

FEEDS

Automated Determination of Crude Protein, Phosphorus, Calcium, Iron, and Magnesium in Feeds by Using Stopped-Flow Analyzer

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Methods are described for determination of crude protein, phosphorus, calcium, iron, and magnesium in feeds, using an automated microprocessor-based stopped-flow analyzer. Crude protein is determined by a reaction-rate procedure based on the ammonia-sodium phenate-hypochloride Berthelot reaction. Phosphorus determination is based on a phosphomolybdenum blue reaction-rate method. *o*-Cresolphthalein complexone, ferrozine, and calmagite are used as colorimetric reagents for calcium, iron, and magnesium, respectively. The methods are precise with relative standard deviations less than 1%, rapid with analysis rates of 110-266 samples per hour, sensitive, and require less than 1 mL sample and reagent volumes. Results for feed samples are comparable with those obtained by official AOAC methods.

The large number of animal feed samples presented for determination of several constituents in research, industrial, and regulatory laboratories has led to an increasing demand for rapid and precise analytical procedures. Various automated methods based on air-segmented continuous flow or flow-injection analysis have been developed in the routine analysis of agricultural products, and some of these have been adopted as official methods. In this paper, the use of a relatively simple stopped-flow analyzer is described for automated routine analyses of animal feeds. The stopped-flow technique has been shown in recent years to be a generally applicable analytical system, especially since microcomputer-controlled stopped-flow systems have been developed (1-5). The main advantages of the stopped-flow system are the rapid and thorough mixing of the reagent and sample, the ability to obtain measurement in a short time after mixing, the use of kinetic methods with all their advantages (6), and the high overall precision.

In this investigation, colorimetric methods for the determination of crude protein, phosphorus,

calcium, iron, and magnesium in feeds have been adapted to an automated stopped-flow analyzer and evaluated in the analysis of AAFCO check feed samples. The importance of determining these analytes in feeds is based on the fact that they are essential nutrients needed for various cellular processes and perform functions in animal metabolism.

The determination of crude protein is based on a reaction-rate method developed for a stopped-flow system (7) and uses the ammonia-sodium phenate-hypochloride reaction. Phosphorus is determined using the phosphomolybdenum blue reaction in a reaction-rate procedure (8). For the determination of calcium, the *o*-cresolphthalein complexone reaction, introduced by Kessler and Wolfman (9) and improved by Gitelman (10), Moorehead and Biggs (11), and others, for the determination of calcium in serum is adapted to the stopped-flow analyzer.

The reaction of ferrous ions with ferrozine, a new sensitive reagent introduced by Stookey (12), is used for the determination of iron. This reagent is now widely used in the determination of serum iron (13-15). Magnesium determination is based on the magnesium-calmagite complex formation, a reaction introduced by Gindler and Heth (16) for the determination of magnesium in human blood serum. The method was introduced commercially by the Pierce Chemical Co. and has been adapted to a centrifugal analyzer (17).

METHODS

Principles

(1) *Crude protein.*—Samples are digested and the produced ammonia is determined by the Berthelot reaction in which ammonia reacts with hypochloride to form chloramine which, in turn, reacts with phenol under alkaline conditions to form indophenol. The stopped-flow analyzer aliquots and thoroughly mixes about 150 μ L sample digest (premixed with phenol) with an

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equal volume of hypochloride and rapidly transfers the reaction product to the observation cell, where the reaction-rate measurement is performed on the indophenol product at 630 nm during 15 s after a 10-s delay.

(2) *Phosphorus*.—The method involves the reaction of phosphate with molybdenum to form 12-molybdophosphoric acid and a subsequent reduction by ascorbic acid, to form phosphomolybdenum blue. Sample digests are premixed with ascorbic acid and the stopped-flow unit is used to aliquot and mix equal volumes of molybdenum reagent and samples and transfer the reaction product to the observation cell. The absorbance change is measured at 650 nm for 4-s measurement time after 2-s delay time.

(3) *Calcium*.—Calcium is complexed with *o*-cresolphthalein complexone in the presence of 8-quinolinol to avoid interference of magnesium and cyanide by excluding heavy metals from the reaction. 2-Amino-2-methyl-1-propanol is used as buffering agent which maintains the pH at 10. Absorbance is measured at 580 nm during 1 s after a 5-s delay time.

(4) *Iron*.—Iron in the sample digests is reduced to ferrous form with ascorbic acid in an acid solution. The stopped-flow analyzer is used to mix equal volumes of pretreated samples with ferrozine reagent in a pH 4.5 acetate buffer. Absorbance is measured at 560 nm during 2.5 s after a 10-s delay time.

(5) *Magnesium*.—The method involves the reaction of magnesium with the mettalchrome dye calmagite which forms a pink complex with magnesium in alkaline medium. Ethyleneglycol-bis(*p*-aminoethylether) *N,N'*-tetraacetic acid (EGTA) virtually eliminates calcium interference, and cyanide complexes all heavy metals. The absorbance is measured at 520 nm during 2.5-s measurement time 5 s after the mixing of the solutions.

Apparatus

(a) *Stopped-flow analyzer*.—The instrument was designed in our laboratory (18). The entire system is automated using a Rockwell AIM 65 microcomputer (Rockwell International Co., Anaheim, CA 92803) for control of all operations, data acquisition and reduction, display, and printout of results. The sampling/mixing module of the analyzer is used to aliquot and mix ca 150 μ L reagent and sample from the turntable, with a ratio accuracy better than 0.1%, and transfer the solution to an observation flow cell with a pathlength of 1.00 cm. Bandpass interference filters of about 10 nm half width are used

in the photometer module to isolate the desired wavelength. Investigative equilibrium and reaction-rate programs are used to optimize each procedure, and interactive or dedicated routine programs are used for routine analysis.

In the routine reaction-rate program, the slope of the absorbance vs time, in the desired measurement time interval, is calculated by regression analysis using 30 points and is printed out as rate in milliabsorbance units per second (mA/s). In the routine equilibrium program, the absorbance measured is the average of a number of integrations in the desired time interval.

(b) *Dispenser/diluter*.—Model 25000 automatic pipet (Micromedic Systems Inc., Philadelphia, PA 19105), or equivalent.

(c) *Block digester*.—Labconco, Kansas City, MO; Tecator, Inc., Boulder, CO; or equivalent.

(d) *Volumetric digestion tubes*.—75 or 250 mL (Model 1007-021, Tecator, Inc., or equivalent).

Reagents

Use deionized water for preparing solutions.

(1) *Crude protein*.

(a) *Phenol reagent*.—Mix 5 g phenol, 0.656 g NaOH, and 2 g sodium potassium tartrate in 100 mL water; prepare day before use. Sodium potassium tartrate is added to eliminate interferences to Berthelot reaction from side reactions.

(b) *Hypochlorite solution*.—2 g NaOH/100 mL commercial bleach containing sodium hypochlorite (e.g., Clorox, Clorox Co., Oakland, CA 94623). Prepare fresh daily.

(c) *Buffer solution*.—pH 11; 6.44 g sodium phosphate dibasic and 0.364 g NaOH/100 mL water.

(d) *Hypochloride-buffer reagent*.—Equal volumes of reagents b and c.

(e) *Potassium sulfate-mercuric oxide reagent mixture*.—95.5 + 4.5 w/w, respectively (e.g., Mixture No. 5, Pope Kjeldahl Mixtures, Inc., Dallas, TX 75221).

(f) *Nitrogen standard solutions*.—12.5–75.0 mg N/250 mL. Prepare 250 mg N/250 mL stock solution by adding 4.7172 g (NH₄)₂SO₄ primary standard (dried 2 h at 105°C before use) into 1 L volumetric flask. Add 37.68 g reagent e and 34.8 mL H₂SO₄ (so that standard is in same matrix as samples after digestion) and dilute with water. Prepare working standard solutions by appropriate dilutions with reagent g.

(g) *Blank digest solution*.—37.68 g reagent e + 34.8 mL H₂SO₄/L water.

(2) *Phosphorus*.—All standards and reagents must be stored in polyethylene bottles to prevent leaching of silicon from volumetric glassware.

(a) *Molybdenum reagent*.—1.209 g sodium molybdate dihydrate/100 mL 0.1M H₂SO₄. Prepare day before use to ensure equilibrium of different forms of molybdenum in solution.

(b) *Ascorbic acid reagent*.—Prepare daily with 0.53 g ascorbic acid, 0.656 g NaOH, and 5 g KI (to avoid precipitation of mercury in the diluted digest by complex formation) in 100 mL water.

(c) *Phosphorus standard solutions*.—20–100 ppm P. Prepare 100 ppm stock solution by adding 0.439 g potassium dihydrogen phosphate, primary standard (Baker Chemical Co., Phillipsburg, NJ 08865), dried 2 h at 105°C, in 1 L volumetric flask. Add 37.68 g potassium sulfate-mercuric oxide mixture (reagent 1(e)) and 34.8 mL H₂SO₄ (so that standard is in same matrix as samples after digestion) and dilute with water. Prepare working standard solutions by appropriate dilution with reagent 1(g).

(3) Calcium.

(a) *o-Cresolphthalein complexone reagent*.—Prepare stock solution by adding 0.0375 g *o*-cresolphthalein complexone (Sigma Chemical Co., St. Louis, MO 63178) to 30 mL water in 100 mL volumetric flask. Add 0.85 g 8-quinolinol and 2 mL HCl to 40 mL water, add to same volumetric flask, and dilute with water. Store in polyethylene bottle.

(b) *Aminomethylpropanol base solution*.—0.25 g KCN + 22 g 2-amino-2-methyl-1-propanol (Aldrich Chemical Co., Milwaukee, WI 53233) diluted with water to 100 mL. Store in polyethylene container.

(c) *Cresolphthalein-base working reagent*.—Mix equal volumes of reagent a and b to form red solution. Store in acid-washed glass bottle. (Glassware used for preparation of this solution must be washed with dilute nitric acid to remove any trace amounts of calcium. Rinsing all glassware with working reagent ensures freedom from contamination.)

(d) *Calcium standard solutions*.—1–5 ppm. Prepare 1000 ppm stock solution by adding 2.4970 g calcium carbonate primary standard in 2 L beaker with 50 mL water. Add 7 mL HCl dropwise and boil solution 3–4 min to remove carbon dioxide. Transfer to 1 L volumetric flask washed by dilute nitric acid, and dilute with water. Prepare working solutions by appropriate dilutions with water.

(4) Iron.

(a) *Ferrozine reagent*.—Dissolve 0.080 g Ferrozine iron reagent (Hach Chemical Co. Loveland, CO 80537) in 100 mL 2M acetate buffer, pH 4.5.

(b) *Ascorbic acid reagent*.—1% in water.

(c) *Iron(III) standard solutions*.—0.5–10 ppm. Prepare 1000 ppm stock solution by dissolving 1.000 g analytical grade iron wire (99.88%) with aqua regia (3 mL HNO₃ + 9 mL HCl). Add 3 mL HClO₄ and evaporate solution almost to dryness. Dilute to 1 L with water. Prepare working standard solutions by appropriate dilutions with 0.3M HClO₄ (so that standards are in same matrix as samples after digestion).

(d) *Blank digest solution*.—0.3M HClO₄.

(5) *Magnesium*.

(a) *Calmagite reagent*.—Dissolve 0.020 g calmagite (Sigma) and 7.46 g KCl in 100 mL water. Store in polyethylene container.

(b) *Base solution*.—1 g KCN, 4.964 g KOH, and 0.700 g EGTA (Sigma)/L water. Store in polyethylene container.

(c) *Calmagite-base working solution*.—Equal volumes of reagents a and b. Prepare fresh daily and store in polyethylene bottle closed when not in use.

(d) *Magnesium standard solutions*.—0.2–2 ppm. Prepare 1000 ppm stock solution by dissolving 8.4485 g MgCl₂·6 H₂O (99%) in 1 L water. Prepare working standard solutions by appropriate dilutions with water.

(e) *Methyl red indicator solution*.—0.2 g/100 mL alcohol.

(f) *Sodium hydroxide*.—0.1N.

Digestion

(a) *Crude protein and phosphorus determination*.—Digest samples as described in official method 7.031 (19), using reagent 1(e).

(b) *Calcium, iron, and magnesium determinations*.—Digest samples with HNO₃-HClO₄ as described in official method 7.097 (19), using 2.5 g feed samples. Be sure that NO₂ fumes have been driven off, because nitrites in high concentration interfere with iron-ferrozine procedure.

Procedures

(1) *Crude protein*.—Put 630 nm filter in photometer module of analyzer and load reaction rate routine program from cassette recorder into computer memory. Using dispenser/diluter, mix 1 mL blank digest solution 1(g), each nitrogen standard, and digested samples with 4.00 mL phenol reagent 1(a) in 5 mL polyethylene cups and load them on turntable. Use one channel of stopped-flow analyzer to aliquot hypochlorite-buffer reagent 1(d) and the other to aliquot standards and samples. During program run,

Table 1. Typical nitrogen results, using reaction-rate method^a

Std, mg N/250 mL ^b	Rate, mA/s ^c	RSD, %
12.5	7.01	0.6
25.0	11.50	0.8
37.5	16.36	0.7
50.0	20.76	0.8
62.5	26.52	0.8
75.0	31.64	0.4

^a Working curve: slope = 0.394, intercept = 1.70, $r = 0.9991$.

^b Final concentration in observation cell is the 1/8.

^c Average of 4 determinations on a single standard with 10-s delay and 15-s measurement time. $\lambda = 630$ nm.

Table 2. Typical phosphorus results, using reaction rate method^a

Std, ppm P ^b	Rate, mA/s ^c	RSD, %
20.0	26.90	0.2
40.0	47.65	0.2
60.0	70.04	0.5
80.0	91.63	0.4
100.0	113.96	0.3

^a Working curve: slope = 1.091, intercept = 4.60, $r = 0.9992$.

^b Final concentration in observation cell is the 1/8.

^c Average of 5 determinations on a single standard with 2-s delay time and 4-s measurement time. $\lambda = 650$ nm.

0 and 100% transmittances are set using reagent and blank, and operator is asked time parameters, delay and measurement time, number of flushes to change from sample to sample, number of measurements to be averaged, number and concentration of standards, and number of samples to be analyzed. Set delay time of 10 s, measurement time of 15 s, 4 flushes, and 3 measurements. The analyzer sequences through standards and calculates calibration curve, each sample is measured, and value is printed. Use mg N/250 mL found for each sample to determine percent protein nitrogen, using following equation:

$$\% \text{ Protein} = (\text{mg N/250 mL}) \times (6.25/\text{mg sample}) \times 100$$

(2) *Phosphorus*.—Use 650 nm filter. Mix 1 mL blank digest solution 1(g) and each standard and sample with 4.00 mL ascorbic acid reagent 2(b). Use molybdenum reagent 2(a) and proceed as in crude protein procedure, using delay time of 2 s and measurement time of 4 s. Calculate percent phosphorus from ppm P found for each sample, using following equation:

$$\% \text{ P} = [(\text{ppm P}/4)/(\text{mg sample})] \times 100$$

(3) *Calcium*.—Put 580 nm filter in photometer and load equilibrium routine program in computer. Dilute samples with water to 1–5 ppm range. (For samples analyzed in this work, dilutions of 1/25, 1/50, and 1/100 were required.) Use deionized water as blank and cresolphthalein-base working reagent 3(c). Set delay time of 5 s and measurement time of 1 s (two 0.5-s integrations). From ppm Ca found for each sample and dilution factor D , calculate percent calcium from following equation:

$$\% \text{ Ca} = (\text{ppm Ca}/4) \times (D/\text{mg sample}) \times 100$$

(4) *Iron*.—Use 560 nm filter. Dilute samples to 0.5–10 ppm range using 0.3M HClO₄ (so standards and all samples will be in same matrix).

Samples analyzed in this work required dilutions of 1/2 or 1/5. Mix 2 mL blank digest 4(d) and each standard or sample with 2 mL ascorbic acid reagent 4(b). Use equilibrium routine program with 10-s delay and 2.5-s measurement time (5 integrations). Use equation similar to calcium equation to calculate percent Fe for each sample.

(5) *Magnesium*.—Use 520 nm filter. Dilute samples with water, using 50 mL volumetric flasks and at the same time roughly neutralize with 0.1N NaOH, using 2 drops of methyl red indicator (change from red to yellow). Samples in this work required 1/25 or 1/50 dilutions. Use equilibrium program with 5-s delay and 2.5-s measurement time (5 integrations). Calculate percent Mg for each sample, using equation similar to calcium equation.

Measurements for all procedures were carried out in an air-conditioned laboratory maintained at a nominal temperature of 25°C.

Results and Discussion

The reaction-rate procedures for the determination of protein nitrogen and phosphorus described earlier (7, 8) were used in this work without any modification. Because the pH of the measured solution in the observation cell is an important parameter in these reaction-rate determinations, the variation in the sulfuric acid concentration in the digested samples was examined by titration with NaOH standard solution. The acid remaining was $47 \pm 6\%$ of the original. This loss is overcome by preparing the nitrogen and phosphate standards in the same matrix as the samples after digestion.

Typical results for working curves are shown in Tables 1 and 2 for crude protein and phosphorus, respectively, and demonstrate the excellent precision and linearity obtained for the standards.

Table 3. Typical calcium results, using equilibrium method^a

Std, ppm Ca ^b	Absorbance ^c	RSD, %
1.00	0.159	0.7
2.00	0.354	0.2
3.00	0.615	0.2
4.00	0.837	0.1
5.00	1.072	0.1

^a Working curve: slope = 0.231, intercept = -0.085, $r = 0.9993$.

^b Final concentration in cell is the 1/2.

^c Average of 5 determinations on a single standard with 5-s delay and 1-s measurement time (2 integrations). $\lambda = 580$ nm.

The reaction of calcium with *o*-cresolphthalein complexone is complete in less than the dead time of the stopped-flow unit (200 ms) so that an equilibrium procedure with a short delay time can be performed. A delay time of 5 s was chosen to avoid nonreproducible absorbance behavior noted when the working reagent is mixed with the standard and sample solutions. A measurement time of 1.0 s (two 0.5-s integrations) gave excellent precision. The cresolphthalein complexone concentration (0.147 mM final) was chosen as a compromise of low absorbance blank and good linearity of the working curve. Final concentrations of 1.5 mM for 8-quinolinol and 9.6 mM for CN⁻ ensure elimination of magnesium and heavy metals interference. Because of the relatively high concentration of calcium in feeds, an extensive dilution of the sample digest solutions is required. The acid concentration of the diluted samples is low and a 0.62M aminomethyl propanol concentration ensures pH stability. Typical results for the calcium working curve are shown in Table 3, again with good precision and linearity.

The reaction of iron (II) with ferrozine is also rapid and an equilibrium procedure is proposed.

Table 4. Iron results using Fe(II) and Fe(III) standards

Std, ppm Fe ^a	Absorbance	
	Fe(II)	Fe(III)
0.25	0.068	0.068
0.50	0.119	0.125
1.00	0.238	0.243
3.00	0.728	0.719
5.00	1.196	1.194
Slope	0.239	0.237
Intercept	0.003	0.006
Corr. coeff. (r)	0.99994	0.999994

^a In 0.3M HClO₄ and 1% ascorbic acid solution.

Table 5. Typical iron results, using equilibrium method^a

Std, ppm Fe ^b	Absorbance ^c	RSD, %
0.500	0.056	1.0
1.00	0.118	0.4
5.00	0.588	0.1
7.50	0.892	0.2
10.00	1.176	0.1

^a Working curve: slope = 0.118, intercept = -0.001, $r = 0.99996$.

^b Final concentration in observation cell is the 1/4.

^c Average of 5 determinations on a single standard with 10-s delay and 2.5-s measurement time (5 integrations). $\lambda = 560$ nm.

A small increase in the absorbance is noted during the first few seconds of the reaction, so a 10-s delay time was used to ensure stability of the absorbance values. A mixed ferrozine-ascorbic acid reagent was tried with good results for aqueous standard Fe(III) solutions but with a little lower results for samples. So the pre-addition of ascorbic acid in the samples was preferred. The efficiency of the iron(III) reduction with ascorbic acid was checked by obtaining results using iron(II) and iron(III) standards. The results as shown in Table 4 are similar. A measurement time of 2.5 s (5 successive 0.5-s integrations) gave the best precision. The standards and the dilutions of the sample digest solutions are made with 0.3M HClO₄, which is the average concentration of the digest solutions, to ensure matrix similarity of samples and standards. The ferrous complex of ferrozine is stable in the pH range 4-10 (12) so an acetate buffer pH 4.5 with a 1M final concentration was chosen. Typical results for iron calibration curves are shown in Table 5.

The magnesium-calmagite reaction is also fast and an equilibrium procedure with 5-s delay

Table 6. Typical magnesium results, using equilibrium method^a

Std, ppm Mg ^b	Absorbance ^c	RSD, %
0.200	0.070	0.3
0.500	0.189	0.6
1.00	0.364	0.4
1.50	0.539	0.2
2.00	0.690	0.2

^a Working curve: slope = 0.345, intercept = 0.012, $r = 0.9992$.

^b Final concentration in observation cell is 1/2.

^c Average of 5 determinations on a single standard with 5-s delay and 2.5-s measurement time (5 integrations). $\lambda = 520$ nm.

Table 7. Comparison of reaction-rate stopped-flow method with official AOAC method for determining crude protein

Sample	Crude protein, %		
	Stopped-flow ^a	Official ^b	Diff., ^c %
Swine feed (7828)	41.63 ± 0.20	40.77 ± 0.46 D	+0.86
Pig feed (7829)	18.96 ± 0.12	18.38 ± 0.26 D	+0.58
Poultry ration (7831)	19.68 ± 0.05	18.44 ± 0.30 D	+1.24
Soybean meal (7921)	46.28 ± 0.35	45.83 ± 0.68 D	+0.45
Dairy ration (7922)	19.05 ± 0.26	18.38 ± 0.28 D	+0.67
Dried molasses (7923)	10.81 ± 0.06	10.48 ± 0.20 D	+0.33
Swine feed (7924)	22.64 ± 0.09	22.14 ± 0.29 D	+0.50
Milk replacer (7925)	20.49 ± 0.13	20.50 ± 0.26 D	-0.01
Beef cattle sup. (7926)	56.94 ± 0.12	55.27 ± 0.49 D	+1.67
Swine feed (7928)	15.89 ± 0.12	15.76 ± 0.23 D	+0.13
Pig feed (7929)	17.24 ± 0.04	17.07 ± 0.27 D	+0.17
Beef feed (7930)	44.06 ± 0.30	44.85 ± 0.57 D	-0.79
Chick starter (7931)	19.38 ± 0.12	20.06 ± 0.27 D	-0.68
Swine feed (7932)	14.94 ± 0.14	14.38 ± 0.25 D	+0.56
Dairy feed (8021)	43.42 ± 0.34	43.49 ± 0.56 K	-0.07
Dairy feed (8022)	33.70 ± 0.19	34.05 ± 0.46 K	-0.35
Broiler ration (8025)	20.59 ± 0.12	20.09 ± 0.26 K	+0.50
Dried molasses-NaNO ₃ (8024)	12.29 ± 0.08	12.39 ± 0.39 K	-0.10
Swine feed (8025)	20.82 ± 0.10	18.99 ± 0.30 K	+1.83
Cattle sup. (8027)	39.29 ± 0.14	39.63 ± 0.55 K	-0.34

^a Average of 4 determinations performed on single digestion of check feed ±1 SD.

^b Values reported in AAFCO Check Feed Sample Program by 74-90 laboratories during 1978-80, ±1 SD. Official methods 7.015 (20) (Kjeldahl method (K)), and 7.016 (Dumas method (D)).

^c Stopped-flow average - official method average.

time and 2.5-s measurement time gave excellent precision. The calcium interference was checked with mixed magnesium-calcium standards. The EGTA concentration used (0.46 mM final) eliminated any interference up to 1:10

concentration ratio. A 0.25M final KCl concentration provides ionic-strength similarity of standards and samples. A rough neutralization of the acid-digested samples is required to keep the solution at the desired pH with the 0.022M

Table 8. Comparison of reaction-rate stopped-flow method with official AOAC method for determining phosphorus

Sample ^a	Phosphorus, %		
	Stopped-flow ^b	Official ^c	Diff., ^d %
7828	1.944 ± 0.024	1.925 ± 0.094	+0.019
7829	0.620 ± 0.003	0.626 ± 0.031	-0.006
7831	0.634 ± 0.006	0.628 ± 0.035	+0.006
7921	0.659 ± 0.006	0.639 ± 0.034	+0.020
7922	0.863 ± 0.004	0.799 ± 0.055	+0.064
7923	0.101 ± 0.001	0.129 ± 0.018	-0.028
7924	0.690 ± 0.008	0.678 ± 0.031	+0.012
7925	0.837 ± 0.003	0.833 ± 0.040	+0.004
7926	1.295 ± 0.009	1.251 ± 0.061	+0.044
7928	0.594 ± 0.004	0.584 ± 0.030	+0.010
7929	0.769 ± 0.006	0.735 ± 0.035	+0.034
7930	1.090 ± 0.005	1.114 ± 0.063	-0.024
7931	0.782 ± 0.005	0.793 ± 0.046	-0.011
7932	0.628 ± 0.005	0.622 ± 0.033	+0.006
8021	1.395 ± 0.012	1.425 ± 0.052	-0.030
8022	1.160 ± 0.007	1.143 ± 0.047	+0.017
8023	0.766 ± 0.003	0.758 ± 0.029	+0.008
8024	0.078 ± 0.004	0.088 ± 0.016	-0.010
8025	0.774 ± 0.003	0.773 ± 0.035	+0.001
8027	1.280 ± 0.011	1.281 ± 0.051	-0.001

^a Samples identified in Table 7.

^b Average of 5 determinations performed on single digestion of check feed ±1 SD.

^c Values reported in AAFCO Check Feed Sample Program by 19-56 laboratories during 1978-80, ±1 SD. Official method, 7.120 (20) (molybdovanadate photometric method).

^d Stopped-flow average - official method average.

Table 9. Comparison of stopped-flow method with official AOAC method for determining calcium

Sample ^a	Calcium, %		Diff., ^d %
	Stopped-flow ^b	Official ^c	
7828	3.993 ± 0.007	3.859 ± 0.289	+0.134
7829	0.763 ± 0.002	0.737 ± 0.058	+0.026
7831	0.802 ± 0.001	0.882 ± 0.042	-0.080
7921	0.303 ± 0.002	0.297 ± 0.016	+0.006
7922	1.018 ± 0.002	1.042 ± 0.075	-0.024
7923	0.724 ± 0.002	0.783 ± 0.043	-0.059
7924	0.887 ± 0.002	0.854 ± 0.042	+0.033
7925	0.863 ± 0.002	0.820 ± 0.067	+0.043
7926	2.594 ± 0.004	2.594 ± 0.148	0.000
7928	0.747 ± 0.002	0.711 ± 0.063	+0.036
7929	0.995 ± 0.001	1.039 ± 0.084	-0.044
7930	6.128 ± 0.011	6.292 ± 0.403	-0.164
7931	0.941 ± 0.001	0.992 ± 0.067	-0.051
7932	0.810 ± 0.003	0.802 ± 0.038	+0.008
8021	0.893 ± 0.001	0.982 ± 0.062	-0.089
8022	4.468 ± 0.007	4.524 ± 0.207	-0.056
8023	1.096 ± 0.003	1.113 ± 0.079	-0.017
8024	0.430 ± 0.001	0.451 ± 0.037	-0.021
8025	0.811 ± 0.001	0.864 ± 0.077	-0.053
8027	4.637 ± 0.011	4.439 ± 0.273	+0.198

^a Samples identified in Table 7.^b Average of 5 determinations performed on single digestion of check feed ±1 SD.^c Values reported in AAFCO Check Feed Sample Program by 16-55 laboratories during 1978-80, ±1 SD. Official method, 7.091 (20) (atomic absorption spectrophotometry).^d Stopped-flow average - official method average.

final concentration of KOH. The yellow methyl red indicator does not interfere. Addition of Bion NE-9 and Bion PVP (16) in the calmagite reagent, which prevents the shifting by serum proteins of the maximum of the absorption

spectrum of Mg-calmagite complex, showed no effect in the analysis of digested feeds. Typical results for magnesium working curves are shown in Table 6.

To evaluate the accuracy of the proposed

Table 10. Comparison of stopped-flow method with official AOAC method for determining iron

Sample ^a	Iron, %		Diff., ^d %
	Stopped-flow ^b	Official ^c	
7828	0.0828 ± 0.0002	0.0900	-0.0072
7829	0.0347 ± 0.0002	0.0365 ± 0.0040	-0.0018
7831	0.0225 ± 0.0002	0.0288 ± 0.0029	-0.0063
7921	0.0165 ± 0.0004	0.0178 ± 0.0044	-0.0013
7922	0.0993 ± 0.0004	0.1171 ± 0.0085	-0.0176
7923	0.0524 ± 0.0002	0.0536 ± 0.0047	-0.0012
7924	0.0462 ± 0.0007	0.0539 ± 0.0208	-0.0077
7925	0.0161 ± 0.0001	0.0180 ± 0.0019	-0.0019
7926	0.1051 ± 0.0010	0.1025 ± 0.0167	+0.0026
7928	0.0233 ± 0.0001	0.0240 ± 0.0012	-0.0007
7929	0.0333 ± 0.0003	0.0343 ± 0.0018	-0.0010
7930	0.1412 ± 0.0020	0.1561 ± 0.0166	-0.0149
7931	0.0220 ± 0.0003	0.0235 ± 0.0024	-0.0015
7932	0.0309 ± 0.0001	0.0307 ± 0.0045	+0.0002
8021	0.0597 ± 0.0002	0.0582 ± 0.0039	+0.0015
8022	0.0581 ± 0.0003	0.0572 ± 0.0042	+0.0009
8023	0.0314 ± 0.0001	0.0312 ± 0.0043	+0.0002
8024	0.0344 ± 0.0002	0.0348 ± 0.0051	-0.0004
8025	0.0281 ± 0.0001	0.0268 ± 0.0036	+0.0013
8027	0.0693 ± 0.0001	0.0706 ± 0.0049	-0.0013

^a Samples identified in Table 7.^b Average of 5 determinations performed on single digestion of check feed ±1 SD.^c Values reported in AAFCO Check Feed Sample Program by 1-8 laboratories during 1978-80, ±1 SD. Official method, 7.077 (20) (atomic absorption spectrophotometry).^d Stopped flow average - official method average.

Table 11. Comparison of stopped-flow method with official AOAC method for determining magnesium

Sample ^a	Magnesium, %		
	Stopped-flow ^b	Official ^c	Diff., ^d %
7828	0.2774 ± 0.0015	0.2745 ± 0.0158	+0.0029
7829	0.1385 ± 0.0007	0.1439 ± 0.0105	-0.0054
7831	0.1472 ± 0.0013	0.1314 ± 0.0073	+0.0158
7921	0.3019 ± 0.0013	0.2824 ± 0.0276	+0.0195
7922	0.3615 ± 0.0015	0.3508 ± 0.0162	+0.0107
7923	0.2708 ± 0.0011	0.2808 ± 0.0427	-0.0100
7924	0.1961 ± 0.0006	0.1953 ± 0.0143	+0.0008
7925	0.1230 ± 0.0004	0.1245 ± 0.0070	-0.0015
7926	0.3543 ± 0.0003	0.3397 ± 0.0405	+0.0146
7928	0.2354 ± 0.0007	0.2398 ± 0.0202	-0.0044
7929	0.2259 ± 0.0009	0.2218 ± 0.0169	+0.0041
7930	0.5281 ± 0.0005	0.5236 ± 0.0421	+0.0045
7931	0.2429 ± 0.0009	0.2347 ± 0.0273	+0.0082
7932	0.2236 ± 0.0010	0.2124 ± 0.0169	+0.0112
8021	0.4055 ± 0.0003	0.4007 ± 0.0286	+0.0048
8022	0.3770 ± 0.0001	0.3582 ± 0.0301	+0.0188
8023	0.1862 ± 0.0010	0.1740 ± 0.0278	+0.0122
8024	0.1092 ± 0.0004	0.1151 ± 0.0416	-0.0059
8025	0.2088 ± 0.0010	0.2218 ± 0.0157	-0.0130
8027	0.4177 ± 0.0009	0.4350 ± 0.0499	-0.0173

^a Samples identified in Table 7.^b Average of 5 determinations performed on single digestion of check feed ± 1 SD.^c Values reported in AAFCO Check Feed Sample Program by 3-12 laboratories during 1978-80, ± 1 SD. Official method, 7.077 (20) (atomic absorption spectrophotometry).^d Stopped-flow average - official method average.

methods, a series of samples assayed by official methods by other laboratories were analyzed; see Tables 7-11. The data demonstrate that the accuracy of the proposed methods is within the reported uncertainty of the official procedures. There is a small positive bias on the determination of protein.

Statistically summarized data of the comparison

Table 12. Regression equations for comparison of stopped-flow (SF) methods with official AOAC (20) methods (20 samples)

Y(SF) (range)	X(AOAC)	Slope	Intercept (%)	Corr. coeff. (r)
Crude protein (10.8-56.9%)	7.015 (Kjeldahl)			
	7.016 (Dumas)	0.9992	0.38	0.9987
Phosphorus (0.078-1.94%)	7.120 (photo-metric)	1.0089	-0.001	0.9986
Calcium (0.30-4.64%)	7.091 (AAS)	1.0023	-0.012	0.9989
Iron (0.016-0.141%)	7.077 (AAS)	0.9063	0.0021	0.9929
Magnesium (0.109-0.528%)	7.077 (AAS)	1.0048	0.0023	0.9955

are shown in Table 12. All the methods show excellent correlation, with slopes of 0.9063-1.0089 and correlation coefficients of 0.9929-0.9989.

Using 4 flushes to change from one solution to another (flush cycle time about 1.5 s), one measurement per sample and assuming 1 s for the turntable position increment, and 0.5 s for the computer calculation and printing time, the analysis rates shown in Table 13 can be obtained by the methods reported. The total sample or reagent volume consumed is 0.75 mL.

The proposed methods reported here provide rapid, precise, and accurate determination of protein, phosphorus, calcium, magnesium, and

Table 13. Routine analysis rates

Detn	Anal. rate (samples/h)	Delay time, s	Measurement time, s
Crude protein (reaction-rate)	110	10	15
Phosphorus (reaction-rate)	266	2	4
Calcium (equilibrium)	266	1	5
Iron (equilibrium)	180	2.5	10
Magnesium (equilibrium)	240	2.5	5

iron and have the distinct advantages of high sample throughput and utilization of only one relatively simple automated system.

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