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^{1}H NMR ANALYSIS OF THE AQUEOUS-ALCOHOLIC MIXTURES, PREPARED IN DEMINERALIZED BY REVERSE OSMOSIS WATER

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Abstract: The aim of the publication is to study the mechanisms of transformation of ethanol protons (ethyl rectified spirit - ERS) and water (demineralized by reverse osmosis) in the process of creating aqueous-alcoholic mixtures (AAM). The methods used in the work: ^{1}H nuclear magnetic resonance (NMR) spectroscopy of AAM; methods of evaluation of physicochemical and organoleptic characteristics of water, ethanol, AAM. In this paper, we have established fundamentally new features in the process of creating AAM that are directly dependent on the time of contact with demineralized water and ERS. We have experimentally established the dependence rate of achievement of thermodynamic equilibrium and its character, as well as obtaining optimal organoleptic characteristics of AAM prepared with demineralized water and ERS.
Keywords: aqueous-alcoholic mixtures, demineralization, reverse osmosis water, ethanol protons, water.

Introduction

NMR spectroscopy is widely used in physics research. NMR accounts for about 90% of all research of the proton magnetic resonance spectroscopy (^{1}H NMR). Most of them operate in the Fourier transform mode.

The first ^{1}H NMR spectra of H_2O were obtained in 1946 (Bloch et al. 1946). The first ^{1}H NMR spectra of ethanol C_2H_5OH were developed in 1951 (Arnold et al. 1951). At the first glance, it may seem that these are fairly simple organic molecules, at the same time NMR spectroscopy exhibits grate variety (Nose et al, 2005; Richards, Hollerton, 2011; Roberts, 2002; Hu et al, 2010) in such characteristics as chemical shift, spin-spin interactions and the effect of chemical exchange.

An ethanol molecule consists of 6 protons located in a 3 proton-containing groups: methyl (CH_3), methylene (CH_2) and hydroxyl (OH) with a relative intensity characteristic CH_3:CH_2:OH - 3:2:1. This characteristic is proportional to the number of protons in each group.

Nuclear spin-spin interaction is observed between the three proton-containing groups of ethanol, all of which have different resonant frequencies (Roberts, 2002). “N” number of equivalent protons of one group split the signal of the nearest group into (n+1) lines with the intensity of a Pascal triangle (Richards and Hollerton, 2011). The ability to observe spin-spin interactions depends on the rate of the intermolecular proton exchange. Wherein the hydroxyl proton (OH) of ethanol can interchange with free hydrogen ions. The hydrogen ions are generated due to self-dissociation of water or traces of acids, alkalis or dissociated ethanol. The concentration of free ions is characterized by pH level.

In accordance to the requirements of the normative documents of Ukraine (DSTU 3297:95) vodka – is an alcoholic drink with strength from 37,5% to 56% (DSTU 4256:2003), obtained by mixing ERS (DSTU 4221:2003) with water, prepared in accordance with SOU 15.9-37-237:2005, and treated with activated carbon BAU-A, with addition of non-volatile ingredients or without them.

Previously, we have conducted primary research of ^{1}H NMR AAM, which were described in the work of Kuzmin et al, 2013. The obtained results give grounds to assert a fundamental difference in the behavior of the AAM prepared from the alcohol and water passing through various processes. This may indicate the presence of such features as separate signals of OH-protons of H_2O and EtOH. Also abnormal waveforms of CH_3 and CH_2 characterize a product with a lower tasting properties. The presence of the combined signal of H_2O+(EtOH) and rational form of CH_3 and CH_2 signals (triplet - for CH_3, quartet - for CH_2) - characterizes

the AAM with the best tasting properties. In this regard, we have established the system with steady and unsteady balance depending on transformation of hydroxyl protons of ethanol and water. Unsteady balance typical for AAM used with ERS «Lux» and drinking water, with a tasting score – 9,43 points. This also includes the AAM made from ERS «Lux» and demineralized water by reverse osmosis, with tasting score – 9,30 points. Systems with a steady balance that are typical for AAM made of ERS «Lux» and water softened by Na- cationization, with tasting score – 9,49 points were defined.

Thus, in the work of Kuzmin O. etc. (2013) established experimental evidence of instalment nature / (non-instalment) of thermodynamic balance, taking into account the organoleptic characteristics of AAM in dependence on water treatment method and time of system's functioning. However, the questions related to internal mechanism's specification and the rate of establishment of thermodynamic balance depending on type of water used in the process of creating the AAM are remain unsolved.

Therefore, the additional research is required for a detailed study of internal mechanism of thermodynamic balance and insurance in obtaining high quality vodka products - for each type of water separately.

Therefore, the aim of this work is implementation of a next stage - detailed study of mechanisms of transformation of ethanol protons (ERS) and water (demineralized by reverse osmosis) in the process of AAM creation.

Method

^{1}H NMR analysis of the AAM has been conducted in a certified laboratory of the Institute of Physico-Organic Chemistry and Coal Chemistry named after L.M. Litvinenko NAS Ukraine (Donetsk city). Physicochemical and organoleptic qualities of alcohol, water and AAM were carried out in laboratory of the following enterprises: LLC «Donetsk factory of liquor-vodka «Lik», Donetsk regional water test center.

^{1}H NMR analysis of AAM was conducted with the usage of the following: FT-NMR Bruker Avance II spectrometer (400 MHz) with operating frequency at ^{1}H - 400 MHz; specially shaped capillary with acetone-d_6 $(CD_3)_2CO$; high accuracy ampoules № 507-HP for high resolution NMR's spectroscopy (400 MHz); dispenser; ERS of class «Lux» as per DSTU 4221:2003, used at LLC «Donetsk factory of liquor-vodka «Lik»; demineralized water in accordance with SOU 15.9-37-237:2005 prepared at "Donetski factory of liquor-vodka "Lik". Specifications"; AAM from EAR of the class "Lux" and water demineralized by means of reverse osmosis.

Work methodology: 0,3 ml of a AAM prepared with a volumetric pipette with a predetermined strength (40,0 ± 0,2)% vol. External standard separated from the testing substance which is required for LOCK's system operation (deuterium solvent (acetone-d_6) of NMR's deuterium stabilization spectrometer) is added into an ampoule of special form capillary. ^{1}H NMR spectra records and data processing were performed according to the instruction of FT-NMR Bruker Avance II (400 MHz) spectrometer.

Results

The following characteristics were determined for water demineralized by means of reverse osmosis in accordance with SOU 15.9-37-237:2005 "Specifications for water prepared for liquor-vodka production": solid residual – 15 mg/dm^3; electrical conductivity – 20,7 μS/cm; pH – 5,05; ORP – "+" 393 mV; total hardness – <0,05 mM/dm^3; permanganate oxidability – 0,46 mg O_2/dm^3; MC of sodium – 11,60 mg/dm^3; MC of potassium – <2,0 mg/dm^3; MC of ammonium – <2,0 mg/dm^3; MC of calcium – <2,0 mg/dm^3; MC of magnesium – <2,0 mg/dm^3; total alkalinity – 0,15 mM/dm^3.

Characteristics of EAR of class "Lux" according to DSTU 4221:2003 "Specifications of Ethyl alcohol rectified." As follows: content of aldehydes in anhydrous alcohol, in recalculation on acetic aldehyde – 1,3 mg/dm^3; content of fusel oils in anhydrous alcohol: propyl, isopropyl, butyl, isobutyl and isoamyl – 1,5 mg/dm^3; content of esters in anhydrous alcohol, in recalculation on acetic-ethyl ether – 1,3 mg/dm^3; content of methanol in anhydrous alcohol – 0,0022 % vol.

AAM made of EAR of the class "Lux" and process water – demineralized by the reverse osmosis has the following characteristics: alcoholic strength – 39,92 % vol.; electrical conductivity – 3,5 μS/cm; ORP – "-" 98 mV; pH level – 7,60; content of aldehydes in anhydrous alcohol, in recalculation on acetic aldehyde – 1,8 mg/dm^3; content of fusel oils in anhydrous alcohol: propyl, isopropyl, butyl, isobutyl and isoamyl – 1,4 mg/dm^3; content of esters in anhydrous alcohol, in recalculation on acetic-ethyl ether – 1,4 mg/dm^3; content of methanol in anhydrous alcohol – 0,0021 % vol.; alkalinity – 0,4 cm^3 0,1 M of hydrochloric acid for titration

of 100 cm^3 sorting; oxidability test – 9 min.; taste evaluation – 9,30pointa (appearance – colourless liquid without sediment; odor – strong alcoholic; taste – sour and bitter, pungent).

The figure 1 shows the proton group's ^1H NMR spectras of freshly prepared AAM sample and a sample taken after few days, with an interval of 2-3 days with indication of chemical shift. The generalized characteristics of the spectra and the organoleptic characteristics of AAM are presented in table 1.

Table 1

Characteristics of the most important parameters for evaluation of chemical structure of AAM spectra in ^1H NMR spectroscopy

Characteristics	Signal	Time (τ), h					
		0	48	120	192	264	312
The chemical shift (δ), ppm	CH_3	1,08	1,08	1,07	1,08	1,08	1,08
The chemical shift (δ), ppm	CH_2	3,54	3,55	3,54	3,54	3,55	3,55
The chemical shift (δ), ppm	H_2O+(EtOH)	4,73	-	-	-	-	4,82
The chemical shift (δ), ppm	H_2O	-	4,78	4,75	4,74	4,80	-
The chemical shift (δ), ppm	EtOH	-	5,35	5,33	5,36	5,38	-
The chemical shift (δ), ppm	Acetone-d6	2,75	2,75	2,75	2,75	2,75	2,75
Organoleptic evaluation the AAM, point		9,30	9,28	9,28	9,28	9,28	9,30
- appearance		Colorless liquid with no sediment					
- the smell		Harsh, alcohol					
- the taste		Sweet and bitter, pungent					

Discussion

Will examine spectrum of hydroxyl group of water (H_2O), ethanol (EtOH), AAM (H_2O+EtOH) made of demineralized water prepared by means of osmosis and ERS of «Lux» at a different instants of system's operation (life after mixing) (h).

At the initial instant of AAM formation (τ=0 h) hydroxyl group of protons is represented as a single summary peak in the form of joint singlet H_2O+(EtOH) with a chemical shift of $\delta_{H2O+(EtOH)}$=4,73 ppm. Waveform is distorted Gaussian curve with a broadened base. The absence of separate peaks of OH ethanol (EtOH) and water (H_2O) may indicate that at the initial stage of AAM formation (τ=0 h) balanced structure of water in alcohol can be observed.

At the second spectrum (τ=48 h) the presents of two separate signals - OH of ethanol (C_2H_5OH) and water H_2O is observed in the area of hydroxyl protons of ethanol and water. Multiplet component of hydroxyl (OH) proton of ethanol (C_2H_5OH) is presented in a form of a barely noticeable bulge (early stage of signal generation) The bulge is based in a weak field with a chemical shift of δ_{OH}=5,35 ppm. Signal of water (H_2O) protons is presented as an elongated singlet of symmetrical shape with a broad base which is located at δ_{OH}=4,78 ppm. The difference in chemical shifts of OH proton (C_2H_5OH) and H_2O proton at this stage (τ=48 h) is $\Delta\delta_{OH}$=0,57 ppm.

The third spectrum (τ=120 h) is also characterized by the two single peaks - OH of ethanol (C_2H_5OH) and H_2O. The component of OH proton's multiplet C_2H_5OH is in the form of broad singlet with a chemical shift of δ_{OH}=5,33 ppm. H_2O component is in the form of symmetrical singlet with the broadened base and a chemical shift of δ_{OH}=4,75 ppm. The difference in a chemical shifts of OH proton C_2H_5OH and H_2O is $\Delta\delta_{OH}$=0,58 ppm.

The fourth spectrum (τ=192 h) is also characterized by the two single peaks - OH of ethanol and water. The OH component of proton C_2H_5OH is in a form of broad singlet with a chemical shift of δ_{OH}=5,36 ppm. Component of H_2O protons is in the form of symmetrical shaped singlet with a broadened base and an apex of irregular shape, with a bulge in the left part of a chemical shift of δ_{OH}=4,74 ppm. The difference in a chemical shifts of OH proton C_2H_5OH and H_2O is $\Delta\delta_{OH}$=0,62 ppm.

The fifth spectrum (τ=264 h) is also characterized by a separation of peaks - OH of ethanol and water. OH proton's component of multiplet C_2H_5OH is presented in a form of as barely noticeable bulge, which is located in a weak field with a chemical shift of δ_{OH}=5,38 ppm. H_2O component is presented in a form of almost symmetrical shaped singlet with a broadened base and apex of irregular shape due to a significant bulge in the left part of the signal with a chemical shift of δ_{OH}=4,80 ppm. The difference in the chemical shifts of OH proton C_2H_5OH and H_2O is $\Delta\delta_{OH}$=0,58 ppm.

A characteristic feature of ethanol (EtOH) and water (H_2O) during the interval of (τ=48...264 h) is that the spectra located separately from each other with a difference in a chemical shifts of Δδ=0,57...0,62 ppm.

This may indicate that there were no conditions created to form a water composition with hydroxyl proton of alcohol. Therefore, it proves that thermodynamic equilibrium is absent at τ=48...264 h.

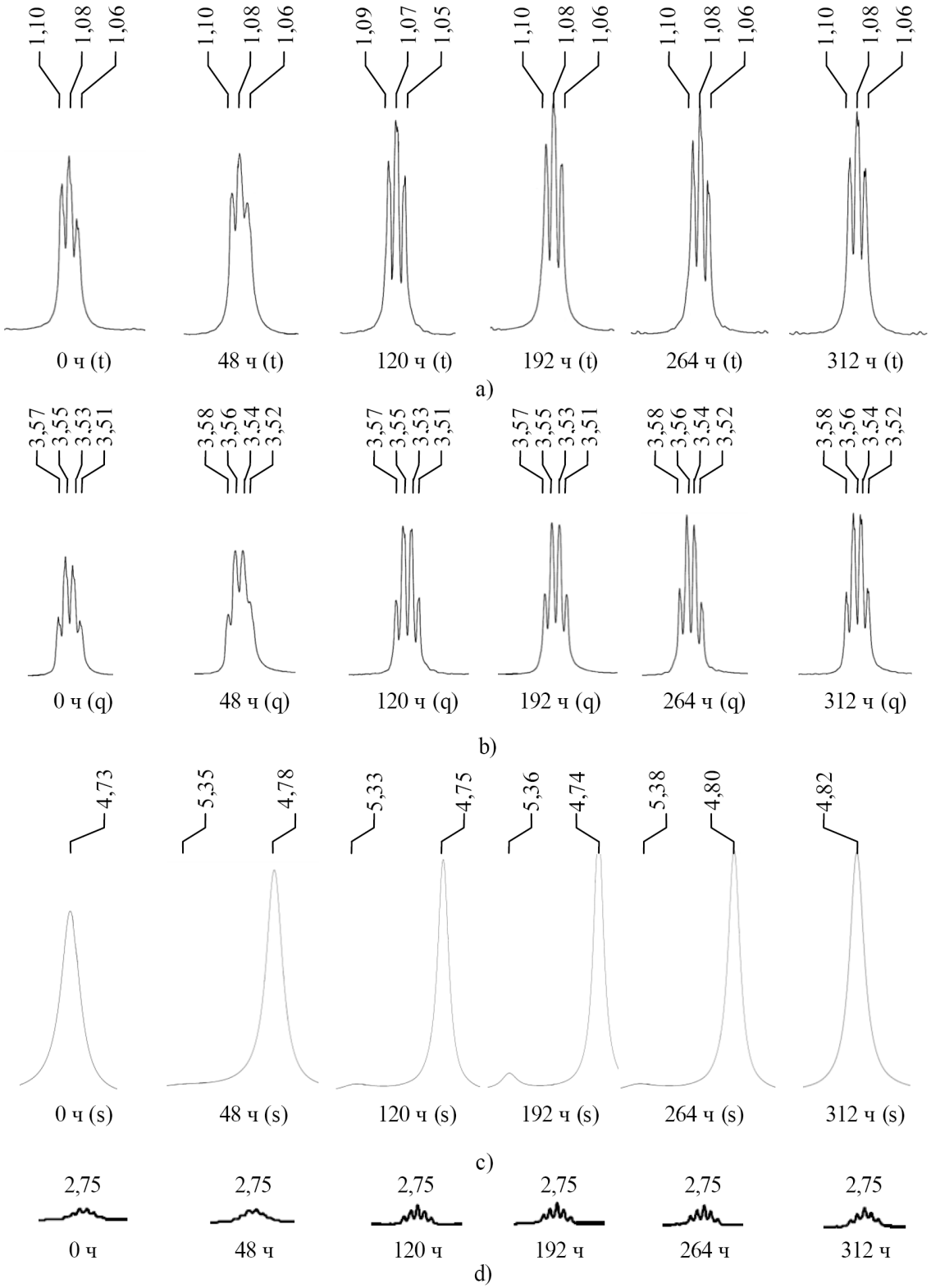

Fig. 1. Modifications of ^{1}H NMR spectra of proton groups of AAM, prepared in demineralized by reverse osmosis water and EAR «Lux»: a - CH_3; b - CH_2; c - H_2O+(EtOH); d - external standard (acetone-d6), dependent from system's functioning time.

The sixth spectrum (τ=312 h) is characterized by a single summary peak - H_2O+(EtOH), in the form of broadened singlet with a chemical shift of δ_{OH}=4,82 ppm.

The analysis of ^{1}H NMR spectra of AAM methyl group's protons (CH_3) states the following:

τ=0. Methyl group of protons (CH_3) is represented as a triplet (t) in the initial time of system's operation. Triplet is formed by the spin-spin interaction with protons of adjacent methylene group (CH_2). The intensity ratio is (1:2:1) according to the Pascal triangle. Not a single group of protons can affect methyl group's spectrum (CH_3) besides the methylene group (CH_2). Thus, the methyl group of protons (CH_3) is located in a strong field with an average value of the chemical shift as δ_{CH3}=1,08 ppm. Individual characteristics of peaks' chemical shift are δ_{CH3}=(1,10; 1,08; 1,06) ppm. The distance between each triplet is 0,02 ppm.

τ=48 h. The methyl group of protons (CH_3) did not change its position comparatively to the initial position (τ=0 h) (position is stable). The average value of chemical shift is δ_{CH3}=1,08 ppm. It has the following individual characteristics of chemical shift of signal's peaks δ_{CH3}=(1,10; 1,08; 1,06) ppm; distance between the peaks - 0,02 ppm: waveform - triplet (t).

τ=120 h. Methyl spectrum has shifted from its original position (τ=0 h) by 0,01 ppm. The average value of chemical shift is δ_{CH3}=1,07 ppm. Waveform - triplet (t). It indicates its stabilization based on spin- spin interaction with protons of methylene (CH_2) group. Individual peaks of triplet have chemical shift of δ_{CH3}=(1,09; 1,07; 1,05) ppm.

τ=192...312 h. The methyl group of protons (CH_3) did not change its position comparatively to the initial position (τ=0 h) (position is stable). The average value of chemical shift is δ_{CH3}=1,08 ppm. It has the following individual characteristics of chemical shift of peaks' signals δ_{CH3}=(1,10; 1,08; 1,06) ppm; distance between the peaks - 0,02 ppm; waveform - triplet (t).

The following initial conclusion can be made: the range (0...312 h) is characterized by a complete structuring of signal of methyl group (CH_3) as a triplet (t) as its form and by the middle positioning of a chemical shift - δ_{CH3}=1,07…1,08 ppm, which remains unchanged. There is no abnormal change in the spectra's structure during this period of time. Its position can be described as stable. The distance between the peaks also remain unchanged - 0,02 ppm.

The analysis of ^{1}H NMR spectra of methylene group (CH_2) reveals the following:

At the beginning of the formation of AAM (τ=0 h) methylene group of protons (CH_2) is presented as a quartet (q), which is confirmed by the spin-spin interaction of protons of methyl (CH_3) groups, that should split signal of the methylene group (CH_2) into four components, form a quartet (q) with intensity ratio of 1:3:3:1. In turn, protons of hydroxyl (OH) group should cleave every component of methylene (CH_2) group's quartet into two components to form a double quartet. The absence of spin-spin interaction between hydroxyl (OH) and methylene (CH_2) groups due to chemical exchange would have to ascertain that the signal of the methylene (CH_2) group must remain as quartet.

Methylene group of protons (CH_2) has an average value of a chemical shift as δ_{CH2}=3,54 ppm, with individual chemical shifts of quartet's peak δ_{CH2}=(3,57; 3,55; 3,53; 3,51) ppm; the distance between each peak of quartet is 0,02 ppm.

τ=48 h. Methylene spectrum with an average value of chemical shift δ_{CH2}=3,55 ppm is shifted to a weak field by 0,01 ppm relatively to its initial position (τ=0 h). Waveform - quartet (q), which is typical for the above proton group, on the assumption of spin-spin interaction with protons of the methyl (CH_3) group and chemical exchange between the hydroxyl (OH) and methylene (CH_2) groups.

τ=120...192 h. Methylene group of protons (CH_2) is in a stable position relatively to its initial position with an average value of chemical shift as δ_{CH2}=3,54 ppm and a distance between the peaks - 0,02 ppm. Waveform is quartet (q).

τ=264...312 h. Methylene spectrum with an average value of chemical shift δ_{CH2}=3,55 ppm is shifted to a weak field by 0,01 ppm relatively to its initial position (τ=0 h). Waveform - quartet (q), which is typical for the above proton group, on the assumption of spin-spin interaction with protons of the methyl (CH_3) group and chemical exchange between the hydroxyl (OH) and methylene (CH_2) groups.

The following initial conclusion can be made: the range (0...312 h) is characterized by a complete structuring signal of the methylene group (CH_2) as quartet (q) in its form and by the middle positioning of the chemical shift - δ_{CH2}=3,53…3,54 ppm, which remains unchanged. There is no abnormal change in the spectra's structure during this period of time. Its position can be described as stable. The distance between the peaks also remain unchanged - 0,02 ppm.

As a results we have evidence of a complex dynamic of achievement processes of solution equilibrium for AAM prepared in demineralized by reverse osmosis water with pH = 5,05 and ERS «Lux». At the same time pH of obtained AAM is pH = 7,60 i.e. alkalescent medium. A «restoration» of location of hydroxyl proton's signal (OH) of ethanol is observed during the time interval of 48 to 264 h after the mixing, with a constant alcohol concentration (AAM's strength – 39,92 % vol.) and system's temperature control (t=23,5 ºC). It can be assumed that the initial division of the signals related to the process of reconstruction of water structure that was destroyed by demineralization by reverse osmosis. Next follows a standard process of sustainable system of alcohol/water formation with a high degree of protons' socialization and specific for system exchange rates. The low exchange rate (separately observed signals of hydroxyl and water) can be related to a significant microheterogeneity of the system and relevant barrier effect that reduces the effective rate of protons' exchange.

Besides the presence of divided signal of OH protons and H_2O EtOH describes a product with a low tasting qualities (9,28 points). The presence of a common signal of H_2O+(EtOH) describes AAM with a best tasting qualities (9,30 points). The external appearance is a colorless liquid with no sediment; odor – sharp and alcoholic; taste – sweet and bitter, pungent.

Thus, this article describes mechanisms of transformation of ethanol's protons and water demineralized by reverse osmosis in the process of creating AAM. The paper presents experimental evidence of speed and nature of determination of thermodynamic equilibrium's dependence as well as dependence of obtainment of maximized organoleptic characteristics of AAM from contact time of demineralized water and alcohol after its mixing.

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