

Chemiluminescence Detection of Nitrite in Nonfat Dried Milk Powders

ROBERT C. DOERR, ROBERT A. GATES, and WALTER FIDDLER

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118

Determination of nitrite in nonfat dry milk powders by chemiluminescence detection was compared with a colorimetric method specifying Griess reagents. The chemiluminescent technique requires no sample preparation, is free from apparent interferences, and is sensitive to 25 ppb nitrite. Statistical analysis shows no difference at $P = 0.05$ between the colorimetric and chemiluminescent methods, based on the analysis of 16 commercial nonfat dry milk samples.

Nitrite in nonfat dry milk (NFDM) powder results from nitrogen oxides generated by the direct-fired dryers now commonly used in the United States. Recent reports (1, 2) of the presence of dimethylnitrosamine in NFDM have placed new importance on the determination of nitrite because of its role as a precursor in nitrosamine formation.

Nitrite in milk is generally determined colorimetrically using some form of Griess reaction, which is the basis of the method proposed by the International Organization for Standardization (ISO) in 1979 (3). However, the colorimetric method requires considerable sample preparation and is subject to error resulting from solution turbidity, color caused by pigment formation, and naturally occurring reductants. A chemiluminescence detection (CLD) method, unaffected by these problems, has been reported by Walters et al. (4) for nitrite analysis in a simulated food matrix. Cox (5), using sodium iodide as a reductant, determined nitrite in water solutions by CLD. Dahn and Loewe (6) studied the quantitative reduction of nitrite by ascorbate and reported a 92% conversion to nitric oxide at pH 5. Doerr et al. (7) used sodium ascorbate as a reductant to measure nitrite in cured meat slurries by CLD. The purpose of the present study is to determine whether the CLD method is suitable for measuring low levels of nitrite in NFDM.

Experimental

Milk

Two and one-half g of commercial NFDM powder was reconstituted with double deionized water to 25 mL. Two drops of Dow Corning

Antifoam A silicone defoamer were added to prevent excessive foaming during mixing.

Reagents

All chemicals were reagent grade or highest purity available and were used without further purification.

Double deionized water was prepared by passing tap water through a charcoal filter, a mixed bed ion exchange resin, a Barnstead standard ion exchange cartridge, and, finally, a Barnstead organic removal column.

Chemiluminescence Method (7)

Two mL of reconstituted milk was added to the reaction flask containing ca 56 mg solid sodium ascorbate, 44 mg solid tartaric acid, and 20 μ L silicone antifoam agent and stirred vigorously. The evolved nitric oxide was injected into a thermal energy analyzer (Model 502, Thermo Electron Corp., Waltham, MA) by means of a Carle No. 5518 6-port minivalve (Carle Instruments, Inc., Anaheim, CA) as described elsewhere (7). Peak areas were measured by the integrator supplied with the thermal energy analyzer. Sodium nitrite concentrations were calculated by comparing sample peak areas with those obtained from aqueous standard solutions of sodium nitrite at similar concentrations. Standards were measured several times a day to assure good repeatability. Solvent and reagent blanks were analyzed daily and contained insignificant levels of nitrite. However, all sample calculations were corrected for these low values. A minimum of 3 injections for each sample was used to determine the mean injection value.

Colorimetric Method

Solutions.—(a) *Carrez I.*—17.2 g potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) diluted to 100 mL with water.

(b) *Carrez II.*—53.5 g zinc sulfate ($ZnSO_4 \cdot 7H_2O$) diluted to 100 mL with water.

Received September 15, 1981. Accepted November 20, 1981.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Table 1. Nitrite in commercial nonfat dry milk powders

Sample	Chemiluminescence			Colorimetric (Griess)		
	NaNO ₂ , ^a ppb	SD	CV, %	NaNO ₂ , ^a ppb	SD	CV, %
1	35	7	20	350	0	0
2	425	120	28	720	170	24
3	990	127	13	1400	148	11
4	1090	191	17	1670	226	13
5	1110	85	7.6	1280	0	0
6	1510	148	9.8	1510	7	0.5
7	1630	57	3.5	1780	7	4.0
8	2160	49	2.3	2350	198	8.4
9	2550	106	4.1	2370	49	2.1
10	2580	7	0.3	2800	64	2.3
11	2610	325	12	3120	403	13
12	3480	99	2.8	3440	290	8.4
13	3710	516	14	3740	134	3.6
14	3910	361	9.2	4200	311	7.4
15	4280	84	2.0	4230	156	3.7
16	8670	955	11	7750	290	3.7

^a Average of duplicate analyses.

(c) *Buffer*.—5 mL concentrated HCl added to 50 mL solution containing 10 mL concentrated NH₄OH and 40 mL water. Resulting solution was diluted to 100 mL with water.

(d) *Griess reagent*.—0.133 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED) and 0.333 g sulfanilamide dissolved in 100 mL 15% acetic acid solution.

Procedure.—Twenty mL reconstituted milk was added to a 50 mL centrifuge tube; 4.0 mL Carrez II, 4.0 mL Carrez I, and 3.0 mL buffer were added in sequence. The solution was stirred thoroughly after each addition and left to stand 5 min after the final addition. The solution was centrifuged at 15 000 rpm for 15–20 min, and the supernatant liquid was filtered through Whatman No. 50 paper which had been previously washed for 30 min with deionized water to remove residual nitrite and then dried in a 115°C oven for 1.5 h. One mL Griess reagent was added to 5 mL filtrate, and the solution was diluted to 10 mL. After a color developing time of 20 min, the absorbance was measured at 515 nm with a Sargent Welch Model 6-550 UV/visible spectrophotometer. The nitrite concentration was calculated by comparing the final absorbance to a calibration curve. The final absorbance was determined by measuring absorbance of the sample plus Griess reagent vs Griess alone and subtracting from this value the absorbance of undiazotized milk. It was necessary to make this correction because the milk samples had a slight color that would be measured erroneously as nitrite.

The colorimetric procedure used in this study is similar to the ISO method for nitrite in dried milk except that we add a centrifugation step before filtration, and our final sample concentration is approximately 3 times greater than that recommended. The higher concentration was necessary to measure nitrite at the low ppb level. As a result, the volume of precipitate formed represented a significant part of the total volume, thereby making it necessary to measure the volume of supernatant liquid after centrifugation in order to calculate the final nitrite concentration.

Results and Discussion

Recovery of Added Nitrite

Milk powder dried by indirect heat and containing low apparent nitrite was spiked with sodium nitrite to yield a final concentration of 250, 2000, and 10 000 ppb. Recoveries of duplicate analyses were 75% at the 250 ppb level, 81% at the 2000 ppb level, and 91% at the 10 000 ppb level for CLD, and 82 and 88% at the 2000 and 10 000 ppb levels, respectively, for the Griess method.

Linearity, Sensitivity, Repeatability

The nitric oxide response was linear over a range equivalent to 25–20 000 ppb sodium nitrite in NFDM powder. The theoretical detection limit for a 10 mL sample and assuming a 2:1 signal-to-noise ratio was 2.5 ppb in the reconstituted milk. From a practical point of view, 25 ppb

Table 2. Analysis of variance for CLD vs colorimetric methods for nitrite

Source	DF	SS	MS	F	Signif. level
Sample	15	22.4 × 10 ⁷	14.9 × 10 ⁶	220	***
Method	1	24.3 × 10 ⁴	24.3 × 10 ⁴	3.58	NS ^a
Sample × method	15	18.1 × 10 ⁵	12.1 × 10 ⁴	1.78	NS
Error	32	21.7 × 10 ⁵	67.7 × 10 ³	—	—
Total	63	22.8 × 10 ⁷	—	—	—

*** $P < 0.001$.

^a NS = not significant.

sodium nitrite was considered the minimum level of reliable measurement, based on 75% average recovery from spiked samples containing low levels of normally incurred nitrite.

For the determination of instrument repeatability, 16 determinations ($n = 16$) of 500 ppb NaNO₂ standard solutions were made over a period of several weeks. The standard deviation (SD) and coefficient of variation (CV) were 23 ppb and 4.6%, respectively. During the same period, a commercial milk sample was analyzed 5 times and was determined to contain 600 ± 16 ppb sodium nitrite (CV 2.7%).

Nitrite Stability

Great care should be exercised when using very dilute standard nitrite solutions. We found that, even for samples stored under ideal conditions (brown bottle, cool environment), sudden loss of nitrite can occur without apparent reason. In several cases, after days of remaining at a constant value, a solution of 500 ppb sodium nitrite decreased in concentration by 90% overnight. Therefore, it is recommended that standards, particularly very low concentrations, be checked regularly to ensure that no loss has occurred.

Comparative Determination of Nitrite

Table 1 lists the mean results of duplicate nitrite analyses, uncorrected for percent recovery, of 16 commercial nonfat dry milk samples by the CLD and colorimetric methods. A comparison of the mean differences and coefficients of variation by a 2-tailed paired *t*-test indicates no significant differences at the $P = 0.05$ level.

Table 2 shows the results of analysis of vari-

ance of the comparison of the CLD and Greiss nitrite analyses. The data show that over a wide range of sample levels there is no significant ($P = 0.05$) difference between the 2 methods. In addition, the interaction between the range of sample levels and the different methods shows no significant effect statistically.

Since significant differences were not apparent in the statistical tests ($P = 0.05$), it was concluded that the results of the 2 methods were equivalent.

These results indicate that inorganic nitrite can be determined reliably in powdered milk by CLD with no sample preparation at a minimum detectable level of 25 ppb that is unattainable by other methods.

Acknowledgment

The authors thank Jay B. Fox, Jr, for his advice and assistance in carrying out this study and Thomas Fazio and Donald Havery, FDA, Bureau of Foods, for supplying the nonfat dry milk powder samples.

REFERENCES

- (1) Libbey, L. M., Scanlan, R. A., & Barbour, J. F. (1980) *Food Cosmet. Toxicol.* **18**, 459-461
- (2) Lakritz, L., & Pensabene, J. W. (1981) *J. Dairy Sci.* **64**, 371-374
- (3) Draft Proposal ISO/DP No. 6736 (1979) (Derived from ISO No. 4099), ISO, Geneva, Switzerland
- (4) Walters, C. L., Downes, M. J., Hart, R. J., Perse, S., & Smith, P. L. R. (1978) *Z. Lebensm. Unters. Forsch.* **167**, 229-232
- (5) Cox, R. D. (1980) *Anal. Chem.* **52**, 322-325
- (6) Dahn, H., & Loewe, L. (1960) *Helv. Chim. Acta* **43**, 287-333
- (7) Doerr, R. C., Fox, J. B., Lakritz, L., & Fiddler, W. (1981) *Anal. Chem.* **53**, 381-384