Preliminary Study on the Analysis of Forages with a Filter-Type Near-Infrared Reflectance Spectrometer

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Near-infrared reflectance spectroscopy with a tilting filter spectrometer was evaluated for the analysis of forages. The results from this instrument along with those from a spectrocomputer (monochromator type instrument) are presented. The analyses were determined for warm- and cool-season grasses. The tilting filter instrument and the spectrocomputer both gave adequate standard deviations for calibration for the crude protein (±1.1%), acid detergent fiber (±1.75%), and permanganate lignin (±0.65%) analysis. The analysis of in vitro dry matter digestibility (±3.0%) and neutral detergent fiber (±3.5%) was not considered adequate. Also results showed that warm- and cool-season grasses should be analyzed separately with different regression equations.

Near-infrared (NIR) reflectance spectroscopy has been previously used for the rapid determination of oil, protein, and moisture in grains and oilseeds (Norris and Hart, 1965; Ben Gera and Norris, 1968; Hymowitz et al., 1974; Rinne et al., 1975). The technique has also been used to predict the nutritive value of feedstuffs (Norris and Barnes, 1976; Shenk et al., 1976). More recently, the composition of forages has been investigated with a monochromator NIR reflectance spectrometer (Norris et al., 1976; Shenk and Hoover, 1976; Shenk et al., 1977, 1978).

The main objective of this study was to determine the feasibility of using a "tilting filter" NIR reflectance spectrometer, specifically the Neotec FQA-51, to analyze forages.

The second objective was to compare the standard deviation of calibration for the FQA-51 and a spectrocomputer for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), true protein (TP), permanganate lignin (PML), and in vitro dry matter digestibility (IVDMD). These results would establish the precision of the method for the two types of spectrometers.

A third purpose was to examine tropical and temperate grasses together and separately with the NIR reflectance spectrometer to see if differences in their spectra would have an effect on the regression equation. Barton et al. (1976) found that IVDMD could be predicted from chemical composition with multiple linear regression techniques and that separate regression equations were ncessary to predict with good accuracy the digestibility of tropical and temperate grasses. Since the NIR reflectance spectrometer regresses compositional data against an absorbance value at a certain wavelength, we wanted to determine whether separate equations would be required for the NIR reflectance spectrometer method as well.

MATERIALS AND METHODS

Description of Grass Samples. A total of 38 grass samples, harvested at 4 and 8 weeks regrowth and obtained and handled as previously described (Barton et al., 1976), were used in these studies. These samples were as follows: Bermuda grass (*Cynodon dactylon* (L.) Pers.); with 28 total samples of cultivars Coastal (8), Coastcross-1 (7), Common (2), Alicia (2), Callie (2), Tifton-44 (2), Cultivars-68 (2), 393 (1), 1-9 (1), and 2-10 (1); "Southland" bromegrass (*Bromus inermis* Leyss.) (2); "Boone" orchardgrass (*Dactylis glomerota* L.) (2); Tall fescue (*Festuca arundinacea* Schreb.), with four total samples of cultivars "Kentucky"-31 (2) and "Kenhy" (2); "Kentucky" bluegrass (*Poa pratensis* L.) (2); and "Clair" timothy (*Phleum pratense* L.) (2). In the first experiments 24 samples, 10 temperate and 14 tropical, were used to calibrate the instrument. These samples were used collectively (i.e., all 24) and as sets of tropical (14) and temperate (10). In the bermudagrass experiments 28 samples were used (24 on the FQA-51 and 28 on the spectrocomputer) to calibrate the FQA-51 and the spectrocomputer.

Chemical Analysis. Determination of CP, NDF, ADF, PML, and IVDMD were made as previously described (Barton et al., 1976). The TP analysis was determined as 6.25 × Kjeldahl nitrogen on the forage residue following extraction with hot 5% trichloroacetic acid.

Sample Preparation for NIR Reflectance Analysis. The dry (<6% moisture) ground grass samples were reground in a Tecator/UDY cyclone mill through a 1.0-mm screen and packed into a Neotec sample cup.

NIR Reflectance Instruments. The NIR reflectance data were obtained with two types of instruments: (1) the Neotec FQA-51 "tilting filter" spectrometer with an Intel 808 computer and (2) the Neotec spectrocomputer with a Cary 14 monochromator and a Data General Nova Computer.

Operating Parameters. *FQA-51.* The spectral range of the FQA-51 is 1.50–2.36 μm and six discrete sections of that range are scanned. Each section is 0.06–0.11 μm. Therefore, a discontinuous spectrum over the total range of approximately 0.5 μm is obtained. A total of 2000 data points is assigned to the filter wheel with 120 points per filter for a total of 720 usable data points. The remaining 1280 points are in the dark periods, between filters. The log reflectance ($1/R$) values at each point were stored in the computer. The second derivative of each point was calculated from either 60 or 30 (20 or 10 points per segment of the three-segment equation, respectively) data points such that the spectrum was compressed to 60 or 90 data points, respectively. For a more thorough explanation, see Norris et al. (1976) and Shenk et al. (1978). The second derivative values at each wavelength were regressed on the laboratory data for the respective grass sample. This resulted in multiple regression equations of the type described by Norris et al. (1976) together with the standard deviation for the calibration of each forage constituent. A maximum of 25 samples can be used in the calibration sample set. A Coors ceramic disk (97104) installed in the instrument by the manufacturer was used as a reflectance

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Table I. Ranges in Percentage Chemical Composition for Samples Analyzed by NIR Reflectance

	N^a	NDF	ADF	PML	CP	hemicellulose
bermudagrass	28	58.2-71.6	25.0-40.0	3.2-6.5	8.0-19.2	24.5-31.6
temperate	10	43.9-59.8	23.9-33.6	2.1-5.6	11.8-23.2	20.0-26.2

[a] N is the number of samples in the set.

Table II. Commercial Configuration of the Neotec FQA-51

filter position	pulse point	filter wavelength, μm	scanning range, μm	primary constit.
1	168-287	1.68	1.596-1.677	none (ref.)
2	502-621	1.58	1.501-1.577	urea
3	835-954	1.97	1.842-1.936	moisture
4	1168-1287	2.22	2.108-2.216	protein
5	1501-1620	2.31	2.243-2.308	crude fiber
6	1834-1953	2.33	2.263-2.328	oil

standard, and the instrument was calibrated before each spectrum was taken. A nomograph provided by Neotec was used to convert data points to wavelength.

Spectrocomputer. The spectral range was 1.50–2.40 μm, with 300 data points assigned. Data were obtained every 4.5 nm at a scan rate of 10 nm/s. Barium sulfate was used as a standard and its spectrum stored in the computer to correct the forage spectral curves. Since a monochrometer was used, the entire spectrum was recorded. The spectral data were expressed as log $(1/R)$ and the second derivative of log $(1/R)$ in order to compare results with the same mathematical data treatment of the FQA-51. However, the number of points in each segment of the second derivative calculation was not fixed as in the FQA-51, so 12 points per segment were selected. Also, the multiple linear regression analysis was quite different for the spectrocomputer. The program examined all 300 wavelengths and combinations of wavelengths, predicted the best wavelength, and gave the coefficients of the regression equation and the standard deviations of predicted components in the calibration samples. The program also provided for eliminating from the calibration set those samples which caused the greatest deviation from the regression line. Results obtained with the spectrocomputer could then be transferred to the FQA-51 by converting the memory addresses with the nomograph based on the equations obtained from Neotec. With the FQA-51, data for the log $(1/R)$ vs. wavelength were obtained with a computer program routine called "Versidump". Wavelengths of minimum and maximum absorbances were subjectively chosen for correlation with analytical data. This procedure for determining wavelengths of best correlation was not as efficient a predictive tool as that of the spectrocomputer.

RESULTS AND DISCUSSION

Chemical Data. The ranges in chemical composition of the forage samples used in these studies is shown in Table I. The bermudagrasses were higher in fiber than the temperate grasses, as measured by NDF. Most of this difference in fiber content between the bermudagrasses and the temperate grasses is in the readily hydrolyzable polysaccharide (hemicellulose) fraction. Differences in composition among our samples were similar to those among the samples evaluated in the NIR reflectance studies of Norris et al. (1976); hence the results of our and their NIR studies should be comparable.

FQA-51 Commercial Configuration. The Neotec FQA-51 was originally designed for use with grains and mixed feeds and was delivered with the specific filters and programs for that purpose. The filters and their positions are described in Table II. Although any filter may be placed in any filter position, each position is associated with a specific set of "pulse points" (Neotec's designation of data point or computer memory address). For example, any filter in position 4 will have pulse points from 1168–1287. In the commercial configuration filter position 1 is unique and is used as a reference because over that scanning range, 1.60–1.68 μm, the NIR spectrum is relatively quiet and nonabsorbing for feed grains. The spectral data accumulated are essentially background noise; and when the data from the other filters are divided by the data from first filter a signal to noise (S/N) enhancement results. This reference filter technique was found to improve the measurement of protein in wheat and was part of Neotec's standard programming. The wavelength ranges of the remaining filters were selected on the basis of previous work with grains (Norris and Hart, 1965; Ben Gera and Norris, 1968).

Table III. Standard Deviations for NIR-Reflectance and Percentage Wet Chemical Analyses

	protein	lignin	NDF	ADF	IVDMD
tropical (10)	0.6	0.4	1.9	1.6	3.6
temperate (14)	0.9	0.7	2.3	1.6	3.1
tot. samp. (24)	1.0	0.8	3.9	2.5	6.4
chem. anal.	0.5	0.5	1.0	0.8	2.7

Tropical and Temperate Grasses Experiment. The first experiment was conducted with a set of samples comprising both tropical and temperate grasses. The standard deviations were determined and are designated "Tot Samp." in Table III. Next, the set was divided into temperate and tropical grasses and the instrument recalibrated with the subsets. In all cases the standard deviations were better when tropical and temperate species were analyzed separately.

The standard deviations in Table III were obtained at wavelengths selected with the FQA-51 Versidump 1 and 2 programs. Versidump-1 prints the log $(1/R)$ for each of 120 data points and these points when plotted became a spectrum for that filter and that specific sample. When a variety of samples representative of the range of the particular constituent being measured are examined, a point reflecting a minimum or maximum can be chosen as the data point on that filter and used for a measurement. This process is repeated for each filter and each constituent to be measured. Versidump-2 is identical except that each of 60 second derivative log $(1/R)$ is printed.

The wavelengths chosen by the Versidump method in the experiment are given in Table IV as "original" wavelengths. Versidump plots for filters I, II, and IV are shown in Figures 1–3. These plots were made with Versidump-2 for four temperate grasses, in this case fescue (broken line), and four bermudagrass (solid line) samples. The division

Table IV. Best Wavelengths (μM) for FQA-51 in Original and Revised Mathematics and for the Spectrocomputer

	FQA-51 original		FQA-51-revised		spectro-computer
CP	2.105		2.094		2.090
	2.179		2.168		1.050
	2.334		2.330		
TP			CP + 1.674		
NDF	1.539		1.674		1.705
	1.661		2.070		1.975
	2.334		2.294		
ADF	1.539		1.555		1.710
	1.661		1.702		1.615
PML	1.563		1.555		
	1.667		1.666		1.705
	2.089		2.355		1.630
IVDMD	1.541	2.179	1.555	2.170	2.170
	1.650	2.250	1.674	2.294	1.300
	2.097	2.343	2.070	2.355	

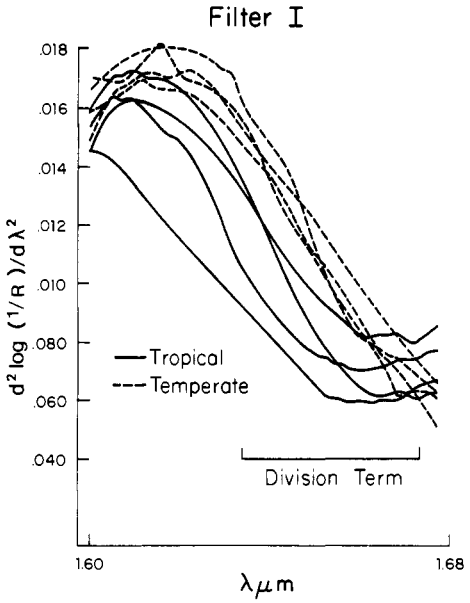

Figure 1. Plot of $d^2 \log (1/R)d\lambda^2$ vs. λ in micrometers for the first filter (1.60–1.68 μm).

term mentioned earlier is denoted on Figure 1. Figure 1 shows some real absorbances as denoted by a minimum in the spectrum of each bermudagrass sample and that the wavelength maxima (λ max) and minima (λ min) for the fescues and bermudagrasses do not coincide. If the division term is used in the case of bermudagrasses where real absorbances occur (Figure 1), it will not be random noise and no S/N enhancement will be gained. Instead, computer-generated chatter as shown in Figure 2 (arrows) will result and selecting points from the plot would not be possible with any degree of confidence. The adverse effect of the division term was not as pronounced for filter IV (Figure 3), because the $d^2 \log (1/R)/d\lambda^2$ absorbances are three times larger, but the differences between λ max and λ min for fescue and bermudagrass were quite different. Filter IV is used primarily for protein measurements. From this experiment it was concluded that for a set of samples containing both tropical and temperate grasses, data on forage compositional or animal performance could not be predicted with high accuracy. Thus, it appears that regression equations may have to be determined separately on temperate and tropical grasses.

Bermudagrass Experiment. In a second experiment a number of bermudagrass samples (24 for the FQA-51 and 28 for the spectrocomputer) were used to generate the regression equations and find the standard deviations of

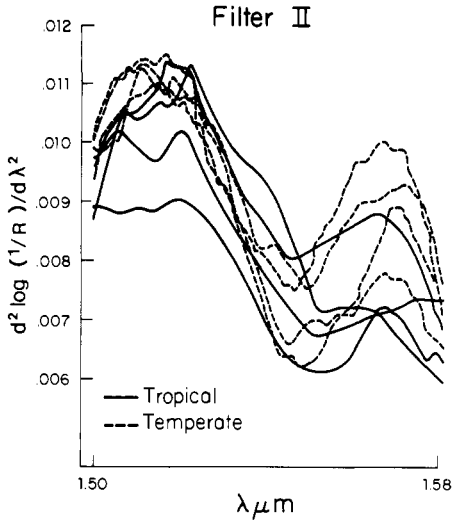

Figure 2. Plot of $d^2 \log (1/R)/d\lambda^2$ vs. λ in micrometers for the second filter (1.50–1.58 μm).

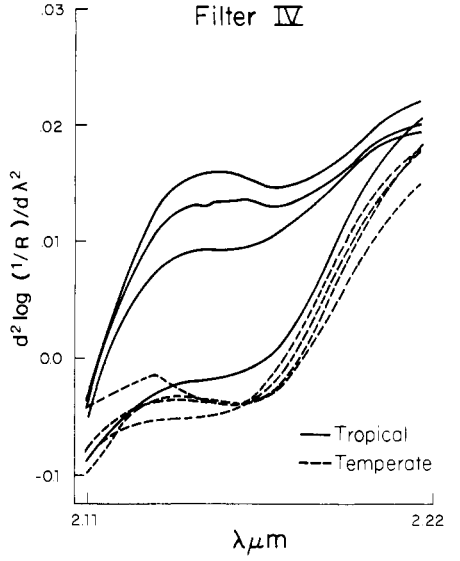

Figure 3. Plot of $d^2 \log (1/R)/d\lambda^2$ vs. λ in micrometers for the fourth filter (2.11–2.22 μm).

calibration. For this experiment the program board of the FQA-51 was modified by Neotec such that the division term was deleted and the number of data points used in the calculation of the second derivative was reduced to 30 (in this case Versidump-2 would print 90 points). A survey was made of all known wavelengths which have been shown by different investigators to correlate with forage constituents (Figure 4). The original filters in the FQA-51 were changed and a total of 11 different filters (FA in Figure 4) in sets of six were evaluated by Versidump 1 and 2 in order to obtain correlations with the laboratory of the samples. NIR data also were obtained on the same samples with the spectrocomputer. The wavelengths chosen by the spectrocomputer also are given in Table IV. The primary wavelength or that of highest correlation for the spectrocomputer was generally similar to some wavelengths which appeared to be best for the FQA-51 for all forage parameters except PML and IVDMD. The secondary wavelengths of the spectrocomputer were not as consistent.

Optimum wavelengths for forages were chosen for the FQA-51 from the wavelengths selected by the spectrocomputer and those obtained with the FQA-51 Versidump routines. These selections are shown in Table IV in the "Revised" column. No distinction can be made for wave-

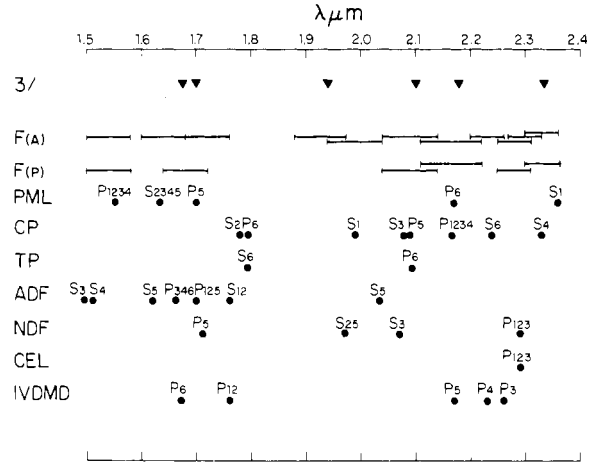

Figure 4. Pictogram showing the range of NIR wavelengths scanned with all filters evaluated (FA) and those picked as best (Fp) for bermudagrasses. Also shown are wavelengths chosen by Norris et al. (1976) (3) to determine each of the compositional factors listed from PML to IVDMD. The primary (P) and secondary (S) wavelengths of correlation are shown as points for each compositional factor. The numbers refer to the following references: (1) Shenk and Barnes (1977), (2) Shenk (1977a), (3) Norris et al. (1976), (4) Barton and Burdick (1977a), (5) Barton and Burdick (1977b), and (6) Shenk (1977b).

Table V. Standard Deviations of Calibration for 24 Bermudagrass Samples for Percentage Composition

constituent	FQA-51 original	FQA-51 revised	spectro-computer
CP	1.23	1.12	1.21
TP		0.87	
NDF	3.17	3.88	3.02
ADF	2.18	1.80	1.63
PML	0.65	0.69	0.61
IVDMD	3.58	3.60	3.00

lengths of primary correlation since the choice of wavelengths was subjective based on all data considered and resulting minimum standard deviation. The standard deviations of calibration for the set of bermudagrass samples is shown in Table V for the FQA-51, in the original and revised configuration and the spectrocomputer. All the standard deviations in the table are quite similar. The slight differences noted may be due to differences between the two instruments. For example, the spectrocomputer calculates the second derivative of log $1/R$ from a linear wavelength array, whereas the FQA-51 performs this operation on a trigonometric array (the arcsin θ, where θ = 5–27°. This difference could shift the calculated wavelength maxima and minima on the NIR spectrum's complex wave forms.

Figure 5 is a plot of the correlation coefficient (r) for crude protein of the bermudagrass samples and wavelength. The portion from 1.50 to 1.95 μm shows six peaks for the six largest absolute values of r. These peaks of maximum positive and negative correlation are very sharp over narrow wavelength regions. The sharp narrow peaks may mean that the wavelength of correlation may be dependent upon the particular set of samples used for calibration, and/or that the magnitude of log $1/R$ (absorbance) is not great enough (i.e., S/N) in that region to allow better correlation. In the wavelength region between 1.95 and 2.40 μm, the areas of maximum correlation are quite broad and have r values over |0.75|. In this region the correlations may not be as sample dependent.

Finally, if filter instruments are to be used, some standardization or referencing of the wavelengths by data point will be necessary (i.e., is pulse point 1203 equal to 2.168

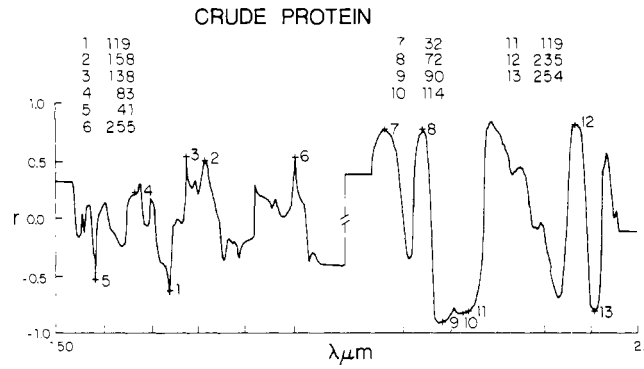

Figure 5. Plot of correlation coefficient (r) vs. λ in micrometers for NIR reflectance data regressed on crude protein. The plot is split into two segments (1.50–1.95 μm and 1.95–2.40 λ) of 300 data points each.

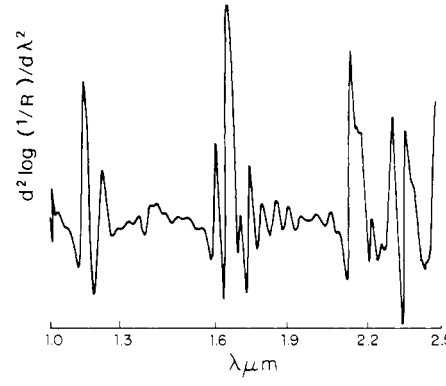

Figure 6. Plot of $d^2 \log (1/R)/d\lambda^2$ vs. λ in micrometers for polystyrene beads in a sealed cup.

μm on all instruments?). Polystyrene has been used as a standard with infrared spectrometers and its spectrum is well known. The NIR has been examined, characterized, and reviewed by Kaye (1954, 1955), and certain polystyrene bands have been identified. Figure 6 is a spectrum ($d^2 \log (1/r)d\lambda^2$) of polystyrene, showing numerous intense peaks which could be used to calibrate each filter. It is proposed that polystyrene beads in a sealed sample cup be used as the standard for calibrating the pulse point (data point) setting for wavelength in NIR filter instruments such as the Neotec FQA-51.

On the basis of the above experiments, six filters were chosen for bermudagrasses. They are designated Fp in Figure 4 and represent a compromise. Specific wavelengths for each component and IVDMD are given in Table IV. Ideally a filter would be chosen where the center of the absorption band is in the center of the filter. This was not possible in all cases with the bermudagrass samples. Four of the six original wavelengths chosen by Norris et al. (1976) and Shenk et al. (1977) were on the filters chosen as best in this study. Since the tilting filter instrument was limited to six filters and one set of wavelengths per product (the manufacturer has programmed the files to be a 6 × 6 array of equations × wavelength set, where the wavelengths for the six equations must be the same), it was sometimes necessary to run the sample in more than one "product" file to achieve the best results. For certain forage components (e.g., crude protein), the standard deviation of calibration was approximately the same as that of the wet chemical (i.e., Kjeldahl) method. However, for other components (e.g., neutral detergent fiber), the error for the NIR method was two–three times as large. This initial study indicates that much research still has to be accomplished before NIR can be widely

adopted to routinely predict forage quality with high accuracy.

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Industrial Whole Animal Blood. Characterization Studies and Quantitative Protein Removal by Chemical Coagulation

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Industrial whole animal blood samples were obtained from processors and analyzed for total nitrogen, volatile nitrogen, nonprotein nitrogen, and ammonia nitrogen levels by Kjeldahl analysis. Blood samples obtained from a renderer displayed greater protein decomposition than samples obtained from a packer. Whole blood samples were treated, under conditions of low dilution and at acidic pH values, with the protein coagulants sodium polyphosphate, ferric chloride, lignin, or sodium lignosulfonate. Sodium polyphosphate or ferric chloride quantitatively removed blood protein, while lignin or sodium lignosulfonate treatment resulted in near quantitative protein removal, under the conditions tested. The potential use of chemical coagulants for industrial reclamation of blood proteins is discussed.

Protein derived from animal blood is reclaimed by the meat packing and rendering industries and is used to supplement animal feed products (Waibel et al., 1977). Blood protein may be reclaimed quantitatively by cooking whole blood to dryness, or nonquantitatively by steam coagulation of whole blood, followed by separation of the coagulated material from the resulting effluent. Each of these methods creates problems for the meat packer and renderer. In the case of whole blood drying (vat drying), the high water content of blood necessitates a large investment in heat energy to dry the product. In addition, vat drying results in severe reduction in the levels of lysine, methionine, and cystine, as well as diminished digestibility of the protein product (Waibel et al., 1977; Kramer et al., 1978). Steam coagulation of blood, followed by separation of the coagulated solids, often results in nonquantitative removal of protein from blood producing an effluent serum which is high in biochemical oxygen demand (BOD). The serum effluent contains nitrogen, in the form of colloidal protein and suspended solids, as well as soluble nonprotein containing compounds. The most common approach to the serum effluent problem is to develop coagulation–flocculation techniques for removal of protein from the effluent before it leaves the processor as sewage (Hopwood and Rosen, 1972; Sanders, 1948).

A different approach to the recovery of blood protein lies in the development of techniques to remove the protein directly from blood using chemicals. Chemical coagulation of blood protein could be competitive with conventional methods if: (1) the chemical treatment did not require heat or diminished the amount of heat currently used; (2) the chemicals were cost competitive; (3) the chemicals were nontoxic or nutritionally desirable; (4) little residual chemicals were released in the effluent produced; (5) the nu-

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