Japanese Journal of Applied Physics

BRIEF NOTE

Enrichment of amino acids from its aqueous solution by ultrasonic atomization and ultrafine bubbles

Japanese Journal of Applied Physics **61**, SG1009 (2022) **BRIEF NOTE**
https://doi.org/10.35848/1347-4065/ac43e5

Enrichment of amino acids from its aqueous solution by ultrasonic atomization and ultrafine bubbles

Keiji Yasuda[1*], Koji Hamada[1], and Yoshiyuki Asakura[2]

[1]*Department of Chemical Systems Engineering, Graduate School of Engineering, Nagoya University, Nagoya 464-8603, Japan*
[2]*Honda Electronics Co., Ltd., Toyohashi, Aichi 441-3193, Japan*

[*]E-mail: yasuda.keiji@material.nagoya-u.ac.jp

Received November 2, 2021; revised December 10, 2021; accepted December 15, 2021; published online March 22, 2022

The enrichment characteristics of amino acids by ultrasonic atomization were investigated. Samples were aqueous solutions of L-phenylalanine and L-tyrosine. The ratio of amino acid concentration in the mist to that in the solution was defined as the enrichment factor. As the flow rate of carrier gas became higher, the collection mass of mist increased and the enrichment factor decreased. The enrichment factor depended on the solution pH. The enrichment factor increased with decreasing amino acid concentration in the solution and enhanced by the addition of ultrafine bubbles. © 2022 The Japan Society of Applied Physics

Ultrasonic atomization generates a mist including fine droplets at room temperature.[1–4)] When an ethanol aqueous solution is atomized by ultrasound, the ethanol is enriched in the mist.[5–9)] Ethanol enrichment is attributed to the hydrophobic interaction of ethanol molecules in water.[8)] Separation by ultrasonic atomization also has been reported on ketones,[10)] amino acids,[11,12)] surfactant,[13)] fine particles,[14)] and ion liquid.[15)] Amino acids, which have surface active properties, are often used in the pharmaceutical and food industries. The authors separated phenylalanine by ultrasonic atomization.[12)] Advantages of ultrasonic atomization separation are that it is simple to operate, free from maintenance, and available to heat-sensitive materials. Recently, Kozuka et al. reported that the attachment of a horn above a transducer enhanced the mist generation of ultrasonic atomization.[2,3)]

Fine bubbles of less than 1 μm in diameter are termed as ultrafine bubbles.[16,17)] Ultrafine bubbles are able to stay in water for more than a few months[18)] because their rise velocity due to buoyancy is negligibly small. They also have a very large specific surface area, are biologically active,[19)] and are electrically charged at the surface.[20)] Ultrafine bubbles water is attracting attention in many fields such as cleaning,[21)] medicine,[22)] and surface treatment.[23)] In this study, ultrasonic atomization separation was conducted to aqueous solutions of amino acids using an atomizer with the horn. As amino acids, L-phenylalanine and L-tyrosine were used. Effects of the flow rate of carrier gas, the solution pH and the amino acid concentration in the solution, and the addition of ultrafine bubbles on the enrichment characteristics of amino acids were investigated.

Figure 1 shows the outline of the experimental apparatus. A cylindrical vessel was made from transparent acryl resin. The inside diameter and height of the vessel were 80 and 250 mm, respectively. A disc-shaped PZT transducer with a horn (Honda Electronics, HMC-2400) was attached at the middle of the vessel bottom. The diameter of the transducer was 20 mm and the dimension of the horn was presented elsewhere.[2)] The ultrasonic frequency was 2.4 MHz and the ultrasonic irradiation time was 30 min. L-Phenylalanine and L-tyrosine were purchased from Kanto Chemical Co. Inc. and FUJIFILM Wako Pure Chemical Corporation, respectively. These reagents were used without further purification. The initial volume and height of the sample in the vessel were 200 ml and 43 mm, respectively. Dry nitrogen in a cylinder was used as carrier gas and flowed into the vessel center. The mist generated by ultrasonic atomization was accompanied by the carrier gas, exited the vessel from the middle of the vessel top and was collected in a 100 ml graduated cylinder immersed in liquid nitrogen. The concentrations of phenylalanine and tyrosine in the collected mist were measured by an ultraviolet–visible spectrophotometer (UV-1900, Shimadzu). Mass change of the sample solution by ultrasonic atomization was measured by an electric balance to determine the mass of mist generation. Ultrafine bubbles water was produced from ultrapure water (Milli-Q Reference and Elix Essential UV5, Merck) and air by pressurized dissolution method (ultrafineGaLF, IDEC). The number concentration and mean diameter of ultrafine bubbles measured by nanoparticle tracking method (NanoSight, Malvern) were about 5×10^9 ml^{-1} and 100 nm, respectively. The solution pH was changed using sodium hydroxide (FUJIFILM Wako Pure Chemical Corporation).

Figure 2 shows the effect of the flow rate of carrier gas on the collection mass of mist. Samples were aqueous solutions of phenylalanine (0.2 mmol l^{-1}) and tyrosine (0.01 mmol l^{-1}). The solution pH was 7. In both samples, the collection mass of mist increased with the flow rate of carrier gas. This is because there is a size distribution of atomized droplets[1)] and large droplets accompany by the carrier gas with a high flow rate. The difference in the collection mass of mist between samples was small. The collection ratio of mist, which is the ratio of the collection mass of mist to the mass change of solution by ultrasonic atomization, was plotted in Fig. 2. As the flow rate of carrier gas became higher, the collection ratio of mist increased and became almost constant at 70%–75%.

The enrichment factor is defined as the ratio of the amino acid concentration in the collected mist to that in the solution. Figure 3 shows the effect of the flow rate of carrier gas on the enrichment factor. In both samples, enrichment factors were higher than 1. This result indicates that ultrasonic atomization can concentrate phenylamine and tyrosine in the aqueous solution. The enrichment factor increased with decreasing flow rate of carrier gas. In a previous paper,[8)] we performed ultrasonic atomization of ethanol solution and reported that the ethanol concentration in small droplets was higher than that in large droplets. Since phenylalanine and tyrosine have surface active properties, they adsorb to gas–liquid interfaces of solutions and then enter droplets through ultrasonic atomization. Small droplets are considered to consist of a highly concentrated amino acid solution because the ratio of the surface area to the volume is large.

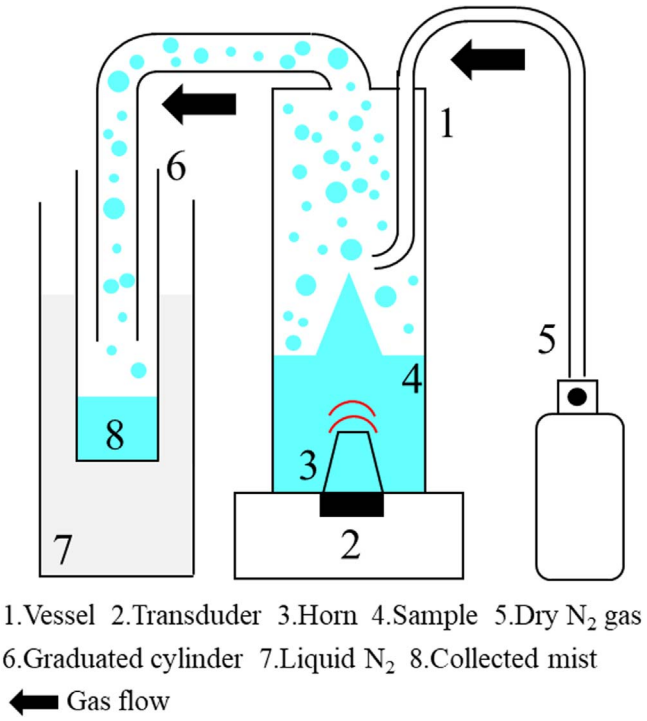

Fig. 1. (Color online) Outline of experimental apparatus.

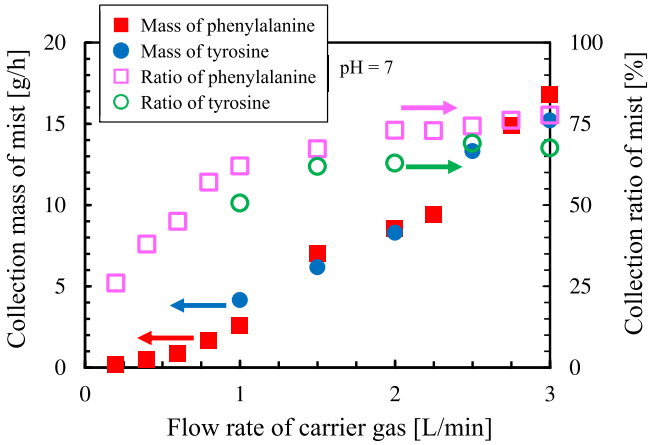

Fig. 2. (Color online) Effect of flow rate of carrier gas on collection mass and collection ratio of mist.

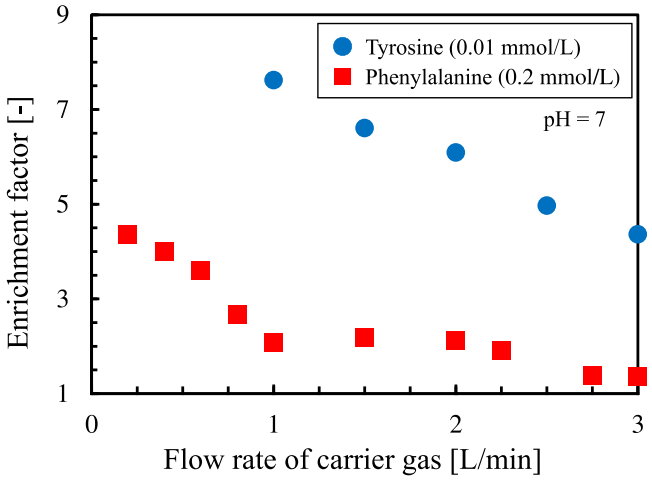

Fig. 3. (Color online) Effect of the flow rate of carrier gas on enrichment factor.

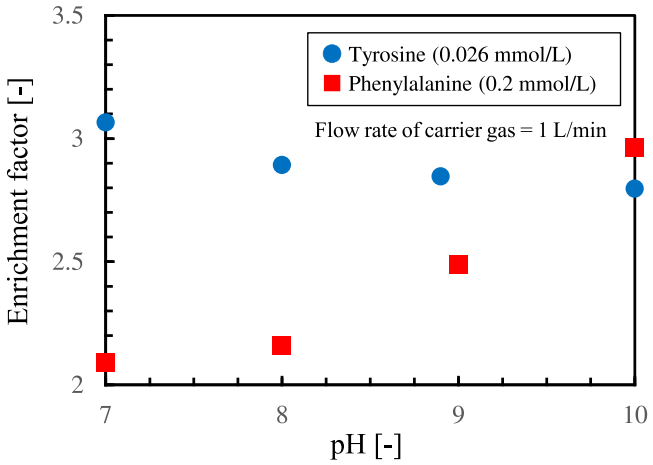

Fig. 4. (Color online) Effect of solution pH on enrichment factor in collected mist.

Figure 4 shows the effect of the solution pH on the enrichment factor. The flow rate of carrier gas was 1 l min^{-1}. In the case of phenylamine, the enrichment factor increased with the solution pH. This is because the adsorption equilibrium constant and the saturated adsorption density was the highest at pH 10.[11] As a result, the concentration in the mist became higher as pH was close to 10. On the other hand, for the case of tyrosine, the enrichment factor slightly decreased with increasing solution pH. This reason may be due to solubility. Although the tyrosine solubility to water is extremely low, tyrosine dissolves in sodium hydrate solution. A higher concentration of sodium hydroxide may reduce the hydrophobic property of tyrosine.

Figures 5(a) and 5(b) show the effect of the amino acid concentration in the solution on the enrichment factor with and without ultrafine bubbles for phenylalanine and tyrosine. The solution pH of phenylalanine and tyrosine were 10 and 7, respectively. Regardless of concentration in solutions, phenylalanine and tyrosine were enriched in the mist by ultrasonic atomization. In both solutions, the enrichment factor increased with decreasing concentration in the solution. This is because as the amino acid concentration in the solution becomes lower, the ratio of the number of amino acid molecules adsorbed on the solution surface against the number of total amino acid molecules in the solution becomes higher. When phenylalanine concentration was the same as tyrosine concentration, the enrichment factor of phenylalanine was higher than that of tyrosine. Tyrosine has a molecular structure that is a hydroxylation of phenylalanine. Compared with phenylalanine, the hydrophobic property of tyrosine may be low since the OH group is possible to be ionized. Furthermore, the enrichment factors with ultrafine bubbles were higher than those without ultrafine bubbles. The authors believe that this reason is explained as follows. Ultrafine bubbles adsorb amino acids since ultrafine bubbles have hydrophobic surfaces.[18] Ultrafine bubbles with amino acids aggregate or coalesce by secondary Bjerknes force under ultrasonic fields, rise in the solution by buoyancy force, and burst at the solution surface.[24] When ultrafine bubble water was used as the sample, the number concentration of ultrafine bubbles in the sample before and after atomization were 5.0×10^9 and 4.4×10^9 ml^{-1}, respectively. The concentration of amino acid increases at the solution surface and many

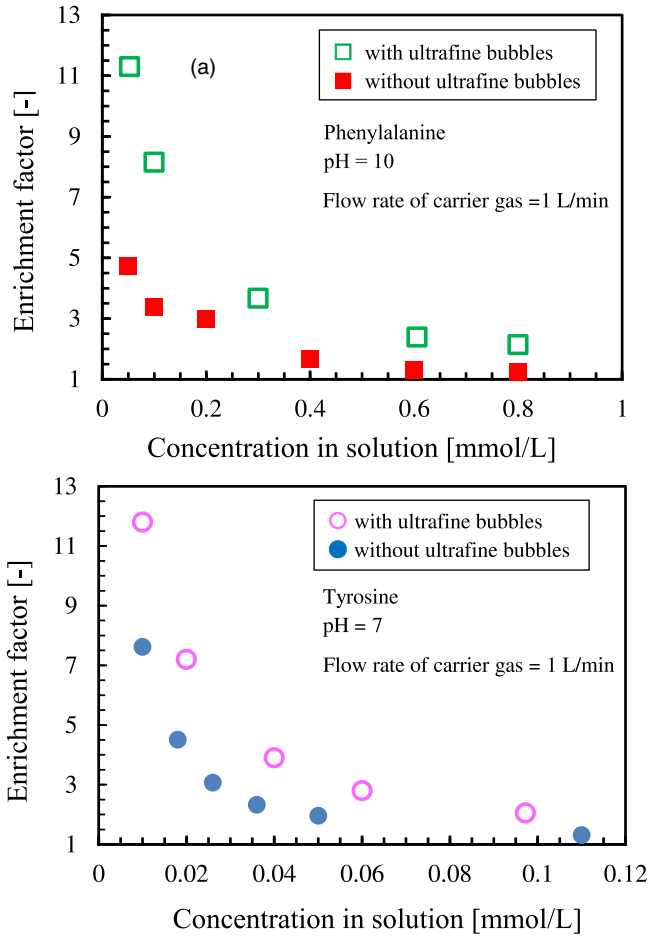

Fig. 5. (Color online) Effect of concertation in solution on enrichment factor with and without ultrafine bubbles for (a) phenylalanine at pH = 10 and (b) tyrosine at pH = 7.

amino acids are contained in droplets. The difference of enrichment factor between with and without ultrafine bubbles became larger as the amino acid concentration in the solution decreased. Since the number of amino acid molecules in the solution is much larger than that of ultrafine bubbles, a part of the amino acids in the solution adsorbs on surfaces of ultrafine bubbles. Therefore, when the concentration of amino acid in the solution is low, the enrichment enhancement effect by ultrafine bubbles is significant. For the future, the authors plan to use ultrasonic atomization and ultrafine bubbles, and effectively separate heat-sensitive bioactive substances such as vitamins in addition to amino acids.

Acknowledgments This work was partially supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant No. JP19H02505.

1) T. Kudo, K. Sekiguchi, K. Sankoda, N. Namiki, and S. Nii, Ultrason. Sonochem. **37**, 16 (2017).
2) T. Kozuka, J. Ando, M. Sato, S. Hatanaka, and K. Yasui, Jpn. J. Appl. Phys. **58**, SGGD18 (2019).
3) T. Kozuka, S. Hatanaka, T. Yoshimoto, M. Sato, and K. Yasui, Jpn. J. Appl. Phys. **59**, SKKD16 (2020).
4) T. Aikawa and N. Kudo, Jpn. J. Appl. Phys. **60**, SDDD13 (2021).
5) M. Sato, K. Matsuura, and T. Fujii, J. Chem. Phys. **114**, 2382 (2001).
6) K. Suzuki, T. W. McCracken, and D. M. Kirpalani, J. Chem. Eng. Jpn. **41**, 855 (2008).
7) K. Hamai, N. Takenaka, B. Nanzai, K. Okitsu, H. Bandow, and Y. Maeda, Ultrason. Sonochem. **16**, 150 (2009).
8) K. Yasuda, K. Mochida, Y. Asakura, and S. Koda, Ultrason. Sonochem. **21**, 2026 (2014).
9) K. Yasuda, Y. Nohara, and Y. Asakura, Jpn. J. Appl. Phys. **59**, SKKD09 (2020).
10) K. Yasuda, Y. Bando, S. Yamaguchi, M. Nakamura, E. Fujimori, A. Oda, and Y. Kawase, J. Chem. Eng. Jpn. **37**, 1290 (2004).
11) A. Suzuki, H. Maruyama, H. Seki, Y. Matsukawa, and N. Inoue, Ind. Eng. Chem. Res. **45**, 830 (2006).
12) K. Yasuda, K. Hamada, and Y. Asakura, Proc. Symp. Ultrason. Electron., **42**, 2Pa4-1 (2021).
13) B. Jimmy, S. Kentish, F. Grieser, and M. Ashokkumar, Langmuir **24**, 10133 (2008).
14) S. Nii and N. Oka, Ultrason. Sonochem. **21**, 2032 (2014).
15) N. L. Mai, Y.-M. Koo, and S. H. Ha, Ultrason. Sonochem. **53**, 187 (2019).
16) M. Alheshibri, J. Qian, M. Jehannin, and V. J. Craig, Langmuir **32**, 11086 (2016).
17) T. Tuziuti, K. Yasui, and W. Kanematsu, Jpn. J. Appl. Phys. **59**, SKKD03 (2020).
18) W. Kanematsu, T. Tuziuti, and K. Yasui, Chem. Eng. Sci. **219**, 115594 (2020).
19) S. Liu, S. Oshita, S. Kawabata, Y. Makino, and T. Yoshimoto, Langmuir **32**, 11295 (2016).
20) N. Nirmalkar, A. W. Pacek, and M. Barigou, Softw. Matter **14**, 9643 (2018).
21) Z. Wu, H. Chen, Y. Dong, H. Mao, J. Sun, S. Chen, V. S. J. Craig, and J. Hu, J. Colloid Interface Sci. **328**, 10 (2008).
22) V. L. Safonov and A. K. Khitrin, Colloids Surf. A **436**, 333 (2013).
23) H. Matsuno, T. Ohta, A. Shundo, F. Fukunaga, and K. Tanaka, Langumir **30**, 15238 (2014).
24) K. Yasuda, H. Matsushima, and Y. Asakura, Chem. Eng. Sci. **195**, 455 (2019).