

Composite Medicine “Azisal” Based on Azithromycin and Salicylic Acid

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Abstract

Salicylic acid essentially is obtained under the pressure by the method of Kolbe-Schmidt. One of the biggest drawbacks of this method is the necessity of synthesizing sodium phenolate in advance which involves considerable technological difficulties: water predistillation under vacuum and also the dry sodium phenolate getting very hygroscopic. It is therefore of interest to look for more convenient alternative pathways for the synthesis of salicylic acid, excluding the use of sodium phenolate and this drawback is eliminated by using sodium and potassium salts of ethyl carbonic acid as carboxylation body. Consequently, according to the more convenient method we obtained the salicylic acid. In medicine, 1% solution of salicylic acid in 70 % alcohol called salicylic alcohol is used as an antiseptic. We investigated the antimicrobial activity of a 1% solution of salicylic acid in various concentrations of ethanol (40%, 50%, 60%, 70%, 80% and 90%) in order to determine the effect of different concentrations of ethanol on the antimicrobial activity of salicylic acid. The experiment proved that 1% solution of salicylic acid in various concentrations of ethanol (40-90%) to the appropriate strains of bacteria acts with the same activity regardless of the concentration of ethanol (40%, 50%, 60%, 70%, 80% and 90%). These actions of the acid are due to its solubility in alcohols of different concentrations and complete disintegration of salicylic acid molecules into ions. Thus, on the basis of antimicrobial research the necessity of preparation of 1% solution of salicylic acid in 40% alcohol is proved as the drug is cheaper and cost-effective to produce. The technology of the new composition of the drug “Azisal” consisting of 0.25 g azithromycin and 1.0 g salicylic acid in 60% ethanol was developed, in a similar way solution of azithromycin in different concentrations 0.25, 0.5, 0.75, 1.0% in 60% ethanol were prepared and their antimicrobial activities were defined. The comparison of their antimicrobial activity shows the effectiveness of the composite product called “Azisal”.

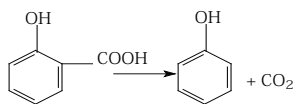
Introduction

Organic synthesis is the most important and complex part of modern pharmaceutical science. Product manufactured by the chemical-pharmaceutical industry is widely used in various fields of medicine. They are the synthetic chemotherapeutic drugs, vitamins, hormones, food additives etc. [1, 2]. Nowadays the synthesis of biologically active substances is extremely popular and has good prospects. In modern medicine there remains important problem of combating pyogenic infection, despite the large arsenal of antibiotics. In the structure of the population with infectious pathologies the local processes of various sites caused mainly by pathogenic microorganisms take an important place. In the treatment of local inflectional processes priority is given to the antiseptics, anti-inflammatory drugs and antibiot-

ics. However, most of them do not have the required properties such as a broad spectrum of antimicrobial activity and at the same time ability to selectively subdue the synthesis of prostaglandins and histamine in inflamed cells, normalize metabolism in tissues of lesion. Therefore the creation of composite formulations offer the opportunities to provide the practical medicine with the active therapeutic remedies, allow the simultaneous treatment of several diseases, expand the range of their application and reduce the dosage of drugs [3-5]. A promising direction is to create the integrated medicines based on a combination of antiseptics, anti-inflammatory drugs and antibiotics with the drugs of the different pharmacological groups. The optimum combination of antiseptics and the drugs of the different pharmacological groups will create synergies and multi-direction of the pharmacological actions and reduces the

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side effects of the latter. There are various methods and techniques of treating lesions but none of them fully meets the requirements of modern surgery [6, 7]. Practically, the most important group of medial means are chemotherapy, antiseptic and anti-inflammatory remedies. Considering above-mentioned, we focus on the drug known as salicylic alcohol or 1% solution of salicylic acid in 70% alcohol. Since this drug is widely used as an antiseptic in many countries and contains of 1% salicylic acid. It is found in the willow bark and its medicinal properties have been known since antiquity. To use the decoction of willow bark as an antipyretic, analgesic, anti-inflammatory remedy had been recommended even by Hippocrates. German chemist Buchner isolated a substance called salicin from the willow bark (from Latin – Salix for willow). Later pure salicylic acid was acquired from salicin and its healing effects were proved. Salicylic acid has found its usage in medicine; its solution salicylic alcohol is used to cure the skin inflammation and is found in the ingredients of many cosmetic lotions. Salicylic acid was obtained first by Piri in 1838 by oxidation of the salicylic aldehyde. The structure of salicylic acid as the 2-hydroxybenzoic acid was found in 1853 by Kolbe, while heating he observed its decomposition into phenol and carbon dioxide.



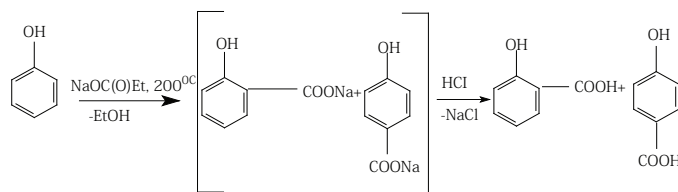
Synthesizing the salicylic acid according to Kolbe-Schmidt method the reaction occurs in the presence of the very hygroscopic reagent sodium phenolate. Consequently, the presence of water in the reaction's environment brings the different complications, reduces the obtainment of salicylic acid.

That is why it is important to find more convenient alternative ways for the synthesis of salicylic acid excluding the use of sodium phenolate.

Experimental

There was only one work in literature containing just fragmentary information about the application of the sodium and potassium salts of ethyl carbonic acid as carboxyl agent in the carboxylation reaction of phenol. By slowly heating up the mixture of phenol with the sodium ethyl carbonate suspension in ethanol to 175 Celsius degrees simultaneously evaporating the solvent (up to 100 Celsius degrees, 1 atm) and the uncreated phenol particles (up to 170 Celsius degrees, low pressure) and following usual treatment of the reactive mixture (treatment with water, extraction with ether, acidification the aqueous phase with acid) 50% salicylic acid was obtained.

When conducting carboxylation of phenol with sodium ethyl carbonate in work conditions [7, 8] we did not acquire the salicylic acid due to the strong resinification of the mixture because of the oxidation processes of condensation and compression. We studied some predictable progressions of carboxylation reaction of phenol with sodium ethyl carbonate and found the optimum conditions to conduct the process excluding above-mentioned unwanted oxidation processes. It is revealed that the sodium salt of ethyl carbonic acid can be successfully used in the carboxylation reaction of phenol and carboxylation goes mainly in the o-position forming salicylic acid. It is found that the reaction should be done in the inert gas environment (argon, carbon dioxide) as in the atmosphere the obtaining of the salicylic acid does not exceed 23-26% due to oxidative condensation. The affection of temperature, pressure and duration of reaction time on the process of reaction was defined.



The optimum condition for carboxylation reaction of phenol with sodium ethyl carbonate were $P_{ArCO_2} = 1.0$ MPa, $T = 200$ °C, $t = 6$ h in which the total input of o- and p-isomers of hydroxybenzoic acid is 87.5% (70% salicylic acid and 17.5% p-hydroxybenzoic acid). A by-product of this process – p-hydroxybenzoic acid is used as a food preservative and for the preparation of polymeric materials and liquid crystal polyesters with high heat resistance. The lab-

oratorial and technological order was worked out to get salicylic acid by the developed new methods in accordance with the requirements of normative and technical documentation. The issues defining the most appropriate optimum technological plans of production, the quality requirements for raw materials, the methods of separation of o- and p-isomers of hydroxybenzoic acid, cleaning the product (salicylic acid) and recovery of uncreated phenol were

worked out. At the chair of Pharmaceutical Chemistry of S.D. Asfendiyarov Kazakh National Medical university salicylic acid obtained by the new method was fully pharmacopoeial analysed. The test of the drug to meet the requirements of the normative and technical documentation for salicylic acid include the following quality indicators: description, solubility, the authenticity, the melting temperature, clarity and colour of the solution, chlorides, sulphates, organic impurities, dyes and phenol, hydroxybiphenyl, sulphate ash and heavy metals, loss of mass while drying and quantifications. All tests were conducted according to the procedures of SP 12th edition. According to the tests' results quality of salicylic acid obtained by the new method is fully corresponds to the VFS requirements. High quality of salicylic acid obtained by the new method is also confirmed by IR, NMR. In the end the simple and convenient way to acquire salicylic acid using carboxylation of phenol with sodium ethyl carbonate was developed. IR spectrums were recorded by UR-20 (Germany) spectrophotometer in KBr tablets; PMR spectrums by "Tesla"-BS 487 (60 MHz) device in the 10% DMCO-d₆ solution; the shifts were given relative to TMS. There are the bands in the IR spectrum of salicylic acid corresponding to aromatic bonds (Ar) in the field of 700, 1610, 3020 cm⁻¹; and C=O bonds (carbonyl) in the field of 1670 cm⁻¹; OH-group in the field of 3240 cm⁻¹ (Table 1).

In the carboxyl group of salicylic acid the hydrogen is in the form of hydride ion, so it cannot be detected by the device "Tesla"-BS 487 (60 MHz). While recording PMR spectrum of salicylic acid its aromatic protons appear as a multiplet (due to no equivalence of protons from aromatic carbon) and is located in the 7.66 ppm area corresponding to the 4th protons. Hydroxyl proton of aromatic carbon appears as a singlet in the 11.7 ppm area corresponding to 1 ppm proton (Table 1).

Table 1
IR-, PMR-spectrums of salicylic acid.

#	Frequency of valence oscillation (γ), cm ⁻¹				Values of chemical shifts (σ), ppm		
	Ar	Alk (CH ₃)	C=O	OH alcohol	Ar	Alk (CH ₃)	OH alcohol
1.	700, 1610, 320	-	1670	3240	7,56 (m)	-	11,7 (s)

Results and Discussion

Salicylic acid is used in medicine as an antiseptic, distracting, stimulating and keratolytic powder

(2-5%) and (1-10%) in ointments, pastes, alcohol (1-2%), as the solvent in all cases 70% ethyl is taken and this preparation is called salicylic alcohol.

By its pharmacological action salicylic acid belongs to the non-steroidal anti-inflammatory remedies, which includes the following as well:

- Acetylsalicylic acid
- Derivatives of anthranilic acid (or o-amino-benzoic acid) – mefenamic acid, flufenamic acid
- Derivatives pyrazolone– butadion, amidopyrine
- Derivatives indoluxus acid – indomethacin
- Derivatives phenylacetic acid– ortofen
- Derivatives phenylpropionic acid – ibuprofen
- Derivatives neftilproiionic acid – naproxen

Non-steroidal anti-inflammatory remedies have anti-inflammatory, analgesic, antipyretic effects.

The anti-inflammatory effect of these drugs is linked to their inhibitory effects on the cyclooxygenase enzyme which is necessary for the synthesis of cyclic endoperoxides. This reduces the production of prostaglandins. This in turn leads to the reduction of the inflammation symptoms such as hyperaemia, swelling, pain.

Non-steroidal anti-inflammatory remedies also have analgesic and antipyretic effects. Analgesic effect of the most medications is due to prostaglandins reducing the sensitizing effect on the sensitive ends by reducing the effects of "inflammatory mediators" (bradykinin) and other types of stimuli on them. Also the non-steroidal remedies reduce the pain. Antipyretic effect of considering drugs is also linked to the inhibition of the biosynthesis of prostaglandins E1 and the reduction of pyrogenic effect on hypothalamic thermoregulation center.

Among the various areas of modern clinical surgery the problems of local treatment of purulent lesions take a special place. Overseas and in our country research and groundwork are intensively conducted into new remedies. Many antimicrobial drugs can cure the various diseases caused by bacteria [11-13] in order to determine the effect of different concentrations of alcohol on the antimicrobial activity of the active ingredient the authors prepared 1% solution of salicylic acid in 6 samples in the different concentrations of ethanol (40%, 50%, 60%, 70%, 80%, 90%). The technological process of the production of 1% salicylic acid solution in the different concentrations of alcohol consists of 2 technological processes, 3 supplementary works, 3 stages of PLS.

Laboratory tests of the antimicrobial activity of salicylic acid were conducted according to the existing methods of studying the disinfecting activity of disinfectants as the test samples were used the microorganisms which are recommended for such research: strains of gram-positive bacteria *Bacillus*

Subtilis, *Staphylococcus aureus*, strains of gram-negative *Escherichia coli* and *Candida albicans* by the diffusion method (holes).

The test results of antimicrobial activity of the solution of salicylic acid (1-6 samples) are shown in the Table 2 and the Fig. 1.

Table 2

#	Strains	1% solution of salicylic acid					
		1 sample	2 sample	3 sample	4 sample	5 sample	6 sample
		40% alcohol	50% alcohol	60% alcohol	70% alcohol	80% alcohol	90% alcohol
1	<i>S.aureus</i>	13 ± 0.2	13 ± 0.2	13 ± 0.2	13 ± 0.2	13 ± 0.3	13 ± 0.2
2	<i>Bacillus Subtilis</i>	13 ± 0.3	13 ± 0.3	13 ± 0.3	13 ± 0.3	13 ± 0.3	13 ± 0.3
3	<i>Escherichia coli</i>	12 ± 0.2	12 ± 0.2	12 ± 0.2	12 ± 0.2	12 ± 0.2	12 ± 0.2
4	<i>Candida albicans</i>	11 ± 0.2	11 ± 0.2	11 ± 0.2	11 ± 0.2	11 ± 0.2	11 ± 0.2

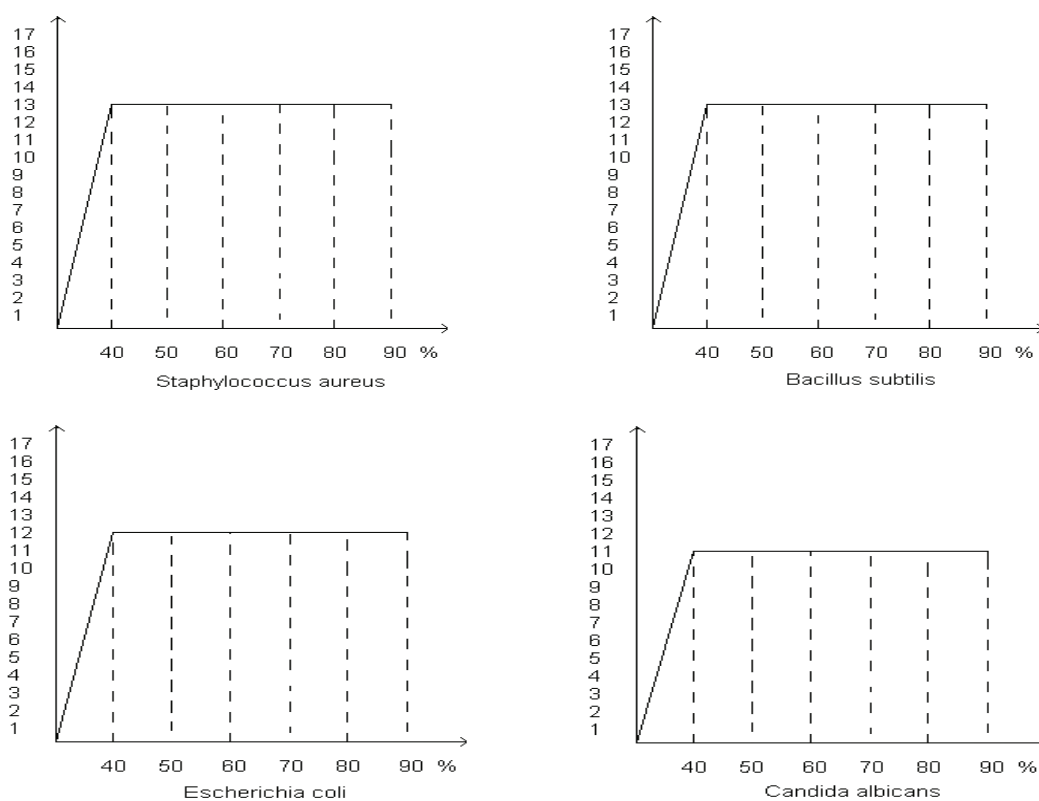


Fig. 1. Graphic representation of the 1% alcohol solution of salicylic acid in various concentrations of ethanol (1-6).

On the basis of biological studies of alcohol solutions of salicylic acid and comparing the data in the Table 2 and the Fig. 1 we found that the 1% solution of salicylic acid affects the appropriate strains of bacteria with the same activity regardless of the concentration of ethanol (40%, 50%, 60%, 70%, 80%, 90%). It is shown in the Table 2 and the Fig. 1. This action of the acid is due to its solubility in dilute alcohol and complete disintegration of salicylic acid molecules into ions. In addition, alcohol and salicylic acid have

the stimulating, anti-inflammatory effects, i.e. synergistic effect. Antimicrobial tests proved the necessity of preparing the 1% solution of salicylic acid in 40% alcohol in order to obtain the cheaper drugs than existing ones, i.e. getting the 1% solution of salicylic acid in 40% alcohol would be more cost effective.

Recently, when the pharmaceutical market is getting filled with the new kind of drugs of the previously unknown manufacturers and suppliers the quality of those remedies is questionable. Therefore

the orientation of domestic pharmaceutical companies to GMP positions ensures the development and production of effective, competitive remedies and will meet the need of public health care. The use of drugs in medical practice involves the use of effective medicines. Rationally chosen LF provides to use the maximal therapeutic effect of the drug with minimal side effects, significantly change the effect of substance, i.e. accelerate or extend the pharmaceutical effect, to regulate the absorption and expelling, allergenic effects, improve the organoleptic properties of LF. In this case the remedies should meet the following requirements such as bioavailability of the medical substance in the given form and appropriate pharmacokinetics, equally dissemination of the drug substances in the supplementary substances and hence the accuracy of dosing them while storing, compliance with microbial contamination, convenience of administration and simplicity of the preparation of the medicine.

Considering all the above-mentioned we focused on the medical solutions as they are widely used in medicine for internal use and also for external use and their sterilized forms are often used parenterally, moreover solutions are prepared easily. Various substances, mainly antiseptics, local anaesthetics, anti-mycotics, anti-bacterial, anti-inflammatory and painkillers can be added as components of the

solution. The widespread use of alcohol solutions is because of their easiness of production, various ways of administration, high stability in comparison with the aqueous solutions [13].

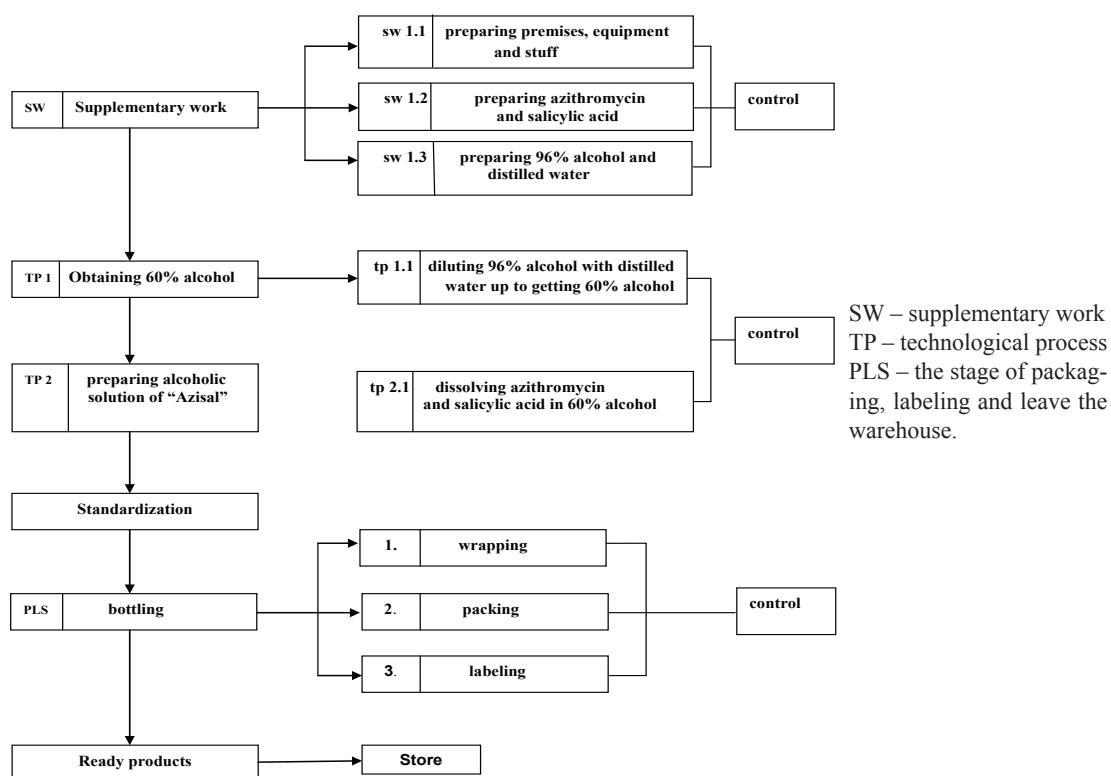
Earlier under the guidance of professor D.K. Kiyashev the composite product "Brisal" based on 40%, 50%, 60%, 70%, 80%, 90% ethanol, consisting of brilliant green and salicylic acid in different proportions of the ingredients was obtained. Continuing the research in this field in order to improve the therapeutic efficacy of the drug we have developed a new composite product called "Azisal" which contains azithromycin and salicylic acid in 60% ethanol to define the antimicrobial activity of the combined alcohol solution.

The literary data are taken for selecting the dosage of ingredients of solution. The component of the alcohol solution "Azisal" is given in the Table 3, the instrumental plan in the Scheme 1.

Table 3

Sample of the composite drug "Azisal"

#	Ingredients	
1.	Azithromycin	0.75 g
2.	Salicylic acid	1.0 g
3.	Ethyl alcohol 60%	100 ml



Scheme 1. Technological scheme of solution's production "Azisal".

Brief description of the research method of antimicrobial activity of the medicine "Azisal" and the azithromycin's solution in the different concentrations of alcohol.

The study of antimicrobial activity of the medicine "Azisal" was conducted in relation to the bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* by the diffusion method (holes). Testing samples were dissolved in 96% ethanol at the concentration of 1 mg/ml, they were grown at 37° Cel-

sus degrees during 18-24 hours. Culture was diluted in 0.9% sodium chloride solution, 1 ml bacteria was inserted into the cups with meet (hole) and *Candida* into Saburo environment, planted until obtaining "lawn". 96% ethanol was dripped into the shaped holes with 6 mm in diameter.

The test results of antimicrobial activity of the drug "Azisal" are given in the Table 4 and the other test results of the antimicrobial activity of azithromycin's solutions in the different concentrations of alcohol are given in the Table 5.

Table 4
Antimicrobial activity of the drug "Azisal"

#	Code	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
"Azisal"	DKK-4	29	7	27	10

Table 5
Antimicrobial activity of the azithromycin's solution in the different concentrations of alcohol

#	Code	Azithromycin in 60% alcohol	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
1.	DK-5	0,25	25	12	14	0
2.	DK-6	0,5	29	7	27	0
3.	DK-7	0,75	26	0	16	0
4.	DK-8	1,0	26	0	25	0

Based on the studies of the antimicrobial activity of azithromycin's solution in the different concentrations of alcohol (0.25-1.0) and combined drug "Azisal", comparing the data of the Tables 4 and 5 we found that the medicine "Azisal" shows maximum efficiency and also candid mycosis reaction to the fungi due to synergism effects of these 2 drugs: azithromycin and salicylic acid.

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