Determination of the sewage indicator, aminoacetone, in aqueous samples and its correlation with faecal bacterial indicators

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ABSTRACT

The potential of aminoacetone (AA) as a sewage indicator compound has already been reported, and the current work comprised of the following: 1) the development of an improved analytical method to determine AA in aqueous solution 2) a pilot study to determine its correlation with faecal indictor bacteria in sewage effluent.

AA was determined in aqueous samples as its hydrazone through reaction with 2,4-dinitrophenylhydrazine (DHPH), using a gas-stripping system connected on-line to a cartridge containing DNPH. The cartridge was eluted with acetonitrile and the hydrazone was determined by HPLC with UV detection. Recoveries were > 90% at the 10 µM level and the detection limit was 18 nM.

A pilot study was then undertaken to estimate the environmental lifetime of AA and its correlation with specific bacterial indicators in a sample of secondary treated sewage effluent. The AA concentration at the commencement of bacterial die-off was 1.5 µM and the analyte was persistent throughout the lifetime of the experiment. Although the data set was small, a correlation between AA concentration and bacterial numbers was evident, with r^2 values of 0.8753, 0.8100 and 0.7986 for AA plotted against total coliforms, faecal coliforms and faecal streptococci, respectively. These results further indicate the potential application of this compound to the quantitative measurement of faecal contamination in surface waters.

INTRODUCTION

EC Directives relating to the quality of bathing and shellfish waters aim to protect public health by means of setting limits on the numbers of bacteria found in water samples. Currently the bacteria included in the Directives are total coliforms, faecal coliforms and faecal streptococci. However, as organisms in these categories can be found outside the human gut, this means that the testing undertaken cannot practically point to the origin of the contamination, i.e. sewage or other sources. This presents real difficulties to water companies and their environmental regulators, when seeking to improve both bathing and shellfish waters. However, if contamination could be 'sourced' to human sewage or other sources, then improvements could be made much more effective

Aminoacetone ($CH_3COCH_2NH_2$, AA) has been identified as a possible sewage indicator compound [1]. It is readily produced by liver mitochondria [2], and is one of only two α-aminoketones that have been identified in humans. AA is produced through degradation of the essential amino acid, L-threonine, and it is thought that its concentrations in urine may be directly linked to L-threonine intake and production [3]. A normal human adult excretes about 0.4 mg of APR per day in the urine, which equates to 3 µM [3].

Although the analytical method for the determination of AA in aqueous samples (involving microdiffusion and GC-NPD) gave reproducible results [1], low recovery of the analyte (20 ± 1%) prevented further studies on its environmental behaviour. Here we describe a newly developed method for the determination of AA via on-line gas stripping and derivatization on a cartridge containing 2,4-dinitrophenylhydrazine (DNPH), with analysis by UV-HPLC. Results of a pilot study, undertaken to determine the persistence of AA in secondary treated sewage effluent with respect to faecal bacterial indicators, are also presented.

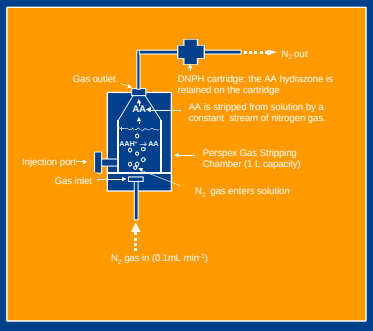

Fig. 1. Schematic representation of the gas stripping and derivatization system used for the determination of AA in aqueous samples

MATERIALS AND METHODS

AA was synthesised in hydrochloride form through modification of a previously published synthetic route [4]. Acetonitrile was supplied by Merck Ltd and DNPH cartridges were supplied by Waters Ltd (DNPH loading on the cartridge was 2.9 mg DNPH g^{-1} silica). Water for the preparation of reagent standards was from a SERADEST S600 system (< 0.1 µS conductivity). Secondary effluent samples were obtained from Thames Water Deephams sewage treatment works (London, UK)

Preparation of the AA Hydrazone

The hydrazone of AA was synthesized for calibration of the HPLC system and in order to determine recovery of aqueous AA using the gas stripping system (Fig. 1) This was a standard reaction of a compound containing a primary amine group with a carbonyl compound to form an imine. Full details of the reaction conditions are given elsewhere [5].

Determination of AA in Water Samples

A stock standard solution of AA (120 µM) was prepared and acidified to pH 1-2 to convert all AA to the protonated form $[AAH]^+$. Working standard solutions were then prepared as required by dilution of the stock standard solution. The on-line gas stripping and derivatization system consisted of a specially-constructed Perspex chamber (1 L capacity), equipped with an injection port and gas inlet (Fig. 1). This chamber was connected to a Waters Sep-Pak® DNPH-silica cartridge using PTFE tubing. An acidified aqueous sample (up to 1 L) was introduced into the extraction chamber using a glass syringe, followed by NaOH (2 mL; 6 M) in order to bring the sample to pH 12. AAH^+ in the sample was deprotonated at this pH, and was stripped from solution by a continuous flow of nitrogen gas and transferred onto the cartridge for derivatization with DNPH. A conical ceiling ensured maximum transfer of gaseous AA from the chamber. N_2 flow rate and purging time were optimised at 0.1mL min^{-1} and 1 hour, respectively, after which time the cartridge was disconnected and removed from the on-line system. The cartridge was eluted using acetonitrile added dropwise (3mL, 2 min), which was then evaporated under a gentle stream of N_2 gas, and the residue made up in a fixed volume of acetonitrile for injection onto the HPLC. Procedural blanks of water were included in the sample batch. 10 mL samples of sewage effluent were taken from the incubation for direct injection into the system.

Determination by HPLC

Cartridge eluent samples were analysed on an LDC Analytical HPLC system. The mobile phase consisted of a 40:60 mixture of deionized water and acetonitrile, respectively, which was degassed by sonication (10 min) before use. The AA hydrazone was resolved on a spherisorb 5mm ODS HPLC column at a solvent flow rate of 1.68 mL min^{-1}, and was detected on a LDC Analytical UV photodiode array detector (λ = 360 nm).

Enumeration of Faecal Coliforms

Initial tests on coliform survival in the secondary treated sewage effluent indicated an optimum water temperature of 15ºC**.** The effluent sample was maintained at 15ºC in a water bath, with stirring, and aliquots of sample taken over a period of 4 days (days 5-8; Table 1) for bacterial enumeration and AA determination. The membrane filtration technique (MFT) was used to enumerate the coliform bacteria, and in order to reduce cell death by osmotic shock, samples were serially diluted in 90 mL of physiological saline water (8.0% NaCl/500mL). The MFT method was chosen for its simplicity, relatively short incubation period (52 hours compared to 72 hours for the most probable number test, MPN) and its relative high precision over the MPN. The MFT is known to be more sensitive to turbid waters, since high concentrations of suspended solids can clog the membrane. Filtration of the effluent used in this experiment did not cause a noticeable problem, however.

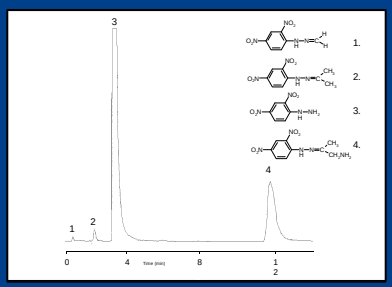

Fig 2. HPLC chromatogram of a primary-treated effluent sample. For conditions see Material and Methods.

RESULTS AND DISCUSSION

Determination of AA in Aqueous Samples

AA was successfully determined as its hydrazone after reaction with DNPH. Other carbonyl compounds, which were already present in the cartridge or were introduced during pre-concentration, also reacted to form hydrazones (Fig. 2). AA was calibrated between 20-120 µM (r^2 = 0.9988), which was well within the range reported for water samples [1]. Recoveries were measured at the 10 µM level and were always > 90%. The detection limit for the method was calculated as 18 nM (4 x σ blank).

AA was shown to be present in sewage effluent at concentrations comfortably above the Method Detection Limit, which confirmed the applicability of the method to 'real environmental' samples. The gas stripping chamber has a capacity of 1 L, so injection of large sample volumes can be injected to improve the detection of AA at low concentrations. Only the hydrazones of methanal and propanone were detected along with AA and unreacted DNPH (Fig. 2). The high pH of the reaction chamber solution meant that potential competing compounds, such as amino acids and ethanoic acid, remained in solution as their anions. Aliphatic amines are also present at high concentrations in wastewater but do not form a derivative with DNPH. The large peak of unreacted DNPH that appeared in all chromatograms of environmental samples indicated that it was always present in large excess so that derivatization with competing ions was unlikely to lead to underestimation of AA concentrations.

This method allows rapid sample processing (i.e. within 2 hours of arrival of the sample in the laboratory). The gas stripping system is mobile and can be used on board ship or for land-based water sampling. The sample can be pre-concentrated, subsequent to elution and solvent evaporation, by making up the sample residue in the required volume of acetonitrile.

Time (days)	Total coliforms (per 100 mL)	Faecal coliforms (per 100 mL)	Faecal streptococci (per 100 mL)	AA (µM)
5	37650	7210	356	1.5
6	6400	710	29	0.85
7	2700	700	34	0.25
8	98	85	3	0.35

Table 1. Changes in bacterial indicator numbers and AA concentrations in secondary treated sewage effluent incubation with time. Sampling for AA was started when bacterial numbers were observed to decrease.

Correlation of AA with Bacterial Indicator Species

The decrease in bacterial numbers with time is shown in Table 1, along with changes in AA concentrations in the effluent. An increase in bacterial numbers was observed from days 0-3, followed by a consistent decrease from days 5-8. Thus, sampling for AA concentrations began on day 5. The number of samples taken during this experiment was limited to ensure both a reasonable sample volume, for pre-concentration of AA, and the viability of the organisms. AA was shown to be persistent in the effluent for 8 days, which is consistent with previous findings [1]. Concentrations were observed to decrease rapidly from days 5-7 (Table 1). The increase from days 7-8 was unexpected, as production of AA has only been reported in higher mammals, and further experiments are required to verify this pattern. Adsorption onto external cell surfaces, followed by desorption, is a possible explanation. No data exists on the solubility of AA in aqueous media and, although the presence of a carbonyl and an amino group will greatly enhance the solubility of the compound, protonation of the nitrogen will also confer the ability to adsorb to solid surfaces.

The objective of the pilot study was to observe the variation in AA concentrations in relation to bacterial numbers, to determine whether any correlation could justify further testing on samples of varying dilution and salinity. The correlation between AA and the three bacterial indicators used is shown in Figs. 3 and 4. The correlation was strongest with total coliforms, while plots of AA against faecal coliforms and faecal streptococci also gave high r^2 values. Thus, although the dataset was small, these results indicate that, based on the trend observed for the effluent sample, the presence of AA in surface waters could serve as a useful quantitative indicator of sewage contamination.

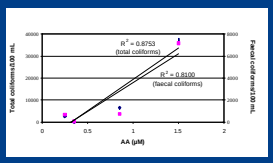

Fig. 3. Correlation of AA with faecal coliforms and total coliforms in secondary-treated sewage effluent

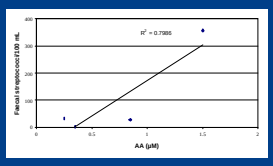

Fig. 4. Correlation of AA with faecal streptococci in secondary-treated sewage effluent

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REFERENCES

1. Fitzsimons M.F., Abdul-Rashid M.K., Riley J.P., Wolff G.A. 1995. Aminopropanone as a marker for raw sewage in natural waters. *Marine Pollution Bulletin* **30**, 306-312.
2. Urata G. and Granick S. 1963. Biosynthesis of alpha-aminoketones and the metabolism of aminoacetone.*Journal of Biological Chemistry* **238**, 811-820.
3. Marver H.S., Tschudy D.P., Perlroth M.G., Collins A. and Hunter G.J.R. 1966. The determination of aminoketones in biological fluids. *Analytical Biochemistry* **14**, 53-60.
4. Ellinger N. and Goldberg A. 1949. A new route to imazoles. *Journal of the Chemical Society*. **1**, 263-266
5. Dawitt, M., Williams, I.D. and Fitzsimons, M.F. (2001) Determination of 1-aminopropan-2-one, a dissolved sewage component, in water samples *Water Research* **35**, 1151-1156.

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http://www.env.plym.ac.uk/pegg/pegg.htm