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Comparative amino acid color constants from hydrindantin–ninhydrin reagents produced with Ni–Pt and $SnCl_2$ reducing agents

Some experiences were recently published concerning a "ninhydrin reactor", which electrochemically produces hydrindantin–ninhydrin reagent for automated amino acid analyzers[1]. One of the advantages claimed for the reactor, improved color yields, was not discussed because "passivation" of the electrodes was then a limiting concern. Subsequently, it became obvious that the color yield of several compounds was markedly different for ninhydrin made with the reactor *versus* that made with $SnCl_2$. For instance, the adjacent peaks of glycine and alanine were nearly equal from ninhydrin containing $SnCl_2$ whereas the glycine peak was visibly larger and the alanine peak much smaller from "reactor" ninhydrin. This led to a closer look at comparative color yields of a range of amino acids.

A reference mixture containing more than 30 ninhydrin positive compounds, including norleucine as the internal standard, was eluted from a single high-resolution column of 60 × 0.9 cm using lithium citrate buffers (0.3 *N* Li at pH 2.80 and 4.10; 0.8 *N* Li at pH 4.90) in step-change sequence. Replicate runs were made using ninhydrin prepared both with the electrolytic nickel–platinum couple and with $SnCl_2$. Color yields of separated compounds were obtained with an electronic "integrator-calculator" (Technicon Corp., Tarrytown, N.Y.)* and color factors (CF) relative to the norleucine internal standard were calculated from the color yields.

TABLE I

COMPARATIVE COLOR FACTORS, CF[a], OF AMINO ACIDS RESULTING FROM "REACTOR" AND "$SnCl_2$" REDUCED HYDRINDANTIN–NINHYDRIN REAGENTS

Amino acid	*Reactor Av*[b]	*$SnCl_2$ Av*[c]	*Amino acid*	*Reactor Av*[b]	*$SnCl_2$ Av*[c]
Phosphoethanolamine	11.07[2]	6.03	Cystathionine	0.64	0.99
Taurine	5.16[2]	2.74	Methionine	0.64	0.95
Hydroxyproline	8.70	11.72	Isoleucine	1.06	0.99
Aspartic acid	1.07	1.22	Leucine	0.86	1.01
Threonine	1.10	1.22	Glucosamine	1.31	1.34
Serine	1.12	1.21	Tyrosine	0.75	1.07
Asparagine	1.55	1.77	Phenylalanine	0.76	1.10
Glutamine	1.01	1.30	β-Alanine	10.08[2]	4.11
Sarcosine	High	46.50	β-Aminoisobutyric acid	6.63[2]	2.50
Proline	4.70	5.73	γ-Aminobutyric acid	1.88[1]	1.07[4]
Glutamic acid	0.90	1.18	Ornithine	0.96[1]	1.10[4]
Citrulline	1.23	1.28	Ammonia	1.31[1]	0.74[4]
Glycine	0.81	1.17	Lysine	0.95[1]	0.93[4]
Alanine	1.51	1.23	1-Methylhistidine	0.67[1]	1.05[4]
α-Aminoadipic acid	1.40	1.39	Histidine	0.51[1]	1.04[4]
α-Aminobutyric acid	1.30	1.25	3-Methylhistidine	0.64[1]	1.09[4]
Valine	1.31	1.26	Arginine	0.93[1]	1.09[4]
Half-cystine	1.22	2.46			

[a] CF = yield of norleucine/yield of specific amino acid.
[b] Average of 3 determinations, except where denoted otherwise by superscript.
[c] Average of 5 determinations, except where denoted otherwise by superscript.

* Mention of trademark or company names is for information purposes and does not imply endorsement or preferential treatment by the U.S. Department of Agriculture.

The results in Table I show that the ninhydrin reactor in our system was as much a disadvantage as an advantage with respect to color yields. Although for many compounds the yield relative to norleucine was higher with the reactor (smaller CF value, *e.g.*, hydroxyproline, glutamine, glutamic acid, glycine, half-cystine, cystathionine, methionine, tyrosine, phenylalanine, histidine and the methylhistidines) for almost as many other compounds the yield was lower (larger CF value, *e.g.*, phosphoethanolamine, taurine, sarcosine, alanine, β-alanine, β-aminoisobutyric acid, γ-aminobutyric acid and ammonia). The much poorer reaction for already poor color yielders (such as phosphoethanolamine, taurine, sarcosine, β-alanine, and β-aminoisobutyric acid) is especially serious when analyzing complex samples that contain numerous compounds of interest besides the common protein amino acids.

That the two methods of reducing ninhydrin did not yield equivalent color constants is less surprising than distressing. To avoid potentially gross errors, it appears necessary to determine color constants on every compound of interest for each different method of preparing hydrindantin–ninhydrin reagent. Harassed analysts would welcome more complete elucidation of the basic chemistry of hydrindantin and ninhydrin as related to their reactions with amino acids and other ninhydrin-positive compounds.

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Sugar borate chromatography using refractometry for monitoring

After the introduction by KHYM AND ZILL[1,2] of the ion-exchange chromatographic separation of neutral sugars as borate complexes, many analytical modifications of this principle have been described[3–15]. The eluates have, as a rule, been analysed colorimetrically. We have found it of interest to try differential refractometry for this purpose. Although less sensitive than the colour reactions, refractometry has advantages in the form of being highly reproducible and easy to run with minimal cost of reagents. Furthermore the components may afterwards be recovered for further identification. The use of refractometry does involve certain difficulties, however, in the form of sensitivity to variations of buffer concentration and lack of specificity. In the present paper we report the successful application of refractometry in the routine analysis of different sugars in SOMOGYI filtrates[16] of food products. The determination of the individual sugars in such products is an essential problem in the food industry.