



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

<sup>1</sup>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.

<sup>1</sup>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 <sup>1</sup>H - 400 MHz; specially shaped capillary with acetone-d<sub>6</sub> (CD<sub>3</sub>)<sub>2</sub>CO; 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<sub>6</sub>) of NMR's deuterium stabilization spectrometer) is added into an ampoule of special form capillary. <sup>1</sup>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<sup>3</sup>; electrical conductivity – 20,7 μS/cm; pH – 5,05; ORP – “+” 393 mV; total hardness – <0,05 mM/dm<sup>3</sup>; permanganate oxidability – 0,46 mg O<sub>2</sub>/dm<sup>3</sup>; MC of sodium – 11,60 mg/dm<sup>3</sup>; MC of potassium – <2,0 mg/dm<sup>3</sup>; MC of ammonium – <2,0 mg/dm<sup>3</sup>; MC of calcium – <2,0 mg/dm<sup>3</sup>; MC of magnesium – <2,0 mg/dm<sup>3</sup>; total alkalinity – 0,15 mM/dm<sup>3</sup>.

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<sup>3</sup>; content of fusel oils in anhydrous alcohol: propyl, isopropyl, butyl, isobutyl and isoamyl – 1,5 mg/dm<sup>3</sup>; content of esters in anhydrous alcohol, in recalculation on acetic-ethyl ether – 1,3 mg/dm<sup>3</sup>; 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<sup>3</sup>; content of fusel oils in anhydrous alcohol: propyl, isopropyl, butyl, isobutyl and isoamyl – 1,4 mg/dm<sup>3</sup>; content of esters in anhydrous alcohol, in recalculation on acetic-ethyl ether – 1,4 mg/dm<sup>3</sup>; content of methanol in anhydrous alcohol – 0,0021 % vol.; alkalinity – 0,4 cm<sup>3</sup> 0,1 M of hydrochloric acid for titration of 100 cm<sup>3</sup> 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  $^1\text{H}$  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  $^1\text{H}$  NMR spectroscopy

Characteristics	Signal	Time ( $\tau$ ), h					
		0	48	120	192	264	312
The chemical shift ( $\delta$ ), ppm	$\text{CH}_3$	1,08	1,08	1,07	1,08	1,08	1,08
The chemical shift ( $\delta$ ), ppm	$\text{CH}_2$	3,54	3,55	3,54	3,54	3,55	3,55
The chemical shift ( $\delta$ ), ppm	$\text{H}_2\text{O}+(\text{EtOH})$	4,73	-	-	-	-	4,82
The chemical shift ( $\delta$ ), ppm	$\text{H}_2\text{O}$	-	4,78	4,75	4,74	4,80	-
The chemical shift ( $\delta$ ), ppm	$\text{EtOH}$	-	5,35	5,33	5,36	5,38	-
The chemical shift ( $\delta$ ), 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 ( $\text{H}_2\text{O}$ ), ethanol ( $\text{EtOH}$ ), AAM ( $\text{H}_2\text{O}+\text{EtOH}$ ) 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 ( $\tau=0$  h) hydroxyl group of protons is represented as a single summary peak in the form of joint singlet  $\text{H}_2\text{O}+(\text{EtOH})$  with a chemical shift of  $\delta_{\text{H}_2\text{O}+(\text{EtOH})}=4,73$  ppm. Waveform is distorted Gaussian curve with a broadened base. The absence of separate peaks of OH ethanol ( $\text{EtOH}$ ) and water ( $\text{H}_2\text{O}$ ) may indicate that at the initial stage of AAM formation ( $\tau=0$  h) balanced structure of water in alcohol can be observed.

At the second spectrum ( $\tau=48$  h) the presents of two separate signals - OH of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) and water  $\text{H}_2\text{O}$  is observed in the area of hydroxyl protons of ethanol and water. Multiplet component of hydroxyl (OH) proton of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) 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  $\delta_{\text{OH}}=5,35$  ppm. Signal of water ( $\text{H}_2\text{O}$ ) protons is presented as an elongated singlet of symmetrical shape with a broad base which is located at  $\delta_{\text{OH}}=4,78$  ppm. The difference in chemical shifts of OH proton ( $\text{C}_2\text{H}_5\text{OH}$ ) and  $\text{H}_2\text{O}$  proton at this stage ( $\tau=48$  h) is  $\Delta\delta_{\text{OH}}=0,57$  ppm.

The third spectrum ( $\tau=120$  h) is also characterized by the two single peaks - OH of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) and  $\text{H}_2\text{O}$ . The component of OH proton's multiplet  $\text{C}_2\text{H}_5\text{OH}$  is in the form of broad singlet with a chemical shift of  $\delta_{\text{OH}}=5,33$  ppm.  $\text{H}_2\text{O}$  component is in the form of symmetrical singlet with the broadened base and a chemical shift of  $\delta_{\text{OH}}=4,75$  ppm. The difference in a chemical shifts of OH proton  $\text{C}_2\text{H}_5\text{OH}$  and  $\text{H}_2\text{O}$  is  $\Delta\delta_{\text{OH}}=0,58$  ppm.

The fourth spectrum ( $\tau=192$  h) is also characterized by the two single peaks - OH of ethanol and water. The OH component of proton  $\text{C}_2\text{H}_5\text{OH}$  is in a form of broad singlet with a chemical shift of  $\delta_{\text{OH}}=5,36$  ppm. Component of  $\text{H}_2\text{O}$  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  $\delta_{\text{OH}}=4,74$  ppm. The difference in a chemical shifts of OH proton  $\text{C}_2\text{H}_5\text{OH}$  and  $\text{H}_2\text{O}$  is  $\Delta\delta_{\text{OH}}=0,62$  ppm.

The fifth spectrum ( $\tau=264$  h) is also characterized by a separation of peaks - OH of ethanol and water. OH proton's component of multiplet  $\text{C}_2\text{H}_5\text{OH}$  is presented in a form of as barely noticeable bulge, which is located in a weak field with a chemical shift of  $\delta_{\text{OH}}=5,38$  ppm.  $\text{H}_2\text{O}$  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  $\delta_{\text{OH}}=4,80$  ppm. The difference in the chemical shifts of OH proton  $\text{C}_2\text{H}_5\text{OH}$  and  $\text{H}_2\text{O}$  is  $\Delta\delta_{\text{OH}}=0,58$  ppm.

A characteristic feature of ethanol ( $\text{EtOH}$ ) and water ( $\text{H}_2\text{O}$ ) during the interval of ( $\tau=48...264$  h) is that the spectra located separately from each other with a difference in a chemical shifts of  $\Delta\delta=0,57...0,62$  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  $\tau=48\dots264$  h.

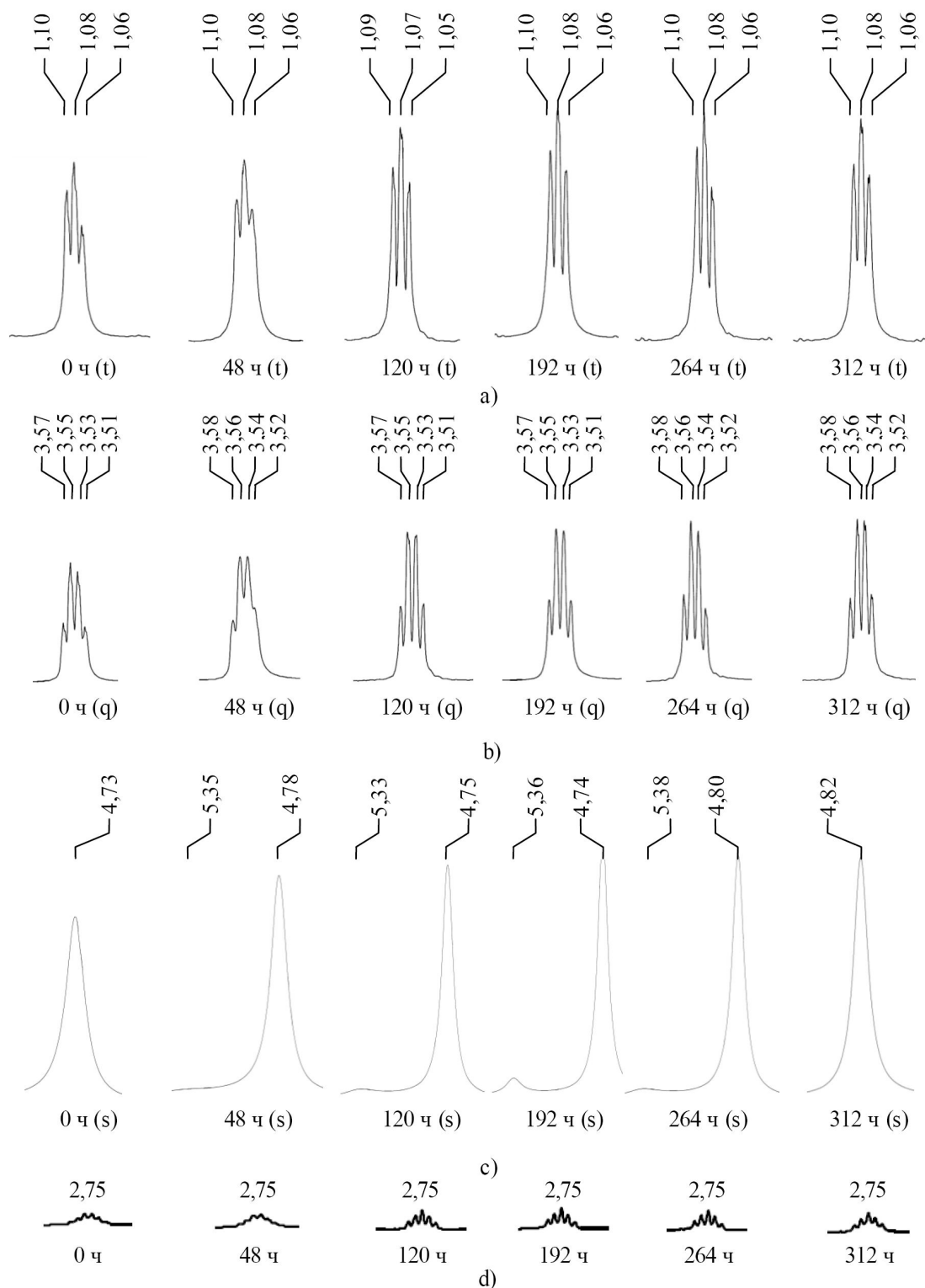


Figure 1. Modifications of  $^1\text{H}$  NMR spectra of proton groups of AAM, prepared in demineralized by reverse osmosis water and EAR «Lux»: a -  $\text{CH}_3$ ; b -  $\text{CH}_2$ ; c -  $\text{H}_2\text{O}+(\text{EtOH})$ ; d - external standard (acetone- $\text{d}_6$ ), dependent from system's functioning time.

The sixth spectrum ( $\tau=312$  h) is characterized by a single summary peak -  $\text{H}_2\text{O}+(\text{EtOH})$ , in the form of broadened singlet with a chemical shift of  $\delta_{\text{OH}}=4,82$  ppm.

The analysis of  $^1\text{H}$  NMR spectra of AAM methyl group's protons ( $\text{CH}_3$ ) states the following:

$\tau=0$ . Methyl group of protons ( $\text{CH}_3$ ) 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 ( $\text{CH}_2$ ). The intensity ratio is (1:2:1) according to the Pascal triangle. Not a single group of protons can affect methyl group's spectrum ( $\text{CH}_3$ ) besides the methylene group ( $\text{CH}_2$ ). Thus, the methyl group of protons ( $\text{CH}_3$ ) is located in a strong field with an average value of the chemical shift as  $\delta_{\text{CH}_3}=1,08$  ppm. Individual characteristics of peaks' chemical shift are  $\delta_{\text{CH}_3}=(1,10; 1,08; 1,06)$  ppm. The distance between each triplet is 0,02 ppm.

$\tau=48$  h. The methyl group of protons ( $\text{CH}_3$ ) did not change its position comparatively to the initial position ( $\tau=0$  h) (position is stable). The average value of chemical shift is  $\delta_{\text{CH}_3}=1,08$  ppm. It has the following individual characteristics of chemical shift of signal's peaks  $\delta_{\text{CH}_3}=(1,10; 1,08; 1,06)$  ppm; distance between the peaks - 0,02 ppm; waveform - triplet (t).

$\tau=120$  h. Methyl spectrum has shifted from its original position ( $\tau=0$  h) by 0,01 ppm. The average value of chemical shift is  $\delta_{\text{CH}_3}=1,07$  ppm. Waveform - triplet (t). It indicates its stabilization based on spin-spin interaction with protons of methylene ( $\text{CH}_2$ ) group. Individual peaks of triplet have chemical shift of  $\delta_{\text{CH}_3}=(1,09; 1,07; 1,05)$  ppm.

$\tau=192...312$  h. The methyl group of protons ( $\text{CH}_3$ ) did not change its position comparatively to the initial position ( $\tau=0$  h) (position is stable). The average value of chemical shift is  $\delta_{\text{CH}_3}=1,08$  ppm. It has the following individual characteristics of chemical shift of peaks' signals  $\delta_{\text{CH}_3}=(1,10; 1,08; 1,06)$  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 ( $\text{CH}_3$ ) as a triplet (t) as its form and by the middle positioning of a chemical shift -  $\delta_{\text{CH}_3}=1,07...1,08$  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  $^1\text{H}$  NMR spectra of methylene group ( $\text{CH}_2$ ) reveals the following:

At the beginning of the formation of AAM ( $\tau=0$  h) methylene group of protons ( $\text{CH}_2$ ) is presented as a quartet (q), which is confirmed by the spin-spin interaction of protons of methyl ( $\text{CH}_3$ ) groups, that should split signal of the methylene group ( $\text{CH}_2$ ) 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 ( $\text{CH}_2$ ) group's quartet into two components to form a double quartet. The absence of spin-spin interaction between hydroxyl (OH) and methylene ( $\text{CH}_2$ ) groups due to chemical exchange would have to ascertain that the signal of the methylene ( $\text{CH}_2$ ) group must remain as quartet.

Methylene group of protons ( $\text{CH}_2$ ) has an average value of a chemical shift as  $\delta_{\text{CH}_2}=3,54$  ppm, with individual chemical shifts of quartet's peak  $\delta_{\text{CH}_2}=(3,57; 3,55; 3,53; 3,51)$  ppm; the distance between each peak of quartet is 0,02 ppm.

$\tau=48$  h. Methylene spectrum with an average value of chemical shift  $\delta_{\text{CH}_2}=3,55$  ppm is shifted to a weak field by 0,01 ppm relatively to its initial position ( $\tau=0$  h). Waveform - quartet (q), which is typical for the above proton group, on the assumption of spin-spin interaction with protons of the methyl ( $\text{CH}_3$ ) group and chemical exchange between the hydroxyl (OH) and methylene ( $\text{CH}_2$ ) groups.

$\tau=120...192$  h. Methylene group of protons ( $\text{CH}_2$ ) is in a stable position relatively to its initial position with an average value of chemical shift as  $\delta_{\text{CH}_2}=3,54$  ppm and a distance between the peaks - 0,02 ppm. Waveform is quartet (q).

$\tau=264...312$  h. Methylene spectrum with an average value of chemical shift  $\delta_{\text{CH}_2}=3,55$  ppm is shifted to a weak field by 0,01 ppm relatively to its initial position ( $\tau=0$  h). Waveform - quartet (q), which is typical for the above proton group, on the assumption of spin-spin interaction with protons of the methyl ( $\text{CH}_3$ ) group and chemical exchange between the hydroxyl (OH) and methylene ( $\text{CH}_2$ ) groups.

The following initial conclusion can be made: the range (0...312 h) is characterized by a complete structuring signal of the methylene group ( $\text{CH}_2$ ) as quartet (q) in its form and by the middle positioning of the chemical shift -  $\delta_{\text{CH}_2}=3,53...3,54$  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 ( $t=23,5$  °C). 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<sub>2</sub>O EtOH describes a product with a low tasting qualities (9,28 points). The presence of a common signal of H<sub>2</sub>O+(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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### Bibliography

- Arnold, J.T., Dharmatti, S.S., Packard, M.E. (1951) 'Chemical effects on nuclear induction signals from organic compounds', *The journal of chemical physics*, (№.19), pp. 507.
- Bloch, F, Hansen, W.W., Packard, M. (1946) 'The nuclear induction experiment', *Physical review*, (v. 70), pp. 474-489.
- DSTU 3297:95 (1995) *Alcoholic beverage industry. Terms and definitions*, Kiev: The State Committee for Standardization Metrology and Certification of Ukraine.
- DSTU 4221:2003 (2003) *Ethyl alcohol rectified. Technical conditions*, Kiev: The State Committee for Standardization Metrology and Certification of Ukraine.
- DSTU 4256:2003 (2003) *Vodka and vodka singular. Technical conditions*, Kiev: The State Committee for Standardization Metrology and Certification of Ukraine.
- Hu, N., Wu, D., Cross, K. and other (2010) 'Structurability: A collective measure of the structural differences in vodkas', *Journal of agricultural and food chemistry*, (№ 58), pp. 7394-7401.
- Kuzmin, O., Sujkov, S., Topol'nik, V. (2013) 'The change of the hydroxyl protons in aqueous alcoholic mixtures under the process of making vodkas', *The advanced science journal*, (Special issue in China), pp. 15-27.
- Nose, A., Hamasaki, T., Hojo, M. and other (2005) 'Hydrogen bonding in alcoholic beverages (distilled spirits) and water-ethanol mixture', *Journal of agricultural and food chemistry*, (№ 53), pp. 7074-7081.
- Richards, S.A., Hollerton, J.C. (2011) *Essential practical NMR for organic chemistry*, Chichester: John Wiley & Sons, Ltd.
- Roberts, J.D. (2002) 'Organic chemistry applications', in Grant, D.M., Harris, R.K. (ed.) *Encyclopedia of nuclear magnetic resonance*. Chichester: John Wiley & Sons, Ltd, pp. 3386-3400.
- SOU 15.9-37-237:2005 (2005) *Water is prepared for production of alcoholic beverage. Technical conditions*, Kiev: Organization standard Ukraine.