

Chromatographic separation of nitrite and nitrate ions

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Abstract. The influence of solvents on the migration of nitrite and nitrate ions on paper chromatograms was studied. The differential migration and separation is based upon differential solubilization of the ions by virtue of differential polarity of the ions and the solvents. The separated ions are estimated photometrically.

Keywords. Paper chromatography; analysis of, nitrites, nitrates.

1. Introduction

The typical valences, 3 and 5 of nitrogen are exhibited by the nitrite and nitrate ions respectively. The estimation of nitrite in the presence of nitrate assumes importance, because the nitrite is more poisonous than nitrate and the two are associated in many instances (Pearson 1970; Tisdale and Nelson 1971). Nitrite produced by partial reduction of nitrate is present in wet brines and dry curing mixtures used in the manufacture of pickled meat products. The characteristic red colour of the pickled meat is due to the presence of the nitrite which combines with the meat pigment, myoglobin. No doubt the nitrites are better preservatives than nitrates, but quantities more than 200 ppm will act as poison in humans by the oxidation of haemoglobin into methaemoglobin. Nitrites are permitted as preservatives in cheese and cheese products, its quantity not exceeding 10 ppm in the presence of 50 ppm of nitrate. Nitrites found in soils, act as toxins to plants but being unstable they seldom lead to any major tragedies. However, the separation and estimation of nitrite and nitrate will be useful in soil fertility test, in preservatives and curing agents.

Nitrogen(III) as nitrous acid is unstable and known only in very dilute solutions, whereas its salts are fairly stable (Remy 1956). Nitrogen (V) is quite stable both in acid and salt forms. From the structural point of view, nitrate is more ionic than nitrite. Consequently nitrite is expected to migrate faster than the nitrate in less polar organic solvents. It is this difference, little though, that has helped in the separation of the two ions.

2. Experimental

Weighed quantities of potassium nitrite and nitrate (A.R.) were used to prepare aqueous solutions of 1 M strength. Nitrite solution was prepared everyday to ensure

Table 1. Chromatographic behaviour of nitrite and nitrate mixture

| Sl. No. | Eluents | Dielectric constant | R_f values | | ΔR_f |
|---------|------------------------------------|---------------------|-----------------|-------------------|--------------|
| | | | NO_2^- | NO_3^- | |
| 1. | Water | 78.54 | 1.0 | 1.0 | zero |
| 2. | Ethylene glycol | 37.7 | 1.0 | 1.0 | zero |
| 3. | Aq. ammonia (25% W/V) | — | 1.0 | 1.0 | zero |
| 4. | Aq. methylamine (25% W/V) | — | 1.0 | 1.0 | zero |
| 5. | Methanol | 32.63 | 0.58 | 0.46 | 0.12 |
| 6. | Ethanol | 24.3 | 0.35 | 0.25 ^d | 0.10 |
| 7. | MeOH: Water (95 : 5) | — | 0.62 | 0.55 | 0.07 |
| 8. | MeOH: Water (90 : 10) | — | 0.64 | 0.58 | 0.06 |
| 9. | MeOH: Aq. ammonia (95 : 5) | — | 0.58 | 0.51 | 0.07 |
| 10. | MeOH: Aq. ammonia (90 : 10) | — | 0.62 | 0.53 | 0.09 |
| 11. | MeOH: <i>n</i> -PrOH: Water | | | | |
| | (a) 89 : 10 : 1 | — | 0.58 | 0.50 | 0.08 |
| | (b) 69 : 30 : 1 | — | 0.50 | 0.40 ^d | 0.10 |
| 12. | MeOH: <i>n</i> -BuOH: Water | | | | |
| | (a) 89 : 10 : 1 | — | 0.59 | 0.47 | 0.12 |
| | (b) 69 : 30 : 1 | — | 0.50 | 0.39 ^d | 0.11 |
| 13. | MeOH: <i>n</i> -AmOH: Water* | | | | |
| | (a) 89 : 10 : 1 | — | 0.57 | 0.38 | 0.19 |
| | (b) 79 : 20 : 1 | — | 0.50 | 0.30 | 0.20 |
| | (c) 69 : 30 : 1 | — | 0.47 | 0.25 ^d | 0.22 |
| 14. | MeOH: <i>n</i> -AmOH: Aq. ammonia* | | | | |
| | (a) 89 : 10 : 1 | — | 0.58 | 0.36 | 0.22 |
| | (b) 79 : 20 : 1 | — | 0.53 | 0.30 | 0.23 |
| | (c) 69 : 30 : 1 | — | 0.49 | 0.24 ^d | 0.25 |

^d-Diffused; *Good and satisfactory separation.

freshness. Whatman No. 1 chromatographic paper of 12 cm dia. was used for separation. Agla micrometer syringe was used for spotting. Rutter's (1948, 1950) technique of circular paper chromatography was adopted. The R_f values were calculated by measuring the distance between the centre of the applied spot and the outer boundary of the chromatographic band, and relating it to the solvent front. The choice of eluents was guided by their polarity, viscosity and solubilization (Paul and Janardhan 1967). Nitrite ion was identified by spraying with 1% sulphanilic acid in 30% acetic acid followed by 0.03% α -naphthyl amine in 30% acetic acid which forms a red dye (Feigl 1958). Nitrate ion was identified by diphenyl benzidine in conc. sulphuric acid by the technique reported by Loach (1961). The results are presented in table 1.

3. Discussion

Polar solvents were unable to distinguish between the trivial difference in the ionic nature of the two valences, and migrated the two ions equally to the solvent front, (see table sl. nos. 1-4). In ammonia and methylamine, the migration of both ions to the solvent front was due to the presence of considerable amount of water in them.

Methanol and ethanol of lower polarity were able to differentiate between the two valences (sl. nos. 5 and 6). Nitrite ions being less ionic than nitrate, migrated faster in organic solvents. However the values of ΔR_f were not sufficiently large to warrant quantitative separations.

In mixed solvents such as methanol-water, ethanol-water, methanol-ammonia and ethanol-ammonia, the nitrite ion moved faster than the nitrate ions, (sl. nos 7-10). With increasing percentage of water or ammonia, migrations of both the ions improved but the values of ΔR_f decreased continuously and eventually touched zero beyond 10% of water or ammonia. Small percentages of water or ammonia up to 5% showed signs of separation of the two ions. However the values of ΔR_f were too small to give quantitative separations.

The presence of higher alcohols, propanol or butanol or amyl alcohol in the solvent mixture made the nitrite and nitrate ions to migrate differentially (sl. nos 11-14). The ΔR_f 's increased from propanol to amyl alcohol. The addition of higher alcohols decreases the polarity of eluents but increases the viscosity. On both these counts, the difference between the R_f values of the two ions is widened. In the mixtures containing amyl alcohol, nitrite ion moved faster than the nitrate. The presence of water and ammonia have almost the same influence on the migration of the ions (enhanced solubilization), however ammonia will be more useful to provide an alkaline condition which is conducive to the stability of the nitrite ion.

3.1. Quantitative estimation

Quantitative estimations of the separated ions were carried out photometrically using sulphanic acid method for nitrite and phenol-disulphonic acid method for nitrate (Taras 1958) to assess the degree of separation of the two ions. Results of quantitative estimation showed that the precision ranged from 1 to 5% when the quantities handled were below 100 μg .

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