A simple method was created and implemented through the technology of ink-jet printing to study the effects of three chemical factors (chemical reagents) to the ninhydrin reaction. The effects of each single reagent and their interactions on the reaction were studied in one experiment. The three reagents all have effects on ninhydrin reaction, and the effects under the different combinations of reagents were presented on a chip. This work was completed efficiently with a smaller experimental workload compared with the traditional method.

**Keywords** Amino acids, ninhydrin, ink-jet printing, high throughput, digitalization

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Four reaction areas were cut off and marked as I, II, III, IV according to designed template, respectively. In the gray image, every pixel corresponded to a micro-reaction unit on the reaction chip. The gray value of the pixel was represented in an integer (a byte) from 0 to 255 (black to gray and white); black is 0 and white is 255, and each gray value corresponded to the color of the experimental result in the pixel.

Some gray values (rows and columns) from the gray image were presented in a 2-D coordinate (Fig. S4, Supporting Information). In Fig. S4(a), in reaction I (condition of A and B), when there is only A (B = 0), the effect on the reaction is not obvious with the change of the A amount. By comparison, when under the interaction of A and B, the effect is obvious. In Fig. S4(b), in reaction III (condition of B and C), when there is only B (C = 0), the effect on the reaction is not obvious with the change of the C amount. By comparison, when under the interaction of B and C, there is an effect on reaction, too.

The contour map is drawn to analyze the results, and four areas (I, II, III, IV) are expressed, respectively, in Fig. 3 ((a) – (d)). In Fig. 3(a), the result demonstrates that in the top-right region (the amount of A is large; B is relatively small), there is no significant effect on the ninhydrin reaction. When the amount of A is small, the gray values are increasing with the C amount changing; there is significant effect on the ninhydrin reaction. In Fig. 3(c), the interaction of B and C has no significant effect on the ninhydrin reaction. In Fig. 3(d), in the above half region, there is an obvious effect on the reaction under the interaction of A, B and C; in this case, the A amount is large compared with the lower half.

Control experiments also were made under the conditions in the absence of the amino acid sample and three reagents (A, B and C) in Fig. S5 (Supporting Information). In Fig. S5(a), there is no color on the chip without the sample. This proves that ninhydrin reaction does not occur in the absence of the amino acid samples. In Fig. S5(b), there is an obvious result on the chip, and the color is uniform (under the condition of ninhydrin and amino acid sample without chemical reagents). Compared with Fig. 2(a), this proves that the ninhydrin reaction is effected by the three reagents. Moreover, the results that appeared as a pattern on the reaction chip are relative to the design rules of the three reagents (on the design template).

In a word, chemical reagent A has no obvious effect on the ninhydrin reaction; however, A with B and A with C have an obvious effect on the reaction. Both B and C have an obvious effect on the reaction, while the interaction of B and C has no obvious effect on the reaction. Under the interaction of A, B and C, the amount of A plays a role on effect to the reaction.

In conclusion, we carried out a new method to study the ninhydrin reaction affected by the interaction of three chemical reagents through a high-throughput experiment and ink-jet printing, and obtained much information by one experiment. This method shortens the experimental workload and completes a difficult problem concerning the traditional experiment.

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Supporting Information

Experimental section; progress of the screening experiment; change rules of the reagents amount on the designed template; error analysis of the experiment results; changes of the gray values; images of the control experiments. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
Fig. 3 Contour map of four reaction areas: (a), (b), (c), (d), corresponding to the reaction areas I, II, III, IV, respectively. X, Changes of the gray values along the lateral displacement; Y, changes of the gray values along the longitudinal displacement.

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