosion products and bind iron ions into complex compounds of chelate structure [4].

In connection with the foregoing, gossypol resin is an effective material against corrosion, provided that the appropriate solvents are selected, and another synergistic enhancing inhibitor. To solve this problem, we have used hexamethylenetetramine  $(\text{CH}_2)_6\text{N}_4$ , monoethanolamine (MEA) and diethanolamine (DEA), which is one of the most well-known representatives of acid corrosion inhibitors [5].

The fractionation study of gossypol resin, the identification of physicochemical and mechanical characteristics have formed the basis for the development of sustainable anticorrosion compositions

of a complex nature. Table 1 shows the main parameters of the influence of the molar ratio of reagents on the composition of the product upon receipt of a polymeric anticorrosion inhibitor.

Table 1. – The influence of the molar ratio of reagents on the inhibitor composition of the oligomeric antioxidant GHOP. (T = 338°K, t = 2 h)

Molar ratio: Gossypol: HOP.	Output,%	Average mol.mass. (cryoscopic)	Element analysis,%			
			Carbon		Hydrogen	
			Calculated	Found	Calculated	Found
3:1	72.3	2380		68.1		4.8
2:1	87.7	3450		67.9		5.2
1:1	98.8	4860	69.1	68.8	5.7	5.1
1:2	85.6	3820		69.3		4.9
1:3	79.2	3340		69.2		4.5

The inhibitory corrosion composition preparation method based on gossypol resin, 3-chloroxypropane (OCP) and HMTA, monoethanolamine (MEA) and diethanolamine (DEA) (compositions Mir K-1, World K-2, World K-3 and World K-4), which solves the problem of eliminating the disadvantages. A distinctive feature is that the gossypol resin components are available, the preparation technology and its use is simple an intermediate complex. The components can change the nature of the interaction of the metal surface with the surrounding corrosive medium individually or together and thereby, enhance the protective effect of the inhibitors. This technique is of particular relevance for the corrosion protection of metal structures in contact with multicomponent media and acid solutions.

The optimal ratio of the composition components is defined. The corrosion behavior of the metal has been evaluated by an electrochemical method in accordance with the standard CЭB 4421–83 on samples made of carbon steel (St. 3) 40x40x160 mm in size, without corrosive lesions (standard), with corrosive lesions (thickness of corrosion products from 150 to 300 microns). The corrosion behavior of the samples has been evaluated by the nature of the anodic polarization curves. The obtained data have also been confirmed by the results of chemical analyzes. The rate of general corrosion has been estimated by weight loss per unit area per unit of time ( $g/m^2 \times h \cdot 10^{-3}$ ) (Table 2).

Table 2. – Test samples for corrosion resistance in the presence of anti-corrosion coating based on gossypol resin

Inhibitor HS, HOP, MEA.	The difference in the sample mass, g (day).			Corrosion rate, g / $m^2 \times 10^{-3}$ (day).			Protective ef- fect,% (day).			The appearance of the sample after 90 days.
	7	28	90	7	28	90	7	28	90	
<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>	<i>10</i>	<i>11</i>
Without processing.	0.0105	0.0567	0.0696	44.4	56.16	22.08	–	–	–	High corrosion.
91:2:0:2,0:4,5:0,5	0.0014	0.0067	0.0742	5.91	7.13	4.48	86.4	88.2	89.6	Medium corrosion.
90:2:2,0:5,5:0,5	0.0010	0.0089	0.0013	4.03	9.22	4.35	90.6	94.3	90.1	Traces of corrosion.
89:2:2,0:6,0:1,0	0.0001	0.0005	0.0009	0.41	0.46	0.29	99.0	99.2	98.2	Clean.

1	2	3	4	5	6	7	8	9	10	11
88:2:2,0:6,5:1,5	0.0007	0.0008	0.0014	3.47	5.27	1.64	98.2	98.1	97.2	Traces of Corrosion.
87:2:2,0:5,5:1,5	0.0014	0.0019	0.0025	5.1	7.35	3.19	97.2	96.5	95.5	Low Corrosion.

The test results show that the composition of the World K system has the properties of reliable protection at concentrations of components, masses.% property Evaluation of the composition as a rust converter modifier has been carried out on the basis of GOST-6992 visually, according to an eight-point scale. Coatings has been applied on unalloyed (a)

and rusted (b) steel surfaces, about 1.0 mm thick, resistance to the effects of distilled water and 3% solution NaCl has been determined (Table 3). Weathering resistance has been measured in the atmosphere of the Aral Sea region, which has been considered to be a medium aggressive environment for two years.

Table 3.– The stability of the protective properties of coatings in time in points

№	The name of the test	Time, Day	Coverage.									
			1		2		3		4		5	
			a	b	a	b	a	b	a	b	a	b
1	Resistance of coatings to static exposure to water at $T = 20 \pm 2$ °C.	3	1	2	2	1	2	1	1	2	3	2
		5	2	1	2	1	2	1	2	1	2	3
		7	2	1	2	1	2	1	2	1	3	2
		10	1	2	2	1	2	1	2	2	3	3
		14	1	1	1	1	1	1	2	1	3	3
2	The resistance of coatings to the static effects of a NaCl solution $T = 20 \pm 2$ °C.	3	1	2	2	2	1	2	2	2	3	4
		5	1	1	2	2	1	2	1	2	3	4
		7	1	2	2	2	2	2	3	2	4	4
		10	2	2	2	2	2	2	2	2	3	4
3	Resistance of coatings to atmospheric influences in the city.	182	1	1	2	2	1	2	1	2	3	4
		365	1	1	2	2	1	2	1	2	4	4
		547	2	2	2	2	2	1	3	2	3	4
		730	2	1	2	2	3	2	3	3	4	4

The rate of general corrosion has been estimated on a ten-point scale: corrosion rate  $<0.001$  mm / year (perfectly resistant), 2 – from 0.001 to 0.003 mm / year, 3 – from 0.003 to 0.01 mm / year (very resistant), 4 – from 0.01 to 0.03 mm / year, 5 – from 0.03 to 0.1 mm / year (resistant), etc. (with each point, the corrosion rate increases by about 3 times). The 10th mark corresponds to a corrosion rate greater than 10 mm / year (non-resistant).

All obtained coatings are characterized by 1–2 points with water resistance up to 14 days, resistance to 3% solution NaCl to 10 days, and weather resistance up to two years. For coating No. 5, salt re-

sistance is characterized by at least 120 hours, but water resistance up to 14 days and salt resistance up to 7 days have been practically observed.

In all cases, the coating durability was studied when applied to a rusty surface. At the same time, it should be noted that on samples of coatings deposited on rusty surfaces and tested in atmospheric conditions in accordance with the allotted time of the experiments, no partial rust clearing has been observed.

Probably, there was a modification of rust, due to the formation of carbon compounds, due to which, on a rusty surface, pre-treated with developed coatings,

it is possible to apply paintwork material, which is the main positive feature of the coating as a rust modifier.

The physical-mechanical indicators of anti-corrosion coatings based on gossypol resin, calcium oxide,

zinc oxide, phosphoric acid, and hex methylene tetramine are shown in (Table 4).

Table 4.– Physical and mechanical properties of anti-corrosion coatings based gossypol resin

№	The name of indicators	Norm of indicators
1	Colour	From light brown to brown
2	Appearance.	Tar-like.
3	Smell	SPECIFIC
4	Flash point, °C	315, without solvent
		ON with Nefras solvent
5	The adhesion strength metal shear (adhesion), MPa, not less	4,0
6	Impact strength, p / gp, not less	1.9
7	Bend, tgp, no more	7.0
8	The interval of ambient temperature during application, °C	4–45
9	Hydrogen ion index (pH)	5.6–6.1
10	Crystallization temperature, °C	Minus 40
11	Hiding power, g/m <sup>2</sup> , not more	100.0
12	Water absorption,%, not more	0.1
13	Drying time, h, not more	24
14	Expected protection period, days, in atmospheric conditions, not less	1000.0
15	Time of complete formation of the protective layer, days, not more	4–5

Based on the analysis of data (table 4) it can be stated that the obtained coatings meet the requirements for anti-corrosion coatings by their basic indicators. For example, fast drying time, impact strength, bending elasticity, high adhesion, and the possibility of applying a paint and varnish material on these coatings.

Based on the foregoing, it can be considered that, due to its specific property, the interaction products

of gossypol's resin with other inhibitors can form a thin, stable anticorrosive coating with the carbon steel surface.

Thus, on the basis of the multi-tonnage waste of the fat-and-oil industry – gossypol resin can be easily used as new raw material for the production of anticorrosive polymer composite materials. Coatings have been tested with a positive result in conditions of high salinity at «Mubarekneftegaz» LLC.

#### Список литературы:

1. Алибеков Р. С. Антиккоррозионные покрытия на основе госсиполовой смолы со свойствами модификаторов ржавчины: Автореф... дис. канд. хим. наук. – Т.: 2002 г., –23 с.
2. Юлдашев Н. Х., Жуманиязов М. Ж., Дюсебеков Б. Д., Ходжаев О. Ф., Курамбаев Ш. Р. Разработка антикоррозионного покрытия на основе госсиполовой смолы для защиты оборудования йодо-бромной промышленности // Тез. докл. Межд. науч. конф. «Инновация-2003», – Т.: 2003. – С. 110–111.

3. Специальный технический регламент «Требования безопасности смолы госсиполовой» утвержден и введен в действие постановлением Узбекского Агентства стандартизации, метрологии и сертификации от 31.12.10 г. – № 1. – С. 19–23.
4. Алибеков Р. С., Дюсебеков Б. Д., Усенова С. О. Стандартизация физико-химического метода антикоррозионной устойчивости покрытия на основе госсиполовой смолы // Вестник Казанского технологического университета, 2013. – № 2. – С. 143–148.
5. Жуманиязов М. Ж., Курамбаев Ш. Р., Жуманиязова Д. М. Антикоррозионный состав для модификации ржавчины металлов // Журн. «Композицион материаллар», 2015. – № 4. – Ташкент. – С. 47–49.

## Section 5. Chemistry

<https://doi.org/10.29013/AJT-20-5.6-32-36>

*Azimova Gulmira Zayniddinovna,  
Base doctoral student, National University of Uzbekistan, Tashkent*

*E-mail: gulmira.azimova.84@mail.ru*

*Yuldasheve Mukhabbat Razzokberdiyevna,  
Doctor of chemical sciences, dosent,  
National University of Uzbekistan, Tashkent*

*Tadjimuhamedov Khabibullo Sayfullaevich,  
candidat of chemical sciences, professor,  
National University of Uzbekistan, Tashkent*

### INFLUENCE OF A NANOSTRUCTURAL CATALYST IN THE SYNTHESIS OF ALLYLNAPHTHOLS

**Abstract.** A new methodology was developed for the allylation of naphthols with an agents of allylation in the presence of a  $\text{FeCl}_3/\text{TiO}_2\text{-SiO}_2$  nanostructured catalyst, it was found that the  $\text{FeCl}_3/\text{TiO}_2\text{-SiO}_2$  catalyst is the most effective catalyst for the synthesis of 1-allyl-2-naphthol.

**Keywords:** allylation, thin layer chromatography, naphthol, allyl alcohol, allylacetate.

#### Introduction

Derivatives of naphthols are known by the uniqueness of their biological properties, including they show antitubercular activity [1] and the inhibitory effects on cyclooxygenase I and II [2; 3]. Different compounds among naphthols are useful as intermediates in organic synthesis. In order to synthesize biologically active allyl derivatives of naphthols, Indian scientists used the widely used catalyst Amberlyst-15 [4]. When using the Amberlyst-15, only C-allylation products-2-allyl-naphthol-1 and 1-allyl-naphthol-2- are mainly formed, the formation of O-allyl products under these conditions was not observed. Allylation reactions of naphthol-1 and naphthol-2 with allyl alcohol were carried out in the  $\text{Pd}\cdot\text{Et}_3\text{B}$  catalyst system and selective methods for the synthesis of C-allyl product were developed.

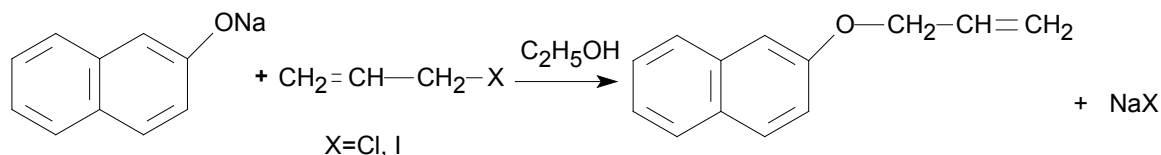
However, the very high consumption of allyl alcohol and the yields are low in these reactions [5]. When the using following catalysts  $[\text{Rh}(\text{nbd})(\text{CH}_3\text{CN})_2]\text{PF}_6$ ,  $[\text{Rh}(\text{nbd})\text{Cl}]_2$ ;  $[\text{Rh}(\text{nbd})_2]\text{BF}_4$ ,  $\text{RhCl}(\text{PPh}_3)_3$ ,  $[\text{Ir}(\text{cod})\text{CH}_3\text{CN}_2]\text{PF}_6$ , the disadvantage is the low reaction yields (up to 33%) [5]. In the reaction, the allylation of  $\beta$ -naphthol is allyl tosylate [6] in the presence of the catalyst  $[\text{Rh}(\text{nbd})(\text{CH}_3\text{CN})_2]\text{PF}_6$ , 1-allyl-2-naphthol and methyl-dihydronaphthofuran are formed in 38% and 32% yield.

#### Results and discussion

In this work, we have developed a procedure for the preparation of allylnaphthols using  $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$  and  $\text{FeCl}_3/\text{SiO}_2\text{-TiO}_2$  catalysts. Allylated naphthol reactions were studied under various reaction conditions with various reagents: allyl bromide, allyl acetate and allyl alcohol. At the first stage, sodium



$\beta$ -naphtholate was obtained by the reaction of  $\beta$ -naphthol with sodium ethylate, from its reaction, allyl bromide in ethanol synthesized naphthylallyl ether in high yield (90%).



When using allyl iodide instead of allyl bromide, a decrease in the duration of the reaction was observed.

Table 1. – The results of the reaction of sodium naphtholate with allyl halides

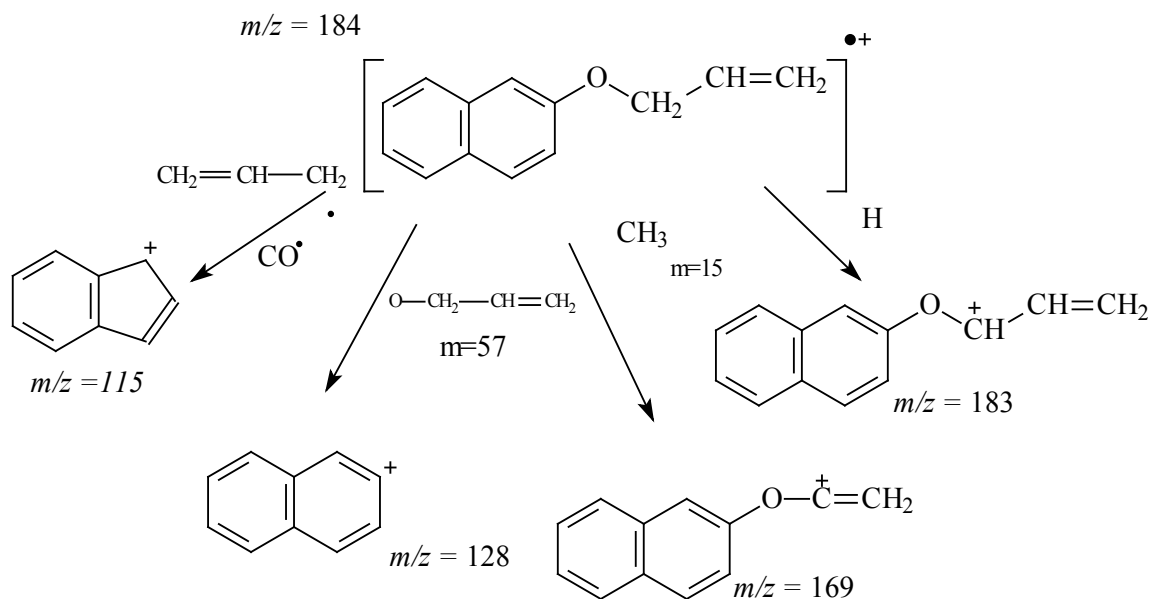
Agents of al-lylation	Solvent	Reaction duration	The molar ratio of sodium naphtholate and allyl halide	Yield,%
Allyl bromide	ethanol	3 hour	1:1	90
Allyl iodide	ethanol	1hour	1:1	92

If sodium naphtholate is reacted with allyl halide in non-polar solvents, benzene, C-allyl products are formed as a result of the reaction in high yield. The structure of the obtained ether was conformed by IR spectroscopy.

Table 2. – IR absorption areas,  $\text{cm}^{-1}$

	$\nu_{\text{c-c}}$ in arom. ring	$\delta_{\text{CH}}$ monosubs. arom. ring	$\delta_{\text{c-o-c}}$	$\delta_{\text{c=c}}$
	1600 1629	702.8	1216.34 1264.45	811.08 838.25

The results of the chromate-mass spectral analysis of naphthylallyl ether (MassHunter/GCMS/1/597. Agilent technologies)

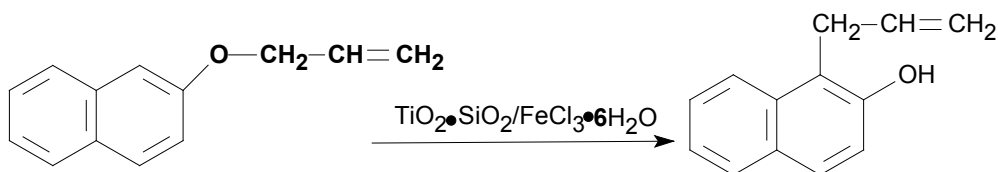


At the second stage, the rearrangement reaction of the obtained ether in the presence of a catalyst was studied. From literature data it is known that allyl aryl ethers are rearranged according to

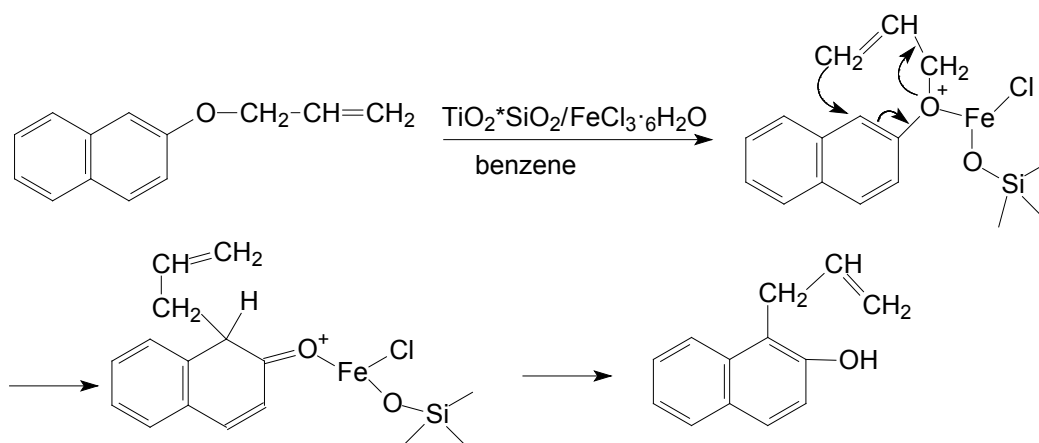
Claisen rearrangement at high or low temperature by Lewis acids, but in the presence of Lewis acid, polyallylation side reaction occur, and resinous products are formed at high temperatures. Therefore, we carried out the isomerization reaction at low temperature in benzene using nanostructured oxides of metallic titanium and silicon as a carrier of iron (III) chloride. With this catalyst, the reaction proceed under mild conditions at the boiling point of the benzene for 3 hours. TLS (thin layer

chromatography) in an ethyl acetate: hexane: chloroform (1:4:1) solvent system on a Silufol-UV<sub>254</sub> plate determined the progress of the reaction at intervals of one hour and determined  $R_f$  ( $R_f=0.84$  naphthylallyl ether), ( $R_f=0.1$  1-allyl-2-naphthol). As a result of the reaction, the formation of C-allyl and O-allyl products was established. The mixtures were separated using Claisen solution (solution of potassium hydroxide in methanol). Yield was 68%.

Reaction equation



The reaction mechanism can be proposed as follows



Allylation reaction of 2-naphthol under catalytic conditions with allyl alcohol and allyl acetate have also been studied.

Table 3. – Reaction conditions of 2-naphthol with various allylating agents and the results obtained

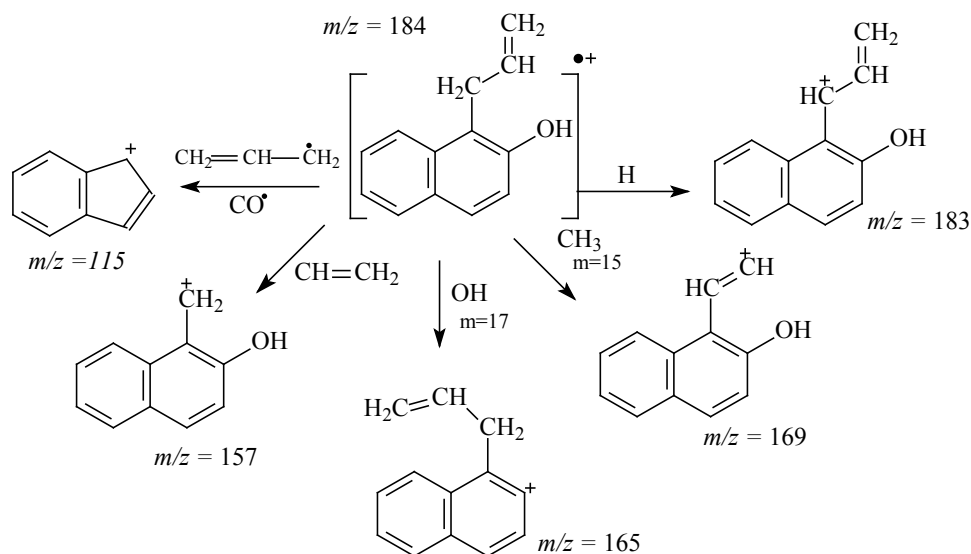
Agents of allylation	The molar ratio of 2-naphthol: reagent: catalizator = 1:1:10 <sup>-4</sup>	Conditions of reaction	Products	Yield,%
Allyl alcohol	FeCl <sub>3</sub> · 6H <sub>2</sub> O	benzene, 3 hour	naphthylallyl ether: 1-allyl-2-naphthol = 1:1	35%
Allyl alcohol	TiO <sub>2</sub> *SiO <sub>2</sub> /FeCl <sub>3</sub> · 6H <sub>2</sub> O	benzene, 3 hour	naphthylallyl ether: 1-allyl-2-naphthol = 1:3	55%
Allyl acetate	FeCl <sub>3</sub> · 6H <sub>2</sub> O	benzene, 3 hour	naphthylallyl ether: 1-allyl-2-naphthol = 1:1	76%
Allyl acetate	TiO <sub>2</sub> *SiO <sub>2</sub> /FeCl <sub>3</sub> · 6H <sub>2</sub> O	benzene, 3 hour	naphthylallyl ether: 1-allyl-2-naphthol = 1:3	80%

It was observed that iron (III) chloride with a nanostructured catalyst has a higher selectivity for the production of mono- and orto- products, according to the ratio of iron (III) chloride itself is used as a catalyst. This can be explained by the fact that iron (III) chloride is adsorbed on the surface of the nanocatalyst and forms unstable complexes with allylating agent and this reduce the polyallylating ability of the reagent. Based on the results obtained, it can be said that the reactivity of agents of

allylation increases in the series allyl alcohol, allyl halide, allyl acetate.

The formation of C-allyl and O-allyl products also depends on the nature of the solvent, in the presence of aprotic solvents C-allyl products are charged; in protic solvents O-allyl products are formed.

**The results of the chromate-mass spectral analysis of 1-allyl-2-naphthol (MassHunter/GCMS/1/597. Agilent technologies)**



#### The experimental part

A thin layer chromatography plate (TLC) DC-Fertigfolien ALUGRAM® Xtra SIL G/UV<sub>254</sub> (Germany) was used, the appearance of spots was determined under the influence of a UV lamp on the plate. The reaction products were identified by IR analysis (Perkin Elmer Spectrum IR, Version 10.06.1) and NMR H<sup>1</sup> and C<sup>13</sup> (Unity 400 plus ICPSASR Uz, Si(CH<sub>3</sub>)<sub>4</sub> standart) Solvent C<sub>6</sub>D<sub>6</sub>. MassHunter / GCMS/1 instruments were used for chromatographic mass spectrometry /597 Agilent technologies)

#### Experimental part

**Preparation of catalysts from nanostructured metal oxides and iron (III) chloride FeCl<sub>3</sub>/TiO<sub>2</sub>-SiO<sub>2</sub>**

The catalytic system FeCl<sub>3</sub>/TiO<sub>2</sub>-SiO<sub>2</sub> was prepared as follows FeCl<sub>3</sub> · 6H<sub>2</sub>O and TiO<sub>2</sub>-SiO<sub>2</sub> [7] were weighed on an analytical balance in an amount

of 2 · 10<sup>-4</sup> grams. In powder form, both substances were melted and mixed in a mortar. It was dried in the oven at 50–60 °C for 1 hour, then at 100–120 °C also for 1 hour.

#### Synthesis of allylnaphthyl ether

An alcohol solution of sodium naphtholate is poured into a three-necked round bottom flask connected to a stirrer, a dropping funnel and a reflux condenser and, with a working stirrer, allyl bromide is introduced from the dropping funnel (molar ratio of sodium naphtholate and allyl bromide = 1:1). The dropping funnel is replaced thermometer, the reaction mixture is heated for one hour in a water bath at a temperature of 70–80 °C. As a result of the reaction, a white precipitate of NaBr is formed. The reaction products were identified by TLC (thin layer chromatography) one spot with R<sub>f</sub> = 0.84, which corresponds to the R<sub>f</sub> value of naphthylallyl ether.

After cooling the reaction mixture, the precipitate was filtered. Ethyl alcohol was distilled under normal conditions from the filtrate. The remaining precipitate is naphthylallyl ether. Melting point=31–33 °C.

### Synthesis of 1-allyl-2-naphthol

The reaction was carried out in a three-necked round bottom flask a capacity of 250 ml. The flask is connected to a thermometer and a reflux condenser closed by a calcium chloride tube. A solution of naphthylallyl ether in benzene is poured in a volume of 6ml,  $2 \cdot 10^{-4}$  g of  $\text{FeCl}_3/\text{TiO}_2\text{-SiO}_2$  are added. For 3 hours, the mixture is heated at a temperature of 70–80 °C. The second spot was identified by TLC (system: ethylacetate: hexane: chloroform=1:4:1), the  $R_f$  value of which corresponded to o-allylnaphthol  $R_f = 0.1$ . The reaction mixture was separated using Claisen solution and column chromatography (eluent= hexane: ethylacetate = 5:1) Melting point = 53–54 °C. Analysis

of the PMR spectrum: (400 MHz,  $\text{C}_6\text{H}_6$ , chemical shift of protons,  $\delta$ , ppm) 3.85 (dm,  $J = 5.8$  Hz, 2 H), 5.05 (dm,  $J = 17.0$  Hz, 1 H), 5.10 (dm,  $J = 10.2$  Hz, 1 H), 5.13 (s, 1 H), 6.06 (ddt,  $J = 10.2, 17.0, 5.8$  Hz, 1 H), 7.08 (t,  $J = 8.6$  Hz, 1 H), 7.32 (t,  $J = 8.6$  Hz, 1 H), 7.47 (t,  $J = 8.6$  Hz, 1 H), 7.66 (d,  $J = 8.6$  Hz, 1H), 7.78 (d,  $J = 8.6$  Hz, 1 H), 7.89 (d,  $J = 8.6$  Hz, 1H).

**$\text{C}^{13}$  spectrum** 100MHz,  $\text{CDCl}_3$   $d = 29.3, 116.0, 116.8, 118.0, 123.0, 123.2, 126.5, 128.4, 128.6, 129.5, 133.3, 135.8, 151.2$

$m/z$   $\text{C}_{13}\text{H}_{12}\text{O}$ : 184.0888; 184.0891 ( $M^+, 100$ ), 170 (7), 169 (58).

### Thanks

We thank for the synthesis of nano-structured  $\text{TiO}_2\text{-SiO}_2$  to professor of the Turin Polytechnic Institute in Tashkent O. Ruzimuradov.  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  belong to Aldrich.

### References:

1. Das S. K., Panda G., Chaturvedi V., Manju Y. S., Gaikwad A. K., Sinha S. Design, synthesis and antitubercular activity of diarylmethylnaphthol derivatives. *Bioorganic and Medicinal Chemistry Letters*, 2007.– P. 17, 5586.
2. Feixas J., Jimenez J. M., Godessart N., Puig C., Soca L., Crespo M. I. Naphthalene derivatives: A new series of selective cyclooxygenase-2 inhibitor. *Bioorganic and Medicinal Chemistry Letters*, 2001.– P. 11, 2687.
3. Kongkathip B., Sangma C., Kirtikara K., Luangkamin S., Hasitapan K., Jongkon N., Hannongbua S., Kongkathip K. Inhibitory effects of 2-substituted-1-naphthol derivatives on cyclooxygenase I and II. *Bioorganic and Medicinal Chemistry Letters*, 2005.– P. 13, 2167.
4. Das B., Veeranjanyulu B., Krishnaiah M., Balasubramanyam P., Benzylolation and Allylation of Naphthols Using Amberlyst-15. *Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry*, 2009.– P. 1929–1935.
5. Kimura M., Fukasaka M., Tamaru Y., Palladium-Catalyzed Triethylborane-Promoted C-Allylation of Naphthols and Benzene Polyols by Direct Use of Allyl Alcohols, *Synthesis*,– New York, 2006.– No. 21.– P. 3611–3616.
6. Tsukada N., Yagura Y., Sato T., Inoue Y., Rhodium- and Iridium-Catalyzed Allylation of Electron-Rich Arenes with Allyl Tosylate. *Synlett*,– New York, 2003.– No. 10.– P. 1431–1434.
7. Рuzимурадов О. Н. Синтез титановых мезопористых золь-гель материалов с использованием полиэтиленгликоля в качестве органического темплата. *Узб. хим. ж.*,– № 6. 2008.– С. 24–26.

<https://doi.org/10.29013/AJT-20-5.6-37-43>

Ziyaeva Sitora F.,  
postgraduate student, Department of Chemistry  
Samarkand State University  
E-mail: Sitoraziyaeva@mail.ru

Vasina Svetlana M.,  
Ph.D., Associate Professor, Department of Chemistry,  
Samarkand State University  
E-mail: vasin2003@mail.ru

## PHYSICO-CHEMICAL FUNDAMENTALS TECHNOLOGY PRODUCTION OF MULBERRY JUICE CONCENTRATE

**Abstract:** The chemical composition of white and black mulberries has been determined. A comparative analysis of different mulberry varieties shows that the white mulberry fruits are characterized by a higher content of pectin substances and organic acids than black varieties. It has been proposed to use the hot fermentation method to break down pectin substances. Based on the studies, the amount of enzyme necessary for the breakdown of pectin substances has been proposed. The enzymatic activity of amylase and pectinase in the wastes of apple juice after ultrafiltration. Was studied it was found the in the waste a significant amount of enzymes remains that retain their activity, which made it possible to assume their repeated use after regeneration.

**Keywords:** berry, white and black mulberry, pectin substances, hot fermentation, amylase, pectinase, regeneration.

### Introduction

One of the most important areas in meeting the demand of the population for high-quality food products is the search and use of little-used and non-traditional types of local plant raw materials. From this point of view, mulberry culture, widespread in Uzbekistan and practically not used in food production, can serve as a promising raw material for the production of various products. The purpose of this work is to study the physicochemical fundamentals of the mulberry juice production technology [9].

Mulberry is sweet or sweet-sour juicy fruit with a delicate aroma. It is used mainly in raw form. In order to use mulberry fruits for food production, their chemical composition was investigated.

### Objects and research methods

The objects of the research were the fruits of white (*morus albalinn*) and black (*morus nugalinn*)

mulberries common in Uzbekistan, as well as the waste of mulberry juice after fermentation. The subject of the research is amylase (Amylase AG 300 L) and pectinase (Pectinex Ultra AFP Pectinase) contained in the waste fruit juices after ultrafiltration.

Analysis and processing of the research results was carried out by the methods of physicochemical analysis.

The applied method for determining the mass concentration of carotene is based on photometric measurements after extraction from products with organic solvents and using column chromatography to purify the associated coloring matter. The mass fraction of soluble solids was determined using a refractometer. The quantitative content of vitamin C was calculated by the results of the potentiometric titration after extraction with a solution of hydrochloric acid. The content of vitamins B1 and B2 was

determined by the method of acid-enzymatic hydrolysis using columns with cation exchangers and by the measurement of fluorescence intensity using a fluorometer. The fixation of organic acids in the studied samples was carried out by High Performance Liquid Chromatography.

When conducting the researches on the content of pectin substances, the conventional methods of analysis were used: the mass fraction of pectin substances was determined by the calcium-pectate method, the content of free and methoxylated carboxyl groups by the titrimetric method; total number of carboxyl groups and degree of esterification – by calculation method [1].

The results of the pectolytic activity of enzymes were obtained on the basis of the method for determining the products of pectin hydrolysis, which is not precipitated by zinc sulfate under the action of enzymes of the pectolytic complex under standard conditions. To evaluate the activity of amylase the Volgemuta method was used [3].

#### Experimental part

The fruits of white (*morus albalinn*) and black (*morus nugalinn*) mulberries were taken for the investigation. The content of water, carbohydrates, organic acids, minerals and vitamins were taken as the main indicators. The obtained results are presented in (table 1).

Table 1. – The chemical composition of mulberry fruits, % in wet weight

Indicator	Mulberry varieties	
	white	Black
Carbohydrates:	10.54	8.57
Pectic substances	1.7	1.3
Organic acids	1.1	0.8
Minerals	0.45	0.38
Vitamins. mg %		
Vitamins. mg %: Carotenes	0.02	0.04
B <sub>1</sub>	0.04	0.05
B <sub>2</sub>	0.02	0.04

As the studies have shown that the main components of the dry substances of all of mulberry vary in the range of 8.57–10.54%. The content of pectic substances in all samples studied is about 1%. The fruits contain a sufficient amount of minerals, vitamins.

A comparative analysis of different varieties of mulberry shows that the white fruits of mulberry are characterized by a higher content of pectic substances and organic acids compared with black

fruits. The black fruits are superior to the whites on the content of vitamins. Soluble pectin is a polysaccharide consisting of interconnected residues of galacturonic acid, which is presented in it in the form of methyl ether.

Under the influence of soluble pectin with the diluted alkaline enzyme pectase, methoxyl groups can be easily split off – methyl alcohol and free pectic acid, which is a polygalacturonic acid, are formed.

Table 2. – Physico – chemical indicators of pectic substances of mulberry fruits, % of dry matter

Indicator	Mulberry Variety	
	White	Black
Galacturonic acid content	70.3	69.8
Degree of methoxylation	64.8	63.9
Molecular mass	22000	16000

To determine the possibility of using mulberry fruits, their pectic substances were investigated. The obtained data are presented in (table 2).

The analyses showed that the pectic substances of mulberry fruits are characterized by low molecular weight, with a not very high degree of methoxylation and with a sufficiently high degree of purity (the content of galacturonic acid is 70.3–69.8%).

The results of chemical analysis of mulberry fruits allow us to conclude that it can be used in the production of juices.

Enzymes are used to produce high-quality and persistent juice concentrates [3–5]. As enzymes,

it is possible to use amylase and pectinase brands Amylase AG 300 LK.F. and Pectinex Ultra AFP contains KF.

It is known [6] that the protein of nature provides enzymes with the ability of proteins to change activity depending on various factors. The degree of activity change from various factors can be determined from the rate of the enzymatic reaction. We studied the effect of temperature and pH on the rate of enzymatic reactions of the enzymes used. When studying the influence of any factor on the rate of the enzymatic reaction, all other factors remained unchanged and had optimal values.

Table 3. – The effect of temperature on the amylase activity of K.F.

Incubation temperature, °C	Coloring with Iodine	Name of the products detected on the basis of color
0	blue	No decomposition detected
22–30	Orange	Incomplete decomposition
45–50	yellow	Complete decomposition
85	blue	Enzyme inactivation

To determine the effect of temperature on the activity of amylase, a known method was used [7]. The obtained results are presented in (Table 3).

To determine the effect of temperature on pectinase activity, ethanol test for pectin was used [8]. Table 4 presents the experimental data on the effect of temperature on pectinase activity.

Table 4 – The effect of temperature on the activity of pectinase K.F.

Temperature incubation, °C	Detection index	Name of products detected on the basis of color
0	gelformation	No decomposition detected
22–30	Heavy precipitation	Incomplete decomposition
45–50	Homogeneous system	Complete decomposition
85	gelation	Enzyme inactivation

Based on the obtained data, it was found that the optimum temperature value at which the reaction proceeds at maximum speed is above 50 °C. Increasing above the optimum temperature leads to a decrease and then stopping of the enzyme action, which is associated with denaturation. During the transition from optimal to low temperatures, the rate of the enzymatic reaction drops and stops at 0°C. The reason is a decrease in the speed of movement of

the molecules of the substrates and enzymes, which slows down the formation of the enzyme-substrate complex and the reaction. As the temperature rises from negative values, the effect of the enzymes is restored, and the rate of the catalyzed reactions increases.

The effect of pH on the activity of enzymes was studied. The obtained results are presented in (tables 5 and 6).

Table 5. – The effect of pH on amylase activity

Indicators	Test tube numbers				
	1	2	3	4	5
pH	2.87	4.10	5.33	6.59	7.96
Coloring with iodine	brown	orange	yellow	orange	brown

*It is established that the most optimal is the pH value of 5.33, at which the reaction proceeds at maximum rate.*

Table 6. – The effect of pH on pectinase activity KF

Indicators	Test tube numbers				
	1	2	3	4	5
pH	2.87	4.10	5.33	6.59	7.96
detection	Gelation	Large pre- cipitation	Homogenous system	Flake-like precipitation	Gel plug

Taking into account that the properties of pectin have studied the effect of the quantitative content of to form jelly adversely affect the yield of the juice, we the enzyme on the yield of the juice.

Table 7. – The effect of enzyme concentration on the physico – chemical indicators of mulberry

Indicator	The content of the enzyme% wet weight			
	0.005	0.01	0.03	0.05
Content of pectic substances.%	1.04	1.1	1.30	1.30
Decomposition degree.%	800	86	100.0	100

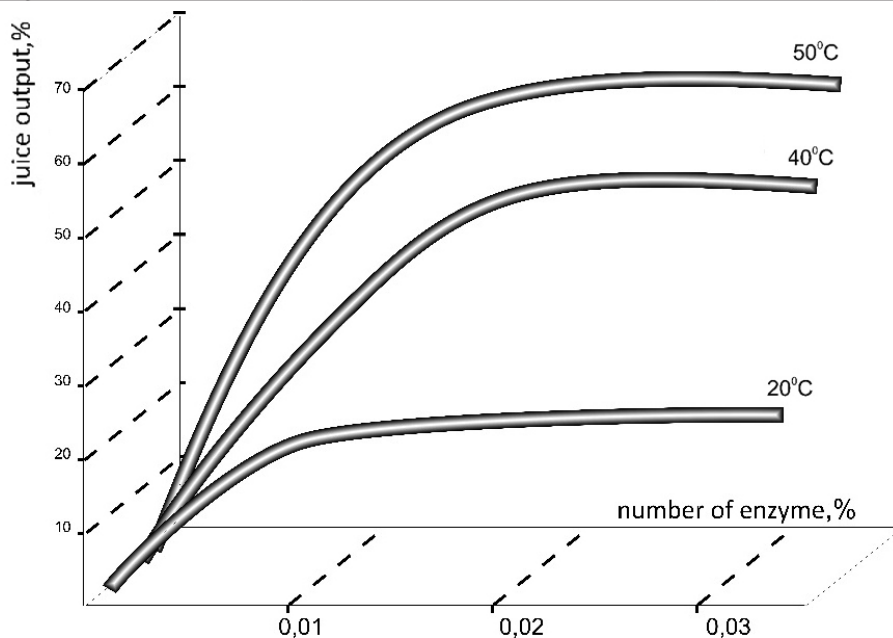


Figure 1. The dependence of the yield of black mulberry juice on the amount of enzyme injected and temperature

It is known from the literature data that for carrying out the fermentation process, the amount of enzyme injected varies in the range from 0.01 to 0.05%,

depending on the amount of pectin substances. The effect of the quantitative content of the enzyme on the processing process of black mulberry at room



temperature was studied. Pectinase was used as a pectolytic enzyme.

On the basis of the obtained data, it was established that an increase in the amount of the enzyme does not affect the juice yield, it is only the temperature that affects. In this connection, it is recommended to use the method of hot fermenta-

tion for the decomposition of pectic substances. For this method, it is necessary to heat up to the temperature of 50 °C and treat the enzyme at the same temperature for 30 minutes. Then the juice should be separated by pressing. The effect of pressure and speed of pressing on the juice output was investigated.

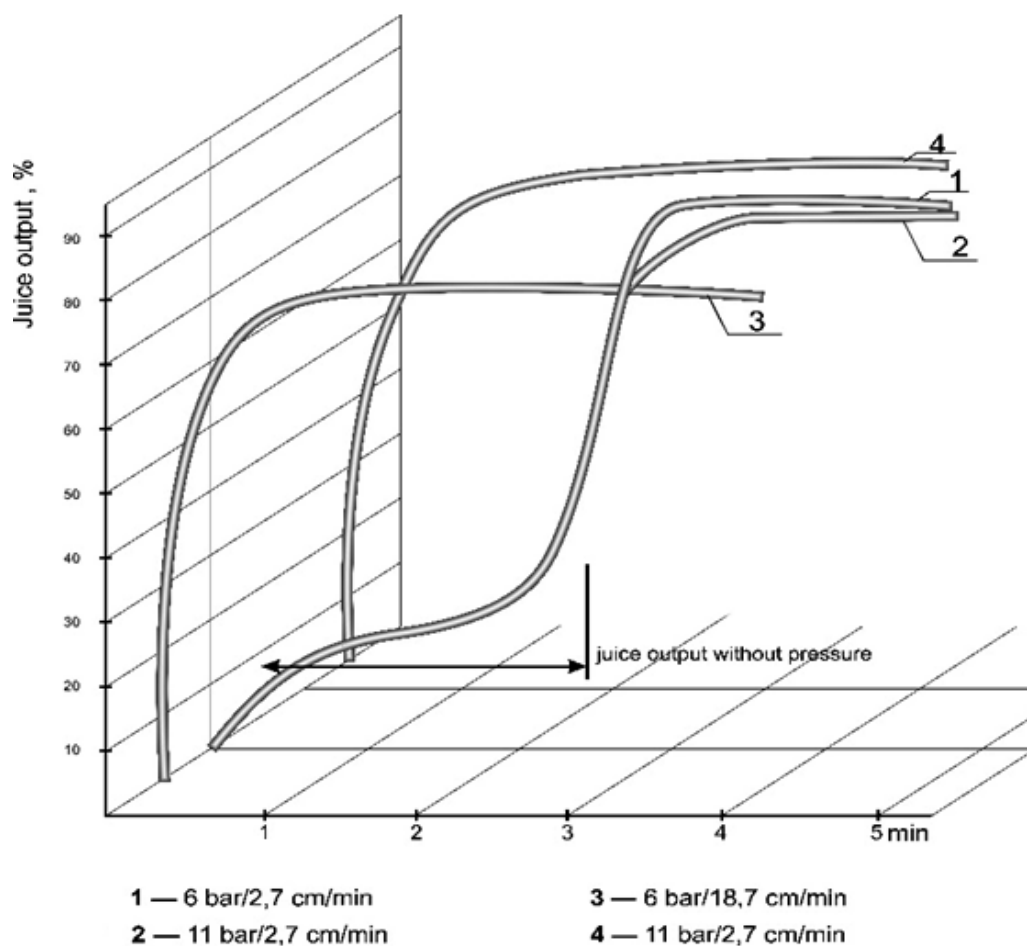


Figure 2. The dependence of the juice yield on the pressure and speed of pressing

The obtained results showed that the pressing process should be carried out slowly, which leads to a better result. Pressing should be carried out at a pressure of 6 bar and a speed of 2.7 m/min for 3 minutes. A further increase in the duration of pressing does not affect the juice yield. The influence of the pressing speed becomes more noticeable in the area of the maximum values of the juice yield. And the result of pressing does not depend on pressure.

The juice obtained after pressing was cooled and clarified in order to remove fine suspensions and colloidal substances to obtain a transparent product. For that, gelatin from 0.005 to 0.02% was added in the form of a 1% solution to the juice, mixed thoroughly and aged for 2 hours. The optimum temperature for processing the juice is 20 °C. When this occurs, additional decomposition of the remaining pectin substances due to the neutralization of negatively charged particles of pectic substances by positively

charged particles of gelatin. Then the juice was filtered through a filter. After filtration, the juice was clear. The resulting semi-manufactured juice is sent to further technological operations.

Based on the research conducted, a technology for producing semi-manufactured juice of black mulberry was proposed.

The physicochemical parameters obtained using the proposed mulberry juice technology were determined on state standard methods.

Table 8. – Organoleptic characteristics of semi-manufactured mulberry juice

Name of indicators	Characteristics of clarified juice	Characteristics of aromatic substances
Appearance	Liquid, syrupy like with dark burgundy shade	Clear liquid
Taste and smell	Well pronounced, peculiar to the fruits of which the juice is made, without foreign smell and taste	Well pronounced, peculiar to the fruits of which the juice is made, without foreign smell and taste
Water solubility	Full, without repeated sedimentation	Full, without repeated sedimentation

In the production of the juice after ultrafiltration, a waste is formed, which is a mixture of carbon adsorbent of enzymes and residues of the juice. We have extracted the sorbent, which is formed as a result of ultrafiltration, from the production line and studied the enzymatic activity of pectinase, adsorbed in it.

Pectolytic activity in the juice waste was determined using the interferometric method. For per unit of pectolytic activity was taken the amount of enzyme that catalyzes the hydrolysis of 1 gram of pectin to products, which is not precipitated by zinc sulfate when carrying out hydrolysis under strictly defined conditions: the temperature 50 °C, the hydrolysis time 1 hour, the pH value of the reaction medium 4. The pectolytic activity was expressed by number unit in 1 gram of the preparation tested (table 9).

Table 9. – The activity of pectinase KF in the waste after ultrafiltration

№ of test-tubes	Pectinase activity	
	PKC, u/g	%
1	77.5	98.7
2	77.3	98.5
3	77.6	98.9

Thus, it was established that after the ultrafiltration process, the fermentative activity of the enzyme

in the waste of juice production is preserved, which allows to propose using it again after regeneration. The method of regeneration of the enzyme used has been developed. The application of the developed technology allows to increase the efficiency of juice production by precipitating and regenerating enzymes from sorbents.

### Conclusions

A comparative analysis of different varieties of mulberry shows that white mulberries are characterized by a higher content of pectic substances and organic acids compared to black varieties. Based on the obtained data, the application of the method of hot fermentation for decomposition of pectic substances has been suggested. It is established, that the process of pressing should be carried out at pressure of 6 bar and at a speed of 2.7 cm/min. It is shown that the enzymatic activity of pectinase after filtration in the waste of juice production is preserved and it is possible to reuse it after regeneration.

On the basis of the conducted research, a technological scheme for producing black mulberry semi-manufactured juice is proposed. The main technical indicators of the semi-finished juice produced by the proposed technology have been identified.

**References:**

1. Avdeenko V. Innovations in biotechnology // Innovation – June, 2002. – 270 p.
2. Asatiani V. S. Enzymatic methods of analysis. – M. Nauka, 1985. – 740 p.
3. Golubev V. N., Zhiganov I. N. Food biotechnology – M. Deliprint, 2001. – 423 p.
4. Ivanova A. A., Voyno L. I., Ivanov I. S. Food biotechnology. – M. Kolos, 2008. – 427 p.
5. Kochetkova A. A., Kolesnov A. E., Gorenkov S. State of International project to develop Codex Alimentarius Uniform Standard for fruit juices and nectars, including methods of analysis and sampling // International Congress “Water, beverages and juices”. – M: OCE, 2004. – 237. – 306 p.
6. Kislukhina O. V. Enzymes in food production. Publisher, – M. Deliprint, 2002. – 309 p.
7. Korolev A. P. Handbook for laboratory work on biochemistry. – M. Nauka. 1994. – 96 p.
8. Kolesnov A. Y. European Union association of industry juices and nectars from fruits and vegetables “Set of Rules for assessing the of fruit and vegetable juices” (AJIN) 2004. – P. 219, 235.
9. Parfenova T. V., Kudryasheva P. A. “Ways of rational use of fruit and berry rawmaterials. Storage and processing of agricultural raw materials”. – No. 11. 2000. – P. 46–47.

<https://doi.org/10.29013/AJT-20-5.6-44-48>

*Kabulova Lola Baltamuratovna,  
Doctor, Tech. sciences (PhD), Nukus state Pedagogical Institute,  
Republic of Karakalpakstan, Nukus, Uzbekistan  
E-mail: Kabulova1806@mail.ru*

*Xojametova Bibimaryam,  
Doctor, Tech. sciences, Karakalpak state university,  
Republic of Karakalpakstan, Nukus, Uzbekistan*

## ACTIVATED TUFFITE AS AN ACTIVE POZZOLANIC ADDITIVE

**Abstract.** The article discusses the possibility of obtaining composite cements based on Portland Cement clinker and calcined tuffite. The results of standard and special physical-mechanical tests of Portland cement with heat-treated tuffite additives are presented as a hydraulic additive. It is shown that the active mineral additive tuffit, calcined at 600 °C is suitable for production sulfate resistance cement.

**Keywords:** portlandcement, pozzolan, methods of testing additives, heat-treated tuffits, slaked lime, active additives.

### Introduction

Construction and technical properties of the portland-pozzolana cement depends by nature and amounts of the active mineral additive, which is contained in it. The activity of the additive and the nature of products of its interaction with the hydrated minerals of the Portland cement have significant effect on durability, density, water resistance and a salt resistance of the hardening portland-pozzolana cement.

Due to the expansion of amount of the materials applied as active mineral additives interest in studying of the mechanism and chemist of an action of tuffit, hydraulic active mineral additives of a new type increases. Pocket-tuffit breeds owing to specificity of chemical and mineralogical composition (high percent of calcareousness and existence of a significant amount of a clay component) are a new type of active mineral additives. The specificity also consists in the low content of silicon dioxide and rather high content of alumina. At the same time was the possibility of their use as active mineral additives of natural origin is proved.

For the final conclusion on use of the burned tuffit standard physical-mechanical tests of activity of tuffit additives, burned at 600 °C were carried out.

1. Determination of activity of the burned tuffit testing in mix with a Portland cement. At the same time that additive which, in the optimum dosage being added to a Portland cement is considered more active, gives the last through the smallest period the same mechanical durability, which the Portland cement taken for testing has. Results of the specified physical-mechanical tests are given in (tab. 2).

2. Determination of activity of the burned tuffit in mix with extinguished lime. The method is based on determination of mechanical durability of solutions from mix of the knitting substance with the studied additive, and the last is considered that more active, than solution at an optimum dosage of additive has big mechanical durability. That dosage for which the sum of values of strength at a bend in 28 days, 3 months and 6 months will be the greatest is considered an optimum dosage of additive and extinguished lime.

Results of physical-mechanical tests are given in tab. 3. The received results of standard tests show that the tests of lime applied in work represent the construction air fast-extinguished lime of the first sort.

Table 1. – Determination of activity of additives testing in mix with a portland cement

Name of components of mixes	Composition of mixture	Volume weight. kg/m <sup>3</sup>	Grindingsub tlety			Normal density at%	Grabbing terms in an hour -min.		Strength in mix MPa at												The sum of indicators of strength at a bend in 28 days. 3 and 6 months
			The rest on a 008	Passthrough a sieve 008	test solution		Begin	End	compression				bend								
									day	month	day	month	day	month	day	month					
<b>I</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>	<b>21</b>	
PC Tuffit	80 20	907	0.56	3.10	96.34	25.75	7.44	3–50	5–00	26.1	48.5	50.3	51.9	59.7	2.6	3.5	3.9	4.6	3.9	12.1	
PC Tuffit	70 30	900	0.56	2.98	96.46	26.20	7.10	3–20	4–20	27.0	41.8	44.9	59.4	67.1	2.4	3.1	4.0	4.3	4.2	11.5	
PC Tuffit	60 40	867	0.53	2.38	97.09	25.00	7.05	3–10	4–55	28.0	47.2	51.0	62.1	55.1	2.6	3.2	4.4	4.1	4.1	11.8	
PC Tuffit	50 50	840	0.51	2.08	97.41	26.75	7.50	3–25	5–15	15.8	3.8	37.7	43.4	59.5	2.2	3.1	3.8	4.1	4.3	11.1	
PC Tuffit	40 60	808	0.49	1.78	97.73	27.50	7.67	3–29	5–23	10.0	21.6	22.1	34.0	40.6	1.5	2.0	3.7	3.9	4.0	9.7	

PC – Portland Cement; Tuffit–burnt tuffit at 600 °C

3. Determination of activity of the burned tuffit in mix with extinguished lime and plaster. When studying activity of the burnt tuffit in mix with hydrate lime and plaster, two -water plaster served (5% in terms of 100% of CaSO<sub>4</sub>·2H<sub>2</sub>O). Results of the specified studying are given in (tab. 3).

The high-level overview of results of physical-mechanical tests active finely dispersed the burned tuffit both in mix with a Portland cement, and with extinguished lime shows that educated knitting do not show abnormal properties and the received indicators of physical-mechanical definitions quite keep within existing rules (volume mass, normal density of the test, grabbing terms, mechanical durability, etc.)

Processes of solidification of each look knitting, put trials, submit respectively to patterns of solidification of portland-pozzolana cement or limy and puzzolan-knitting.

In terms of identification of the nature of the studied additives, their tests in mix with only one extinguished lime, certainly, are of the greatest interest (tab.2). Consideration of these results regarding indicators of mechanical durability, first of all, indicates the continuous growth of durability in time, and to the annual age samples show increase in durability on average 3–5 times against branded (at the identical content of lime in mixes).

In case of tests of three-component mixes (additive + extinguished lime + plaster) some increase in absolute measures (a plaster role in three-component mixes with participation of “kipelka”, certainly, much more considerably) durability is observed.

Table 2. – Determination of activity of additives testing in mix with extinguished lime

Name of components of mixes	Composition of mixture	Volume weight, kg/m <sup>3</sup>	Grinding sub-tlety			Normal density at% test	Grabbing terms in an hour -min.		Strength in mix.MPa at												The sum of indicators of strength at a bend in 28 days. 3 and 6 months
			The rest on a 02	The rest on a 008	Pass through a sieve 008		End	Be- gin	compression				bend								
									day	month	day	month	day	month	day	month					
<b>I</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>	<b>21</b>	
Extinguished lime (pr.№ 1) Bunrt tuftit	15 80	629	0.82	1.78	97.40	31.25	8.01	2–15	HeyAOBA.	3.4	8.4	21.3	28.0	33.4	0.6	1.5	2.8	3.2	3.3	7.5	
Extinguished lime (pr.№ 2) Bunrt tuftit	20 80	620	0.24	2.38	97.38	32.50	8.32	4–00	—	2.4	8.5	24.3	29.0	30.2	0.9	1.2	2.9	2.9	3.1	7.1	
Extinguished lime (pr.№ 3) Bunrt tuftit	25 75	617	0.26	2.42	97.32	27.00	6.95	-	—	2.9	8.1	23.8	26.3	30.2	0.8	1.3	2.3	2.8	3.5	6.4	
Extinguished lime (pr.№ 4) Bunrt tuftit	30 70	612	0.75	1.52	97.73	28.75	7.38	3–55	—	2.9	9.1	22.9	23.5	31.4	0.3	0.8	2.2	3.2	3.9	6.3	
Extinguished lime (pr.№ 5) Bunrt tuftit	40 60	610	0.48	2.00	97.52	29.00	7.45	4–05	—	3.0	7.1	20.3	24.7	29.8	0.1	0.7	2.3	3.0	3.6	6.1	

Table 3. – Determination of activity of additives testing in mixes with extinguished lime and plaster

Name of components of mixes	Composition of mixture	Volume weight. kg/m <sup>3</sup>	Grinding subtlety			Normal density at%	Grabbing terms in an hour -min.	Strength in mix MPa at												
			The sum of indicators of strength at a bend in 28 days. 3 and 6 months					compression				bend								
			The rest on a 02	The rest on a 008	Pass through a sieve 008			test	solution	Begin	End	day	month	day	month	day	month			
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>	<b>21</b>
Extinguished lime (pr.№ 1) Bunrt tuffit	15 80	627	0.36	0.96	98.68	32.50	8.33	4–10	6–00	1.1	13.3	25.7	29.3	31.2	1.4	1.9	3.0	2.4	1.8	7.3
Extinguished lime (pr.№ 2) Bunrt tuffit	20 80	617	0.34	1.18	98.48	33.00	8.45	4–15	6–20	0.7	12.4	25.9	32.1	37.5	1.2	1.8	3.3	3.8	2.3	9.0
Extinguished lime (pr.№ 3) Bunrt tuffit	25 75	612	0.33	1.20	98.47	35.00	8.95	4–25	неулова.	0.7	12.9	27.9	35.8	33.3	1.3	1.3	3.0	2.0	3.8	6.4
Extinguished lime (pr.№ 4) Bunrt tuffit	30 70	602	0.29	1.32	98.39	38.25	9.76	4–45	– // –	0.4	11.7	23.2	31.0	32.0	0.9	2.1	3.3	2.2	3.7	7.7
Extinguished lime (pr.№ 5) Bunrt tuffit	40 60	588	0.25	1.32	98.43	37.00	9.45	4–00	– // –	0.3	10.5	17.5	32.0	34.1	0.8	1.4	2.5	3.7	3.8	7.6

Note: the third component two-water plaster (5% in terms of 100% of CaSO<sub>4</sub>·2H<sub>2</sub>O)

At tests of additives in mix with a Portland cement in our samples, continuous increase in mechanical durability was observed that should be carried only due to application of tests of the Portland cement, which showed increase increase in mechanical durability was observed that should be carried only due

to application of tests of the Portland cement, which showed increase.

On the basis of the data (tab. 1–3) obtained by us it is possible to recommend the following limits of fluctuations of an optimum dosage of the additives studied by us for knitting, provided in (tab. 4).

Table 4. – Limits of fluctuations of an optimum dosage of additives to knitting

Name of additives	Knitting	
	to Portland cements	to extinguished limes
Bunrt tuffit	20–60	85–60

### Conclusion

The carried-out work allows to draw the following conclusions:

1. Indicators of activity of the tuffit additive burned at 600°C certain standard with physic mechanical tests of the samples made of tuffit mixes with a Portland-cement and extinguished lime con-

vince that standard methods reveal true activity of the studied additive.

2. Data of results of indicators of mechanical durability, first of all, indicate the continuous growth of durability in time, and to the annual age samples show incur ease in durability on average 3–5 times against branded.

### References:

1. Бутт Ю. М., Тимашев В. В. Практикум по химической технологии вяжущих материалов. – М. 1973. – 504 с.
2. Кузнецова Т. Новое испытание для цемента. Журнал «Технологии строительства» 2(64). – М.: 2009. – 64 с.
3. ГОСТ 51795–2001. Цементы. Методы определения содержания минеральных добавок. – М.: МНТСК, 2001. – 45 с.



<https://doi.org/10.29013/AJT-20-5.6-49-53>

*Makhmudov Mukhtor Jamolovich,  
PhD in Institute Bukhara of engineering and technology  
E-mail: makhmudov.mukhtor@mail.ru*

*Axmedov Ulug' Karimovich,  
Professor, Institute of the General and Inorganic chemistry  
Academy of Sciences of the Republic of Uzbekistan, Tashkent*

## MODERN METHODS OF REDUCING THE CONTENT OF AROMATIC HYDROCARBONS IN GASOLINE

**Abstract.** Various refining methods are presented to improve the ecological environment and reduce the amount of aromatic hydrocarbons in motor gasolines in the oil refining industry.

**Keywords:** gasoline, aromatic hydrocarbons, azeotropic distillation, extraction, rectification, adsorption.

Road transport is the main source of air pollution. Pollution occurs as a result of fuel combustion. About 2 billion tons of petroleum fuel are burned annually in automobile internal combustion engines in the world. Moreover, the average efficiency is 23%, the remaining 77% goes to the environment [1].

The following types of transport are distinguished: light, medium and heavy freight, bus, passenger. At the same time, 90% of cars use gasoline as fuel, and 10% – gas, trucks use diesel fuel and gas, 50% of buses use gasoline, and 50% – gas [2].

About 25% of the world's oil is used to produce gasoline, which is the main type of fuel for vehicles [3].

It was found that 30% of urban diseases are directly related to air pollution by exhaust gases. The most dangerous for humans are hydrocarbon compounds of

the carcinogenic group: 1,2-benzanthracene ( $C_{18}H_{12}$ ), 3,4-benzpyrene ( $C_{20}H_{12}$ ), 1,2-benzpyrene ( $C_{20}H_{12}$ ), 3,4-benzofluorantene ( $C_{20}H_{14}$ ). Particularly dangerous is 3,4-benzpyrene, which is a kind of indicator of the presence of other carcinogens in the mixture [4–6].

The range and quality of gasolines are determined recently by environmental requirements for them. Currently, in order to reduce the toxicity of car exhausts, according to the requirements of European standards, restrictions are set on the content of benzene (up to 1%) and total aromatic hydrocarbons (30–35%) in gasolines (table 1). MPC of gasoline = 300 mg / m<sup>3</sup>. Reducing the harmful effects of exhaust gases on the environment and humans can be achieved by reducing the content of aromatic hydrocarbons in gasolines, primarily benzene [7].

Table 1. – Modern requirements for the quality of gasoline

Indicators	Requirements	
	Euro 4 (since 2005)	Euro 5 (since 2009)
Benzene content, not more than,%	1.0	1.0
Sulfur content,%	0.005	0.001
The content of aromatic hydrocarbons,%	35	35
The content of olefinic hydrocarbons,%	14	14
Oxygen content,%	2.7	2.7

Benzene, the most easily boiling among aromatic compounds, is harmful to people directly working with gasoline, as it contributes to the disease with leukemia [8].

There are various methods for the separation of aromatic hydrocarbons:

- Azeotropic distillation;
- Extractive distillation;
- Extraction;
- Adsorption release.

**Azeotropic distillation.** As you know, aromatic hydrocarbons form azeotropic mixtures with boiling paraffin-naphthenic hydrocarbons in cases of significant deviations of the mixtures from the behavior of an ideal system. In fig. Figure 1 shows the dependence of vapor pressure on the composition of the mixture of two components A + B for a system with a minimum boiling point.

The dashed line A + B represents the theoretical or ideal vapor pressure of the solution, calculated according to Raoult's law.

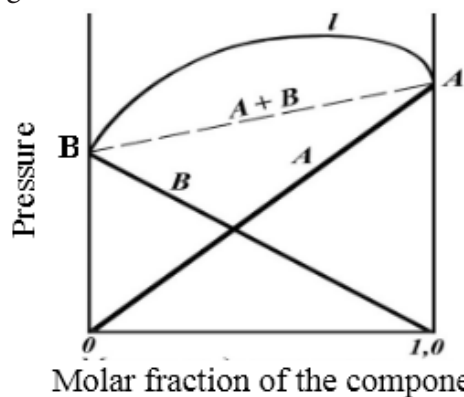


Figure 1. The dependence of vapor pressure on the composition of the mixture with a minimum boiling point

The upper solid curve shows the actual vapor pressure of the mixture. Point *z*, where the curve passes through the maximum, corresponds to the composition of the azeotrope. Since this is the point of maximum vapor pressure, it corresponds to the minimum boiling point of mixture A-B, which is lower than the boiling point of pure components A and B. The mixture corresponding to the composition at

point *z* will be distilled at a constant temperature and without changing the composition.

Aromatic hydrocarbons  $C_6$ ,  $C_7$  and  $C_8$  form azeotropes with paraffin-naphthenic hydrocarbons with only a minimum boiling point. In fig. 2. The composition of the vapor and liquid phases of the cyclohexane-benzene system is given. These hydrocarbons boiling at 80.0 and 80.1 °C, respectively, form an azeotrope with a benzene content of 51.8% by weight. The boiling point of the azeotrope is 77.5 °C. The vapor pressure curve of this system is similar to the curve in (Fig. 1).

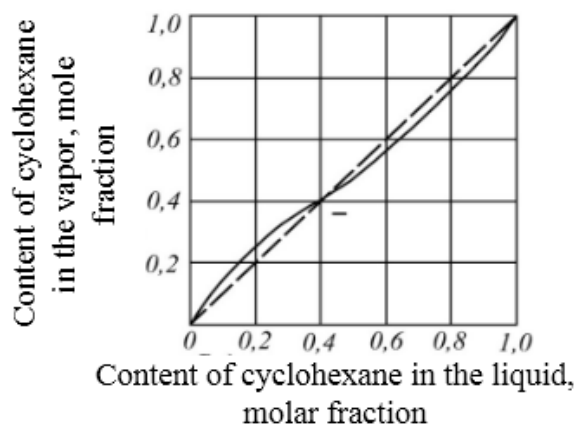


Figure 2. The cyclohexane-benzene system

Since the azeotrope has a minimum boiling point, then for any ratio of components, the azeotropic mixture will be distilled first. But the nature of the residue will already depend on the ratio of the components. So, if the initial mixture contains 20% cyclohexane and 80% benzene, then after distillation of the azeotrope, pure benzene will remain in the residue; if a mixture consisting of 80% cyclohexane and 20% benzene is distilled, pure cyclohexane will remain in the residue. In practice, this method is sometimes used to obtain pure benzene or cyclohexane from mixtures thereof.

When choosing a reagent that forms an azeotropic mixture with one or both of the separated components, the following series of circumstances must be taken into account:

1. When adding the third component, the boiling point of the resulting azeotrope should be quite

different from the boiling points of the other components or mixture.

2. It is desirable that the resulting azeotropic mixture contains the maximum amount of product per unit weight of the distilled reagent and has a low boiling point.

3. The third component must have a low latent heat of evaporation, so that the heat consumption for distilling the resulting product is minimal.

4. The third component should be easily regenerated for further use in the process. For this, several methods are used to separate the reagent and non-aromatic hydrocarbon, such as separation of liquid phases upon cooling, followed by extraction with a solvent, or water washing, distillation at various pressures, and other methods.

5. The third component should be chemically inert, not react with shared hydrocarbons, not corrode the equipment, be thermally stable, non-toxic and available on an industrial scale.

**Extractive distillation** – characterized by the use of a solvent, the boiling point of which is much higher

than the boiling points of the separated components. The solvent is fed to the top of the column. Flowing down, it dissolves one of the constituent parts of the mixture – aromatic hydrocarbons. Non-aromatic hydrocarbons free of solvent are removed from the top of the column. The solvent used to separate the hydrocarbon mixture changes the normal relative volatility of the components. In this case, a deviation of the system from the ideal one is observed.

A large number of solvents have been tested to isolate aromatic hydrocarbons by extractive distillation. In the (table 3) shows data on the relative volatility of a mixture consisting of 50 mol% methylcyclohexane and 50 mol% toluene, in the presence of various solvents (relative volatility of the toluene – methylcyclohexane 1.37 system).

Table 4 shows the relative volatilities of the toluene-non-aromatic hydrocarbon system (1: 1 ratio) in the presence of various compounds. As a non-aromatic hydrocarbon, a specially dearomatized fraction of direct distillation gasoline boiling in the range of 99–113 °C served.

Table 3. – Relative volatility of methylcyclohexane mixed with toluene in the presence of various solvents

Solvent	Solvent boiling point, °C	Solvent content, mol%.	Relative volatility methylcyclohexane to toluene, $\alpha$
Aniline	184.4	65.8	2.71
Ethylene glycol monoethyl ether	135.2	65.8	2.29
Diethylene glycol	197.2	66.5	1.88
Formamide	193	66.7	1.42
Ethylene glycol monomethyl ether	124	66.7	2.72
Phenol	182.2	65.8	2.52
Pyridine	115.3	66.7	2.59

Furfural turned out to be the best selective solvent for the isolation of toluene. However, furfural boiling at a temperature of 163 °C can form azeotropic mixtures with non-aromatic hydrocarbons distilled from toluene, which makes it difficult to regenerate it.

Phenol, being the same selective solvent as aniline, is chemically inactive and quite stable. It also meets the requirements for boiling point, availability and cost. For the above reasons, phenol is the main solvent used for the extraction of benzene and toluene mainly consists of aromatic hydrocarbons.

Table 4. – Relative volatility of non-aromatic hydrocarbons mixed with toluene in the presence of various solvents

Solvent	Solvent boiling point, 0C	The solvent content in the feed, % wt.	Relative volatility methylcyclohexane to toluene, $\alpha$
Furfural	163	50	2.30
Acetonyl acetone	188	50	2.20
Nitrobenzene	212	50	2.16
Nitrotoluene	223	50	2.16
Phenol	182	50	2.10
Aniline	184	50	2.08
Phenol + Cresol (60% + 40%)	193	50	1.98
Phenol + Cresol (40% + 60%)	195	50	1.95
Acetophenone	203	50	1.95
Meta + or paracresol	205	50	1.85
Diacetonglycol	191	50	1.64

**Extraction.** Extractive separation processes are based on the unequal solubility of aromatic and non-aromatic hydrocarbons in various solvents.

The conditions of the extraction process are determined by the nature of the feedstock and solvent, the amount of solvent, temperature and the required number of stages of extraction and the given selection and properties of the aromatic hydrocarbon concentrate.

The solvent should be characterized by the following qualities:

- high selectivity and high dissolving ability in relation to aromatic hydrocarbons;
- stability during long-term operation;
- the difference in density between the solvent and hydrocarbons to facilitate phase separation during extraction;
- ability to easy regeneration;
- to be economically accessible, not to corrode the equipment and not have a pronounced toxic effect.

The choice of the temperature range of extraction depends on the critical temperature of dissolution of the feedstock. Extraction is carried out in the temperature range at which there are two phases – extract and raffinate.

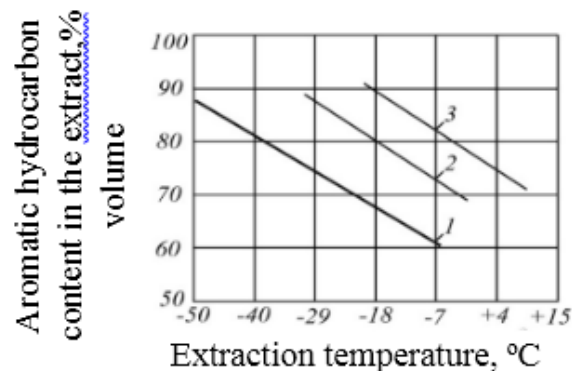


Figure 3. The effect of the temperature of extraction and the fractional composition of raw materials on the content of aromatic hydrocarbons in the extract: 1 – average boiling point of the fraction 144 °C; 2 – average boiling point of the fraction 216 °C; 3 – average boiling point of the fraction 276 °C

The amount of solvent is determined by the temperature of the process and a given percentage of extraction of aromatic hydrocarbons from the feedstock. The process must be carried out in such a way as to obtain, possibly, a greater extraction of aromatic hydrocarbons with a minimum consumption of solvent and a practically acceptable number of extraction steps. Diethylene glycol (DEG) and liquid

sulfur dioxide are used as solvents in the evolution of aromatic hydrocarbons in industrial practice.

The influence of the extraction temperature and the fractional composition of the raw material on the content of aromatic hydrocarbons in the extract is shown in (Figure 3).

The curves were obtained on the basis of laboratory studies and the operation of an industrial extraction plant using sulfur dioxide.

**Adsorption release.** Aromatic hydrocarbons are able to adsorb more strongly on specially selected adsorbents than paraffinic and naphthenic hydrocarbons; this is the basis for their isolation from petroleum products.

Separation of complex mixtures using the adsorption method has been used in laboratory practice for a long time – since 1903. Adsorption separation in the liquid phase is used not only to separate aromatic

hydrocarbons, but also to isolate a number of other chemical products [9; 10].

Adsorbents used to extract aromatic hydrocarbons from oil fractions should have high selectivity, mechanical strength, and long service life; they should be easily regenerated, be chemically inert with respect to shared components and economically available.

The industrial design of the process of adsorption of aromatic hydrocarbons is determined by the absorption capacity of the adsorbent, the rate of percolation of the raw material, propellant and desorbent. The necessary amount of propellant and desorbent and the size of the adsorbent granules are also taken into account.

Thus, for the extraction of aromatic hydrocarbons from the composition of automobile fuels in the oil refining industry, the cleaning methods presented in this article can be used.

### References:

1. Ivanov V.N. Ecology and motorization / V.N. Ivanov, V.K. Storchevus. 2<sup>nd</sup> ed., Revised. and additional.– K.: Budivelnik, 1990.– 128 p.
2. Emelyanov V.E., Krylov I.F. Automobile gasoline and other types of fuel: properties, assortment, application.– M.: AST: Profizdat, 2005.– 207 p.
3. Sayfullin N.R., Ishmakov R.M., Abyzgildin A. Yu., Gubaidullin N.M., Gaskarov N.S., Khafizov A.R. Automobile and Ecology.– Ufa: Publishing House of Ufa State Technical University, 1998.– 133 p.
4. Vasiliev V., Pisarev V., Khazin G. Ecology and international relations.– M: Chemistry, 1978.– 240 p.
5. Yakubovsky Yu. Road transport and environmental protection. Translation from Polish.– M: Transport, 1979.– 198 p.
6. Makhmudov M.J., Khalilov A. Kh., Kayitov R. R., Narmetova G. R. Obtaining motor gasoline that meets the requirements of the European standard for benzene content // Chemistry and Chemical Technology. 2017. – No. 1.– P. 66–68.
7. Makhmudov M.J., Khaitov R. R., Narmetova G. R. Modern requirements for motor fuels // Russian journal “Young Scientist”, Kazan, 2014.– No. 21 (80).– P. 181–183.
8. Usakova N.A., Emelyanov V.E., Demina L. V. Oil refining and petrochemicals: OAO “Lukoil”, VNII NP: Trends in the production of gasoline.– M., 1999.– No. 1.– 23 p.
9. Narmetova G. R. Colloidal – chemical basis for the creation of polyphase sorbents for gas – liquid – solid phase chromatography: Abstract. dis. ... doctor of chemical sciences – Tashkent, 1993.– 38 p.
10. Rashidova F.M. Refining of the gasoline fraction of high-paraffin oil in order to obtain motor fuel: Abstract. dis. ... Ph.D.– Tashkent, 1998.– 23 p.

<https://doi.org/10.29013/AJT-20-5.6-54-59>

*Rakhmatova Guzal Botirovna,  
Senior lecturer in Karshi Engineering Economics Institute,  
Uzbekistan, Karshi*

*E-mail: raxmatova85guzal@mail.ru*

*Kurbanov Mingnikul Jumagulovich,  
Doctor of Chemistry Karshi State University  
Uzbekistan, Karshi*

*E-mail: kurbanovmj@mail.ru*

*Turabayeva Nargisa Bekmuradovna,  
Senior lecturer in Karshi Engineering Economics Institute,  
Uzbekistan, Karshi*

*E-mail: turabayeva75nargisa@mail.ru*

*Tursunova Gulnoza Qaxxarovna,  
Senior lecturer in Karshi Engineering Economics Institute  
Uzbekistan, Karshi*

*E-mail: tursunovagulnoza@mail.ru*

## **STUDY OF INSPACTIVE PROPERTIES AGAINST CORROSION OF $\alpha$ -AMINOCETONES AND THEIR PRODUCTS**

**Abstract.** The paper examines the anti-corrosion inhibitory properties of  $\alpha$ -aminocetones such as thiainan and thiachromane. 15% HCl, 20% H<sub>2</sub>SO<sub>4</sub>, 1M HCl and 1M H<sub>2</sub>SO<sub>4</sub> solutions were used as the aggressive medium. As the concentration of the studied compounds in solution increases, so does their protective effect. The increase in temperature showed an increase in the inhibition rate of the inhibitors during the corrosion process. Inhibitors containing morpholine fragment as the most effective inhibitor in aggressive environments were found to have higher inhibition rates.

**Keywords:** benzothiophene, thiainden, thiaindane, thiochroman, hydrochloric acid, sulfuric acid.

Corrosion of various industrial goods and construction materials made of steel around the world due to corrosion is considered to be one of the most urgent problems today. This is causing great economic and environmental damage to any country. It is therefore attracting the attention of chemists, technologists and scientists dealing with socio-economic problems all over the world. In addition, the decline in efficiency in gas and oil refining, gas and oil refineries, which are the mainstays of any country's economy and strategic

priorities, undermines the operation of the plant as a whole.

The use of organic-based inhibitors in addressing the above-mentioned global problem is currently yielding good results. The use of corrosion inhibitors is one of the most effective ways to prevent the corrosion process [1, 16657–16668]. Research on inhibitors of metal corrosion is a long-term important investment and is of strategic importance from an economic and environmental point of view. The world community is conducting international re-

search on the development of toxicologically non-toxic, biodegradable and highly effective inhibitors of corrosion protection of metals, in-depth study of the physicochemical aspects of protective coatings for corrosion protection of metals [2, 82–95].

In studying the anticorrosive properties of the synthesized compounds, we initially used the gravimetric (gravity) analysis method. This method is simple in nature in determining the degree of corrosion of metals, the method is one of the methods that does not require complex and expensive equipment

and can provide a very large amount of data, and has not lost its relevance to date.

In this study, the inhibitory properties of the following synthesized compounds were studied at different temperature ranges and concentrations of different aggressive media. For the study,  $\alpha$ -piperidino-5-acetyl-2-methyl-1-thiaine (AIT-1),  $\alpha$ -morphalino-5-acetyl-2-methyl-1-thiaine (AIT-2),  $\alpha$ -piperidino-6-acetyl-1-thiochroman (AIT-3) and  $\alpha$ -morphalino-6-acetyl-1-thiochroman (AIT).

Table 1.

Ingib. cipher	Ingib. amount mg/l	Time, hour	15% HCl			20% H <sub>2</sub> SO <sub>4</sub>		
			K <sub>r/M<sup>2</sup>h</sub>	$\gamma$	Z%	K <sub>r/M<sup>2</sup>h</sub>	$\gamma$	Z%
1	2	3	4	5	6	7	8	9
AIT -1	50	2	0.84	23.80	95.80	1.84	16.64	93.99
		4	0.89	23.59	95.76	0.97	30.61	96.73
		6	0.84	27.26	96.33	0.95	31.78	96.85
	100	2	0.46	43.47	97.70	0.87	35.97	97.22
		4	–	–	–	0.93	31.93	96.86
		6	–	–	–	0.92	32.82	96.95
	250	2	0.48	41.66	97.60	0.50	62.60	98.40
		4	0.33	55.26	98.12	0.45	66.00	98.48
		6	0.38	60.26	98.34	0.45	67.11	98.50
	500	2	0.48	41.66	97.60	0.48	65.20	98.46
		4	0.36	58.33	98.28	0.36	82.50	98.78
		6	0.31	73.87	98.64	0.39	77.43	98.70
1000	2	0.55	36.36	97.25	0.38	82.36	98.78	
	4	0.45	46.66	97.85	0.34	87.35	98.85	
	6	0.39	58.71	98.29	0.36	83.88	98.80	
AIT -2	25	2	1.15	17.39	94.25	0.95	32.94	96.96
		4	1.16	18.10	94.47	–	–	–
	100	2	0.89	22.47	95.55	0.89	35.16	97.15
		4	0.78	26.92	96.28	–	–	–
	250	2	0.59	33.89	97.05	0.53	59.05	98.30
		4	0.49	42.85	97.66	–	–	–
	500	2	0.48	41.66	97.60	0.34	92.05	98.91
		4	0.39	53.84	98.14	–	–	–
	1000	2	0.35	57.14	98.25	0.20	156.5	99.36
		4	0.38	55.26	98.19	–	–	–

The data in the table show that the inhibitory efficiency of inhibitors increases in parallel with increasing inhibitory concentration and time at 200 °C

in 15% HCl and 20% H<sub>2</sub>SO<sub>4</sub> solutions of AIT-1 and AIT-2. Based on the results, it can be said that the inhibitory properties of AIT-1 and AIT-2 inhibitors

in sulfuric acid solution were higher than in hydrochloric acid solution. The melting rate of the metal decreases rapidly when a sample of steel grade St.3 is added to a solution of 15% HCl and 20% H<sub>2</sub>SO<sub>4</sub> at a temperature of 200 °C with a concentration of 10<sup>-4</sup> mol/l (25 mg/l) of inhibitors AIT-1 and AIT-2. Showed that the inhibition coefficient remained the same when the inhibitor concentration was increased from 4 × 10<sup>-3</sup> to 2 × 10<sup>-3</sup> mol / l (100 to 500 mg/l).

It has been shown that the effectiveness of inhibitors in sulfuric acid solution increases in parallel with the increase in inhibitor concentration. Showed that the inhibition coefficient of AIT-1 and AIT-2 inhibitors 1 × 10<sup>-4</sup> and 4 × 10<sup>-4</sup> mol/l increased with time. Table 2 below provides information on the protection levels of AIT-3 and AIT-4 inhibitors.

Efficacy of AIT-3 and AIT-4 inhibitors in 15% HCl and 20% H<sub>2</sub>SO<sub>4</sub> solution at 20 °C

Table 2.

Ingib. ciper	Ingib quantity, mg/l	Time hour	15% HCl			20% H <sub>2</sub> SO <sub>4</sub>		
			K <sub>r/m<sup>2</sup>ч</sub>	γ	Z%	K <sub>r/m<sup>2</sup>ч</sub>	γ	Z%
AIT -3	50	2	1.77	11.31	91.15			
		4	1.50	14.00	92.86			
		6	1.17	19.61	94.90			
	1000	2	0.94	21.29	95.30	0.40	78.25	98.72
		4	0.68	30.88	96.76	0.35	84.86	98.82
		6	0.59	38.88	97.42	0.52	58.12	98.91
AIT -4	50	2	0.68	29.42	96.60	0.44	71.14	98.59
		4	0.58	36.20	97.23	0.26	114.2	99.12
		6	0.48	47.79	97.90	0.33	91.58	98.91
	1000	2	0.50	40.02	97.50	0.17	184.0	99.46
		4	0.30	70.00	98.57	0.15	198.0	99.49
		6	0.30	76.46	98.69	0.14	215.8	99.54

During the study, a mass reduction in the temperature range of 60–95.50S in a working system of 15% HCl and 20% H<sub>2</sub>SO<sub>4</sub> at a concentration of 1000 mg/l for 6 hours in a St.3 sample of AIT-3 and AIT-4 inhibitors was determined by gravimetric measurement. Measurement of the mass reduction of the sample before the working solution and after a certain time in the working solution is useful in evaluating parameters such as corrosion rate (g) and the effectiveness

of inhibitors (Z%). The following equation was used to determine these parameters and the results of the study were summarized in where: m<sub>1</sub> is the weight of the sample before the study, in grams, m<sub>2</sub> is the weight of the sample after the study, in grams, s is the area of the sample in square meters (m<sup>2</sup>) and t is the duration of the experiment, in hours. Efficacy results of inhibitors at different temperatures and 15% HCl and 20% H<sub>2</sub>SO<sub>4</sub> environments

Table 3.

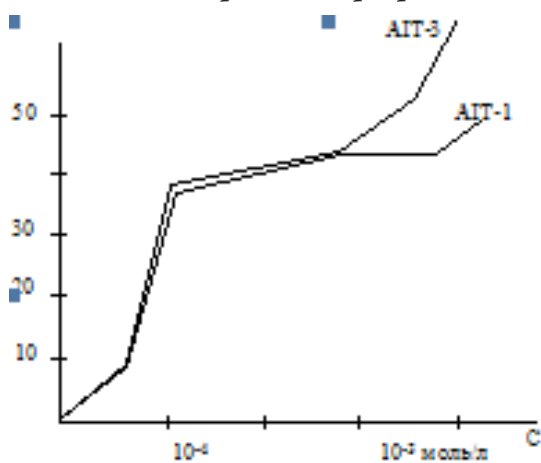
Ingib. ciper	Environment%	Inh Amomr/Δ	Time hour	60 °C			95,5 °C		
				K <sub>r/m<sup>2</sup>ч</sub>	γ	Z%	K <sub>r/m<sup>2</sup>ч</sub>	γ	Z%
1	2	3	4	5	6	7	8	9	10
AIT -3	HCl 15%	1000	1	–	–	–	61.74	51.4	88.05
			2	3.16	106.50	99.06			
			4	3.01	122.53	99.18			
			6	3.81	106.91	99.06			



1	2	3	4	5	6	7	8	9	10
AIT -4	HCl 15%	1000	1	-	-	-	68.66	46.19	79.84
			2	7.07	51.46	98.06			
			4	6.06	63.14	98.42			
			6	6.74	59.20	98.31			
AIT -3	H <sub>2</sub> SO <sub>4</sub> 20%	1000	1	-	-	-	5.38	543.5	99.82
			2	1.28	338.64	99.70			
			4	1.35	372.07	99.73			
			6	1.20	394.78	99.75			
AIT -4	H <sub>2</sub> SO <sub>4</sub> 20%	1000	1	-	-	-	8.30	351.6	99.72
			2	3.01	144.01	99.31			
			4	3.37	149.05	99.33			
			6	2.90	163.36	99.39			

Raising the temperature to 600S leads to an increase in the inhibition rate even in 15% HCl and 20% H<sub>2</sub>SO<sub>4</sub> media of all inhibitors under study. However, raising the temperature to 95.5 °C and above indicates that the inhibitors being studied in the hydrochloric acid environment are shifting in the direction of decreasing inhibition. However, an increase in temperature to 95.5oC was found to give a positive result (99.72%) for the AIT-4 inhibitor in a sulfuric acid environment. The protective properties of the metals

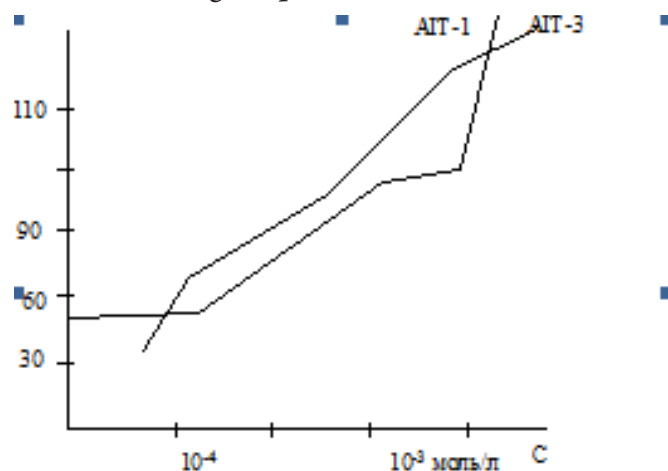
of the compounds under study are stable over time in hydrochloric and sulfuric acid environments. The data obtained show that the inhibitors studied slow down the corrosion rate by 12–32 times in an aggressive environment of hydrochloric acid at a dose of 50 mg/l, while at a rate of 1000 mg / l slow down the corrosion rate by 75–140 times. Similarly, it was found that the protective properties of the inhibitors under study in hydrochloric and sulfuric acids increase periodically with increasing temperature.



1-grafik. Tiindan va tiaxroman aminoketonlari qatori ingibitorlarining HCl muhitidagi korroziyani tormozlash koeffitsentining konsantrsiyaga bog'liqligi

Figure 1. shows the dependence of the inhibitor of the series of thiachromanic anions on the corrosion inhibition coefficient in the H<sub>2</sub>SO<sub>4</sub> medium

Figure 1 below shows a graph of the correlation inhibition coefficient of the corrosion inhibitors of



2-grafik. Tiindan va tiyaxroman aminoketonlari qatori ingibitorlarining H<sub>2</sub>SO<sub>4</sub> muhitida korroziyani tormozlash koeffitsentining konsantrsiyaga bog'liqligi

Figure 2. shows the dependence of the inhibitor of the series of thiachromanic anions on the corrosion inhibition coefficient in the H<sub>2</sub>SO<sub>4</sub> medium

the corrosion inhibitors AIT-1 and AIT-3 containing the thian and thiachroman fragment in the

hydrochloric acid environment. The graph of the corrosion inhibition coefficient of the inhibitors studied above in the sulfuric acid environment depends on the inhibitor concentration.

The process of time-dependent reduction of the mass in the presence of an inhibitor in a 20%

$H_2SO_4$  solution of the St.3 sample obtained for the study can be represented by the image in Fig. 1. As can be seen from the figure, the melting rate of a steel sample in a solution with an inhibitor relative to a solution without an inhibitor significantly reduced.

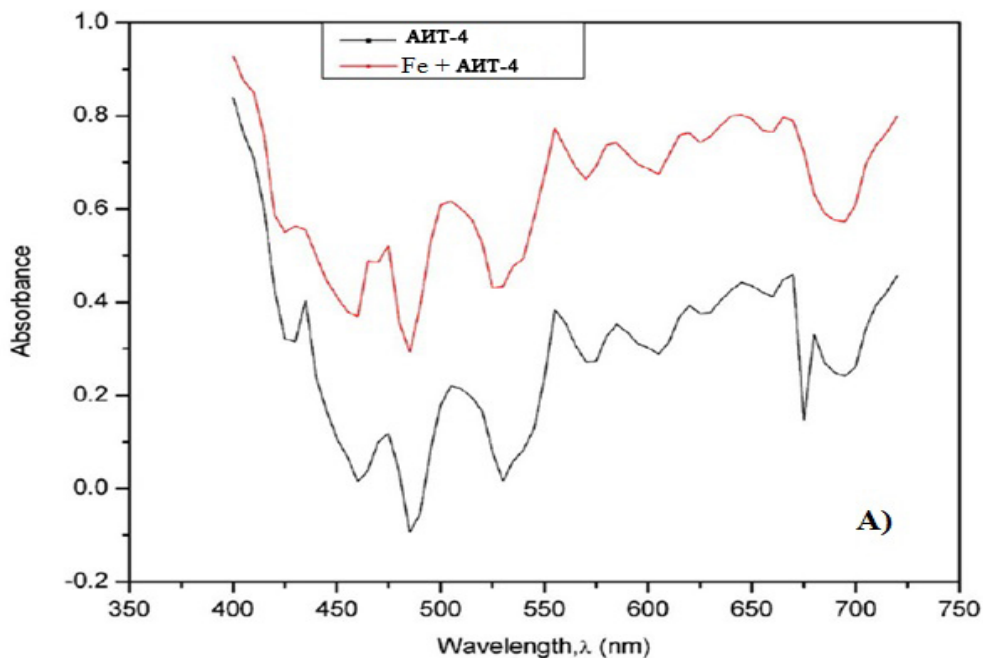
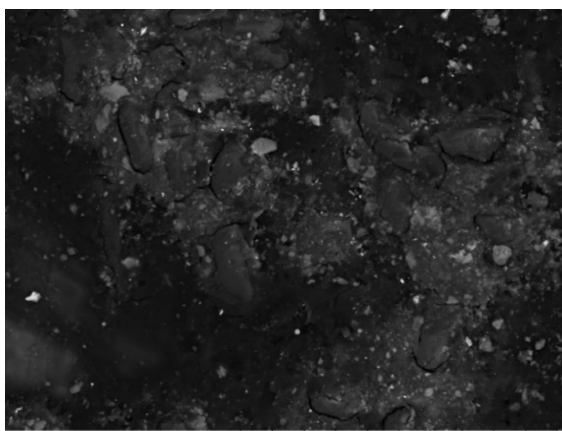


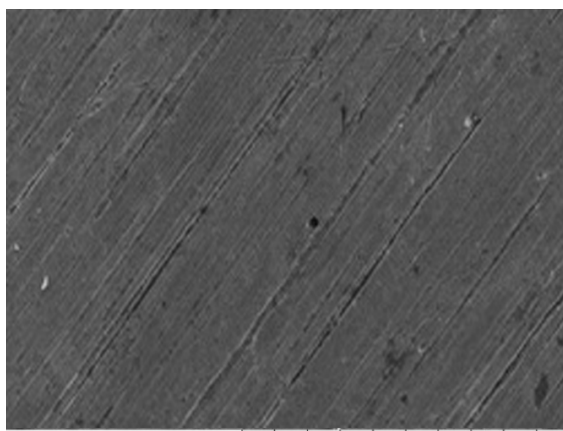
Figure 1 Decrease in mass of steel St.3 in 20%  $H_2SO_4$  solution over time

The UV spectra of this solution were analyzed to confirm the formation of an In-Fe complex in a system containing 1000 mg/l AIT-4 inhibitor in 1 molar sulfuric acid obtained as a working solution.

UV spectra were obtained in the absence of an inhibitor in the working solution and 6 h after the addition of the inhibitor. Comparative images of these UV spectra are shown in (Figure 2).



Without inhibitors in a solution



1 M HCl with Inhibitor AIT-4 of 1M NCl

Figure 3. SEM-microphotography of Pulat St.3 sample

The figure shows the absorption values and the deviation of their intensity. After immersion of the steel in the solution, an increase in absorption for the AIT-4 inhibitor is observed. This indicates the formation of a mutually stable complex between the  $Fe^{2+}$  ions and the functional-active groups of the inhibitor. The formation of this complex may be the cause of the observed deviation in the values of the absorption process and its intensity, which in turn leads to the anticorrosive activity of the inhibitor. In order to confirm the effectiveness of AIT-4 as a corrosion inhibitor, the images in (Figure 3) were obtained using SEM. As can be seen from the figure, the image of a steel sample lowered into the working system without the addition of an inhibitor reflected many cracks and

abrasions on the steel surface. The image of the same steel ngamuna in the working solution of the inhibitor-added system shows that there are no cracks of the above appearance and that the erosion is not noticeable at all.

Thus, during the gravimetric study of the anti-corrosive properties of the inhibitors studied, it was found that they are inhibitors that give good results to the corrosion process that takes place in the acidic environment. The binding of the sulfur atom in all synthesized inhibitors to the aromatic ring on the one hand and the poluaromatic ring on the other shows that the inhibitor molecules studied in these corrosive aggressive systems are capable of forming strong chemisorption-type bonds with structural materials.

#### References:

1. Kokalj A., Peljhan S., Finšgar M., Milošev I. What determines the inhibition effectiveness of ATA, BTAH, and BTAOH corrosion inhibitors on copper., *J Am. Chem. Soc.* – 132. 2010. – P. 16657–16668.
2. Finšgar M., Kek D., Merl, 2-mercaptobenzoxazole as a copper corrosion inhibitor in chloride solution: electrochemistry, 3D-profilometry, and XPS surface analysis, *Corros. Sci.* – 80. 2014. – P. 82–95.
3. Nešić S., Sun W. 2.25 – Corrosion in acid gas solutions, in: J.A.R. Tony (Ed.), *Shreir's Corrosion*, Elsevier, Oxford, 2010. – P. 1270–1298.

<https://doi.org/10.29013/AJT-20-5.6-60-67>

*Turaeva Khurshida Kamalbaevna,  
doctoral student*

*National university of Uzbekistan, the Faculty of Chemistry  
E-mail: torayevah@mail.ru*

*Yuldasheva Mukhabbat Razzoqberdievna,  
Doctor of Chemical Sciences, docent*

*National university of Uzbekistan, the Faculty of Chemistry  
E-mail: ymuxabbat@bk.ru*

## REACTIONS OF NITROMEZITILENE WITH SUBSTITUTED ALKYLMALEINIMIDES

**Abstract.** Amidoethylation reactions of nitromesitylene with  $\beta$ -hydroxyethylmaleinimide in the presence of concentrated catalysts of a mixture of concentrated sulfuric acid and phosphoric acid, and with  $\beta$ -chloroethylmaleinimide in the presence of a small amount of  $\text{FeCl}_3 \times 12\text{H}_2\text{O}$  catalyst were studied. The results showed that amidoalkylation reactions in the presence of Lewis acids were spatially selective, resulting in a single mono- substituted product with high yields. The influence of various factors on the course of the reaction, the yield of the product and their composition was determined. The structure of the synthesized substances was confirmed using physicochemical research methods.

**Keywords:** Nitromesitylene,  $\beta$ -hydroxyethylmaleinimide ( $\beta$ -GEMI),  $\beta$ -chloroethylmaleinimide, malein anhydride (MA),  $\text{FeCl}_3 \times 12\text{H}_2\text{O}$ .

**Introduction.** Maleic acid and its derivatives in the production of polymeric materials [1], various monomers [2], as a binding agent [3], in the production of various adhesives, as a means of reducing corrosion and increasing durability in the paint industry [4], modifying bitumen [5], aviation technology [6–7], in medicine as an antiviral and antibacterial agent [8–9]. N-Methylolmaleinimide is used in the amidomethylation of aromatic compounds and in the production of surfactants. The products obtained from the amidoalkylation reactions of aromatic compounds with N-methylolmaleinimide differ from the products obtained on the basis of other amidoalkylating reagents by the double bond in the maleinimide group, which is used effectively in subsequent syntheses. Reactions leading to double bonding can proceed on the basis of radical or electrophilic cou-

pling reactions, depending on the conditions selected. If a maleinimide derivatives are to be used as a sewing agent, reactions involving light and temperature. Electrophilic coupling reactions are carried out on the double bond for the synthesis of surfactants. This, expands the scope of application of maleic acid derivatives. N-hydroxyalkylmaleinimides are amidoalkylating reagents and may in turn be starting products for the synthesis of alkylamines.

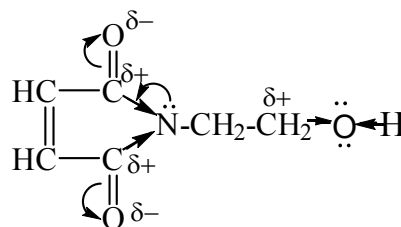
The electrophilic substituted reactions are a widely used synthetic method in the aromatic series, used in the production of various mono-, di- and polysubstituted aromatic compounds [10].

It is known that N-hydroxyalkylamides, N-hydroxyalkylimides are among the amidoalkylating reagents due to their ability to be synthesized under normal laboratory conditions, high yield and resistance

to acids. Using these electrophilic reagents, they are possible to synthesize other types of electrophilic reagents, such as haloalkylamides, ethers, N, N'-alkylene-bis-amides. The reactivity of N-alkylolimides at room temperature in the presence of sulfuric acid varies, among which N-alkylolimides have been shown to have high electrophilic properties. We know that intermolecular amidoalkylation reactions are also important, they are possible to synthesize heterocyclic compounds that are difficult to obtain [11].

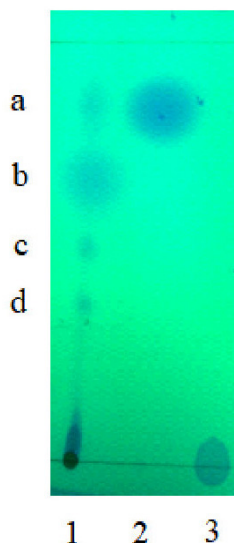
**Results and discussion.** This work is devoted to the course of the amidolalkylation reaction on the basis of the new amidoalkylating reagent  $\beta$ -hydroxyethylmaleinimide and  $\beta$ -chloroethylmaleinimide. For the amidoalkylation reaction,  $\beta$ -hydroxyethylmaleinimide was initially selected as the alkylating electrophilic reagent. Based on the literature and taking into account that  $\beta$ -hydroxyethylmaleinimide is the primary alcohol, the amidoalkylation reaction of nitromesitylene was carried out in the presence of a mixture of proton acids, sulfate, phosphoric acid. Despite the fact that the  $\beta$ -hydroxyethylmaleinimide of our choice is the

primary alcohol, the amidoalkylation reactions with it proceed under relatively mild conditions. This is due to the presence of two carbonyl groups in the  $\beta$ -hydroxyethylmaleinimide molecule, or the attraction of the electron cloud density of the functional groups to:



The reaction of nitromesitylene with  $\beta$ -hydroxyethylmaleinimide was studied in the presence of sulfuric, phosphoric acid mixtures. Reagents for the reaction were nitromesitylene:  $\beta$ -GEMI: con. $H_2SO_4/H_3PO_4$  in soluble benzene in a ratio of 1:1 mol, carried out for 3–4 hours.

Analysis of the reaction product by thin-layer chromatography revealed the formation of three new substances on the plate. A 3:1 molar ratio of benzene and acetone were used as the solvent for thin layer chromatography (Figure 1).



1. Reaction product:

- a) nitromesitylene,
- b) 2,4,6-trimethyl-3- ( $\beta$ -maleinimidoethyl) nitrobenzene,  $R_f = 0.64$
- c) 2,4,6-trimethyl-3,5-bis- ( $\beta$ ,  $\beta'$ -maleinimidoethyl) nitrobenzene  $R_f = 0.55$
- d) ester of  $\beta$ -hydroxyethylmaleinimide  $R_f = 0.41$

2. Nitromesitylene

3.  $\beta$ -hydroxyethylmaleinimide

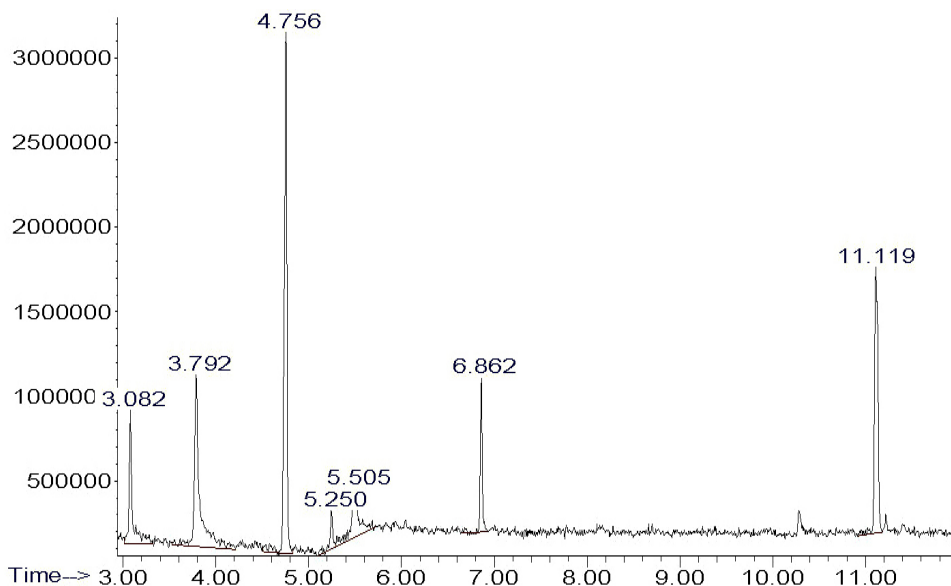
thin-layer chromatography (TLC) with plates (Pre-coated TLC sheets ALUGAM® Xtra SIL G/UV254), mobile phase-benzene: acetone 3:1.

Figure 1. Thin layer chromatography analysis

One of these substances are 2, 4, 6-trimethyl-3- ( $\beta$ -maleinimidoethyl) nitrobenzene ( $R_f=0.64$ ), the others are 2,4,6-trimethyl-3,5-bis-( $\beta$ ,  $\beta'$ -maleini-

midoethyl) nitrobenzene ( $R_f=0.55$ ), a ester of  $\beta$ -hydroxyethylmaleinimide ( $R_f=0.41$ ) as an additive was confirmed by chromato-mass spectrum (Figure 2).

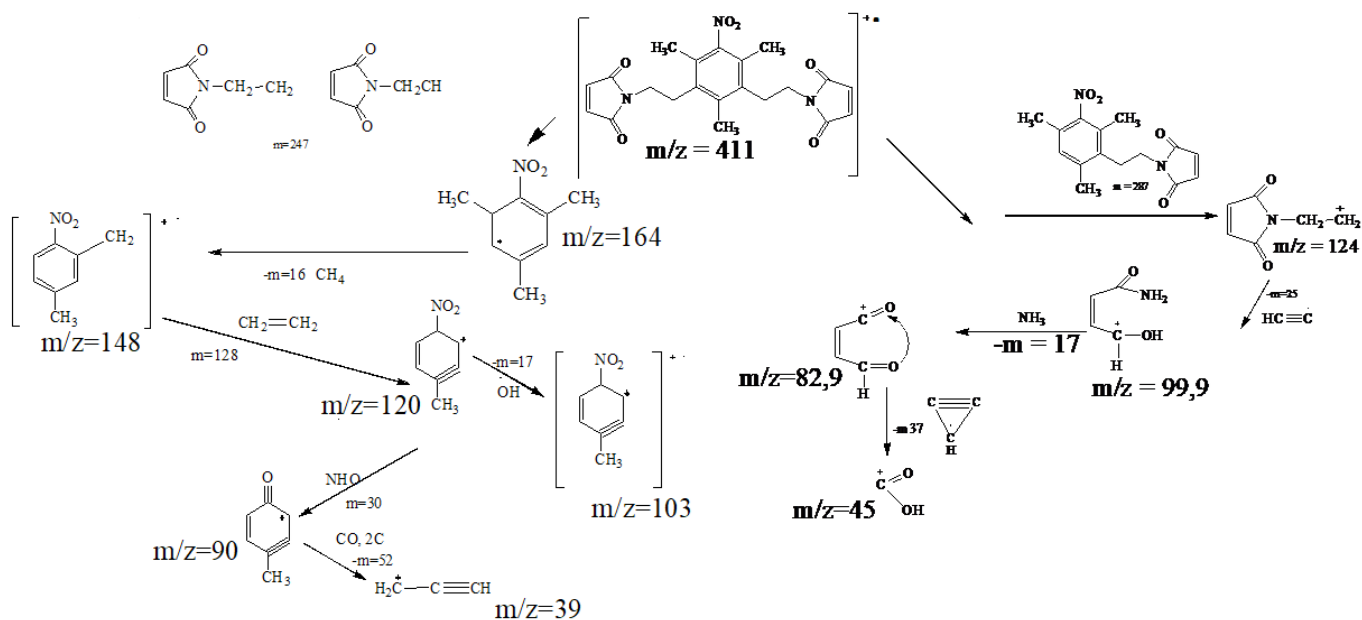
### Results of the analysis of the chromatomass spectrum of the substance obtained from the reaction of nitromesitylene with $\beta$ -GEMI in the device GC-MS



Peak	Ret Time	Width	Area	%	
1	3.082	0.047	19555992	9.68	1) Solvent benzene, 2) 2,4,6-trimethyl-3,5-bis-( $\beta$ , $\beta'$ -maleinimidoethyl) nitrobenzene, 3) 2,4,6-trimethyl-3-( $\beta$ -maleinimido-ethyl) nitrobenzene, 4) ester of $\beta$ -hydroxyethylmaleinimide, 5) nitromesitylene
2	3.792	0.093	41587922	20.03	
3	4.756	0.056	64766582	32.05	
4	6.862	0.029	13780932	17.66	
5	11.119	0.065	40480336	20.58	

Figure 2. Results of chromatomass spectrum analysis in GC-MS

### Formation of fractions of ions of 2,4,6-trimethyl-3,5-bis-( $\beta$ , $\beta'$ -maleinimidoethyl) nitrobenzene



$\beta$ -hydroxyethylmaleinimide is selected as the reagent of amidoalkylation, and forms simple esters, such as primary alcohols.  $\beta$ -hydroxyethylmaleinimides form a simple ether of benzene at a boiling point of 80–85 °C. It is known from the studied literature that the simple esters formed by reagents of amidoalkylation containing the hydroxyl group can also be reagents of amidoalkylation for the reaction. However, simple esters are reagents with low electrophilic ability compared to amidoalkylating alcohols. In order to activate the simple esters formed in amidoalkylation reactions. To increase the reactivity, it is necessary to add an extra amount of catalyst to the reaction

mixture. While part of the extra catalyst is used to decompose the ordinary ether, the other part acts as a catalyst for the amidoalkylation reaction.

However, increasing the catalyst in the reaction requires slightly milder conditions, as the reaction undergoes a polymerization reaction due to the active double bond of the amidoethylation reagent  $\beta$ -hydroxyethylmaleinimide. The reaction resulted in the formation of two new substances – 2,4,6-trimethyl-3-( $\beta$ -maleinimidoethyl) nitrobenzene, 2,4,6-trimethyl-3,5-bis-( $\beta$ ,  $\beta'$ -maleinimidoethyl) nitrobenzene are seen.

The reaction equation is as follows:

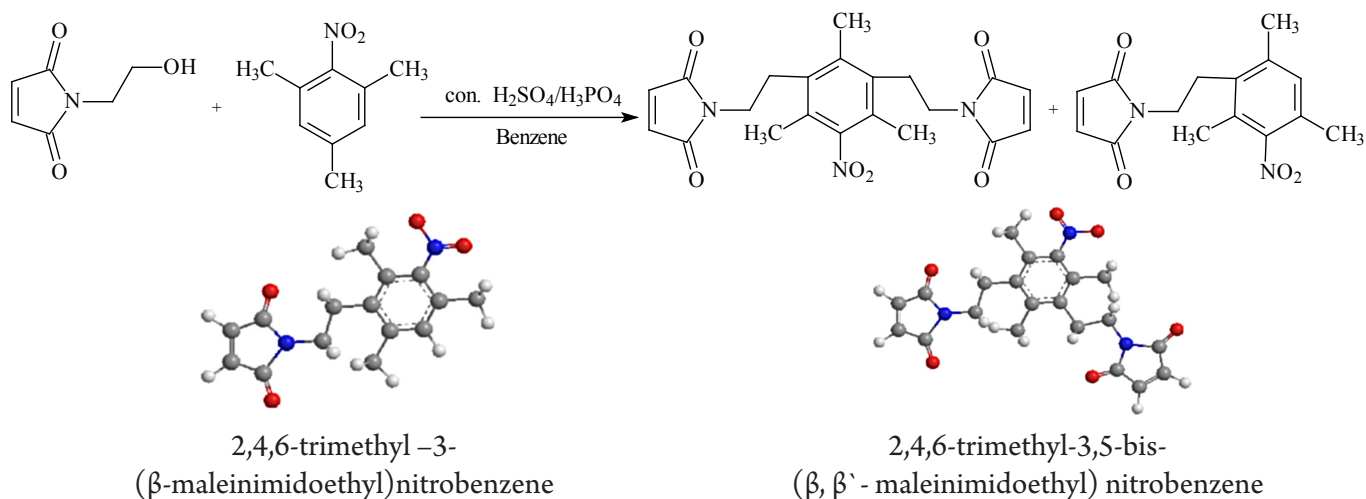
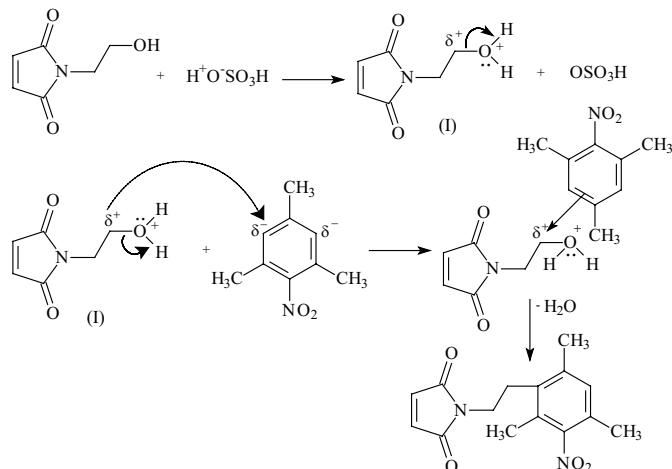


Figure 3. 3D view of the items

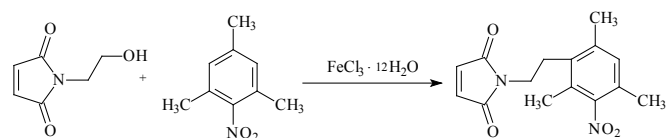
C-amidoalkylation reactions of aromatic compounds follow the  $S_EAr2$  electrophilic substitution mechanism, such as alkylation and acylation reactions.

The reaction mechanism is as follows:



Subsequent amidoalkylation reactions were performed with  $\beta$ -chloroethylmaleinimide. For the amidoalkylation reaction of nitromesitylene with N-XEMI in the presence of a catalyst  $FeCl_3 \cdot 12H_2O$  was carried out at a ratio of 3:1:  $2.64 \times 10^{-4}$  moles, at 100 °C. The reaction revealed that only the mono-substituted product 2-(N-maleinimidoethyl) –1,3,5-trimethyl nitrobenzene was formed with 58% yield, no additional substance was formed. The reaction conditions are given in Table 1.

The reaction equation is as follows:



The reaction mechanism is as follows:

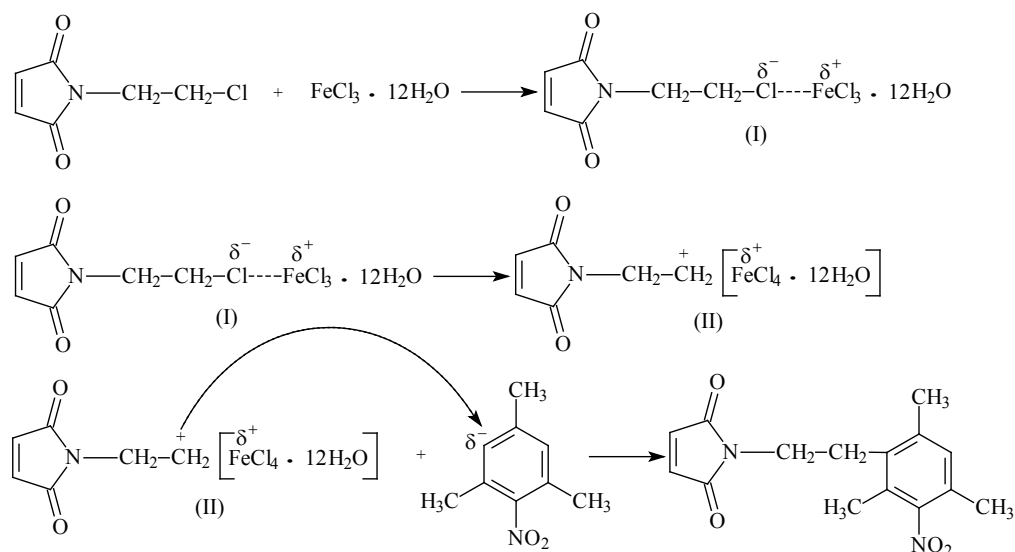


Table 1. – Nitromesitylene with  $\beta$ -chloroethylmaleinimide  $\text{FeCl}_3 \cdot 12\text{H}_2\text{O}$  conditions of amidoalkylation of reaction in the presence of a catalyst

№	Substrate	Mole ratios of reagents substrate: $\beta$ -ChEMI: cat.	Reaction temperature, °C	Reaction duration, hours	The product formed with $\beta$ -chloroethylmaleinimide substrate	
					%	T <sub>liquid</sub>
1.	Nitromesitylene	3:1:2,64×10 <sup>-4</sup>	100	3	58	128–129
2.	Nitromesitylene	2:1:2,64×10 <sup>-4</sup>	100	3	55	128–129
3.	Nitromesitylene	1:1:2,64×10 <sup>-4</sup>	100	4	50	128–129

It can be seen from the table data that increasing the amount of substrate from 1 to 3 and increasing the reaction time leads to an increase in yield. If the reaction is carried out at a temperature above 100°C, the reaction will lead to resinization of the mixture. This is due to the fact that the polymerization is tolerated at

the expense of the active double in maleinimide. When the reaction mixture was examined on the skirt layer chromatography, it was seen that a single new product was formed. No additional reaction was found. A 3:1 molar ratio of benzene and acetone was used as the solvent for thin-layer chromatography. (Figure 4)

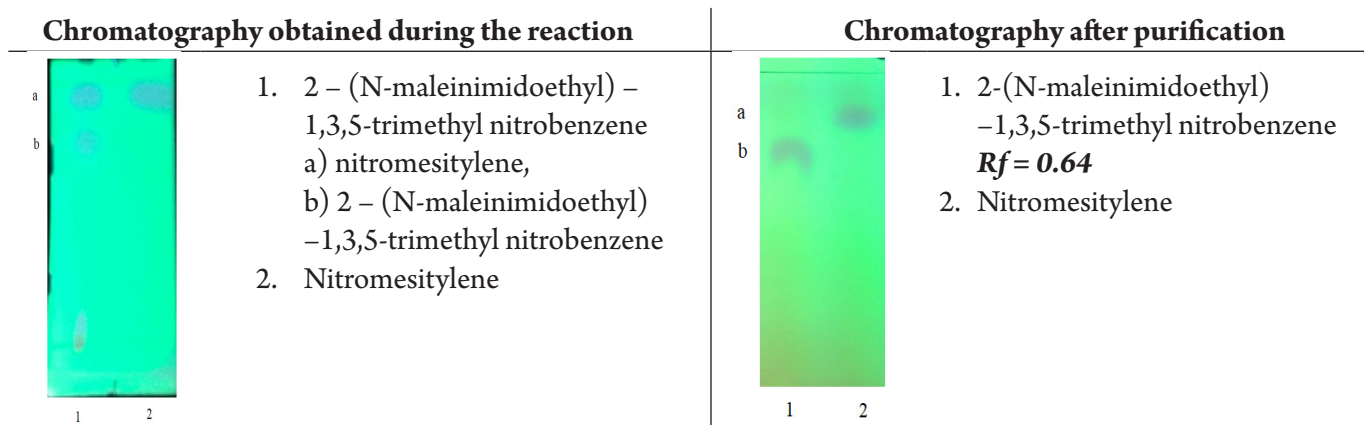


Figure 4. Thin layer chromatography analysis



The reaction substance was recrystallized in ethyl alcohol and chromatographed again.

### IR spectral analysis

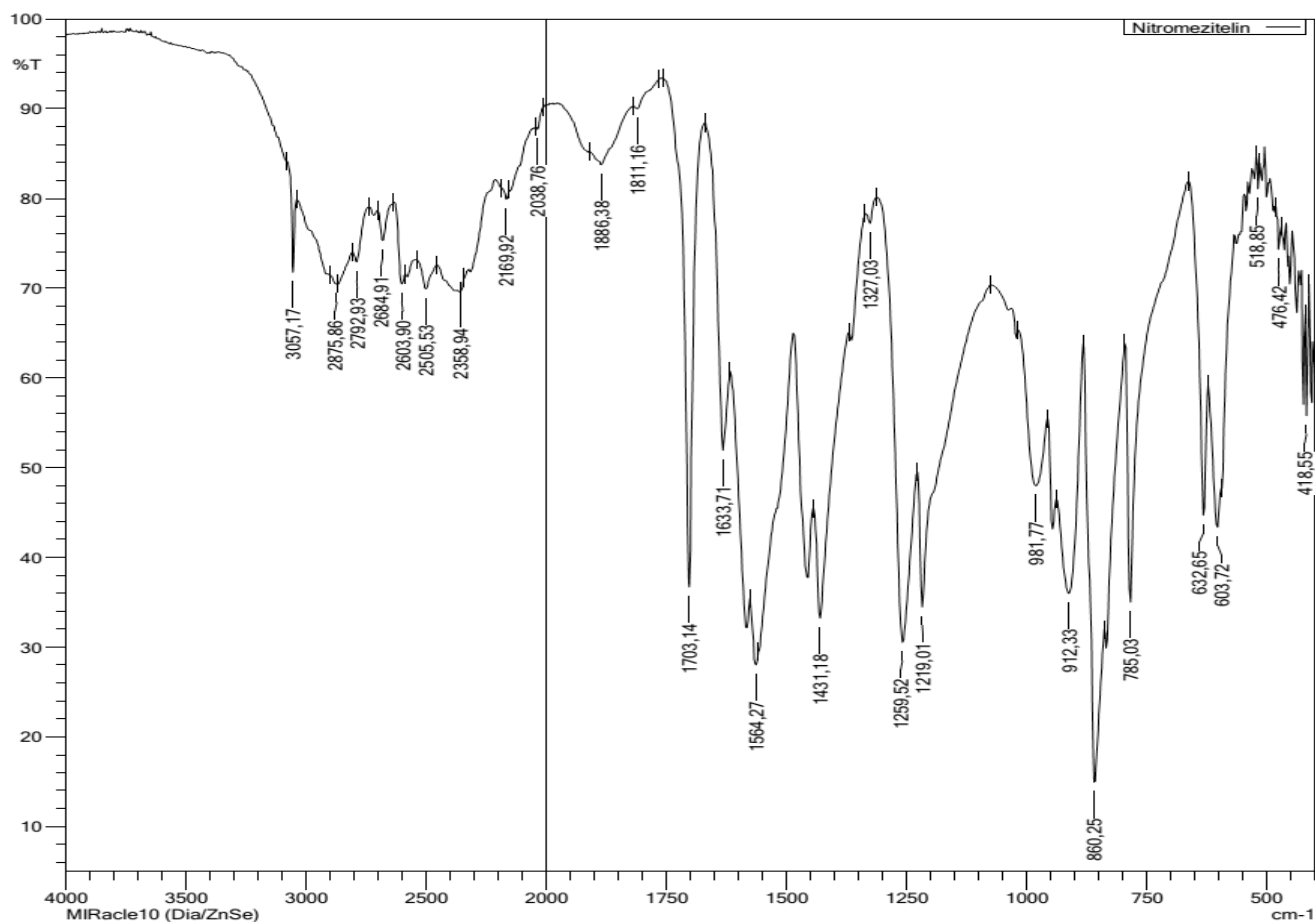


Figure 5. IR spectrum of 2-( $\beta$ -maleinimidoethyl)nitromesitylene

**IR spectrum:** Infrared Fourier spectrometer “IRTracer-100” (SHIMADZU CORP., Japan 2017) in complete with the prefix broken total internal reflection (NIP) MIRacle-10 c prism diamond/ZnSe (spectral range on the scale of wave numbers-4000÷400  $\text{cm}^{-1}$ ; resolution – 4  $\text{cm}^{-1}$ , sensitivity signal-to-noise ratio-60,000:1; scanning speed-20 spectra per second).

When we studied the IR spectrum, we found that there were the following absorption lines: deformation and valence oscillations specific to the aromatic ring in the fields 785, 860, 912, 981 and 1633 ( $\text{cm}^{-1}$ ); Deformation and valence oscillations of the  $\text{HC}=\text{CH}$  group in the branches 1219, 1259,

1654 and 2505 ( $\text{cm}^{-1}$ ), deformation and valence oscillations of the methylene groups in the branches 1431 and 2875, 2792 ( $\text{cm}^{-1}$ ), 1633, 1703 ( $\text{cm}^{-1}$ ). in the presence of valence oscillations of the nitrogen-bound carbonyl group. One of the frequencies with an intense appearance was the oscillation of nitro compounds ( $\text{NO}_2$ ) in this aromatic ring in the valence symmetric 1259.52  $\text{cm}^{-1}$  regions (Figure 5).

**Results of analysis.** As a result of electrophilic substituted reaction of nitromesitylene with  $\beta$ -chloroethylmaleinimide in the presence of iron (III) chloride crystal hydrate mono product with 58% yield 2-(N-maleinimidoethyl)-1,3,5-trimethyl nitrobenzene and  $\beta$ -hydroxyethylmaleinimide in

the presence of sulfate with sulfate as a result, mono with 39,05% yield and bis product with 20,03% yield were formed to form 2,4,6-trimethyl-3,5-bis-( $\beta$ ,  $\beta'$ -maleinimidoethyl)nitrobenzene. While the dialysis product was formed during the amidoethylation reaction of nitromethylene with  $\beta$ -hydroxyethylmaleinimide, the formation of a mono product was determined by the amidoethylation with  $\beta$ -chloroethylmaleinimide.

**Conclusion and suggestion.** Electrophilic substituted reactions in nitromethylene, which contains one electron acceptor and three electron donor groups in the agreed state, were carried out with derivatives of maleinimide occupied by halogen and

hydroxyl groups. Changes in product yield and composition were determined according to the type of electrophilic reagent obtained for the reaction. It can be concluded that if there are only strong reference electron donor groups in the aromatic ring and the negative charge value in the benzene ring increased, conducting reactions in the presence of strong proton catalysts such as sulfuric acid at low temperatures gives good results. Additionally, if the aromatic ring contains an electron-accepting group, such as a nitro group, which reduces the negative charge value of the ring along with the electron-donor groups, more stringent conditions are required for the reaction to proceed.

### References:

1. Mikhaylin Yu. A. Heat-resistant polymers and polymeric materials. SPb.: Profession. 2006.– P. 528–623.
2. Hiran B.L., Barna Shivira, Singh Divya, Jain Sarna. Synthesis of new maleimide monomers and their copolymerization. *Der Pharma Chemica*, 2011.– 3 (2).– P. 5–12.
3. Mikhaylin Yu.A., Miychenko I. P. Maleimide binders (review). *Plastics*. 1992.– No. 5.– P. 56–64.
4. Svetlichny V. M., Kudryavtsev V. V. Polyimides and the problem of creating modern structural composite materials. *High molecular weight compounds. Series B*. 2003.– Vol. 45.– No. – 6.– P. 984–1036.
5. Jyoti Chaudhary<sup>1</sup>, Swati Purohit<sup>1</sup>, Supriya Dadhich, Radha Chaudhary // Synthesis and Characterization of Maleimide-Epoxy Resins and Composites Formation/*Der Pharma Chemica*, 2017.– 9(3).– P. 1–4.
6. Kablov E. N. Innovative developments of FSUE “VIAM” SSC RF on the implementation of “Strategic directions for the development of materials and technologies for their processing for the period up to 2030” // *Aviation materials and technologies*. 2015.– No. 1. (34).– P. 3–33. DOI: 10.18577 / 2071–9140–2015–0–1–3–33.
7. Kablov E. N. Chemistry in Aviation Materials Science // *Russian Chemical Journal*. 2010.– T. LIV.– No. 1.– P. 3–4.
8. Yang Y. et al. ‘OFF-ON’ fluorescent probe for specially recognize on Cys and its application in bioimaging // *Dyes and Pigments* – 114. 2015.– P. 105–109.
9. Ana Maria Scutaru, Maxi Wenzel, Ronald Gust Bivalent benadamine and melphalan derivatives as anticancer agents // *European Journal of Medicinal Chemistry*,– 46. 2011.– P. 1604–1615.
10. Yuldasheva Mukhabbat, Turaeva Khurshida, Kholiqov Tursunali. Amidoalkylation of aromatic compounds. *Bulletin of National University of Uzbekistan: Mathematics and Natural Sciences*.– Vol. 1.– Issue 4. 2018.– P. 150–161.
11. Vovk M. V., Bolbut A. V. Intramolecular amidoalkylation of N-(1-aryl-2,2,2-trifluoroethylidene) -O-(3-diethylaminophenyl) urethanes // *J. Org. Chemistry*. 1998.– T.-34.– Issue 4.– P. 628–629.

---

---

---

## Contents

<b>Section 1. Biology</b> .....	<b>3</b>
<i>Bagdasaryan Samvel, Babayan Bella, Melkumyan Marina, Kinosyan Marina</i> POLYSORBATES BIODEGRADATION POTENTIAL AND PLASMID STABILITY OF SOIL PSEUDOMONAS .....	3
<b>Section 2. Information technology</b> .....	<b>8</b>
<i>Fesokha Vitalii Viktorovich, Subach Ihor Yuriiovich, Kubrak Volodymyr Oleksandrovych,</i> <i>Mykytiuk Artem Viacheslavovych, Korotaiev Stanislav Oleksandrovych</i> ZERO-DAY POLYMORPHIC CYBERATTACKS DETECTION USING FUZZY INFERENCE SYSTEM .....	8
<b>Section 3. Medical science</b> .....	<b>14</b>
<i>Hruzevskyi Olexander</i> A STATUS OF CELLULAR IMMUNITY IN BACTERIAL DYSBIOSIS AND BACTERIAL VAGINOSIS .....	14
<i>Tabatadze Nino, Tsomaia Irma, Chikovani Anton, Gigoshvili Tamar</i> BIOLOGICALLY ACTIVE SUBSTANCES OF CEPHALARIA GIGANTEA GROWING IN GEORGIA .....	22
<b>Section 4. Technical sciences</b> .....	<b>26</b>
<i>Abdulkarimov Mirzohid Muratovich, Yodgorov Normahmad, Jalilov Abdulahad Turobovich</i> SYNTHESIS OF NEW ANTICORROSION COATINGS BASED ON GOSSIPOL RESIN .....	26
<b>Section 5. Chemistry</b> .....	<b>32</b>
<i>Azimova Gulmira Zayniddinovna, Yuldasheva Mukhabbat Razzokberdiyevna,</i> <i>Tadjimuhamedov Khabibullo Sayfullaevich</i> INFLUENCE OF A NANOSTRUCTURAL CATALYST IN THE SYNTHESIS OF ALLYLNAPHTHOLS .....	32
<i>Ziyaeva Sitora F., Vasina Svetlana M.</i> PHYSICO-CHEMICAL FUNDAMENTALS TECHNOLOGY PRODUCTION OF MULBERRY JUICE CONCENTRATE .....	37
<i>Kabulova Lola Baltamuratovna, Xojametova Bibimariyam</i> ACTIVATED TUFFITE AS AN ACTIVE POZZOLANIC ADDITIVE .....	44
<i>Makhmudov Mukhtor Jamolovich, Axmedov Ulug` Karimovich</i> MODERN METHODS OF REDUCING THE CONTENT OF AROMATIC HYDROCARBONS IN GASOLINE .....	49
<i>Rakhmatova Guzal Botirovna, Kurbanov Mingnikul Jumagulovich,</i> <i>Turabayeva Nargisa Bekmuradovna, Tursunova Gulnoza Qaxxarovna</i> STUDY OF INSPACTIVE PROPERTIES AGAINST CORROSION OF $\alpha$ -AMINOCETONES AND THEIR PRODUCTS .....	54
<i>Turaeva Khurshida Kamalbaevna, Yuldasheva Mukhabbat Razzoqberdiyevna</i> REACTIONS OF NITROMEZITILENE WITH SUBSTITUTED ALKYLMALEINIMIDES .....	60