

## Confirmation of polymerisation effects of sodium chloride and its additives on acrylamide by infrared spectrometry

EMIL KOLEK · PETER ŠIMKO · PETER ŠIMON · ANTON GATIAL

### Summary

Acrylamide was applied onto two additives of table salt - potassium ferrocyanide and potassium iodate - and heated in a glass reaction vessel within temperature range 102–180 °C with heating rate of 2 °C.min<sup>-1</sup> to study the effect of these inorganic salts on acrylamide elimination. For comparison, the same experiment was carried out also with chemically pure sodium chloride. As found, the amount of acrylamide applied onto chemically pure sodium chloride decreased only by 13%, while the amounts of acrylamide applied onto potassium ferrocyanide and potassium iodate decreased much more considerably - by 61% and 88%, respectively. Comparing infrared spectra of pure acrylamide and the product formed during the experiments, it was found that all the salts under study brought about the polymerization of acrylamide through the formation of C-C backbone polymer while the carbonyl and amino groups remained unchanged. As concluded, potassium ferrocyanide and potassium iodate exhibit a much stronger effect on acrylamide polymerization than pure sodium chloride itself. For this reason, they could strengthen considerably the efficiency of acrylamide elimination in a real food matrix with regard to their presence in table salt, or being added directly to the thermally treated food matrix, respectively.

### Keywords

acrylamide; polyacrylamide; sodium chloride; potassium ferrocyanide; potassium iodate; infrared spectroscopy; gas chromatography - mass spectrometry

So far, acrylamide (AA) is generally classified as “probably carcinogenic to humans” and this finding is considered important not only for producers but also for consumers. Although a final assessment of the toxicological risk is not finished yet, experts all over the world declare that AA content in foods should be minimized as much as possible [1]. The highest AA content is repeatedly being found in French fries and potato chips, and thus the attention was focused on heat-processed potato products [2, 3]. First investigations of the reactions associated with the formation of AA revealed that the process is initiated with the reaction between reducing monosaccharides and asparagine which indicates that AA might be a product of the Maillard reactions [4]. As found later, the AA formation from asparagine and reducing monosaccharides can also take place in a solid mixture of the compounds during heating up to 190 °C [5]. Potato products have been associated with AA formation due to relatively high levels of AA precursors as well as the temperature of process-

ing. However, there are also assumptions about other mechanism pathways which indicates that AA formation in a real food matrix is a complex of physico-chemical processes involving a number of reactions and interactions [6]. On the other hand, the AA formation can be limited considerably by some additives such as amino acids, proteins or citric acid, respectively [7]. For example, croquettes prepared from fresh potatoes and coated with egg/breadcrumbs contained considerably reduced AA content after thermal processing [8]. Summarizing up current available information it seems that the AA formation cannot be utterly avoided, especially in a starchy matrix treated within a temperature range of 130–180 °C unless its precursors are effectively removed. From this point of view, a promising way is the application of enzyme L-asparaginase, which is able to hydrolyse asparagine. By this way it was possible to reduce AA content in potato products by 97% [9]. On the other hand, AA is a highly reactive compound which could result in a formation of biologically non-active high

**Emil Kolek, Peter Šimko**, VÚP Food Research Institute, Priemyselná 4, P. O. Box 25, SK-824 75 Bratislava, Slovakia.

**Peter Šimon, Anton Gatial**, Institute of Physical Chemistry and Chemical Physics, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovakia.

*Correspondence author:*

Peter Šimko, tel.: 00 421 2 5557 4622, fax: 00 421 2 5557 1417, e-mail: peter.simko@vup.sk

molecular compounds. Presence of such transformation reactions, characterized by variable AA content during heating has already been indicated [10]. Recently it has also been shown that NaCl, a common food additive, exhibits an inhibiting effect on acrylamide formation in a model system consisting of equimolar mixture of glucose and asparagine [11]. As proved by differential scanning calorimetry, NaCl catalysed considerably the process of AA elimination to be formed from glucose and asparagine during heating [12]. The goal of this work was to study the effects of table salts additives,  $K_4[Fe(CN)_6]$  and  $KIO_3$ , on the elimination of AA applied onto the mentioned inorganic salts and characterise reactions of its elimination.

## MATERIALS AND METHODS

### Chemicals

AA of p.a. purity was purchased from Fisher Scientific (Loughborough, United Kingdom) and 2,3,3-D3 AA (98%) was purchased from Cambridge Isotope Laboratories (Andover, Massachusetts, USA). Chemically pure NaCl ACS Reag. Ph Eur. was purchased from Merck (Darmstadt, Germany). Methanol CHROMASOLV,  $K_4[Fe(CN)_6]$  and  $KIO_3$  were purchased from Sigma-Aldrich (Steinheim, Germany).

### Techniques used

Agilent Technologies 6890 (Agilent Technologies, Palo Alto, USA) gas chromatograph equipped with an Agilent Technologies 5973 inert mass selective spectrometer was used for the determination of the AA amounts. Metal Block Thermostat was purchased from Liebig, Germany, and EcoScan Temp JKT Temperature Meter equipped with Probe 3T520C was obtained from Eutech Instruments Europe B.V., Netherlands. A Nylon filter (0.45  $\mu m$ ) was purchased from Supelco (USA). Mid-infrared spectra as KBr pellets at room temperature in the region 4000–400  $cm^{-1}$  were recorded on Nicolet model NEXUS 470 FTIR spectrometer (Nicolet Corporate Headquarters, Madison, Wisconsin, USA).

### Experiment I

1 g of inorganic compound (NaCl, or  $K_4[Fe(CN)_6]$ , or  $KIO_3$ ) and 100  $\mu g$  AA dissolved in methanol was placed into a 40 ml glass vessel and methanol was removed using a nitrogen stream. Then, the vessels were sealed tightly with PTFE/silicone septa and heated in the thermostat from 102 to 180  $^{\circ}C$  at a heating rate 2  $^{\circ}C \cdot min^{-1}$ . The temperature of reactants inside the vessels

was monitored by a thermometer. Systems were sampled periodically, when three parallel measurements were carried out. After reaching a chosen temperature, the vessels were cooled and their content was dissolved in methanol. Then, 20  $\mu g$  D3-AA dissolved in methanol was added, sonicated for 5 min, filtered and analysed by GC-MS.

### Experiment II

0.5 g of inorganic compound and 0.5 g AA were mixed and homogenised thoroughly in a mortar dish, then the mixture was placed into a glass tube, sealed tightly with PTFE/silicone septa and placed into thermostat heated to 180  $^{\circ}C$  for 20 min. After cooling, 10 ml of water was mixed with tube content and formed gel was centrifuged at 31.4  $rad \cdot s^{-1}$ . To remove completely an inorganic component, the gel washing treatment was repeated ten times, and then dried in a laboratory drier at 80  $^{\circ}C$ . In the final stage, the product (also AA) was mixed with KBr and pressed to pellet forms for analysis by infrared spectroscopy.

### Chromatographic conditions

1  $\mu l$  of the vessel content was applied into a splitless injector (purge time 0.5 min at 250  $^{\circ}C$ ). Separations were carried out using an Agilent 122-3232 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu m$  fused silica capillary column coated with a DB-FFAP phase. The column was held at 50  $^{\circ}C$  for 1 min, then heated to 250  $^{\circ}C$  at a rate of 10  $^{\circ}C \cdot min^{-1}$ . The carrier gas (helium) flow was maintained at 0.8  $ml \cdot min^{-1}$  by an electronic control of pressure. Under these conditions AA and 2,3,3-D3-AA eluted at 13.2 min. The data accumulation was not initiated until 12 min to avoid detection of the large peak of methanol. The AA amount was determined from the ratio of the peak area of AA to the peak area of the known amount of spiked 2,3,3-D3-AA. The detection was carried out by the mass detector working in a selected ion monitoring mode - the ions were obtained by negative chemical ionization procedure using methane as the reagent gas. The mass of the most intense fragments was 70.15 and 73.15  $m/z$ , respectively. From the statistical point of view, there are some data characterising parameters of method as follows: Limit of quantification 20  $\mu g \cdot l^{-1}$ ; relative standard deviation 5%; coefficient of regression  $r^2 = 0.999822$ .

## RESULTS AND DISCUSSION

### Elimination of AA

First experiment was carried out with chemically pure NaCl. As follows from Fig. 1, effect of

this salt to AA elimination was not very strong and the AA content lowered only by 13%. However, much more intense effects on AA elimination had the other two compounds. As follows from Fig. 1, under the same experimental conditions,  $K_4[Fe(CN)_6]$  brought about a decrease of AA content by 61% and  $KIO_3$  more than by 88%. From practical point of view it is very important that both compounds are used frequently in table salt production as its additives.  $K_4[Fe(CN)_6]$  is the compound to be permitted for usage in food industry and its number in international numbering system is 535. The same number has the compound also in the European food legislation and it is being added to table salt up to  $20 \text{ mg.kg}^{-1}$  as an anti-caking agent since it adsorbs water and so prevents the table salt from forming clumps and being difficult to pour. In some countries,  $KIO_3$  is used for iodination of table salt up to  $35 \text{ mg.kg}^{-1}$  instead of KI, because iodide can be oxidized to iodine under wet conditions by oxygen and subsequently sublimates out of the salt. Moreover,  $KIO_3$  is occasionally used as a maturing agent in baking technology. As these results show, both compounds could strengthen considerably the effect of NaCl on the AA elimination in a real food matrix.

#### Identification of the product

Fourier transform infrared (FT IR) spectra of AA and the product to be believed as polyacrylamide (PAA) in the mid infrared region  $4000\text{--}400 \text{ cm}^{-1}$  are shown in Fig. 2. The frequencies of bands with their relative intensities are given in Table 1. Characterization of IR and Raman spectra of AA were first reported by Jonathan

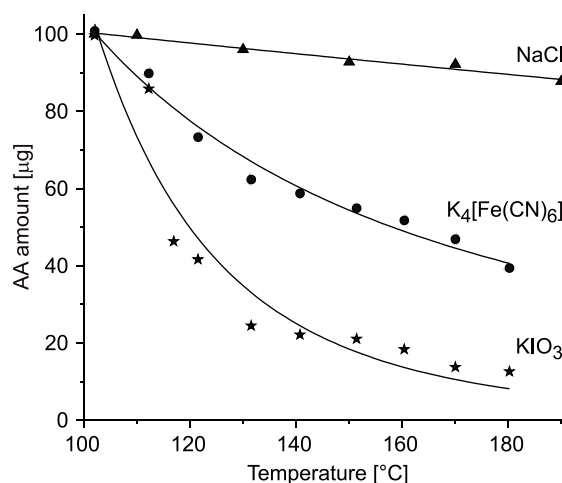


Fig. 1. Decrease of amounts of acrylamide heated in presence of inorganic salts.

[13] and from that time they have been a subject of numerous experimental and theoretical studies, also in the connection with the vibrational study of PAA [14–20]. Despite that, there are still many discrepancies among authors in the assignment of individual bands, especially below  $800 \text{ cm}^{-1}$ . Therefore, in the assignment we made use also of the results of our unpublished quantum-chemistry *ab initio* calculations at MP2 level in 6-31G\*\* basis set. Generally, the AA assignment in Table 1 is very similar to that from refs. [18, 20] and the assignment of PAA bands based on the results of papers [17, 19] was taken from [21]. IR spectrum of the product obtained in experiment II is very similar to the spectrum of PAA presented in [21].

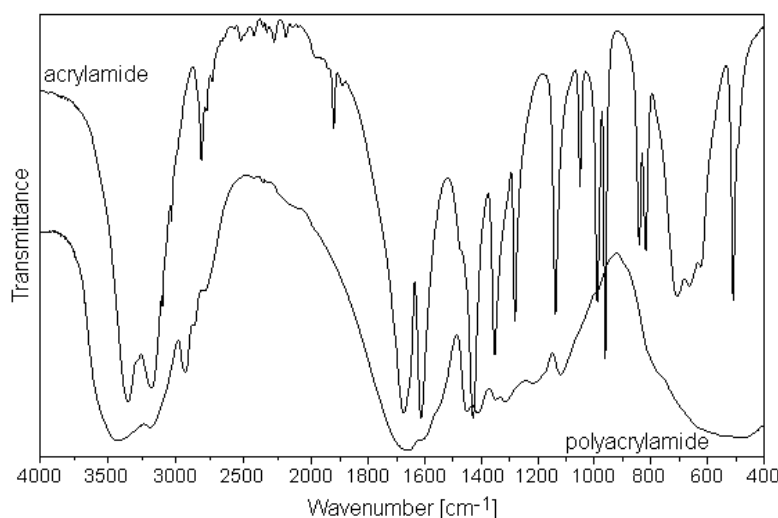


Fig. 2. IR spectrum of acrylamide (top) and isolated product - polyacrylamide (bottom) in KBr pellet at room temperature.

**Tab. 1.** Observed frequencies [ $\text{cm}^{-1}$ ] and assignments of infrared bands of acrylamide and the product - both in solid state.

Acrylamide		Product - Polyacrylamide	
Frequency <sup>a</sup>	Assignment <sup>b</sup>	Frequency <sup>a</sup>	Assignment <sup>b</sup>
3454 vs	NH <sub>2</sub> as	3423 vs	NH <sub>2</sub> as
3184 vs	NH <sub>2</sub> ss	3191 vs	NH <sub>2</sub> ss
3102 w	=CH <sub>2</sub> as		
3034 w	=CH s		
3012 vw	=CH <sub>2</sub> ss		
		2952 vw,sh	>CH <sub>2</sub> as
		2932 m	>CH <sub>2</sub> ss
		2867 w	CH s
1921 m	2 x 962		
1674 vs	C=O s	1654 vs	C=O s
1648 w,sh	C=C s		
1613 vs	NH <sub>2</sub> $\delta$	1613 vs	NH <sub>2</sub> $\delta$
		1450 m	>CH <sub>2</sub> $\delta$
1429 vs	=CH <sub>2</sub> $\delta$		
1353 s	C-N s	1412 m	C-N s
		1348 w	>CH <sub>2</sub> wa
1281 s	=CH ro	1315 w	CH $\delta$
		1215 w	NH <sub>2</sub> ro
1137 s	NH <sub>2</sub> ro	1175 w,sh	C-C s
1051 m	=CH <sub>2</sub> ro	1120 m	C-C s
990 s	=CH wa	993 w, sh	NH <sub>2</sub> tw
962 s	=CH <sub>2</sub> wa		
841 m	C-C s		
818 m	C=O wa, CH=CH wa		
		~757 w, sh	
706 m	C=O ro		
664 m	NH <sub>2</sub> tw		
624 m	CH=CH wa, C=O wa	~623 sh, br	
		~550 sh, br	
510 m	CCN $\delta$		

<sup>a</sup> s - strong; m - medium; w - weak; v - very; sh - shoulder; br - broad.<sup>b</sup> s - symmetric; a - antisymmetric; s - stretching,  $\delta$  - deformation; ro - rocking; wa - wagging; tw - twisting.

The character of polymer with assumed structure  $[-\text{CH}_2-\text{CH}(\text{CONH}_2)-]_n$  can be confirmed by IR spectrum of the product which is similar to the standard infrared spectrum of PAA and noticeably different from AA monomer. The AA bands at 3454 and 3184  $\text{cm}^{-1}$  assigned as NH<sub>2</sub> stretching antisymmetric and symmetric modes, respectively, remain practically at the same wave numbers in the polymer spectrum and with the similar shape and intensities. On the other hand, AA bands cor-

responding to the C-H stretching modes are above 3000  $\text{cm}^{-1}$  which is typical for olefinic hydrogens and normally indicate the presence of  $\text{sp}^2$  hybridized carbon in CH bonds. In the product, these bands disappeared and the new ones at 2952, 2932 and 2867  $\text{cm}^{-1}$ , respectively, have appeared. The area below 3000  $\text{cm}^{-1}$  is typical for C-H stretching modes of saturated hydrocarbons with secondary and tertiary bonded carbon in methylene  $-\text{CH}_2-$  and methine CH groups, respectively.

Thus,  $\text{NH}_2$  group can be identified from this part of the spectrum of product and, in comparison with AA, disappearing of vinyl group and presence of saturated carbon chain. IR spectrum of amide group is characteristic for several modes described in the terms of amide vibrations as amide I ( $\text{C}=\text{O}$  stretching), amide II ( $\text{NH}_2$  deformation or scissoring), amide III ( $\text{C}-\text{N}$  stretching), amide IV ( $\text{C}=\text{O}$  deformation or rocking), amide V ( $\text{NH}_2$  wagging) and amide VI ( $\text{C}=\text{O}$  wagging). In the condensed-state spectra, the amide I and II modes appear as strong bands in the regions  $(1680 \pm 40) \text{ cm}^{-1}$  and  $(1610 \pm 30) \text{ cm}^{-1}$ , respectively [22]. Hence, the assignment of AA bands at  $1674 \text{ cm}^{-1}$  and  $1614 \text{ cm}^{-1}$  as  $\text{C}=\text{O}$  stretching and  $\text{NH}_2$  deformation modes is obvious.  $\text{C}=\text{C}$  stretching mode occurring in this region at  $1648 \text{ cm}^{-1}$  is very weak in IR spectrum and, consequently, not useful for diagnostic purposes. The spectrum of the product in this region is very similar to the AA spectrum indicating that both amide modes are present. It means that the amide group is unchanged in the product. Frequency upshift of  $\text{C}-\text{N}$  stretching mode (amide III) at  $1412 \text{ cm}^{-1}$  and  $> \text{CH}_2$  deformation mode at  $1450 \text{ cm}^{-1}$  were used in [23] as a proof of AA polymerization under the influence of  $\gamma$ -irradiation. The changes regarding the vinyl group can be monitored through its very characteristic three wagging modes including all three hydrogens in  $-\text{CH}=\text{CH}_2$  group. In two of them, hydrogen atoms in *trans* and *cis* positions participate. They appear in a narrow region  $(975 \pm 35) \text{ cm}^{-1}$  and in a wide one  $(565 \pm 165) \text{ cm}^{-1}$  [22], respectively and they are also known as  $\text{CH}=\text{CH}$  *trans* and *cis* wagging. Especially first of them is usually present as a strong band and often is marked as  $=\text{CH}$  wagging. Mainly both ending hydrogen atoms participate in the third wagging mode appearing in the region  $(895 \pm 85) \text{ cm}^{-1}$ . This mode is marked as  $=\text{CH}_2$  wagging. It is usually present as a strong band and additionally accompanied by the very characteristic medium intensive overtone around  $1900 \text{ cm}^{-1}$ . This mode is sensitive to  $\alpha$ -bonded atom and appears in the region  $(950 \pm 30) \text{ cm}^{-1}$  in compounds with  $\text{C}=\text{O}$  bond [22]. Therefore, the strong absorptions at  $\approx 975 \text{ cm}^{-1}$  and  $\approx 950 \text{ cm}^{-1}$  with the overtone at  $\approx 1900 \text{ cm}^{-1}$  are the most useful infrared bands to elucidate the  $-\text{CH}=\text{CH}_2$  structure. In the IR spectrum of AA there is  $=\text{CH}$  wagging mode at  $990 \text{ cm}^{-1}$  and  $=\text{CH}_2$  wagging mode at  $962 \text{ cm}^{-1}$  with the overtone at  $1921 \text{ cm}^{-1}$ . Absence of these three bands in the IR spectrum of the product indicate the transformation of vinyl group into saturated carbon chain similarly as the above-mentioned results for N-H and C-H stretching regions.

## CONCLUSIONS

The results and findings of this work lead to the following conclusions:

1. Inorganic salts  $\text{K}_4[\text{Fe}(\text{CN})_6]$  and  $\text{KIO}_3$ , used as table salt additives, exhibit much stronger effects on AA elimination during thermal processing than pure NaCl.
2. During the heating, AA elimination is brought about by its polymerisation, what is accelerated considerably by inorganic salts. However, various salts exhibit different catalytic effects on the extent of AA polymerisation.
3. Formation of PAA has been proven by FT IR spectroscopy, which confirmed formation of C-C backbone polymer while the carbonyl and amino groups remained unchanged.
4. Addition of table salt in optimal content of NaCl,  $\text{K}_4[\text{Fe}(\text{CN})_6]$  and  $\text{KIO}_3$  could solve considerably problems associated with AA presence in thermally processed salted food products.

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## REFERENCES

1. Weisshaar, R.: Acrylamide in heated potato products - analytics and formation routes. *European Journal of Lipid Science and Technology*, 106, 2004, pp. 786-792.
2. Jung, M. Y. - Choi, D. S. - Ju, J. W.: A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. *Journal of Food Science*, 68, 2003, pp. 1287-1290.
3. Williams, J. S. E.: Influence of variety and processing conditions on acrylamide levels in fried potato crisps. *Food Chemistry*, 90, 2005, pp. 875-881.
4. Mottram, D. S. - Wedzicha, B. L.: Acrylamide is formed in the Maillard reaction. *Nature*, 419, 2002, pp. 448-449.
5. Robert, F. - Vuataz, G. - Pollien, P. - Saucy, F. - Alonso, M. - Bauwens, I. - Blank, I.: Acrylamide formation from asparagine under low-moisture Maillard reaction conditions. 1. Physical and chemical aspects in crystalline model systems. *Journal of Agricultural and Food Chemistry*, 52, 2004, pp. 6837-6842.
6. Friedman, M.: Chemistry, biochemistry and safety of acrylamide. A review. *Journal of Agricultural and Food Chemistry*, 51, 2003, pp. 4504-4526.
7. Rydberg, P. - Eriksson, S. - Tareke, E. - Karlsson, P. - Ehrenberg, L. - Törnqvist, M.: Investigations of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 51, 2003, pp. 7012-7018.



8. Fiselier, K. - Grob, K. - Pfefferle, A.: Brown potato croquettes low in acrylamide by coating with egg/bread crumbs. *European Journal of Lipid Science and Technology*, 219, 2004, pp. 111-115.
9. Ciesarová, Z. - Kiss, E. - Boegl, P.: Impact of L-asparaginase on acrylamide content in potato product. *Journal of Food and Nutrition Research*, 45, 2006, pp. 141-146.
10. Claeys, W. L. - De Vleeschouwer, K. - Hendrickx, M. E.: Kinetics of acrylamide formation and elimination during heating of an asparagine-sugar model system. *Journal of Agricultural and Food Chemistry*, 53, 2005, pp. 9999-10005.
11. Kolek, E. - Šimko, P. - Šimon, P.: Inhibition of acrylamide formation in asparagine/D-glucose model system by NaCl addition. *European Food Research and Technology*, 224, 2006, pp. 283-284.
12. Kolek, E. - Šimko, P. - Šimon, P.: Effect of NaCl on the decrease of acrylamide content in a heat-treated model food matrix. *Journal of Food and Nutrition Research*, 45, 2006, pp. 17-20.
13. Jonathan, N.: The infrared and Raman spectra and structure of acrylamide. *Journal of Molecular Spectroscopy*, 6, 1961, pp. 205-214.
14. Kydd, R. A. - Dunham, A. P. C.: The infrared spectra and structure of acetamide and acrylamide. *Journal of Molecular Structure*, 69, 1980, pp. 79-88.
15. Suh, J. S. - Michaelien, K. H.: Surface-enhanced Raman-spectroscopy of acrylamide and polyacrylamide adsorbed on silver colloid surfaces - polymerization of acrylamide on silver. *Journal of Raman Spectroscopy*, 18, 1987, pp. 409-414.
16. Lin, C. K. - Chen, S. Y. - Lien, M. H.: Site of protonation and proton affinity of acrylamide. A theoretical study. *Journal of Physical Chemistry*, 99, 1995, pp. 1454-1461.
17. Murugan, R. - Mohan, S. - Bigotto, A.: FT IR and polarized Raman spectra of acrylamide and polyacrylamide. *Journal of Korean Physical Society*, 32, 1998, pp. 505-512.
18. Marstokk, K. H. - Møllendal, H. - Samdal, S.: Microwave spectrum, conformational equilibrium,  $^{14}\text{N}$  quadrupole coupling constants, dipole moment, vibrational frequencies and quantum chemical calculations for acrylamide. *Journal of Molecular Structure*, 524, 2000, pp. 69-85.
19. Sundaraganesan, N. - Puviarasan, N. - Mohan, S.: Vibrational spectra, assignment and normal coordinate calculation of acrylamide. *Talanta*, 54, 2001, pp. 233-241.
20. Duarte, A. S. R. - Amorim da Costa, A. M. - Amado, A. M.: On the conformation of neat acrylamide dimers - a study by ab initio calculations and vibrational spectroscopy. *Journal of Molecular Structure: THEOCHEM*, 723, 2005, pp. 63-68.
21. Deng, Y. - Dixon, J. B. - White, G. N. - Loepfert, R. H. - Juo, A. S. R.: Bonding between polyacrylamide and smectite. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 281, 2006, pp. 82-91.
22. Roeges, N. P. G.: Guide to the interpretation of infrared spectra of organic structures. Chichester : John Wiley & Sons, 1994. 356 pp. ISBN 978-0-471-93998-6.
23. Ni, Y. - Ge, X. - Zhang, Z. - Ye, Q.: In situ single-step synthesis of gold/polyacrylamide nanocomposites in an ethanol system. *Materials Letters*, 55, 2002, pp. 171-174.

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