CHAPTER VI

A New Spectrophotometric Method for the Determination of Ammonia and Its Application to the Analysis of Total Nitrogen and Milk Protein

SUMMARY

A new spectrophotometric method is developed for the determination of ammonia. The method is based on the reaction of ammonia with phenol and N-chlorosuccinimide in the presence of nitroprusside-manganese dioxide mixture as catalyst. The colour system obeys Beer's law in the range of 0.9-7.5 µg of ammonia in 100 ml final solution. The indophenol formed has absorption maximum at 635 nm and has apparent molar absorptivity of $2.2 \times 10^5$ 1 mol$^{-1}$ cm$^{-1}$. The method is fairly reproducible with standard deviation and relative standard deviation 0.01 and ±1.5% respectively for 7 replicate analyses of 4 µg of ammonia per 100 ml. Effect of analytical parameters and tolerance limit of diverse ions on colour development reaction have been evaluated. The method has been applied for the determination of free ammonia in air, water, soil and effluents from distillery as well as coke oven. It has also been modified for the determination of total nitrogen in soil, steel, distillery waste and effluent from fertilizer plant as well as for the determination of milk protein. The method has been compared with other popular spectrophotometric methods and has been found to be simple, sensitive and suitable for the routine analysis of ammonia.

(2) Chemia Analyticzna, 38, 123-127, 1993
INTRODUCTION

Ammonia is one of the earliest nitrogen compounds known to man produced originally by destructive distillation of horns of animals. It is an acrid smelling pollutant liberated to the environment as a result of several natural and industrial processes and has ranked forth in the production volume after sulphuric acid, lime and oxygen [WHO, 1984]. It is extensively used in refrigeration, petroleum refining, manufacture of fertilizers, nitric acid and manufacture of other chemicals [Patty, FA 1963]. Ammonia added to earth's surface gets volatilized and escapes to atmosphere at the rate of $10^6$ tonnes per year through natural and biological activity [Kresge, CB, 1960]. Industrial activity causes local and regional elevations in emissions of atmospheric ammonia. Surface waters receive ammonia from point sources such as effluents from sewage treatment plants and industrial units like distillaries, breweries, fertilizer plants, coking plants, paper and pulp, power plants etc. Much more significant quantities arise from the non point sources such as atmospheric deposition decay of vegetation and animal wastes, applied chemical fertilizers and urban run off [Tisdale, L S, 1977; Anderson, O E, 1960]. Reduction of nitrogen containing compounds also add significant quantities of ammonia to environment [Baker J H M, 1959; Blue, W G, 1954].
The environmental levels of ammonia have been estimated in various parts of the world and in different parts of the country as well [Anderson, OE, 1970; Tiwari, S, 1992; Balachandran, N, 1992; Khemani, LT, 1989]. It has been observed that atmospheric concentration vary according to underlying land usage. Urban concentrations are typically in the range of 5-25 \( \mu g \, m^{-3} \) and rural concentrations 2-6 \( \mu g \, m^{-3} \). Areas with intensive manure production or use have been reported to have the concentrations of 100-200 \( \mu g m^{-3} \). Particulate ammonium concentrations above oceans remote from land have been found to be 10-115 \( ng \, m^{-3} \). In surface water the concentrations of ammonia varied both regionally and seasonally. In hydrologically isolated acidified small lakes the total ammonium concentration reach to 3 mg L\(^{-1}\) and up to 12 mg L\(^{-1}\) in intensive farming centres. Ground water usually contains very low concentration of ammonia due to adsorption and nitrification [Tisdale, LS, 1977, Burge, W, 1961, Welch, LF, 1960].

Increased environmental levels of ammonia increases the chance of more ammonia exposure. Unprocessed food, cigarette smoke, certain medicines also contribute to total ammonia intake [WHO, 1987]. Occupational exposure to low levels of ammonia is common but in certain occupations related with steel making, coking, combustion of coal, coal washing, fermentation, and production of ammonium compounds the work place concentration often exceed 100 \( \mu g \, m^{-3} \). Exposure to the
high concentrations of ammonia causes throat irritation, cough, chronic bronchitis and hyper ammonanaemea [Sollmann, J 1944; Lopin, 1941; Henderson, J , 1943]. The chief toxicological symptoms associated with different concentrations of ammonia are tabulated in Table 1. Looking to the toxic effects of ammonia on health the permissible exposure limit value has been set at 35 mg m\(^{-3}\) (50 ppm) whereas short term exposure limit value (STEL) for ammonia is 35 ppm [Ray, C A , 1990].

Higher concentrations of ammonia is highly toxic for fish and causes loss of equilibrium increased breathing, hyper exitability, cardiac output, higher oxygen uptake and in extreme cases results convulsions, coma and death. At lower concentrations, ammonia creates many problems to fish including failure in egg hatching, reduction in growth rate and change in physiology or metabolism. The mean LC 50 values reported for 48h for fresh water fish, invertebrates, marine fish and marine invertebrates are 1.10, 0.56, 0.32 and 0.94 mg L\(^{-1}\) respectively [WHO, 1987; Cooke, I J , 1962]. Ammonia is also found to be toxic to some plant species above defined critical concentrations [Tisdale, L S , 1977].

Ammonia in the environment is a part at natural nitrogen cycles. It volatilizes into the atmosphere and there it undergoes variety of chemical reactions [Owens, L D , 1960]. Photolytic reactions destroy 80% of the ammonia and reaction with sulphurdioxide and ozone results into acrosols which in turn reaches earths
surface through dry or wet deposition. In surface waters ammonia undergoes microbial nitrification which yield hydrogen and imparts acidity to water system as well as catalyzes depletion of oxygen [Court, M N, 1962]. Ammonia in water also reacts with hypochlorous acid resulting from chlorination of water to form monochloramine, dichloramine and nitrogen dichloride which are more toxic than ammonia itself [WHO, 1984].

Table 1 Toxicological symptoms of ammonia

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (mg m⁻³)</th>
<th>Toxic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.5</td>
<td>Organoleptic concentration</td>
</tr>
<tr>
<td>2.</td>
<td>35.0</td>
<td>Irritation</td>
</tr>
<tr>
<td>3.</td>
<td>88.0</td>
<td>Definitely irritating</td>
</tr>
<tr>
<td>4.</td>
<td>98.0</td>
<td>Alteration of respiratory indices, Toxic symptoms starts appearing.</td>
</tr>
<tr>
<td>5.</td>
<td>250.0</td>
<td>Throat irritation.</td>
</tr>
<tr>
<td>6.</td>
<td>1200.0</td>
<td>Cough</td>
</tr>
<tr>
<td>7.</td>
<td>1700.00</td>
<td>Life threatening</td>
</tr>
<tr>
<td>8.</td>
<td>3500.0</td>
<td>High mortality</td>
</tr>
</tbody>
</table>

The significance of ammonia not only as a pollutant but also as one of the important chemical associated with plant and animal metabolism has culminated in a large number of methods over the years for its quantitative estimation. Titrimetric analysis with visual end point determination is one of the oldest
Thomas, P, 1913], Calcium hypochlorite [Vanslyke, PD, 1933], Chloramine-T [Crismer, M R, 1937; Newell, BS, 1967; Haussler, A, 1960; Stegmann, H, 1962], sodium dichloroisocyanurate [Reardon, J E, 1966; Krom, M D, 1980; Seely, J H, 1967] have been used as oxidizing agents in the main reaction. Several other modifications incorporating different catalysts like acetone [Riley, J P, 1957; Crowther, A G, 1957], nitroprusside [Chaney, A L, 1962; Weathurburn, TE, 1967], manganese $^{2+}$ [Riley, J P, 1953; Rossom, J R, 1963; Fenton, J C B, 1962] and potassium hexacyanoferrate [Hampson, B L, 1977; Liddicoat, MI, 1975]. Few methods using hypobromite/thymol as reagents are also reported [Hansen, P A, 1930; Datsko, V G, 1959]. In the above methods since concentration of hypochlorite critically determines the colour development standardization of chlorine sources is necessary [Searle, L P, 1984].

analysis of ammonia in fish, meat, milk, sugar, brewing materials etc. have been reviewed by several authors [Searl, LP, 1984; Matsunga, K, 1970].

Here a new spectrophotometric method based on the use of catalytic mixture containing nitroprusside and manganese dioxide for the determination of ammonia is described. Ammonia is reacted with phenol and N-chlorosuccinimide in the presence of catalytic mixture to form an indophenol dye (fig 1).

\[
\text{NCl} + \text{H}_2 \rightarrow \text{HOCl} \\
\text{NH}_3 + \text{HOCl} \rightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O} \\
\text{OH} + \text{NH}_2\text{Cl} \rightarrow \text{OH} \quad \text{NP} \quad \text{MnO}_2 \rightarrow \text{O} \\
\text{OH} + \text{NCl} \quad \text{NaOH} \rightarrow \text{OH} \quad \text{H}^+ \quad \text{OH}^- \\
\text{fig. 1}
\]
The colour system obeys Beer's law in the range of 0.9-7.5 μg of ammonia in 100 ml of final solution. The blue dye has an absorption maximum at 635 nm and apparent molar absorptivity of $2.2 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$. The optimum reaction conditions and effect of other chemical species have been evaluated. The method has been satisfactorily applied for the determination of free ammonia and total nitrogen in various environmental samples. It has also been modified for the determination of protein in milk. The present method has been compared with other reported popular spectrophotometric methods and found to be more sensitive simple and rapid. The method does not require any sophisticated instruments or toxic reagents.

**EXPERIMENTAL**

**Apparatus**: Carl Zeiss spectrophotometer with 10 mm matched silica cells was used for spectral and absorbance measurements. pH measurements were made with Systronix pH meter model 331. PIMCO make calibrated rotameters and 35 ml midget impingers were used for air sampling.

**Reagents**: All chemicals used were AnalR grade or the best quality available. Double distilled deionized water was used throughout the experiment.

**Ammonia solution**: - 1000 μg ml$^{-1}$ stock solution of ammonia was prepared by dissolving 0.320 g ammonium chloride in 100 ml water. Working standards were prepared
from this stock by appropriate dilution.

**Phenol** :- 5% (W/v) solution of phenol was prepared in 95% ethanol and stored in a cool place.

**N-chlorosuccinimide (NCS)** :- 0.5% (W/v) solution was prepared in water and stored in amber coloured bottle.

**Catalytic mixture** :- 25 mg each of manganese dioxide and sodium nitroprusside were dissolved in 100 ml water and 10 ml of which was again diluted to 100 ml. The solution was filtered before use.

**Sodium hydroxide** :- 25%(W/v) solution of ammonia free sodium hydroxide in water.

**Solutions of diverse ions** :- Solutions of different diverse ions were prepared by the method of west [West, P W ,1941].

**Procedures**

**Procedure for the preparation of calibration graph** :-

To an aliquot of test solution containing not more than 7.5 ug of ammonia 0.5 ml of phenol and 0.5 ml of catalyst mixture were added and shaken thoroughly. Then 1 ml of NCS solution and 0.5 ml of phenol were added and kept in a thermostat maintained at 60°C for a minute. The flask was taken out and 0.5 ml of sodium hydroxide
was added and kept for another two min for complete
colour development. The contents were then diluted to 100
ml with water and absorbance was measured at 635 nm
against a reagent blank.

Procedure for the determination of ammonia in air :-

Two midget impingers each containing 10 ml of
0.01N sulphuric acid as absorbing solution were connected
in series to an air sampling train consisting of a
suction pump and rotameters, Fig. A, Chapter I. The air
was then absorbed through the impingers at a flow rate of
0.250 l/min for 10 min. After sampling solutions of two
impingers were mixed. An aliquot of solution depending up
on the concentration of ammonia was transferred to a 100
ml volumetric flask. The pH was then adjusted to 7 ± 0.5
by dropwise addition of 2.0 ml sodium hydroxide solution
and analyzed as recommended above.

Determination at ammonia in soil :-

To 2g of powdered soil sample taken in a 250 ml
Erlenmeyer flask 25 ml water was added and shaken
thoroughly for 10 min. The contents were then filtered
through a filter paper and centrifuged for 2-3 min. at
3600 rpm. The clear supernatant liquid was then analyzed
as recommended above.

Procedure for determination of total nitrogen:-

To a known quantity of sample (weight or volume)
containing nitrogen, 100 mg of copper sulphate, 250 mg of
potassium sulphate, 25 mg of mercuric sulphate and a pinch of selenium powder were added and digested in 100 ml kjeldahl flask with 15 ml sulphuric acid till the contents of the flask became clear and colourless or for 30 min whichever was earlier. The contents were then allowed to cool and then diluted to 100 ml. The solution was made sufficiently alkaline by adding 25% sodium hydroxide to precipitate metallic ions and filtered through ordinary filter paper. The filtrate was neutralized with acetic acid and final volume was made to 250 ml. An aliquot of neutralized filtrate was then transferred to 100 ml volumetric flask and analyzed as recommended above.

Procedure for determination of milk protein:

To an aliquot of milk sample 1g of each potassium sulphate, copper sulphate and a pinch of selenium powder were added and digested in a kjehdahl's flask by adding 20 ml sulphuric acid for 15 min. The contents were cooled and the clear solution was diluted to 100 ml. 10 ml aliquot was taken, neutralized with sodium hydroxide and diluted to 100 ml with water. An aliquot of neutral sample was then analyzed as recommended for ammonia.
RESULTS AND DISCUSSION

Absorption spectrum: Absorption spectrum of the colour system exhibits maximum absorbance at 635 nm, while reagent blank shows no significant absorbance at this wavelength (fig.2).

Calibration graph: The working range for the determination of ammonia-nitrogen by the proposed method is 0.9-7.5 μg in 100 ml final of solution (0.009-0.075 μg ml⁻¹ or ppm) (fig.3). Colour system obeyed Beers law in the range of 0.6-0.9 μg ml⁻¹ for protein in milk.

Absorptivity and sensitivity: The apparent molar absorptivity of indophenol dye formed by the present method is $2.2 \times 10^5$ L mol⁻¹ cm⁻¹. The Sandell's sensitivity of the method is 0.0001 μg cm⁻².

Sampling parameters: The important sampling parameters like flow rate, sampling time and suitable absorption solution were studied. It was found that flow rate of 0.250 l min⁻¹ was most suitable under the proposed conditions. Sampling time of 10 min was found to be sufficient for the described working range. 0.01N sulphuric acid was found to be the best as absorption solution. This was in consistent with earlier findings [Katz, M, 1969].
Fig. 2 Absorption spectrum

A. 0.05 ppm of NH₃  B. 0.03 ppm NH₃

Fig. 3 Calibration Curve
Effect of pH: The effect of pH on colour development was studied by measuring the absorbance values of the coloured solution at varying pH values. The variation of absorbance with pH is shown in fig.4. Constant and maximum absorbance value was obtained in the pH range of 9-10.5. Absorption maxima of the dye formed was also different at different pH values. However, the wavelength of maximum absorbance shifts to 635 nm with time whenever the initial pH was less than 9.

Effect of temperature, time and stability: The effect of temperature on the reaction was studied by developing the colour at different temperatures. It was observed that the colour developed at higher temperature (>65°C) was less stable. Under optimum conditions temperature range of 45-65°C was most favourable. The time required for complete colour development was 2.5 min. and the dye thus formed was stable for more than 24 h.

Effect of reagent concentration: Keeping the analytical profiles like, temperature, pH etc. optimum the effect of reagent concentrations on colour development were studied. It was found that 1 ml of phenol (added in two steps), 1 ml of NCS reagent and 0.5 ml catalytic mixture were sufficient for complete colour development. Any increase in concentration of reagents caused increase in blank value. The order of addition of reagents was the same as described in the procedure to get the optimum results.
Effect of catalysts: Effect of different catalysts on the colour reaction was studied [Hardwood, J E, 1970b]. Combination of manganese dioxide-nitroprusside was found to be the best. This catalyst combination has been used for the first time here. This has several advantages over other reported catalysts in terms of sensitivity and stability it offers to the colour system.

Effect of diverse ions: The effect of diverse ions likely to interfere with the method was studied by adding known quantity of the ion prior to the colour developing stage as reported [West, P W, 1941]. Most of the metal ions did not interfere when present in about 100-fold excess. 1 ml of EDTA was sufficient to hinder the influence of metal ions added during digestion step in determination of total nitrogen and milk protein. The tolerance limit for 30 diverse ions are listed in table-2.

Reproducibility: Reproducibility of the method was assessed by analysing 4 μg of ammonia in 100 ml solution, over a period of 7 days. The mean, standard deviation and relative standard deviation of absorbance values for 7 replicate analyses were found to be 0.665, 0.01 and ±1.505% respectively. Reproducibility data of the method for milk protein analysis are presented in table-3.

Application: The proposed method has been applied for the determination of free ammonia in soil, polluted river
Table 2 Effect of diverse ions

<table>
<thead>
<tr>
<th>Diverse ion</th>
<th>Tolerance limit* µg/100 ml</th>
<th>Diverse ion</th>
<th>Tolerance limit* µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>30,000</td>
<td>Se^4+, Ca^2+</td>
<td>30,000</td>
</tr>
<tr>
<td>Phenol, Cresol</td>
<td>25,000</td>
<td>Al^3+, Zn^2+, Pb^4+, S^2-</td>
<td>10,000</td>
</tr>
<tr>
<td>Methanol, Formaldehyde</td>
<td>20,000</td>
<td>Ca^2+, Ba^2+, Sr^2+, Co^2-</td>
<td>5,000</td>
</tr>
<tr>
<td>Butanol</td>
<td>15,000</td>
<td>Fe^{2+}, Fe^{3+}, Cu^{2+}</td>
<td>2,500</td>
</tr>
<tr>
<td>Amyl alcohol</td>
<td>10,000</td>
<td>Cr^{3+}, CN^-</td>
<td>2,000</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1,000</td>
<td>Cr^{6+}, Mg^{2+}, Na^+</td>
<td>1,500</td>
</tr>
<tr>
<td>Acetone, Acetic acid</td>
<td>700</td>
<td>NO^-2, NO^-3</td>
<td>800</td>
</tr>
<tr>
<td>Aniline</td>
<td>500</td>
<td>CNS^-, Cl^-</td>
<td>500</td>
</tr>
</tbody>
</table>

* Tolerance limit vary the result by ±2%
Concentration of ammonia - 5 µg

Table 3 Reproducibility data of Protein analyses

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of analyses</th>
<th>Mean µg L^-1</th>
<th>Standard deviation</th>
<th>Relative standard deviation % (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow milk</td>
<td>10</td>
<td>3.10</td>
<td>0.15</td>
<td>4.8</td>
</tr>
<tr>
<td>Buffalo milk</td>
<td>5</td>
<td>3.62</td>
<td>0.165</td>
<td>4.28</td>
</tr>
<tr>
<td>Milk powder</td>
<td>6</td>
<td>13.87</td>
<td>0.52</td>
<td>3.75</td>
</tr>
</tbody>
</table>
water, polluted air, distillery waste, coke oven effluent and effluents from fertilizer plant. As the laboratory air contained no ammonia, ammonia was liberated from liquor ammonia in a fuming chamber by dropwise addition of standard ammonia solution to a conical flask kept in hot plate. The effluent samples and polluted river water were analysed after filtering through whatman filter No.40 and appropriate dilution. The results obtained are presented in table 4 and are found to be in agreement with the reported method.

The method has been suitably extended for the analysis of total nitrogen in distillery waste standard steel sample, soil and sewage. The results obtained are shown in table 5.

The proposed method has been further applied for determination of protein in milk obtained from cow, buffalo and goat. Protein content of milk powder has also been analyzed. The results of analyses are shown in table 6.
Table 4 Results of ammonia analyses

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ammonia found in µg</th>
<th>a</th>
<th>Nessler's method b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil (2 g)</td>
<td>A₁ 8.3</td>
<td>8.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A₂ 0.5</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A₃ 3.6</td>
<td>3.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A₄ 2.9</td>
<td>2.94</td>
<td></td>
</tr>
<tr>
<td>Polluted river water (25 ml)</td>
<td>B₁ 17.5</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B₂ 12.1</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B₃ 9.8</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B₄ 17.8</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>Coke oven effluent (25 ml)</td>
<td>C₁ 30.5</td>
<td>30.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C₂ 18.2</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>Distillery waste (20 ml)</td>
<td>D₁ 50.6</td>
<td>59.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D₂ 39.2</td>
<td>39.3</td>
<td></td>
</tr>
<tr>
<td>Fertilizer effluent (20 ml)</td>
<td>E₁ 100.9</td>
<td>100.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E₂ 62.8</td>
<td>62.6</td>
<td></td>
</tr>
<tr>
<td>Air (10 l)</td>
<td>F₁ 8.60</td>
<td>8.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F₂ 7.3</td>
<td>7.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F₃ 1.5</td>
<td>1.50</td>
<td></td>
</tr>
</tbody>
</table>

a. Mean of three replicate analyses. b. Results obtained after pre separation [Standard methods, 1991].
Table 5  Results of Total nitrogen determination

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nitrogen found µg</th>
<th>Present method</th>
<th>Reported method</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distillary waste (20 g)</td>
<td>14,000.0</td>
<td>13,210.5</td>
<td>13,868.5</td>
<td></td>
</tr>
<tr>
<td>Standard Sheet (10 g)</td>
<td>180.8</td>
<td>176.4</td>
<td>152.1</td>
<td></td>
</tr>
<tr>
<td>Red soil (2 g)</td>
<td>66.2</td>
<td>43.8</td>
<td>60.5</td>
<td></td>
</tr>
<tr>
<td>Sewage (5 g)</td>
<td>320.0</td>
<td></td>
<td>308.6</td>
<td></td>
</tr>
</tbody>
</table>

a. Mean of three replicate analyses
b. [Standard Methods, 1981]
Table 6  Results of milk protein analyses

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein found g/100 ml</th>
<th>Present method</th>
<th>Accepted method^d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow milk(^a)</td>
<td>3.25</td>
<td>3.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.10</td>
<td>3.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.90</td>
<td>2.95</td>
<td></td>
</tr>
<tr>
<td>Buffolow milk(^b)</td>
<td>3.70</td>
<td>3.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.50</td>
<td>3.45</td>
<td></td>
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<td></td>
<td>3.65</td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>3.80</td>
<td></td>
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<tr>
<td>Goat milk</td>
<td>3.30</td>
<td>3.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.05</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.35</td>
<td>3.25</td>
<td></td>
</tr>
<tr>
<td>Milk powder (Amul spray)</td>
<td>13.87</td>
<td>14.4(^c)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Holstein Frisian species,\n\(^b\) Indian water buffalo\n\(^c\) Value given by manufacturer\n\(^d\) [Scarl Le LP,1984].
CONCLUSION

The new method developed for the determinations of free ammonia, total nitrogen and milk protein is simple, sensitive and selective. The stability of the indophenol dye formed is also higher than that of the other reported spectrophotometric methods (Table 7). Manganese dioxide (Mn$^{4+}$) used here for the first time, enables to overcome the turbidity problems associated with the usage of Mn$^{2+}$. The N-chlorosuccinimide used in the method help to get constant concentrations of hypochlorous acid without frequent standardisation. The method is fairly reproducible and does not require any sophisticated instrumentation. The method has been satisfactorily applied for the determination of ammonia and total nitrogen content of several environmental samples. The method has also been applied for the determination of protein in milk. The method can be adopted for the industrial hygiene work.
<table>
<thead>
<tr>
<th>Method</th>
<th>Remarks</th>
<th>Min. Temperature</th>
<th>Max. Temperature</th>
<th>pH Range (ppm)</th>
<th>Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol-NCS/catalyst mixture</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Phenol-hypochlorite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol-naphthyl red</td>
<td></td>
<td></td>
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Table 7: Comparison with other spectrophotometric methods.
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