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Determination of the N-Terminal Amino Acid Residues on Polypeptides in Secondary Wastewaters

Edward L. Creecy
Old Dominion University

DETERMINATION OF THE N-TERMINAL AMINO ACID RESIDUES ON POLYPEPTIDES IN SECONDARY WASTEWATERS

by

Edward L. Creecy
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Approved by:

Frank E. Scully Jr. (Director)

Roy L. Williams (Member)

John R. Donat (Member)

ABSTRACT

DETERMINATION OF THE N-TERMINAL AMINO ACID RESIDUES ON POLYPEPTIDES IN SECONDARY WASTEWATERS.

Edward L. Creecy
Old Dominion University, 1998
Director: Dr. Frank E. Scully, Jr.

The N-terminal amino acid residues on polypeptides and proteins were determined in wastewaters prior to chlorination. The terminal amino groups were first derivatized with the well known derivatizing agent dansyl chloride, and then the resulting dansyl amino acid hydrolyzed from the peptide chain by a propionic acid/ hydrochloric acid mixture. The resulting dansyl amino acids were then separated and detected using reversed phase high performance liquid chromatography with fluorescence detection. The majority of N-terminal residues detected were the more polar amino acids. Concentrations ranged from 1×10^{-10} to 3×10^{-7} moles/liter. It is suggested that the shorter chain length polypeptides present in wastewaters have polar N-terminal amino acid residues. The nonpolar N-terminal amino acid residues do not make a significant contribution to the chlorine demand of organic amino nitrogen in wastewaters.

To the Memory of my father Allen B. Creecy

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INTRODUCTION

Due to increased population growth and continued industrialization, our municipal and commercial water supplies have become more and more threatened by pollution. With increased numbers of impurities present in water supplies more extensive water treatment procedures must be implemented to ensure clean and safe water for public and private water users. Both water being drawn from the supply and water being discharged into the supply should meet minimum requirements.

In order to obtain such goals the United States Congress passed the Federal Water Pollution Control Act of 1970 and enacted the National Pollution Discharge Elimination System (NPDES) of 1977 to ensure water quality in lakes, streams, and reservoirs. The NPDES requires all dischargers of water into lakes, streams, and reservoirs to have and maintain a permit. These permits set regulations, guidelines and limitations on the amount and kind of pollutant that industries and municipalities can discharge into receiving waters. Along with state and local regulations, they also set limits on the number of pathogenic organisms discharged. As a result almost all wastewater containing sewage discharged from industrial and municipal treatment plants is subject to disinfection requirements (1,2).

One major goal of these requirements is to prevent the spread of waterborne diseases such as cholera, typhoid, and dysentery caused by bacteria, viruses, and cysts (1). Equally important is the protection of the areas located around the receiving waters and the marine ecology within the receiving waters. There have been studies showing

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that discharging untreated wastewater containing high bacterial activity can have a detrimental effect on the marine environment (3,4).

Several different methods have been used to disinfect wastewater discharges. These include ozonolysis, chlorination, and ultraviolet irradiation. The predominant method is chlorination due to its availability, ease of handling, low cost and good germicidal properties. It has been used in the United States since 1894 when it was first employed in a municipal wastewater system in Brewster, New York (1).

Chlorine for disinfection can be applied in one of several ways. It may be added to water in gaseous form (Cl_2), or as a solution of sodium hypochlorite, or as solid calcium hypochlorite. The reactions involved are as follows:

$$Cl_2 + H_2O \longrightarrow H^+ + Cl^- + HOCl$$

$$HOCl \longrightarrow H^+ + OCl^-$$

$$Ca(OCl)_2 \longrightarrow Ca^{2+} + 2OCl^-$$

$$NaOCl \longrightarrow Na^+ + OCl^-$$

When ammonia (NH_3) is present, the hypochlorous acid (HOCl) reacts rapidly to form inorganic chloramines (5). The reactions of chlorine with ammonia are as follows:

$$NH_3 + HOCl \longrightarrow NH_2Cl + H_2O$$

$$NH_2Cl + HOCl \longrightarrow NHCl_2 + H_2O$$

$$NHCl_2 + HOCl \longrightarrow NCl_3 + H_2O$$

These reactions are strongly dependent upon pH and on the molar ratios of chlorine to nitrogen (Cl_2:N) present (6). At pH values above 6.5 the formation of monochloramine is practically instantaneous and the resulting monochloramine is very stable and is thus the

predominant disinfection species present in wastewater (8). Inorganic monochloramine is not as potent a disinfecting agent as hypochlorous acid, but it is much less reactive and therefore persists over a longer period of time in the presence of reducing substances such as NO_2^-, Fe^{2+}, Mn^{2+} and HS^-.

There are disadvantages, however, to using chlorine as a disinfectant. Free chlorine species such as hypochlorous acid, hypochlorite, and aqueous chlorine can be toxic to aquatic organisms (3,7,8). The inorganic monochloramines formed when ammonia is present have been found to be even more toxic to fish than free chlorine (9).

If organic amino nitrogen compounds, such as amino acids, are present, aqueous chlorine will react with them forming organic chloramines as follows:

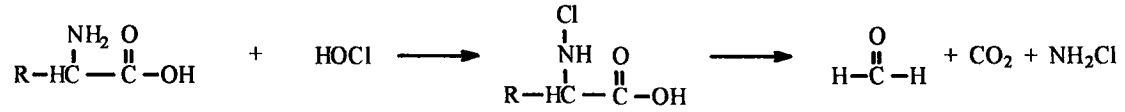
$$RNH_2 + HOCl \longrightarrow RNHCl + H_2O$$

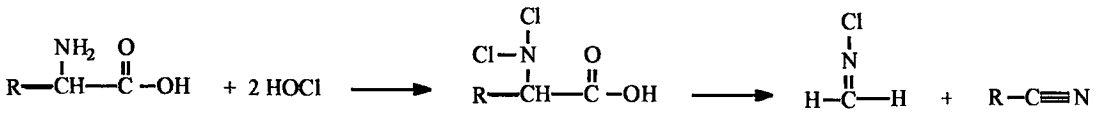
The resulting organic chloramines have been shown to have little if any bactericidal or viricidal activity (10,11,12,13,14,15). Their formation has been shown to occur 2 to 80 times faster than the formation of inorganic chloramines (5). In addition, it has also been shown that inorganic chloramines can transfer active chlorine to organic amino nitrogen compounds (16,17) as follows:

$$RNH_2 + NH_2Cl \longrightarrow RNHCl + NH_3$$

At present it is not possible to distinguish between the bactericidal inorganic chloramines and the non-bactericidal organic chloramines. Because of the formation of these chlorinated organics, some wastewater treatment plants may be overestimating the disinfection effectiveness of the effluent.

It is known that amino acids, proteins and polypeptides are present in wastewaters and represent a major portion of the organic nitrogen-containing compounds present (10,18,19). These compounds react with free chlorine and inorganic chloramines to form organic chloroamines which may react further or decompose to other byproducts. The reactions of the major amino acids present in wastewater have been studied and the initial products identified as N-chloramino acids and N,N-dichloramino acids (5,19,20), which slowly decompose to aldehydes, nitriles and N-chloraldimines (20,21,22,23) as shown below:

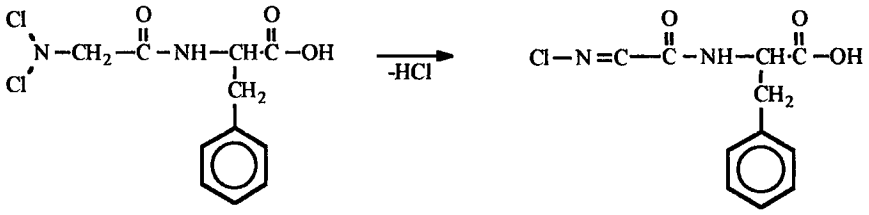




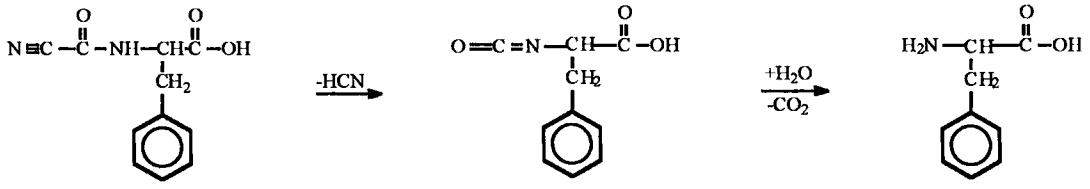
Proteins and polypeptides are a more important and more complex class of compounds found in municipal wastewater. Scully et al. (24), estimated that amino acids accounted for less than 5% of organic amino nitrogen in a primary wastewater and that polypeptides with an average of six amino acid residues per molecule accounted for the vast majority of the amino nitrogen components. To determine the implications of chlorinating wastewaters containing polypeptides, the reactions of the dipeptide glycylphenylalanine have recently been studied and the initial products identified as the

N-chloroglycylphenylalanine and the N,N-dichloroglycylphenylalanine (25). The monochlorinated dipeptide was found to be more stable than the monochlorinated amino acids (19). The chlorinated dipeptides decompose by different pathways because they are unable to undergo decarboxylation like the chlorinated amino acids.

The N,N-dichloroglycylphenylalanine can decompose by a dehydrohalogenation mechanism to a new compound N-choroaldimine (25) as follows:



The N-chloroaldimine can decompose to form a cyanoacylphenylalanine which can lose HCN, leading to liberation of phenylalanine. (25).



These reactions illustrate the diverse products that are formed during the disinfection step in wastewater treatment. Since polypeptides and proteins are found in virtually all water sources in concentrations ranging from a few to several hundreds of

micrograms per liter (10), the products formed during chlorination and their decomposition byproducts formed over time need to be investigated further.

However, there is a large number of possible N-terminal end-group amino acid residues found on polypeptides and proteins. It is not likely that polypeptides and proteins having all the possible end-groups are present in secondary wastewater effluent because of the biological treatment processes upstream. Microorganisms may be able to break down the peptides and proteins only to a certain extent and therefore leave only the most stable peptides and proteins containing a smaller number of N-terminal end-groups. It is the purpose of this study to determine the identity and distribution of the most frequently occurring N-terminal end-group amino acid residues in secondary wastewater effluent. Since only a few of the potential end groups have been studied so far, it is important to determine if certain amino acids have a tendency to be located on the end of the peptide chains in wastewaters. Once these are determined, the chemistry of these end groups can be studied and their influence upon the chlorine demand and disinfection effectiveness can be evaluated.

The polypeptides and proteins were derivatized with a reagent that reacts specifically and quantitatively with N-terminal amino groups. The derivatized solutions were then subjected to acid hydrolysis to break apart the amino acids in the peptide chains, and the identity of the end-group residues were determined by correlation of their retention times with known compounds using high performance liquid chromatography with fluorescence detection.

EXPERIMENTAL

General. All chemicals were reagent grade or better. Sodium carbonate, sodium hydroxide, nitric acid and acetic acid were all ACS certified and purchased from EM Science, Inc. OmniSolv® spectral grade acetonitrile was also purchased from EM Science, Inc. Hydrochloric acid, propionic acid and all pH buffer solutions were purchased from Fisher Scientific Co. 1-Dimethylaminonapthalene-5-sulfonyl chloride (dansyl chloride, DNS-Cl) was purchased from Sigma Chemical. The following dansyl (DNS) amino acids were purchased from Sigma Chemical Co.: DNS-L-alanine, DNS-L-valine, DNS-L-leucine, DNS-L-isoleucine, DNS-L-serine, DNS-L-threonine, DNS-L-phenylalanine, N,O-di-DNS-L- tyrosine, DNS-L-tryptophan, DNS-L-cysteic acid, DNS-L-methionine, DNS-L-asparagine, DNS-L-cystine, di-DNS-L-lysine, DNS-L-aspartic acid, DNS-L-glutamine, DNS-L-proline, DNS-L-γ-amino-butyric acid, DNS-L-glutamic acid and DNS-L-arginine. All aqueous solutions were prepared with water from a MilliQ® water purification system (Millipore). All glassware was cleaned with nitric acid.

Equipment and Analysis. All pH measurements were made on an Orion Research, Inc. digital pH/millivolt meter with a Fisher combination electrode which was calibrated with standard buffers. The Waters Associates Liquid Chromatography System (HPLC), consisted of two M6000A solvent pumps, a temperature control module, a computer system interface module, a UK6 sample injector, and model 474 scanning fluorescence detector (Waters Assoc., Milford MA). The fluorescence detector utilized a xenon lamp with an excitation wavelength of 339 nm, and emission monitored at 470 nm. The analytical column used was a 150 X 4.6-mm, 5-μm C_8 Partisil ODS-3 stainless

steel column (Whatman) . The column was housed in the temperature control module maintained at 25°C. The dansyl amino acids were separated at a flowrate of 1.2 mL/min using a two solvent gradient mobile phase. Solvent A was 90% acetate buffer (containing 1% acetic acid, adjusted to pH 4) and 10% acetonitrile. Solvent B was 90% acetonitrile and 10% of the acetate buffer. The gradient program began with an isocratic elution of 85% A/15% B for 5 minutes followed by a linear gradient from 85% A/15% B to 44% A/56% B over the next 50 minutes, then a linear gradient from 44% A/56% B to 10% A/90% B over the next 10 minutes and finally returned to the initial conditions over the last 10 minutes. The complete analysis time was 75 minutes. Samples were dried under vacuum using a lyophilizer (Labconco Corp). Samples were hydrolyzed in vacuum/hydrolysis tubes using a heat block (Pierce Reacti-Therm). Samples were ultrafiltered using an ultrafiltration apparatus (Amicon, Inc) with nominal 500 molecular weight cutoff ultrafilters.

Description and Handling of Wastewaters. The wastewaters used in this study were obtained from the Army Base Wastewater Treatment Plant, located in Norfolk, Virginia and the James River Wastewater Treatment Plant, located in Newport News, Virginia. Both plants are owned and operated by the Hampton Roads Sanitation District. Both plants utilize the activated sludge process and handle mostly domestic sewage.

Secondary effluent was collected prior to chlorination in 4-liter amber jugs, sealed with PTFE-lined caps and transported directly to the laboratory. Upon arrival, each 4-liter jug was acidified for preservation with 10 mL of high purity sulfuric acid (pH <2) and filtered through Whatman GF-A filters. The total Kjeldahl nitrogen (TKN)

concentration and the ammonia concentration were determined by the Hampton Roads Sanitation District laboratory according to published procedures (26). The filtered wastewater was stored in polypropylene containers at -80 °C until it was analyzed. It was thawed completely and well mixed before an aliquot was removed for analysis and experimentation.

Chromatography of Dansyl Amino Acids. Separate stock solutions of each of the above dansyl amino acids were prepared in acetonitrile. The concentrations ranged from 1.24 mM to 2.74 mM. To determine retention times 50 µl of each were analyzed using HPLC as described above. An additional stock solution containing all of the dansyl amino acids was prepared in acetonitrile. The concentrations ranged from 1.24 mM to 2.74 mM. A 2 µM range standard solution was prepared by pipeting 55 µL of the stock solution into a 50 mL volumetric flask and diluting to the mark with Milli-Q® water. From this standard, standard solutions of all the amino acids were prepared with concentrations known accurately but in the range of 0.5 µM, 0.2 µM, 50 nM, and 20 nM by diluting the 2 µM standard solution. These standards were used to prepare calibration curves for each of the dansyl amino acids. Each standard solution was analyzed three times by HPLC using 250 µL injections. The average peak areas for each amino acid were plotted versus concentration to give the calibration curves.

Method I for the Identification of Terminal Amino Acid Residues. A dansyl chloride derivatizing solution was prepared by dissolving 7 mg DNS-Cl in 5 mL of acetonitrile. A solution comprised of 70% 6N HCl/30% distilled propionic acid was used to hydrolyze the peptide bonds.

To a 100 mL beaker containing a stir bar and a pH probe, 20 mL of the acidified wastewater were pipetted and then three to four drops of saturated sodium carbonate solution were added to adjust the pH to 9.5. HCl was used to adjust the pH if it increased above 9.5. With continuous stirring 150 µL DNS-Cl solution was added quickly. The solution was vigorously stirred for three minutes then allowed to sit in the dark for 45 minutes. After 45 minutes, 1 mL aliquots were transferred to test tubes. An aliquot (250µL) of the derivatized solutions was analyzed for free dansyl amino acids. Their identification was based upon retention times and their concentrations determined by comparison with the standard curves.

One mL was transferred from the test tubes to vacuum/hydrolysis tubes, frozen with liquid nitrogen and lyophilized. One mL of the hydrolysis solution was added and mixed on a vortex mixer (Vortex Genie) to ensure complete redissolution of the dried residue. The hydrolysis tube was placed in the heat block and heated at 130°C for 4 hours. The tubes were then allowed to cool, frozen with liquid nitrogen and lyophilized. The dried residue was redissolved in 1 mL of Mill-Q® water and vortex mixed to ensure complete dissolution. Aliquots of the hydrolyzed solutions (250µL) were analyzed for dansyl amino acids which were the original free dansyl amino acids plus the hydrolyzed N-terminal dansyl amino acids. Components were identified based upon retention times and their concentrations determined by comparison with the standard curves. The N-terminal amino acid residues on the polypeptides were determined by subtracting the free dansyl amino acid concentrations from the free plus N-terminal dansyl amino acid concentrations. This procedure was repeated for all four wastewater samples.

Study of Loss of Dansyl Amino Acids and Dansyl Sulfonic Acid by Ultrafiltration. In order to determine if the dansyl amino acids were going to be lost when ultrafiltered through a 500 MW cutoff filter, and to determine the number of ultrafiltrations required to reduce the dansyl sulfonic acid peak enough to allow detection of the polar dansyl amino acids, an ultrafiltration study was conducted. A 10-mL ultrafiltration apparatus was fitted with 500 nominal molecular weight cutoff filters and rinsed with 10 mL of Milli-Q® water using 75 psi nitrogen gas to pressurize the apparatus. This was done to remove the glycerin used to prevent drying of the membranes in storage. Separate ultrafilters were used to filter different wastewaters.

To determine the loss of dansyl amino acids by ultrafiltration 20-mL sample of a 1 μM solution of dansyl amino acids was ultrafiltered. Because the apparatus could only hold 10 mL, this was carried out in three stages. First 10 mL of the sample were concentrated to 2 mL, 8 mL more were added and the volume reduced to 2 mL. Finally the remaining 2 mL were added and the volume reduced to 2 mL again. The 2 mL of concentrate remaining in the ultrafilter were reconstituted to 10 mL with Milli-Q® water; then a 250 μL sample was analyzed for dansyl amino acids by HPLC. The 9.75 mL remaining were reduced to 2 mL, the remaining concentrate in the ultrafilter was reconstituted to 10 mL with Milli-Q® water, and a second 250 μL sample was analyzed for dansyl amino acids by HPLC. The 9.75 mL remaining were reduced to 2 mL, the remaining concentrate in the ultrafilter was reconstituted 10 mL with Milli-Q® water, and a 250 μL sample was analyzed for dansyl amino acids by HPLC.

To determine the loss of dansyl sulfonic acid by ultrafiltration, a second ultrafilter membrane was prepared as above. To a 100 mL beaker containing a stir bar and a pH probe, 20 mL of Milli-Q® water were pipetted and three to four drops of saturated sodium carbonate solution were added to adjust the pH to 10.75. HCl was used to correct the pH if it exceeded 10.75. With continuous stirring, 150 µL of the DNS-Cl solution were added quickly. The solution was vigorously stirred for three minutes then allowed to sit in the dark for 45 minutes. The sample was ultrafiltered by adding 10 mL of sample and reducing the volume to 2 mL. Then 8 mL more sample were added and the volume reduced to 2 mL. Finally the remaining 2 mL was added and the volume reduced to 2 mL. The 2 mL of concentrate remaining in the ultrafilter were brought up to 10 mL with Milli-Q® water. Then 250 µL was analyzed for dansyl amino acids by HPLC. The 9.75 mL were reduced to 2 mL, the remaining concentrate in the ultrafilter was brought up to 10 mL with Milli-Q® water and a second 250 µL sample was analyzed for dansyl amino acids by HPLC. The 9.75 mL were reduced to 2 mL, the remaining concentrate in the ultrafilter was brought up to 10 mL with Milli-Q® water, and a third 250 µL sample was analyzed for dansyl amino acids by HPLC.

Method II for the Identification of Terminal Amino Acid Residues. A dansyl chloride derivatizing solution was prepared by dissolving 7 mg DNS-Cl in 5 mL of acetonitrile. A solution comprised of 70% 6N HCl/30% distilled propionic acid was used to hydrolyze the peptide bonds.

To a 100 mL beaker containing a stir bar and a pH probe, 20 mL of the acidified wastewater were pipetted and then three to four drops of saturated sodium carbonate

solution were added to adjust the pH to 9.5. HCl was used to adjust the pH if it increased above 9.5. With continuous stirring, 150 µL DNS-Cl solution were added quickly. The solution was vigorously stirred for three minutes then allowed to sit in the dark for 45 minutes.

The 20 mL of derivatized wastewater were ultrafiltered in three stages. First 10 mL of the sample were concentrated to 2 mL, 8 mL more were added, and the volume reduced to 2 mL. Finally, the remaining 2 mL of derivatized wastewater were added and the volume reduced to 2 mL again. The 2 mL of concentrate remaining in the ultrafilter were reconstituted to 10 mL with Milli-Q® water and reduced to 2 mL. The concentrate was reconstituted to 10 mL with Milli-Q® water and reduced to 2 mL a second time. It was reconstituted to 10 mL again and reduced to 2 mL. An aliquot (250 µL) was analyzed for the free dansyl amino acids by HPLC. Their identification was based upon retention times and their concentrations determined by comparison with the standard curves.

One mL of the concentrate was pipetted to a vacuum/hydrolysis tube, frozen with liquid nitrogen, and lyophilized. Then 1 mL of the hydrolysis solution was added and mixed on a Vortex Genie to ensure complete redissolution of the dried residue. The hydrolysis tube was placed in the Reacti-Therm heat block and heated at 130°C for 4 hours. The tubes were then allowed to cool, frozen with liquid nitrogen and lyophilized. The dried residue was redissolved in 1 mL of Milli-Q® water and vortex mixed to ensure complete dissolution. An aliquot (250 µL) of the hydrolyzed solution was analyzed for free plus N-terminal dansyl amino acids. Components were identified by retention times,

and their concentrations determined by comparison with the standard curves. The terminal amino acid residue on the polypeptides were determined by subtracting the concentration of the free dansyl amino acids from the concentrations of the free plus N-terminal amino acids. This procedure was repeated for all four wastewater samples.

RESULTS

Characteristics of Wastewaters. Four secondary effluent samples collected from the treatment plant at a point prior to chlorination were analyzed for total Kjeldahl nitrogen (TKN) and ammonia. Table 1 shows the results. It was found that ammonia is a large contributor to the nitrogen content of the wastewaters. This is not unusual for municipal wastewaters.

Table 1. Concentrations of Total Kjeldahl Nitrogen (TKN) and Ammonia in Wastewaters.

Wastewater	Total Kjeldahl Nitrogen (TKN) mg/L as N	Ammonia mg/L as N
Army Base 1	21.1	18.8
Army Base 2	20.0	20.0
James River 1	1.6	0.6
James River 2	26.9	16.9

Chromatography of Dansyl Amino Acids. All 20 dansyl amino acids were analyzed and their retention times determined. Figure 1 shows the HPLC chromatogram of a 2 μM solution of dansyl amino acids. The dansyl amino acids eluted in order of decreasing polarity. The 20 dansyl amino acids separated into only 16 peaks. The following dansyl amino acids coeluted as pairs: dansyl asparagine and dansyl aspartic acid; dansyl methionine and dansyl proline; dansyl cystine and dansyl tryptophan; and dansyl leucine and dansyl isoleucine. Figure 2 shows an ultrafiltered derivatized wastewater spiked with all 20 dansyl amino acids. As can be seen, dansyl

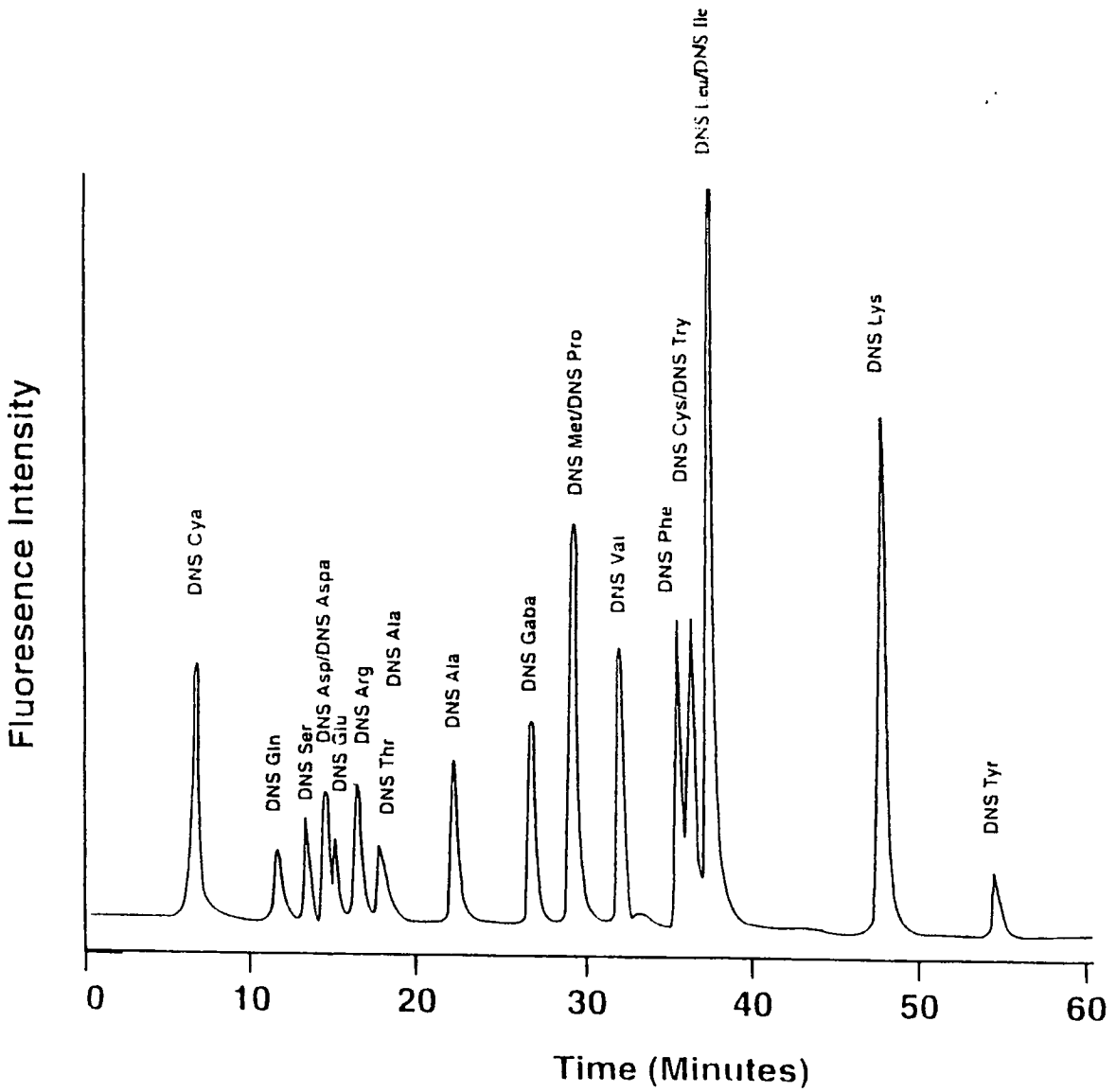


Figure 1. Chromatogram of 2 μM solution of 20 dansyl amino acids.

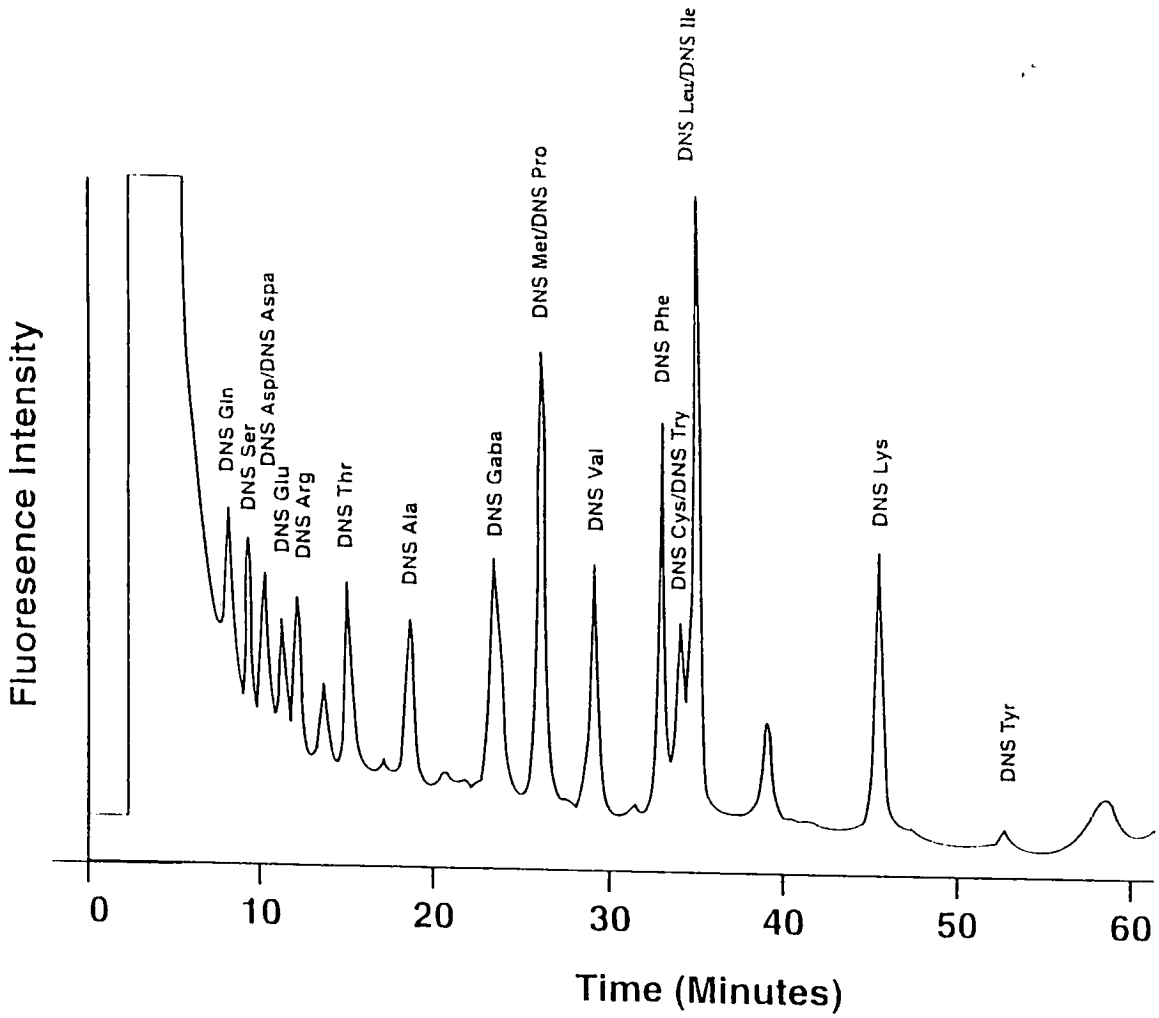


Figure 2. An ultrafiltered, derivatized wastewater spiked with 20 dansyl amino acids.

cysteic acid is the only compound which did not appear because it coelutes with the dansyl sulfonic acid peak, which originates from the hydrolysis of the dansyl chloride derivatizing agent.

Standard calibration curves were prepared by plotting the average peak areas versus concentration from three injections of solutions of five different concentrations. Figure 3 shows the calibration curve for dansyl alanine. It is representative of those obtained for all of the dansyl amino acids studied. Table 2 shows the dansyl amino acids listed in order of elution and the calibration data for each. The calibration curves show that a linear response is followed for each dansyl amino acid. The curves were generated as mixtures of all the dansyl amino acids, the poor correlation for dansyl tryptophan may be a result of this mixture due to degradation or quenching.

Method I for the Identification of Terminal Amino Acid Residues. The free amino acids along with the N-terminal amino acid residues on the polypeptides were derivatized with dansyl chloride, a highly fluorescent handle. The derivatized sample was analyzed for dansyl amino acids. Dansyl amino acids were identified by retention times, and their concentrations determined by comparison with the externally generated standard calibration curves. Figure 4 shows the chromatogram of a representative derivatized wastewater. These concentrations represented only the free dansyl amino acids; the terminal amino acid residue of peptides was still attached to the peptide chain.

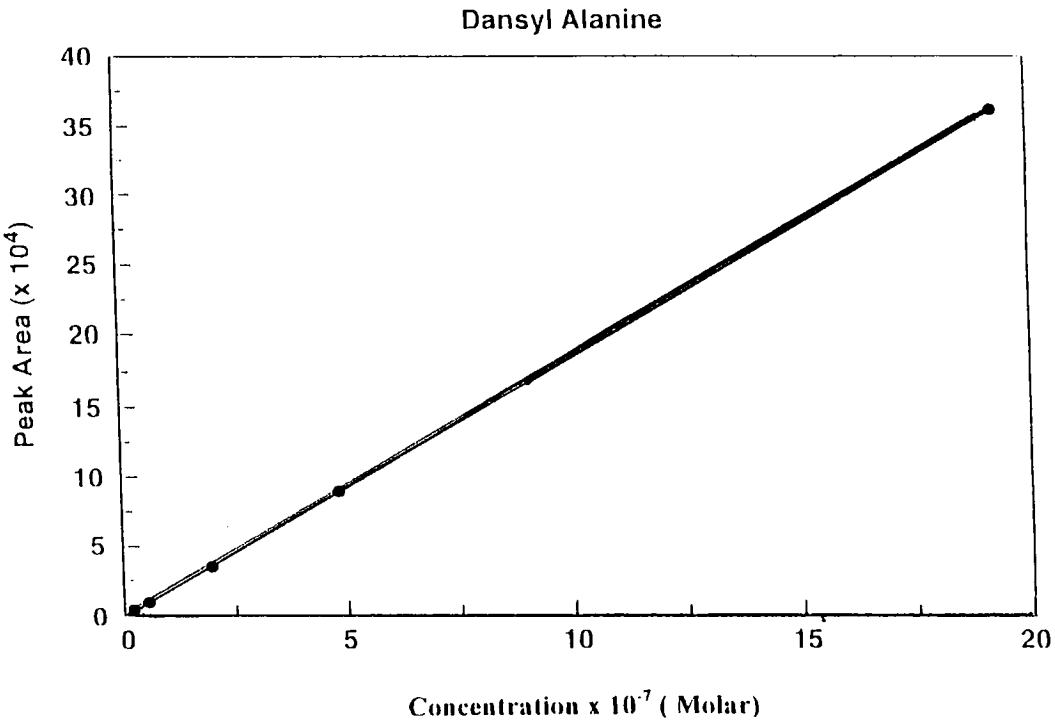


Figure 3. Calibration curve of dansyl alanine. This calibration curve is representative of those obtained for all dansyl amino acids studied.

Table 2. Results of the Determination of Calibration Curves for Dansyl Amino Acids.

Dansyl Amino Acid	Slope (counts/molar)	Correlation Coefficient (r^2)
Dansyl Cysteic Acid	1.25 ± 0.01	0.9999
Dansyl Glutamine	0.80 ± 0.01	0.9999
Dansyl Serine	1.25 ± 0.01	0.9999
Dansyl Asparagine/ Dansyl Aspartic Acid	1.32 ± 0.01	0.9999
Dansyl Glutamic Acid	1.15 ± 0.01	0.9999
Dansyl Arginine	1.51 ± 0.01	0.9999
Dansyl Threonine	1.18 ± 0.01	0.9999
Dansyl Alanine	1.86 ± 0.01	0.9999
Dansyl γ-aminobutyric Acid	2.43 ± 0.02	0.9999
Dansyl Methionine/ Dansyl Proline	2.20 ± 0.02	0.9999
Dansyl Valine	3.07 ± 0.01	0.9999
Dansyl Phenylalanine	2.47 ± 0.01	0.9999
Dansyl Cystine/ Dansyl Tryptophan	2.90 ± 0.01	0.9999
Dansyl Leucine/ Dansyl Isoleucine	4.31 ± 0.01	0.9999
di-Dansyl Lysine	8.56 ± 0.01	0.9999
di-Dansyl Tyrosine	1.66 ± 0.01	0.9959

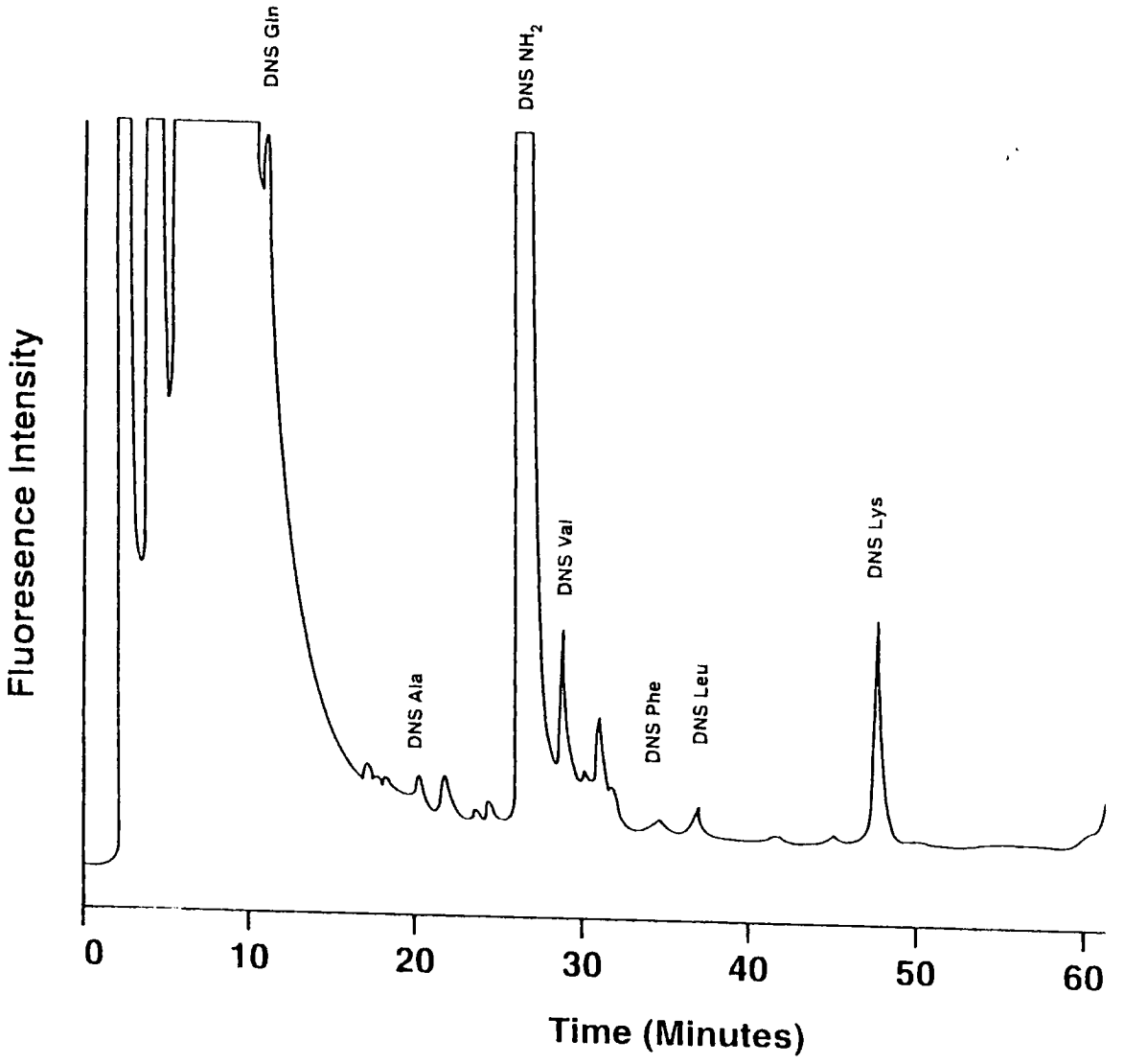


Figure 4. Representative chromatogram of derivatized wastewater showing the free dansyl amino acids found.

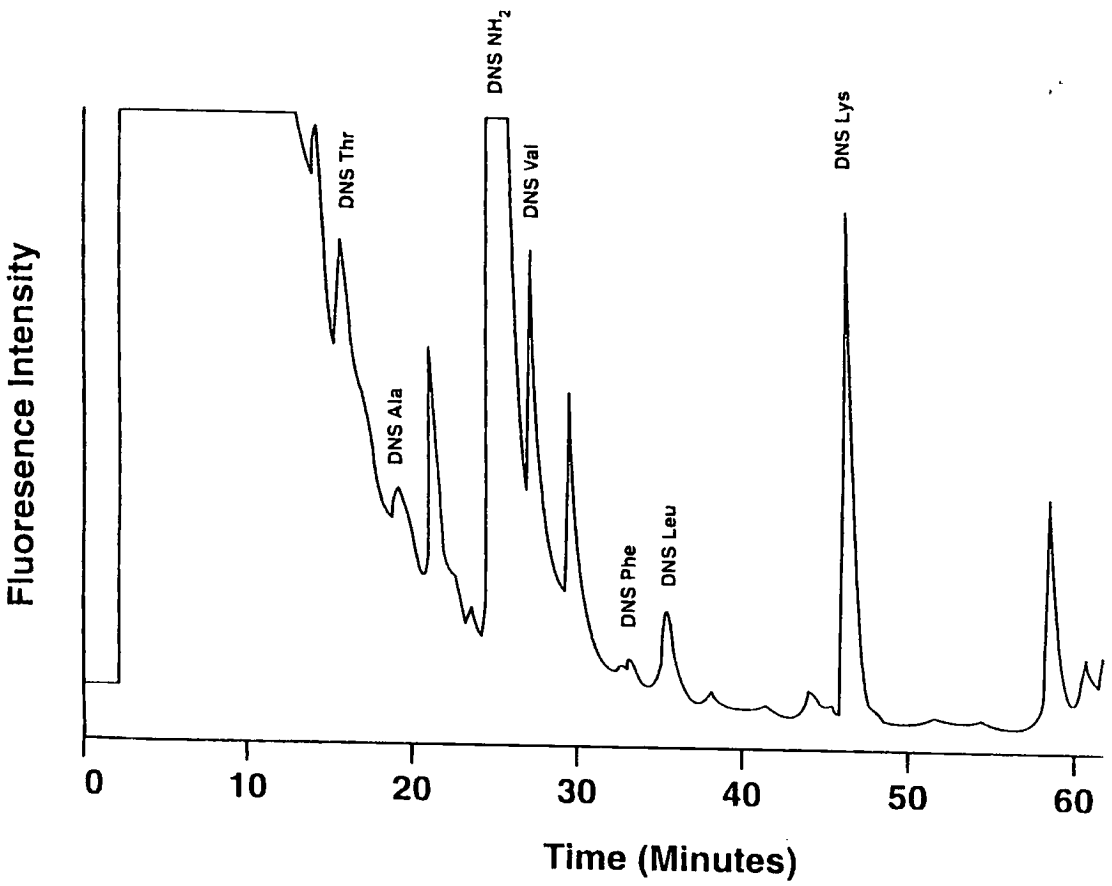


Figure 5. Representative chromatogram of hydrolyzed derivatized wastewater showing the free dansyl amino acids plus the free N-Terminal dansyl amino acid residues found.

The derivatized sample was then subjected to acid hydrolysis and the resulting solution was analyzed for free and terminal dansyl amino acids. Dansyl amino acids were identified by retention times and their concentrations determined by comparison with the externally generated standard calibration curves. Figure 5 shows the chromatogram of a representative sample of derivatized wastewater which had been hydrolyzed. The dansyl amino acids present represent the free dansyl amino acids originally present in the water plus the N-terminal amino acid residues that were released by hydrolysis.

The N-terminal amino acids were identified and their concentrations determined by subtracting the free dansyl amino acid concentrations from the free plus terminal amino acid concentrations. Tables 3, 4, 5, and 6 show the results for the four wastewaters studied. As can be seen in the tables not all of the dansyl amino acids were detected either as free or N-terminal amino acids.

Studies of the Loss of Dansyl Amino Acids and Dansyl Sulfonic Acid by Ultrafiltration. The loss of dansyl amino acids by ultrafiltering them through a 500 nominal molecular weight ultrafilter was studied to determine if significant loss of free amino acids was occurring. This was expected since the dansyl amino acids have molecular weights that may be near the cutoff of the nominal 500 molecular weight ultrafilter. It was assumed that the dansyl peptides and proteins were totally rejected and in this study they were concentrated by a factor of 10. If the dansyl amino acids were passing through the filter, then the reduction in concentration would be 5-fold for each ultrafiltration/reconstitution step. If the dansyl amino acids were being

Table 3. N-Terminal Amino Acid Residues Found in Army Base 1 Wastewater Determined by Method I.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	nd	nd	nd
Dansyl Serine	nd	nd	nd
Dansyl Asparagine/ Dansyl Aspartic Acid	nd	nd	nd
Dansyl Glutamic Acid	nd	nd	nd
Dansyl Arginine	nd	nd	nd
Dansyl Threonine	nd	nd	nd
Dansyl Alanine	88 $\pm$ 2	178 $\pm$2	90 $\pm$3
Dansyl γ-aminobutyric Acid	nd	nd	nd
Dansyl Methionine/ Dansyl Proline	260 $\pm$ 2	342 $\pm$ 2	82 $\pm$ 3
Dansyl Valine	195 $\pm$ 2	195 $\pm$ 2	0 $\pm$3
Dansyl Phenylalanine	nd	nd	nd
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	20 $\pm$ 2	86 $\pm$ 2	66 $\pm$3
di-Dansyl Lysine	nd	nd	nd
di-Dansyl Tyrosine	nd	nd	nd

[a]N-terminal=(column 2 -column1).
[b]nd= not detected= below method detection limit of 2.35nM.

Table 4. N-Terminal Amino Acid Residues Found in Army Base 2 Wastewater Determined by Method I.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	nd	nd	nd
Dansyl Serine	nd	nd	nd
Dansyl Asparagine/ Dansyl Aspartic Acid	nd	nd	nd
Dansyl Glutamic Acid	nd	nd	nd
Dansyl Arginine	nd	nd	nd
Dansyl Threonine	nd	nd	nd
Dansyl Alanine	71 $\pm$2	318 $\pm$2	247 $\pm$3
Dansyl γ-aminobutyric Acid	nd	nd	nd
Dansyl Methionine/ Dansyl Proline	405 $\pm$ 2	405 $\pm$ 2	0 $\pm$ 3
Dansyl Valine	430 $\pm$ 2	430 $\pm$ 2	0 $\pm$ 3
Dansyl Phenylalanine	nd	34 $\pm$ 2	34 $\pm$ 2
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	51 $\pm$ 2	104 $\pm$ 2	53 $\pm$ 3
di-Dansyl Lysine	537 $\pm$ 2	537 $\pm$ 2	0 $\pm$ 3
di-Dansyl Tyrosine	nd	nd	nd

[a]N-terminal=(column 2 -column1).
[b]nd=not detected=below the method detection limit of 2.35nM.

Table 5. N-Terminal Amino Acid Residues Found in James River 1 Wastewater Determined by Method I.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	nd	nd	nd
Dansyl Serine	nd	nd	nd
Dansyl Asparagine/ Dansyl Aspartic Acid	nd	nd	nd
Dansyl Glutamic Acid	nd	nd	nd
Dansyl Arginine	nd	nd	nd
Dansyl Threonine	151 ± 2	169 ± 2	18 ± 3
Dansyl Alanine	125 ± 2	139 ± 2	14 ± 3
Dansyl γ-aminobutyric Acid	nd	nd	nd
Dansyl Methionine/ Dansyl Proline	68 ± 2	68 ± 2	0 ± 3
Dansyl Valine	22 ± 2	22 ± 2	0 ± 3
Dansyl Phenylalanine	nd	nd	nd
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	6 ± 2	26 ± 2	20 ± 3
di-Dansyl Lysine	166 ± 2	238 ± 2	72 ± 3
di-Dansyl Tyrosine	nd	nd	nd

[a]N-terminal=(column 2 -column1).
[b]nd=not detected=below the method detection limit of 2.35nM

Table 6. N-Terminal Amino Acid Residues Found in James River 2 Wastewater Determined by Method I.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	nd	nd	nd
Dansyl Serine	nd	nd	nd
Dansyl Asparagine/ Dansyl Aspartic Acid	nd	nd	nd
Dansyl Glutamic Acid	nd	nd	nd
Dansyl Arginine	nd	nd	nd
Dansyl Threonine	nd	275 ± 2	275 ± 2
Dansyl Alanine	60 ± 2	107 ± 2	47 ± 3
Dansyl γ-aminobutyric Acid	nd	nd	nd
Dansyl Methionine/ Dansyl Proline	nd	nd	nd
Dansyl Valine	140 ± 2	140 ± 2	0 ± 3
Dansyl Phenylalanine	2.58 ± 2	5.29 ± 2	3 ± 3
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	23 ± 2	52 ±2	29 ± 3
di-Dansyl Lysine	173 ± 2	184 ± 2	11 ± 3
di-Dansyl Tyrosine	nd	nd	nd

[a]N-terminal=(column 2 -column1).
[b]nd=not detected=below the method detection limit of 2.35nM

rejected by the membrane, then their concentration would remain the same. It was found that the dansyl amino acids were neither totally rejected nor did they totally permeate the membrane. Their concentrations were reduced by a factor ranging from 1.3-2.5 for each ultrafiltration/reconstitution step.

To determine how many ultrafiltration/reconstitution steps were needed to reduce the dansyl sulfonic acid concentration sufficiently to allow the resolution of the dansyl sulfonic acid peak and the polar dansyl amino acids, a 40 μM DNS-Cl solution was hydrolyzed to DNS-OH and then ultrafiltered. It was found that three ultrafiltration/reconstitution steps were necessary to reduce the dansyl sulfonic acid peak enough to separate the DNS-OH from all of the dansyl amino acids except DNS- cysteic acid. Figure 6 shows the chromatogram of the dansyl sulfonic acid before ultrafiltration and Figure 7 shows the chromatogram after three ultrafiltrations. The dansyl sulfonic acid peak elutes within 8 minutes, allowing resolution between dansyl sulfonic acid and dansyl glutamine. Dansyl cysteic acid still eluted under the dansyl sulfonic acid peak.

Method II for the Identification of Terminal Amino Acid Residues. After derivatization of free and N-terminal amino acid residues with DNS-Cl, the derivatized sample was ultrafiltered through a 500 nominal molecular weight ultrafilter three times. A 250 μL sample was injected and analyzed for free dansyl amino acids. The dansyl amino acids were identified by retention times and their concentrations determined by comparison with the externally generated standard curves. Figure 8 shows the chromatogram of a representative sample of derivatized, ultrafiltered wastewater.

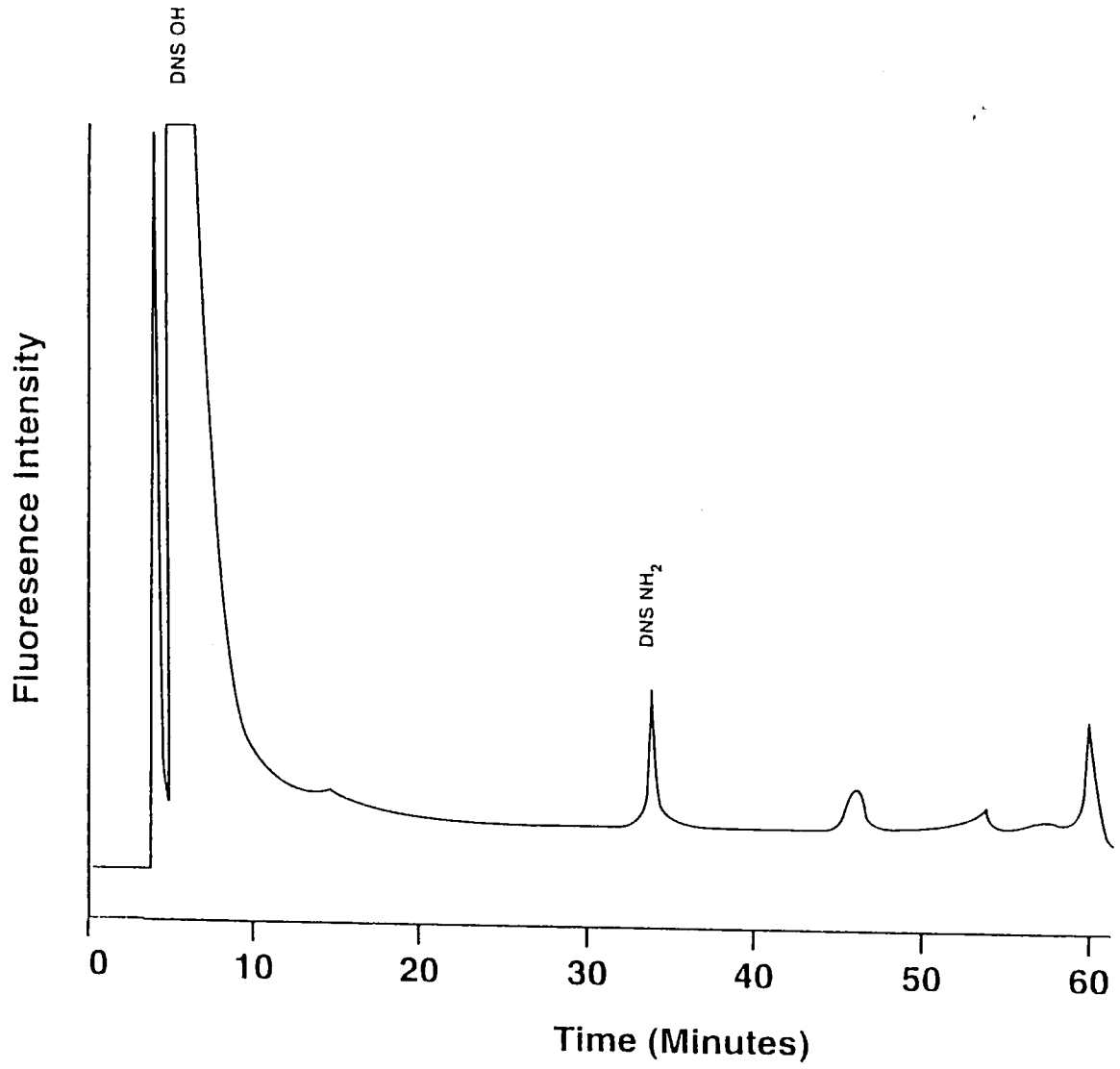


Figure 6. Chromatogram of dansyl sulfonic acid before ultrafiltration.

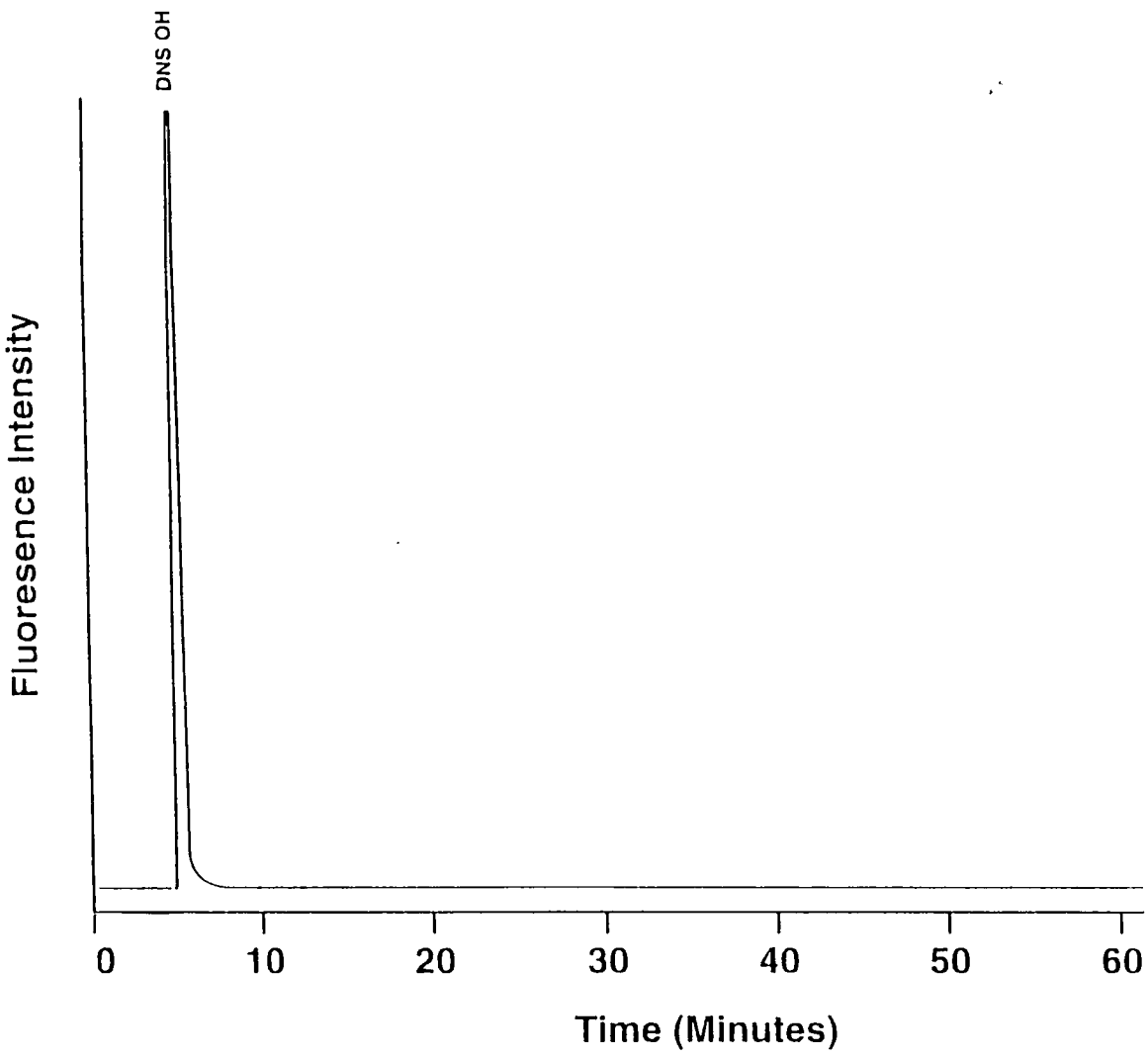


Figure 7. Chromatogram of dansyl sulfonic acid after three ultrafiltrations.

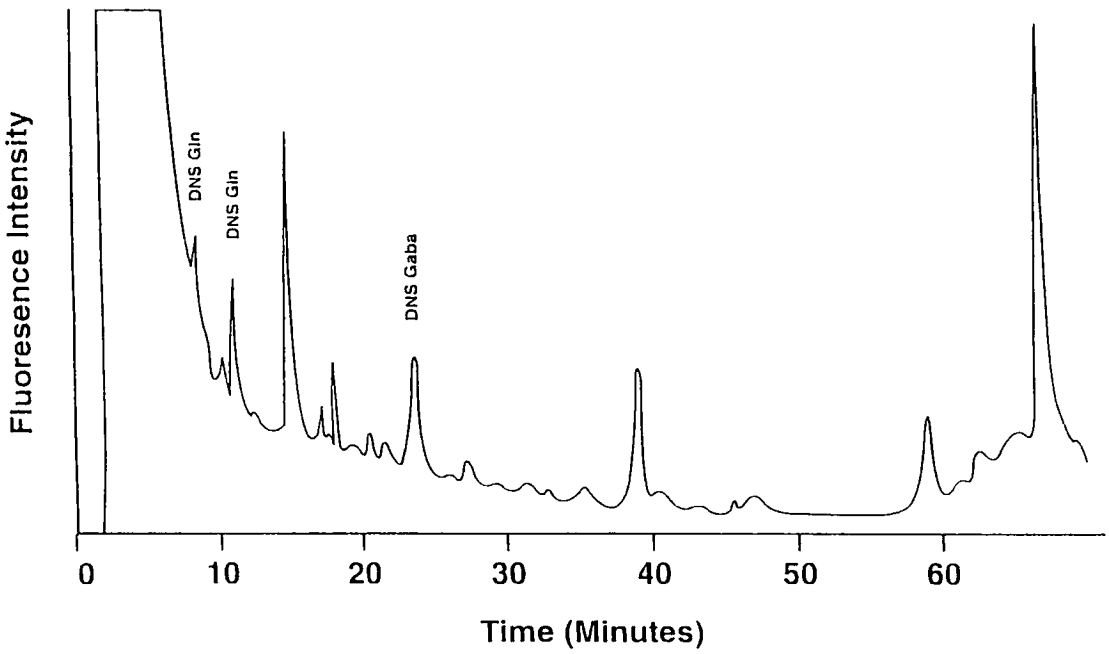


Figure 8. Representative chromatogram of derivatized, ultrafiltered wastewater showing the free dansyl amino acids found.

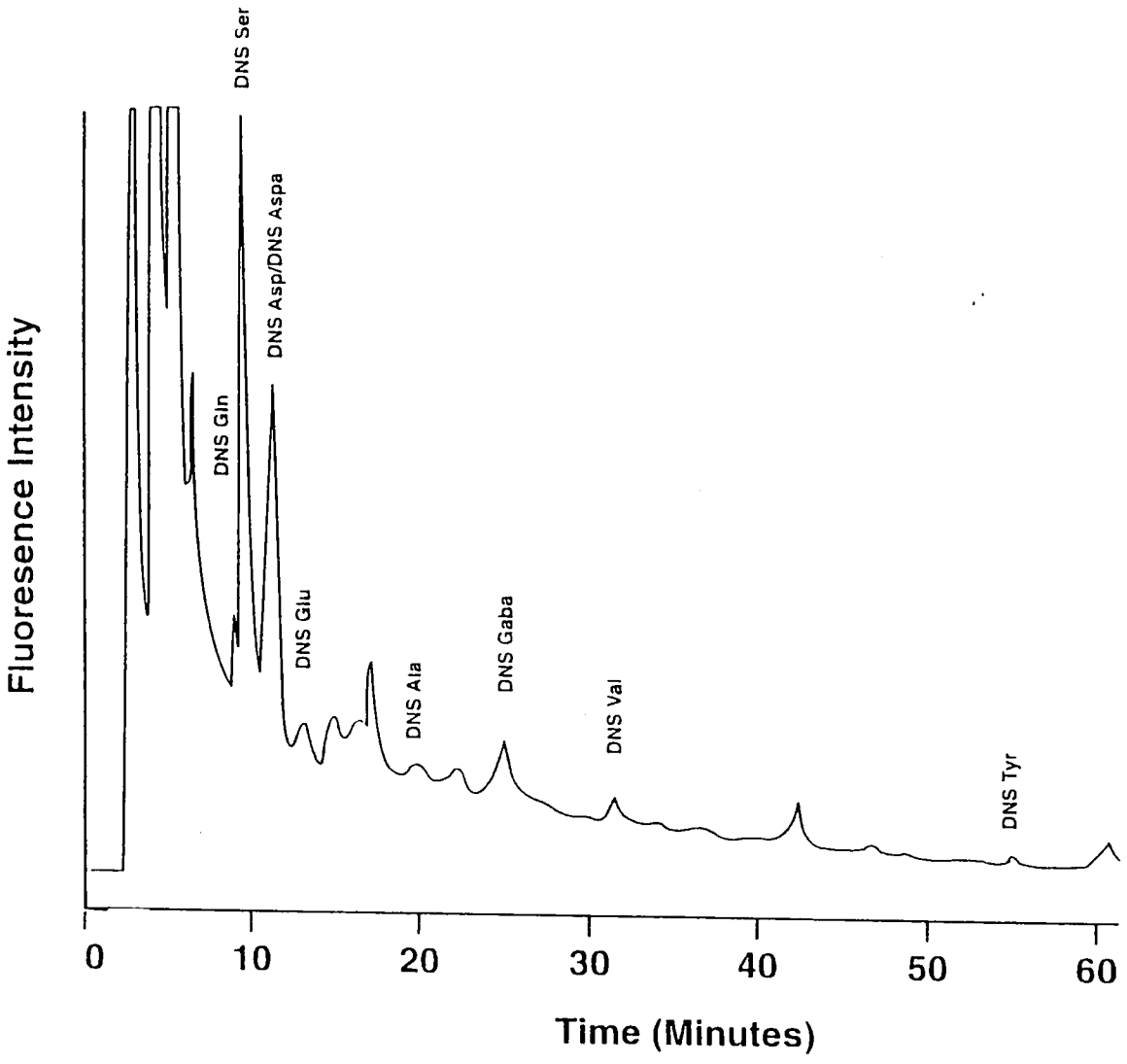


Figure 9. Representative chromatogram of hydrolyzed, derivatized, ultrafiltered wastewater showing the free dansyl amino acids plus the free N-Terminal dansyl amino acid residues found.

These concentrations represented only the free dansyl amino acids, the terminal amino acid residues were still attached to the peptide chain.

The derivatized, ultrafiltered sample was subjected to acid hydrolysis to release N-terminal residues. The hydrolyzed solution was then analyzed for free and terminal dansyl amino acids. The dansyl amino acids were identified by retention times and their concentrations determined by comparison with externally generated standard curves. Figure 9 shows the chromatogram of a representative hydrolyzed wastewater. These concentrations represented the free dansyl amino acids originally present before hydrolysis plus the N-terminal amino acid residues that are now present as free dansylated amino acids.

The N-terminal amino acids were identified and their concentrations determined by subtracting the free dansyl amino acids from the free plus terminal amino acid concentrations then dividing these results by 10 to account for the 10-fold concentration of the peptides. Tables 7, 8, 9, and 10 show the data for the four wastewaters studied. Not all of the dansyl amino acids were found either as free or N-terminal dansyl amino acids but many more of the polar ones were able to be detected.

Table 7. N-Terminal Amino Acid Residues Found in Army Base 1 Wastewater Determined by Method II.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	3.63 ± 2	494 ± 2	49 ± 3
Dansyl Serine	nd	4400 ± 20	440 ± 20
Dansyl Asparagine/ Dansyl Aspartic Acid	nd	3900 ± 20	390 ± 20
Dansyl Glutamic Acid	85 ± 2	365 ± 2	28 ± 3
Dansyl Arginine	nd	nd	nd
Dansyl Threonine	nd	nd	nd
Dansyl Alanine	nd	195 ± 2	20 ± 2
Dansyl γ-aminobutyric Acid	86 ± 2	488 ± 2	40 ± 3
Dansyl Methionine/ Dansyl Proline	nd	nd	nd
Dansyl Valine	nd	6.39 ± 2	0.63 ± 0.2
Dansyl Phenylalanine	nd	nd	nd
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	nd	nd	nd
di-Dansyl Lysine	nd	nd	nd
di-Dansyl Tyrosine	nd	74 ± 2	7.4 ± 2

[a]N-terminal=(column 2 -column1)/10.
[b]nd= not detected= below method detection limit of 2.35nM.

Table 8. N-Terminal Amino Acid Residues Found in Army Base 2 Wastewater Determined by Method II.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	nd	2640 ± 20	264 ± 20
Dansyl Serine	nd	nd	nd
Dansyl Asparagine/ Dansyl Aspartic Acid	5 ± 2	1470 ± 20	146.5 ± 20
Dansyl Glutamic Acid	nd	168 ± 2	17 ± 2
Dansyl Arginine	7.18 ± 2	16.4 ± 2	0.94 ± 0.2
Dansyl Threonine	nd	500 ± 2	50 ± 2
Dansyl Alanine	183 ± 2	251 ± 2	6.8 ± 3
Dansyl γ-aminobutyric Acid	56 ± 2	231 ± 2	17.5 ± 3
Dansyl Methionine/ Dansyl Proline	nd	47 ± 2	4.7 ± 2
Dansyl Valine	28 ± 2	28 ± 2	0 ± 3
Dansyl Phenylalanine	nd	7.01 ± 2	0.7 ± 0.2
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	nd	nd	nd
di-Dansyl Lysine	nd	nd	nd
di-Dansyl Tyrosine	31 ± 2	66 ± 2	3.5 ± 3

[a]N-terminal=(column 2 -column1)/10.
[b]nd= not detected= below method detection limit of 2.35nM.

Table 9. N-Terminal Amino Acid Residues Found in James River 1 Wastewater Determined by Method II.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	nd	996 ± 2	100 ± 2
Dansyl Serine	nd	1500 ± 20	150 ± 20
Dansyl Asparagine/ Dansyl Aspartic Acid	nd	2290 ± 20	229 ± 20
Dansyl Glutamic Acid	nd	1630 ± 20	163 ± 20
Dansyl Arginine	nd	747 ± 2	74.7 ± 2
Dansyl Threonine	nd	834 ± 2	83 ± 2
Dansyl Alanine	216± 2	297 ± 2	7.86 ± 2
Dansyl γ-aminobutyric Acid	nd	282 ± 2	2.82 ± 2
Dansyl Methionine/ Dansyl Proline	nd	8.14 ± 2	0.814 ± 0.2
Dansyl Valine	nd	44 ± 2	4.4 ± 2
Dansyl Phenylalanine	nd	nd	nd
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	nd	nd	nd
di-Dansyl Lysine	nd	nd	nd
di-Dansyl Tyrosine	nd	167 ± 2	16.7 ± 2

[a]N-terminal=(column 2 -column1)/10.
[b]nd= not detected= below method detection limit of 2.35nM.

Table 10. N-Terminal Amino Acid Residues Found in James River 2 Wastewater Determined by Method II.

Dansyl Amino Acid	Free (nM)	Free + N-Terminal (nM)	N-terminal[a] (nM)
Dansyl Cysteic Acid	nd[b]	nd	nd
Dansyl Glutamine	65.2 ± 2	1390 ± 20	133 ± 20
Dansyl Serine	nd	920 ± 2	92 ± 2
Dansyl Asparagine/ Dansyl Aspartic Acid	nd	943 ± 2	94.3 ± 2
Dansyl Glutamic Acid	nd	820 ± 2	82 ± 2
Dansyl Arginine	nd	nd	nd
Dansyl Threonine	nd	443 ± 2	44.3 ± 2
Dansyl Alanine	nd	nd	nd
Dansyl γ-aminobutyric Acid	nd	nd	nd
Dansyl Methionine/ Dansyl Proline	nd	nd	nd
Dansyl Valine	nd	nd	nd
Dansyl Phenylalanine	nd	7.17 ± 2	0.71 ± 0.2
Dansyl Cystine/ Dansyl Tryptophan	nd	nd	nd
Dansyl Leucine/ Dansyl Isoleucine	nd	nd	nd
di-Dansyl Lysine	nd	nd	nd
di-Dansyl Tyrosine	nd	nd	nd

[a]N-terminal=(column 2 -column1)/10.
[b]nd= not detected= below method detection limit of 2.35nM.

FINDINGS AND INTERPRETATIONS

Derivatization of Free and N-Terminal Amino Acids. Dansyl chloride has been used for many years to determine free amino acids (those not bound in peptides and proteins) and N-terminal amino acid residues on peptides and proteins. It is better than the older method of Sanger (27) that utilizes DNFB (dinitrofluorobenzene) to form dinitrophenylamino acids (DNP-amino acids) because it offers a 100-fold increase in sensitivity, and the resulting DNS-amino acids are more stable to acid hydrolysis than are the DNP-amino acids, thus making it applicable to small concentrations of peptides and proteins. (28,29,30).

Dansyl chloride is a typical aromatic sulfonyl chloride and reacts with a wide variety of bases, forming derivatives with varying stabilities. Some of the basic groups that may be present in wastewater, and are present in proteins and peptides include primary and secondary amine groups, aliphatic and phenolic hydroxyl groups, thiols, and imidazoles. Under the pH conditions used in this study, aliphatic hydroxyls are present almost exclusively as their unreactive conjugate acids and will not react with dansyl chloride, and the derivatives from the thiol and imidazole groups are unstable in acidic conditions and will be destroyed in the hydrolysis step (29). In this study sulfonic esters and sulfonamides are the major stable desired reaction products and sulfonamides are of greatest interest.

Sulfonic esters and sulfonamides are not the only products formed on derivatization of wastewater. In order to form the dansyl amino acid derivatives it is necessary to run the reaction at pH 9.5 to ensure that the amino nitrogens are

deprotonated. At this pH the dansyl chloride reacts with OH^- to form dansyl sulfonic acid. Wastewaters also contain high concentrations of ammonia which reacts with dansyl chloride to form dansyl amide. The important reactions are shown below:

$$AA\text{-}NH_2 + DNS\text{-}Cl \longrightarrow AA\text{-}NH\text{-}DNS + HCl$$

$$H_2O + DNS\text{-}Cl \longrightarrow DNS\text{-}OH + HCl$$

where $AA\text{-}NH_2$ is an amino acid or the N-terminal amino group on a protein or polypeptide; DNS-OH is dansyl sulfonic acid, and $DNS\text{-}NH_2$ is dansyl amide.

The high pH favors the formation of the dansyl amino acids but also favors the formation of the dansyl sulfonic acid. An excess amount of dansyl chloride must be added to ensure complete conversion of the amino acids. Much of the dansyl chloride is converted to the sulfonic acid which makes the analysis of the dansyl amino acids difficult. This limitation is evident in Method I of this study and Method II addresses this problem.

In this study it is assumed that the derivatization reaction is complete and the available amino groups form the dansyl derivative. The wastewater matrix is a complex matrix. Tapuhi et al. (31) performed amino acid analysis in a complex matrix containing fermentation broths with whole and fractured cells, proteins, peptides, NH_4Cl, and parts per million levels of various metal ions present and obtained reliable results. Because the wastewaters and the reaction conditions used in this study are similar to the media and conditions used by Tapuhi et al (31), the derivatization was assumed to be quantitative; thus, a conversion study was not performed.

Tapuhi (31) did not use an externally generated calibration curve to obtain the quantitative results they reported. A spike containing various amino acids was added and the increase in concentrations determined. The quantitative results presented in this work are questionable because quantitation was performed using externally generated calibration curves. This approach did not consider the potential for matrix effects of wastewater on the amino acid determinations. The method of standard additions should have been used and compared to the external calibration curves to check for matrix effects. Future studies in wastewaters should compare standard additions to external calibration curves prior to determining the amino acid concentrations. Also, the quantitative results would have been better if replicate sample aliquots had been analyzed. The main goal of this work was the qualitative determination of the N-terminal amino acid residues so that future projects could be formulated.

Comparisons of Methods Used. Both Methods I and II make use of the same reaction conditions during derivatization and hydrolysis. The derivatization reactions are carried out at pH 9.5 with an excess of dansyl chloride present to ensure high conversions of the amino acids, polypeptides, and proteins to their dansyl derivatives. After derivatization it is necessary to analyze for the dansyl derivatives of the free amino acids formed before proceeding to the hydrolysis step because only after hydrolysis are the N-terminal amino acid residues released from their peptide chains. The determination of the N-terminal amino acids are made by subtracting the concentration of dansyl free amino acids from the concentration of dansyl amino acids determined after hydrolysis (free plus N-terminal).

As can be seen in Figure 4, the large peak on the left side of the chromatogram is the dansyl sulfonic acid peak. It requires from 10 to 15 minutes for all of the dansyl sulfonic acid to elute from the column. Many of the polar dansyl amino acid peaks elute during this time and cannot be measured. As a result, the only dansyl amino acids that can be identified are those that are nonpolar, while very few if any of the polar amino acids are identifiable. The chromatogram, shown in Figure 5, still displays this large peak indicating that only the nonpolar terminal amino acid residues can be identified. This is a major limitation with Method I.

Method II addresses this limitation by utilizing ultrafiltration to reduce the dansyl sulfonic acid concentration enough to prevent it from interfering with the responses of the polar dansyl amino acids. Ultrafiltration also minimizes the amount of dansyl amide so that it does not interfere with the analysis of the dansyl amino acids. This technique enabled the successful analysis of polar amino acids in wastewaters. As can be seen in Figure 7, the dansyl sulfonic acid now elutes in less than 9 minutes and the dansyl amide peak has been reduced significantly. Figure 2 shows that all of the dansyl amino acid peaks with the exception of dansyl cysteic acid can be detected in a derivatized wastewater. Figures 8 and 9 show that nonpolar and polar dansyl amino acids can be identified before and after hydrolysis. This makes Method II the method of choice for the determination of N-terminal amino acid residues.

Nevertheless, some information is lost in the ultrafiltration process. Method I allows accurate measurement of the concentrations of the free nonpolar dansyl amino acids in the wastewater, Method II does not. The dansyl amino acids are small enough in

their molecular sizes that they pass through the ultrafilter membrane. Consequently, after the ultrafiltration, the concentrations of free dansyl amino acids determined are not representative of the actual concentrations found in the wastewater but only represent a fraction of the original that was retained by the membrane. It is not likely that the same fraction of dansyl amino acids will remain after each ultrafiltration, so the original concentrations of free dansyl amino acids cannot be back-calculated. This is a drawback to Method II, in that additional information cannot be acquired, but Method II allows the original question of determining the N-terminal amino acids and not the free amino acids in secondary effluent wastewater to be answered.

Originally, it was postulated that Method II would require one less analysis step because the ultrafiltration was thought to filter out all of the free dansyl amino acids and all of the dansyl sulfonic acid, and at the same time concentrate the dansyl peptides. This would eliminate the need to determine the dansyl amino acids before hydrolysis and make Method II even better. It was realized that the ultrafilter used was rated as a nominal 500 molecular weight cutoff filter and that the molecular weights of the dansyl amino acids are close to this arbitrary cutoff point. It was then suspected that a reduction of the dansyl amino acids may occur but that complete removal was not possible. This was proven by the ultrafiltration study. So Method II does require an analysis of the residual free dansyl amino acids before hydrolysis.

Determination of N-terminal Amino Acid Residues in Wastewaters. The N-terminal amino acid residues were identified and their concentrations determined by subtracting the free amino acids found before hydrolysis from the free and N-terminal

amino acids found after hydrolysis, and correcting the results by the appropriate concentration factor.

Because of the limitations mentioned above, the N-terminal amino acids identified by Method I were all nonpolar with concentrations ranging from 2×10^{-9} M to 3×10^{-7} M. Only 3 to 6 nonpolar amino acids were found as terminal residues in any of the wastewaters analyzed. This result raised questions about the relative significance of the polar versus nonpolar N-terminal amino acids and suggested the need to overcome the interference of the dansyl sulfonic acid. Also, the relatively high concentrations of free amino acids subtracted from similarly high free plus N-terminal amino acids concentrations introduced significant error into the determination of the true concentrations of the terminal residues.

Ultrafiltration as used in Method II, increased the concentrations of the N-terminal amino acids and decreased the concentrations of the free amino acids, thus increasing the precision in the measurements. Method II also eliminated interference from the dansyl sulfonic acid allowing analysis of the polar amino acids. Now the same wastewaters were found to contain as many as 12 N-terminal amino acid residues. The concentrations ranged from 1×10^{-10} M to 3×10^{-7} M. It was found that the polar amino acids represent the majority of the N-terminal residues and are at higher concentrations than the nonpolar ones.

Implications for Wastewater Treatment. In the four wastewaters studied it was found that the majority of the N-terminal amino acid residues measured were the more

polar residues. In general glutamine, serine, asparagine, aspartic acid and glutamic acid were found to be the N-terminal residue in the highest concentrations.

Determination of the N-terminal amino acids in small polypeptides would not be affected significantly by the derivatization procedure. However, if the majority of N-terminal amino acids is present in globular proteins, then the method may skew the results in favor of the polar amino acids residing on the surface of the tertiary structure. Gray (26) discussed the need to denature proteins first to accurately determine the nonpolar residues in proteins.

Nevertheless, for similar reasons, reaction of surface-bound terminal amino acids with aqueous chlorine during disinfection of a wastewater is also likely to be far more significant than reaction with terminal residues embedded in the tertiary structure.

It is apparent from this study that monochloroglutamine, monochloroserine, monochloroasparagine, monochloroaspartic acid, and monochloroglutamic acid should be the chlorinated N-terminal groups formed most rapidly. With increased levels of chlorine, the dichloroamino moieties would predominate. Therefore the chemistry of these components in wastewater should be studied.

The disinfection interference in secondary wastewaters caused by organic amino nitrogen associated with proteins in wastewaters may be due solely to the surface amino acid residues and the polar N-terminal amino acid residues. Results presented here suggest that the nonpolar N-terminal amino acid residues do not make a significant contribution to the chlorine demand of organic amino nitrogen in wastewaters.

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VITA

Edward L. Creecy

EDUCATION:

1998	Master of Science Department of Chemistry and Biochemistry Old Dominion University Thesis: Determination of N-Terminal Amino Acid Residues on Polypeptides in Wastewaters Advisor: Frank E. Scully, Jr., Ph. D.
1986	Bachelor of Science Department of Chemistry and Biochemistry Old Dominion University

EXPERIENCE:

1996-Present	Analytical Chemist Ecolochem, Inc. Norfolk, Va. Responsibilities: Perform chemical analysis on surface, ground and wastewaters to determine treatment procedures for ultrapure water production.
1987-1996	Field Service Representative Ecolochem, Inc. Norfolk, Va. Responsibilities: Operate and maintain ultrapure water equipment for Nuclear, Paper, Chemical, Power, Petroleum and Pharmaceutical Industries. Operations included: Coagulation/Flocculation Filtration, Deoxygenation, Reverse Osmosis, Ion Exchange Demineralization, and Electrodemineralization.

PROFESSIONAL ORGANIZATIONS:

1987-Present	American Chemical Society
1996-Present	American Water Works Association