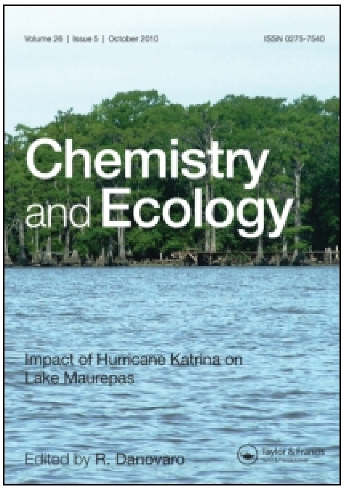

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^{14}C and ^{125}I Labelling of Humic Material for Use in Environmental Studies

P. Warwick[a]; L. Carlsen[b]; Amanda Randall[a]; Rui Zhao[a]; Pia Lassen[b]
[a] Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire [b] National Environmental Research Institute, Roskilde, Denmark

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^{14}C and ^{125}I LABELLING OF HUMIC MATERIAL FOR USE IN ENVIRONMENTAL STUDIES

P. WARWICK,* L. CARLSEN,** AMANDA RANDALL,* RUI ZHAO* and PIA LASSEN**

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire LE11 3TU, National Environmental Research Institute, P.O. Box 358, Frederiksborgvej 399, DK-4000 Roskilde, Denmark***

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Humic and fulvic acids are present in nearly all natural waters. These acids are known to affect the transport of environmental contaminants such as metals and hydrophobic organics through the terrestrial environment. An understanding of their role in the transport of contaminants is therefore essential and is facilitated if the acid is labelled with a suitable radioactive label. This paper reports the use of ^{14}C-methylamine and ^{125}I to label humic acid with either ^{14}C or ^{125}I and investigates factors which affect the yield of these reactions. The stability and mobility of the labelled humic material through sand is also reported.

KEY WORDS Humic acids, fulvic acids, environmental studies, radioactive labelling

INTRODUCTION

Humic and fulvic acids are organic macromolecules which are derived from the decomposition of plant and animal residues. These acids are present in all natural waters except those in which the cation concentration is so high that the humic material precipitates (Carlsen, 1992). These acids are heterogeneous and have a range of molecular weights. The acids are complex, negatively charged, polymers which have a large number of different functional groups attached to an aromatic backbone structure. These groups provide binding sites to which metal cations can bind, and provided that the complexing capacity, that is, the total amount of metal that can be bound by the humic molecules, is not exceeded, the resulting metal-humic complex remains in solution as a negatively charged species. These negatively charged complexes are known to be mobile through the terrestrial environment and therefore these acids are important transport agents for metal contaminants through subsurface and surface water movements. Many studies have investigated the complexation of metals with these acids (e.g. Buffle, 1977, Sposito, 1979, Van den Berg, 1984, Warwick, 1988) and actinides (Nelson, 1986, Nash, 1981, Kim, 1991a, Maes, 1991, Moulin, 1991) and field studies conducted by Champ (1986) have shown that these contaminants have migrated further distances from the source of pollution than can be explained by considering the inorganic speciation of the metals alone.

Several workers have shown that hydrophobic organic contaminants can bind to dissolved humic materials which significantly affect the environmental behaviour of the contaminant. Facilitated transport of the contaminants by organic macromolecules was implied in these studies. Furthermore, recent studies by West (1984) have shown that enhanced transport of hydrophobic contaminants has been observed in laboratory soil columns in the presence of humic material. Magee (1992) investigated the transport of radio-labelled phenanthrene-9-^{14}C in sand columns and showed that, in the presence of water-soluble soil organic material, the phenanthrene eluted 1.8 times faster than in the absence of the organic material. However, these workers also showed that phenanthrene breakthrough was not enhanced through Rhinebeck soil when the organic material was present. Increased solubility of DDT has also been reported by Carter (1982) and by Caron (1985) when humic material is present in solution. PCBs are important environmental contaminants which are currently of major interest to environmental scientists. Henry (1989) has shown that dissolved organic material is the dominant factor in controlling the distribution of these hydrophobic organic molecules in a water column. Degradation, photolysis, volatilization, transfer to sediments and biological uptake may all be affected by the contaminant binding to humic material. Humic materials are therefore of major interest to environmental scientists.

To date, investigations of humic interactions with contaminants have largely relied on the measurement of the free contaminant either by conventional instrumental measurements or by using a radioactively labelled contaminant. However, more information would be gained concerning contaminant-humic interactions if the humic acid could be labelled with a suitable radioactive label. In order to differentiate between the radio-labelled contaminant and the radio-labelled humic material, the radionuclide used to label the humic material should preferably be a different radionuclide to that used to label the contaminant. Reactions of contaminants with humic materials are equilibrium reactions whose stability constants may be low enough to allow dissociation of the contaminant-humic complex when investigating transport, solid phase interactions or the biological uptake of the complex. Thus, the use of a double-labelled complex would enable the fate of the free contaminant, the bound contaminant and the free humic materials to be more easily followed both qualitatively and quantitatively by measuring the relative levels and type of radiation associated with these different species. For metal-humic investigations, the incorporation of ^{14}C into the humic molecule would be a suitable radioactive label. For organic contaminant-humic investigations, where the organic contaminant may already be labelled with ^{14}C, a gamma emitting radionuclide such as ^{125}I or ^{131}I would be a suitable label for the humic material.

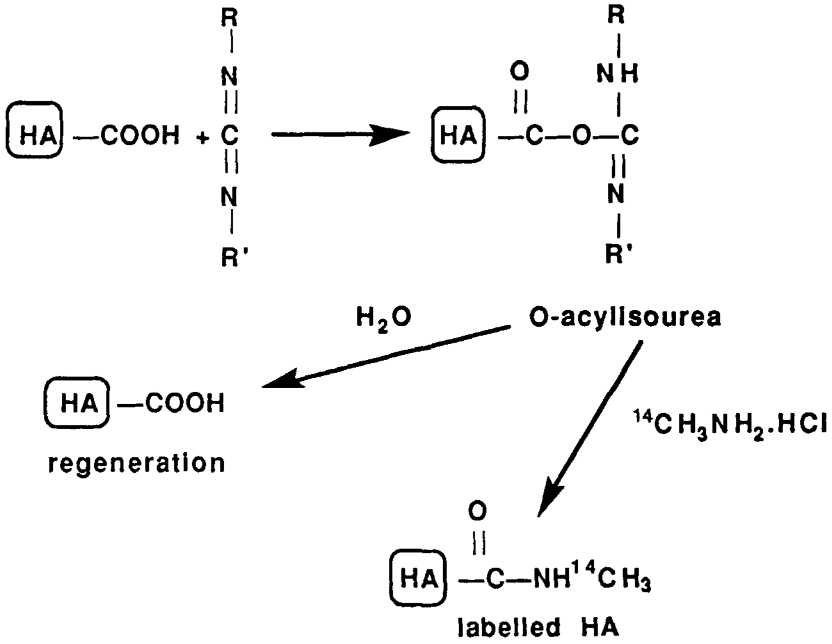
The incorporation of a radioactive label into humic materials must be accomplished by methods which do not significantly change the inherent characteristics of the humic material. These include the size distribution, the charge, and the metal or organic contaminant uptake by the humic material. In order to minimise alteration of the humic material, the methods of labelling employed can be those used in protein chemistry or those thought to take place in the natural environment. An additional requirement for the radio-label is that its incorporation is irreversible, as stability of the labelled material is essential for its use in transport and humic/contaminant investigations. Stability can be achieved by the covalent bonding of the radiolabel to a suitable site, for example within the aromatic part of the humic molecule.

We report here, the labelling of humic materials with ^{14}C and ^{125}I.

^{14}C LABELLING OF HUMIC ACID

Carbodiimides, such as 1-ethyl-3-dimethylaminopropylcarbodiimide, have been used in protein chemistry by Sheehan (1957) and by Hoare (1966) as cross-linking agents and in the determination of carboxylic acid functionality. Reaction at carboxylic acid groups produces an o-acylisourea intermediate which in turn reacts with a nucleophile. When an amine nucleophile is present, an amide is produced with the liberation of a proton and a urea as by-products, and regeneration of the carboxylic acid occurs when water is the attacking nucleophile. We have studied the reaction using ^{14}C labelled methylamine hydrochloride as the amine nucleophile.

The reaction scheme outlined below shows the proposed mechanism for ^{14}C-methylamine incorporation into humic acid using 1-ethyl-3-dimethylpropyl carbodiimide (EDC):



Kim (1991b) reported a figure of 5.43 meq g^{-1} for the total proton capacity of commercially available sodium humate (Aldrich). As the free acid, Peachey (1987) quoted the carboxyl capacity as 4.7 meq g^{-1}. Although these carboxylic acid groups are thought to be important in the binding of metal ions (Gamble, 1980), it should be possible to react a small number of these groups without a significant reduction in the metal-binding capacity of the humic material.

^{125}I LABELLING OF HUMIC MATERIAL

The method employed to label humic material with ^{125}I relies on electrophilic aromatic substitution brought about by the action of the oxidising agent chloramine-T on sodium [^{125}I] iodide. The reaction is widely used in protein chemistry (Hunter, 1962, Greenwood, 1963), in which iodination is primarily directed to the phenolic side chain of tyrosine residues although Bolton (1985) also reported that reaction at histidine, tryptophan and sulphydryl groups may also occur.

Initial studies conducted by Warwick (1991) on ^{125}I labelling with chloramine-T indicated quantitative uptake of the added iodide; however, the resulting label was not stable and a gradual release of activity was observed. A stable iodinated material has now been produced by the addition of the reducing agent, sodium metabisulphite.

EXPERIMENTAL

Materials

^{14}C-methylamine (1.48 GBq mmol^{-1}) and ^{125}I (81.16 TBq mg^{-1}), as a solution of NaI in sodium hydroxide, were purchased from ICN Biomedicals Inc, Irvine, California. Sodium humate, 1-ethyl-3-dimethylpropyl carbodiimide methylamine and chloramine-T were purchased from Aldrich Chemical Company Ltd, Dorset. Rathburn HPLC water was purchased from Analytical Supplies, Derby. Sephadex G10 and G25M gels were purchased from Pharmacia LKB, Milton Keynes. The sand used in the migration studies was taken from Drigg in Cumbria. The sand was eluted with either a synthetic groundwater composed of 6.37 mg l^{-1} Mg, 31.38 mg l^{-1} Ca, 23.62 mg l^{-1} Na, 2.46 mg l^{-1} K, 65.45 mg l^{-1} HCO_3, 58.22 mg l^{-1} Cl, 21.6 mg l^{-1} SO_4, 1.08 mg l^{-1} NO_3, 40 mg l^{-1} of sodium humate, or with 0.45 μm filtered groundwater taken from Drigg in Cumbria. ^{14}C was measured by liquid scintillation counting in a LKB 1215 Rackbeta spectrometer using Ecoscint cocktail. ^{125}I was measured in a Philips PW4800 gamma counter.

SEPARATION OF REACTION MIXTURES

Separation of the ^{14}C reaction mixtures was achieved by size exclusion chromatography using class columns of internal diameter 16 mm and length 250 mm packed with Sephadex G25M gel. The columns were eluted with 0.05 M NaCl at a flow rate of 20 cm^3 h^{-1}. The eluate was monitored continually for UV absorption at 230 nm and then fraction collected for ^{14}C determination in each fraction.

Separation of unreacted ^{125}I and Chloramine-T from the iodinated humic materials was carried out using Sephadex G-10 columns eluted with 0.05 M sodium chloride at a flow rate of 15 cm^3 h^{-1}.

FORMATION OF ^{14}C LABELLED HUMIC MATERIAL

The rate of the labelling reaction was investigated by separating aqueous reaction mixtures containing 1 mg cm^{-3} of sodium humate, with either 2.08 mg cm^{-3} or 0.52 mg cm^{-3} of EDC (equivalent to 1.0 or 0.25 times the total proton capacity of the humic acid) and trace levels of ^{14}C-methylamine. Aliquots of these mixtures were removed and quenched by the addition of 0.1 M sodium acetate prior to separation of the mixture on the gel column.

To determine that the reaction is carbodiimide mediated, a solution of sodium humate (4 mg cm^{-3}) was left in the presence of ^{14}C-methylamine (11.5 kBq mg^{-1} of sodium humate) for two weeks in the absence of EDC and then separated on a gel column.

The effect of concentration of sodium humate on the labelling efficiency of the reaction was investigated by keeping the ratios of the carbodiimide to sodium humate and methylamine to sodium humate concentrations constant. In these experiments, the sodium humate concentrations were varied from 0.5 to 8.0 mg cm^{-3}. In each experiment, EDC was added such that the concentration of EDC was equivalent to twice the total proton capacity of the sodium humate. The concentration of methylamine in each experiment was maintained at 1×10^{-7} moles mg^{-1} of sodium humate using cold methylamine spiked with ^{14}C-methylamine. This methylamine concentration corresponded to 2.1% of the total carboxyl content of the sodium humate in each experiment. Each reaction mixture was separated on a gel column and the eluate fraction collected and counted for ^{14}C activity.

The effect of EDC concentration on the ^{14}C-methylamine labelling efficiency was examined using a concentration of sodium humate of 1 mg cm^{-3} at pH 4.75. ^{14}C-methylamine was added at a concentration of 46 kBq mg^{-1} of sodium humate. This concentration of methylamine corresponds to a maximum consumption of 0.5% of the total carboxyl content of the humic acid. The carbodiimide concentration was varied from 0.5 to 8.0 (2.72 to 43.44 meq g^{-1} of sodium humate) times the total proton capacity of the sodium humate.

Whilst it is desirable to produce labelled humic material of high specific activity, incorporation of high levels of ^{14}C-methylamine at carboxylic sites may induce precipitation of the humic material or prevent its use in metal complexation studies. To investigate the effect of methylamine incorporation, varying concentrations of methylamine (0.5 to 100% of total carboxyl content using cold methylamine spiked with ^{14}C-methylamine) were added to solutions containing 4 mg cm^{-3} of sodium humate and an EDC concentration of twice the total proton capacity.

FORMATION OF IODINATED HUMIC MATERIAL

A mixture of 0.05 cm^3 ^{125}I (50 kBq), 1.0 cm^3 sodium humate solution (2 mg cm^{-3}) and 1.0 cm^3 chloramine-T (1.4 mg cm^{-3}) was prepared. After 30 minutes, 0.2 cm^3 (84.0 mg cm^{-3}) of sodium metabisulphite solution were added to the reaction solution and aliquots of this solution were separated by size exclusion chromatography after known time periods in order to determine the effect of time after addition of sodium metabisulphite solution on the labelling efficiency.

The effect of pH on the iodine labelling efficiency was studied by dissolving ^{125}I, sodium humate and chloramine-T in phosphate buffers of various pHs. All solutions were left for 30 minutes before addition of sodium metabisulphite solution. After a further 5 minutes, each solution was separated on a gel column to determine the labelling efficiency.

STABILITY AND MIGRATION OF LABELLED HUMIC MATERIAL

For a labelled humic material to be used successfully in either laboratory or field experiments, it is essential that the label is irreversibly bound to the humic material. Dissociation of activity from the labelled material during such studies would lead to erroneous results and an incorrect interpretation of the processes under investigation.

An important property of the humic substance is its mobility, which may be assessed by using columns packed with suitable material. Of interest in these experiments is the determination of the percentage of material injected into the column that is recovered from the column because, if labelling is achieved under different reaction conditions, the values observed may indicate that the humic molecules have changed during labelling. Furthermore, the stability of the labelled humic material can also be assessed in the presence of sand. The full nature of the interaction between humic acid and sand is uncertain, but the interaction probably has an electrostatic component due to attraction between the negatively charged silica surface and areas of positive charge on the humic material. This electrostatic attraction may be altered during labelling because of replacement of carboxylic acid groups, which are negatively charged at the pH of most groundwaters, with a neutral amide function. The results of this investigation may also provide evidence for the existence of side reactions, observed in protein chemistry at phenol residues, which introduce positive charge at the phenol site due to carbodiimide binding which is not displaced by weak nucleophiles. Both of these reactions might be expected to increase the attraction between humic acid and sand which should result in lower values being recorded for the percentage recovery of the injected ^{14}C-humic material from the sand column. Iodination of the humic material is not expected to significantly change the mobility of the humic material.

The stability of the labelled humic material is easily determined by separating the labelled humic material from the reaction mixture by using a gel column. The separated, labelled, material can then be re-introduced into the gel column after known time periods in order to determine the rate of dissociation.

The mobility and stability of the ^{14}C labelled or iodinated humic and fulvic materials through sand was investigated by packing glass columns (24 mm diameter, either 150 or 255 mm length) with sand and eluting the columns with 0.45 μm filtered synthetic groundwater at a flow rate of 40 cm^3 h^{-1} for the ^{14}C labelled humic material or, for the iodinated humic material, eluting the column with 0.45 μm filtered groundwater at a flow rate of 15 cm^3 h^{-1}. The labelled humic material (either ^{14}C or ^{125}I labelled) in each solution was first separated from its reaction mixture on a gel column before a pulse of each solution containing the separated, labelled, humic material was injected into the sand column using a 0.5 cm^3 injection loop. The eluate from the sand column was collected and the eluted activity speciated by injecting an aliquot of the eluate containing the highest activity on to a second gel column.

The ^{14}C labelled humic materials were separated from solutions containing different amounts of EDC (with constant amounts of methylamine) and from solutions containing different amounts of methylamine (with constant EDC) by gel column and stored at room temperature in a sealed vial at pH 6.5 in 0.05M NaCl. Samples of these separated solutions were removed at known time intervals and introduced into the gel column in order to determine the bound and free methylamine activity.

Samples (0.5 cm^3) of each of these separated ^{14}C-humic solutions were injected into the sand column and the eluate fraction collected and counted for ^{14}C activity. The percentage recovery of the injected activity was then calculated for each sample.

Sodium metabisulphite treated and untreated samples of iodinated humic material were separated from their reaction mixtures by gel column and stored at room temperature in 0.05M sodium chloride eluent. Samples of the two iodinated humic solutions were introduced periodically into the gel column to determine the percentages of bound and free ^{125}I in each solution.

To investigate the loss of free iodine from a sodium metabisulphite-treated iodinated humic sample further, a sample of sodium metabisulphite-treated iodinated humic material was introduced into the gel column 5 days after initial separation of the iodinated material from the reaction mixture. The peak humic activity was retained and reintroduced into the gel column after a further 5 days.

In order to determine whether the addition of metabisulphite had any effect on the migration behaviour of the iodinated humic material through a sand column, pulses of sodium metabisulphite-treated and untreated iodinated humic material were introduced into the sand column system and the groundwater eluant fractions collected.

RESULTS AND DISCUSSION

^{14}C labelling

Initial labelling experiments indicated that incorporation of ^{14}C-methylamine into humic material can be achieved under ambient conditions. Separation of the labelled product from the reaction mixture is readily achievable by size exclusion chromatography using Sephadex G25M gel. Figure 1 shows the ^{14}C activity elution profile of the reaction mixture and of free ^{14}C-methylamine which was injected into the column separately. This figure shows that the humic acid is labelled with ^{14}C and that the remaining free ^{14}C-methylamine is easily separated from the ^{14}C-humic material. The elution profile also shows that the ^{14}C activity is incorporated across the whole molecular weight range of the humic material. The UV absorption and ^{14}C activity elution profiles are shown in Figure 2.

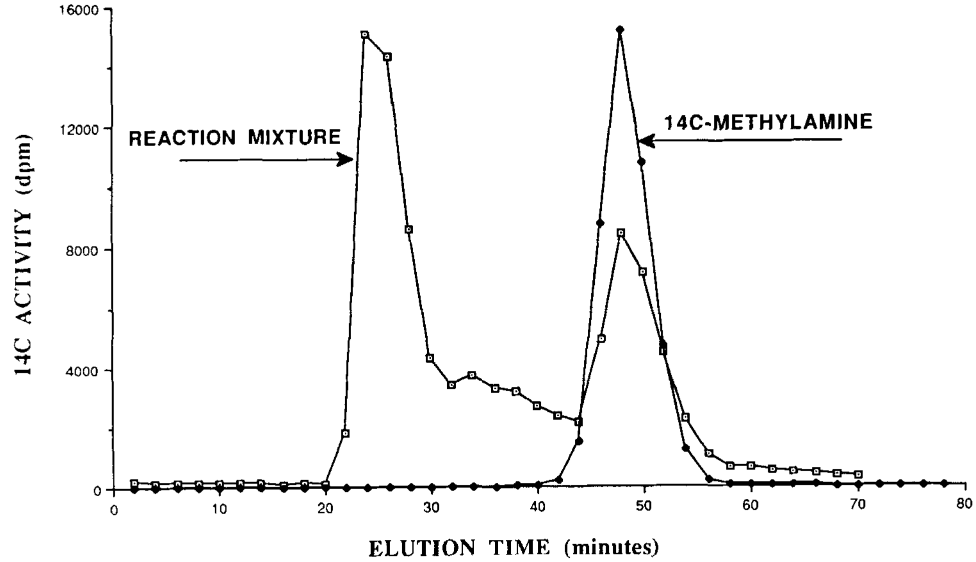


Figure 1 Gel chromatographic elution profiles of the ^{14}C activity of the reaction mixture containing sodium humate, ^{14}C-methylamine and carbodiimide and ^{14}C-methylamine alone.

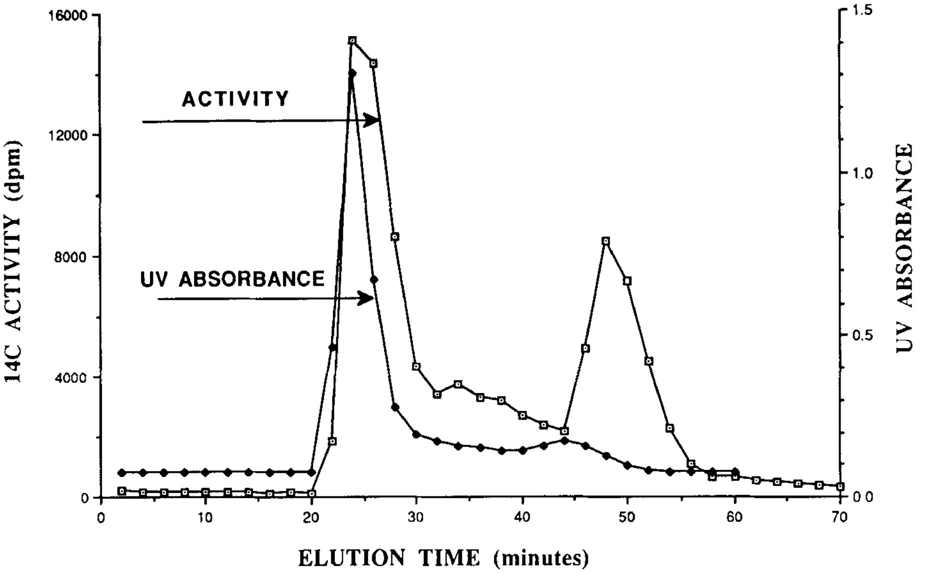


Figure 2 Gel chromatographic elution profiles of the ^{14}C activity and the uv absorbance at 230 nm of the reaction mixture containing sodium humate, ^{14}C-methylamine and carbodiimide.

The results of the investigation of the rate of labelling are shown in Figure 3 which indicates that the reaction is relatively rapid at room temperature and that maximum labelling yield is achieved after about ten minutes. The reaction rate does not vary greatly with the concentration of carbodiimide although the final incorporation of methylamine achieved is dependent on carbodiimide concentration.

In the absence of carbodiimide, 3 to 5% of the ^{14}C activity was eluted with humic material. This apparent incorporation of ^{14}C corresponds to approximately 0.005% of total carboxylic acid groups present in the sodium humate and can probably be attributed to diffusion of the small methylamine molecule into the large three dimensional structure of sodium humate.

Figure 4 shows that increasing the sodium humate concentration leads to a higher incorporation of ^{14}C-methylamine.

The results of the effect of EDC concentration on labelling are also shown in Figure 4. At the highest concentration of EDC, partial precipitation of humate was evident indicating possible damage to the humic molecules. In these samples the supernatant alone was analysed and no free methylamine could be detected. The concentration of carbodiimide has a profound effect on the labelling uptake of methylamine into humic material, and at relatively low humic concentration (1 mg cm^{-3}), complete incorporation of methylamine can be achieved.

Figure 5 shows the percentage labelling as a function of the ratio of the methylamine concentration to total carboxylate concentration. These results show that the pH of the reaction mixture has little effect on the efficiency of labelling and that the incorporation of methylamine appears to plateau at approximately 20% of the total carboxylate groups present in the humic material. Clearly, not all of the carboxyl groups are available for methylamine labelling probably because they are already occupied by metals or because of steric hindrance.

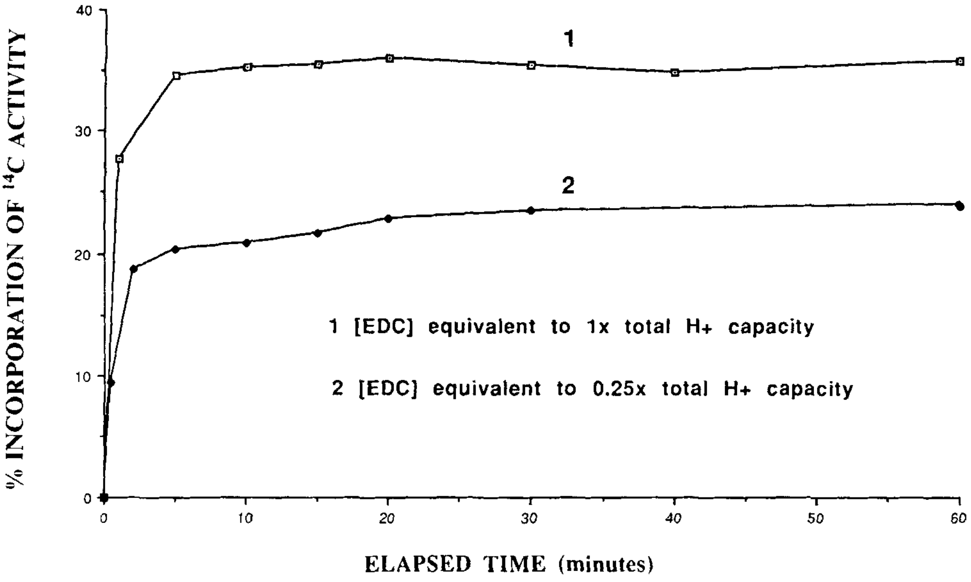


Figure 3 The percentage incorporation of ^{14}C-methylamine as a function of reaction time at two different carbodiimide concentrations.

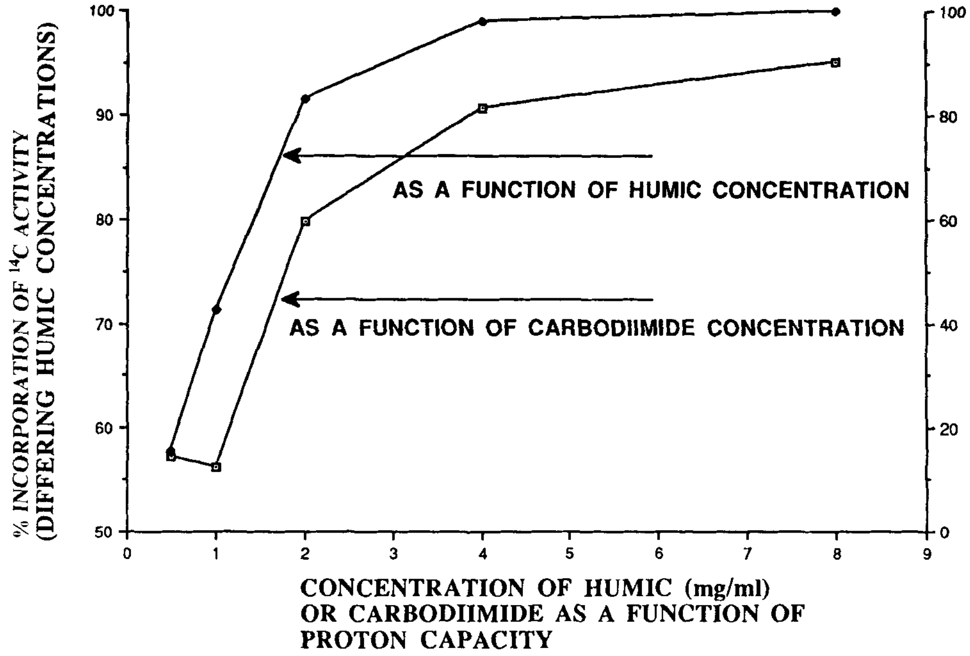


Figure 4 The percentage incorporation of ^{14}C-methylamine as a function of sodium humate or carbodiimide concentrations.

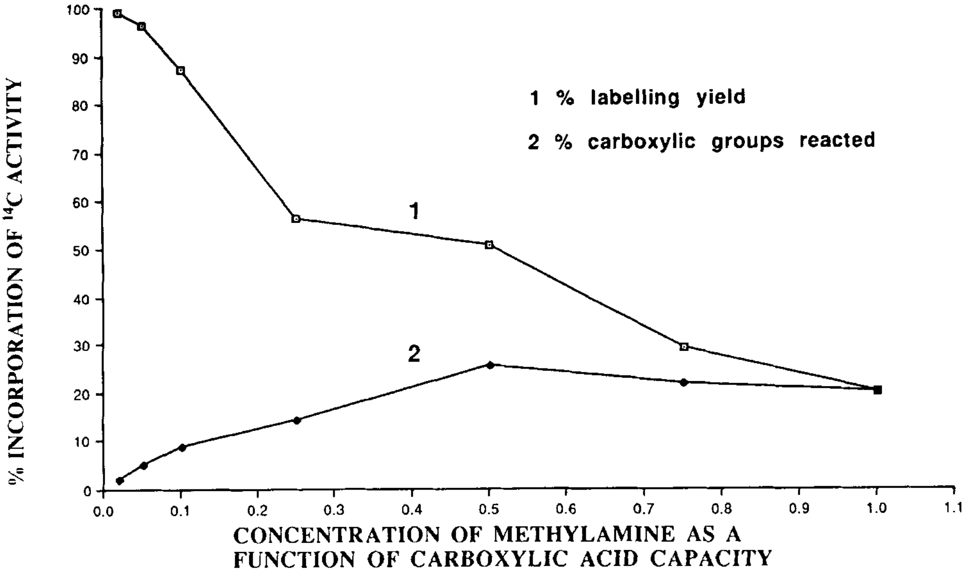


Figure 5 The percentage incorporation of ^{14}C-methylamine as a function of methylamine concentration. 1 shows the percentage labelling yield and 2 shows the calculated percentage of carboxylic acid groups that have reacted.

^{125}I labelling

Figure 6 shows the ^{125}I activity profiles of the reaction mixture containing Na[^{125}I], sodium humate, chloramine-T and sodium metabisulphite and of ^{125}I$^-$injected separately into the gel column. The activity peak eluting at about 60 minutes is the ^{125}I labelled humic material.

Figure 7 shows that there is a rapid drop in percentage yield of iodinated humic material on addition of sodium metabisulphite. Sodium metabisulphite is a reducing agent used in protein iodination by chloramine-T to stop the reaction by converting all reactive iodine species to iodide and consuming any remaining oxidising agent. This indicates that the apparent 100% yield obtained in the presence of chloramine-T alone, which was observed by Warwick (1991), may in part be due to electrostatic interaction between positive iodine species and centres of negative charge on the humic material. This is in addition to the covalent carbon-iodine bond produced by electrophilic substitution into phenolic moieties.

The results of the effect of pH on the ^{125}I labelling efficiency are shown in Figure 8. These results show that the optimum pH for the reaction is pH 7.5, in agreement with conditions used for protein iodination. All further reactions involving chloramine-T were carried out in phosphate buffer of pH 7.5.

Stability and migration studies

Table 1 shows the stability of each solution of labelled humic material prepared from solutions containing varying amounts of EDC. Free ^{14}C-methylamine cannot be

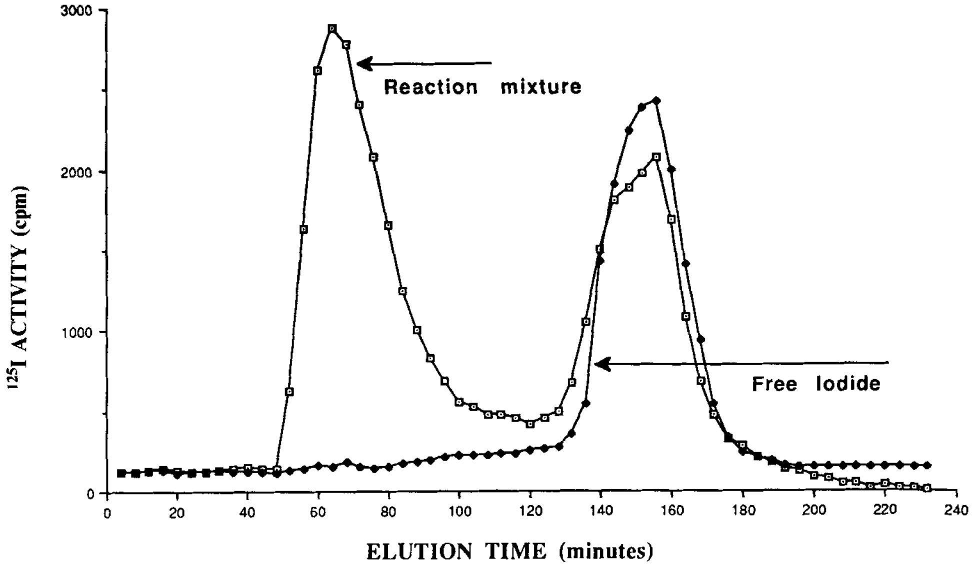


Figure 6 Gel chromatographic elution profiles of the ^{125}I activity of the reaction mixture containing sodium humate, Na(^{125}I), chloramine-T and sodium metabisulphite and Na(^{125}I) alone.

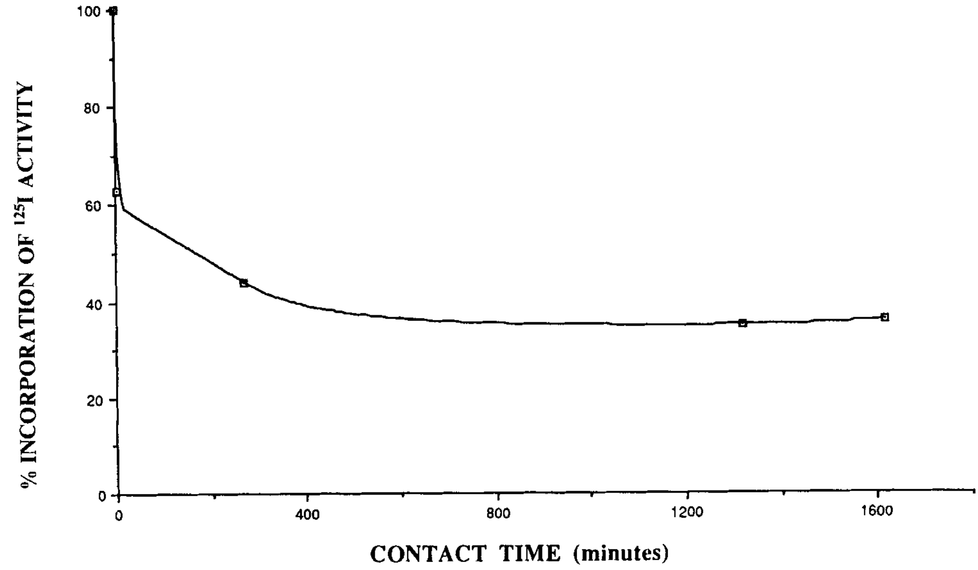


Figure 7 The percentage incorporation of ^{125}I as a function of contact time with sodium metabisulphite solution.

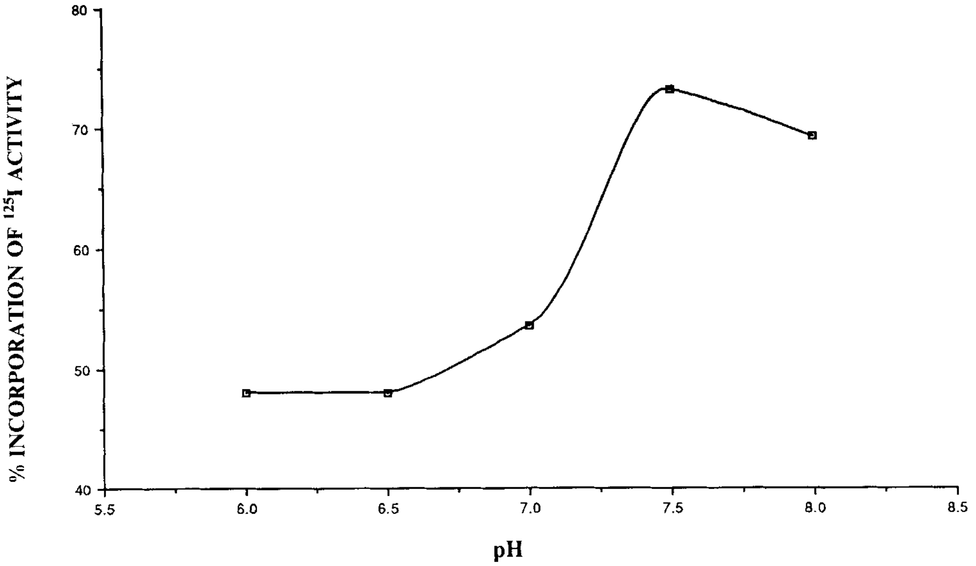


Figure 8 The percentage incorporation of ^{125}I as a function of pH of the reaction mixture.

Table 1 Stability of ^{14}C labelled sodium humate as a function of time, from reaction mixtures containing different concentrations of carbodiimide (EDC).

Ratio of EDC to total proton capacity	*% free methylamine detected*		
	Day 5	*Day 12*	*Day 26*
0.5	0	0	0
1.0	0.4	0	0
2.0	1.7	2.5	0
4.0	3.0	3.7	0
8.0	0.3	0	0

detected in the sample containing the lowest EDC concentration at any time. The maximum dissociation observed during these experiments was 3.7% after the ^{14}C-labelled humic material had been stored for twelve days. However, after 26 days standing, free methylamine activity was not detected in any of the solutions. This is probably due to the fact that free methylamine at pH 6.5 will diffuse out of solution and be lost to the atmosphere.

Table 2 shows that the percentage recovery of the labelled humic material is not affected by the lower concentrations of EDC in the reaction mixture. However, at the highest EDC concentrations where precipitation of the humic material was evident, significantly higher recoveries were obtained from the sand column, both from the supernatant alone and from aliquots of the whole sample which was readily redissolved by the addition of sodium hydroxide. This indicates alteration of the humic molecules. Reaction at phenolic sites, resulting in reduced negative charge on

Table 2 Percentage recovery of separated ^{14}C labelled humic material from a sand column prepared using different concentrations of carbodiimide (EDC).

Ratio of EDC to total proton capacity	*Mean % recovery of activity from the sand column*
0.5	31.5
1.0	35.6
2.0	39.6
4.0	32.6
8.0	48.6*
8.0	66.2

* supernatant

the humic molecule, is likely to decrease the percentage recovery of material from sand, the increased mobility indicating that side reactions at phenolic sites are absent. The enhanced mobility may, in part, be due to the apparent shift to lower molecular weight observed on gel separation of the resuspended sodium humate.

Table 3 shows the stability of each solution of labelled humic material prepared from solutions containing varying amounts of methylamine expressed as the percentage of carboxyl groups which would be reacted at 100% incorporation of the added label. The loss of activity from the solution is also shown after 28 days as a percentage of the initial activity present. The stability of the labelled humic material is seen to decrease as more methylamine is reacted with the sodium humate, both from the amount of free methylamine detected in solution and the loss of activity from solution by diffusion of free methylamine into the atmosphere. This indicates that carboxylic acid groups in different chemical environments react to produce amides of varying stabilities.

Table 3 Stability of ^{14}C labelled sodium humate as a function of time, from reaction mixtures containing different concentrations of methylamine.

Ratio of methylamine to total carboxylate content	*% free methylamine solution*				*% loss of activity in sample*
	Day 0	*Day 6*	*Day 21*	*Day 28*	*Day 28*
0.02	0.00	0.18	0.00	0.39	–
0.05	0.73	0.00	0.76	0.00	0.59
0.10	0	0.57	0.89	0.13	13.57
0.25	1.23	0.60	0.85	1.03	13.59
0.50	–	2.04	1.04	3.33	15.49
0.75	–	7.47	12.71	8.31	21.63
1.00	–	9.43	2.19	9.98	19.44

Table 4 shows that percentage recovery of the labelled material is not affected by the amount of methylamine present in the sodium humate. These results indicate that the reduction in negative charge resulting in the reaction with methylamine is insufficient to alter the mobility of the material through sand.

The results of the investigation of the stability of the metabisulphite treated and untreated iodinated humic material are shown in Figure 9. The results show that after nearly six days standing, only 5 percent of free iodine is present in the solution which contains the treated humic material whereas if metabisulphite is not added

Table 4 Percentage recovery of separated ^{14}C labelled humic material from a sand column prepared using different concentrations of methylamine.

Ratio of methylamine to total carboxyl content	*Mean % recovery of activity from the sand*
0.05	46.10
0.10	42.89
0.25	42.96
0.50	44.37
0.75	48.59
1.00	50.02

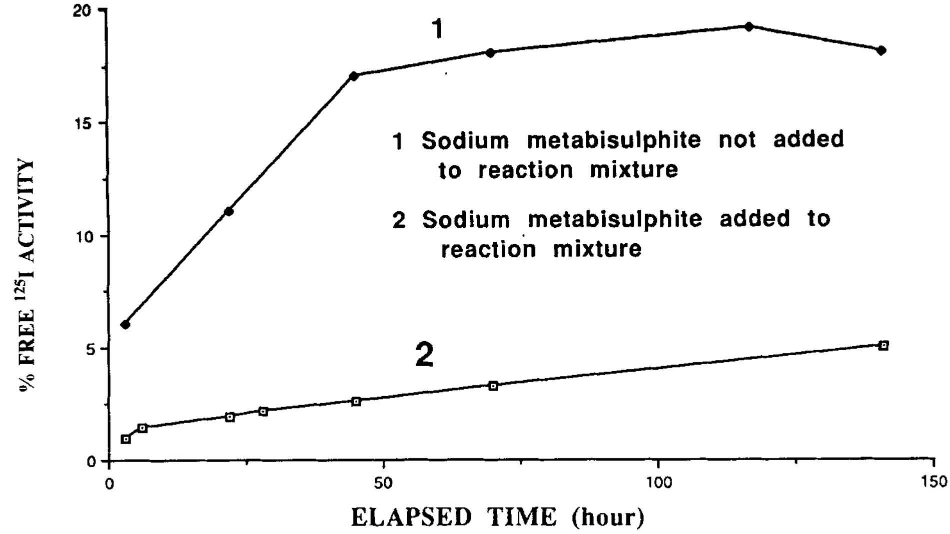


Figure 9 Percentage of free ^{125}I as a function of time after separation of the reaction mixture on the gel column.

to the reaction mixture, about 18 percent of the iodine activity is present in solution as free iodide.

When iodinated humic materials separated from metabisulphite-treated and untreated reaction mixtures were separately introduced into the sand column, both the activity elution profiles and the percentage of activity recovered (30 to 35%) from the sand column were similar. This indicates that the addition of a reducing agent to the humic acid does not further alter its inherent characteristics. Speciation of eluted iodine activity was obtained by introducing a sample of the highest activity fraction of the eluate from the sand column into the gel column. The elution profile of the untreated solution showed that 35 percent of the recovered activity was free iodide, whereas, with the metabisulphite-treated iodinated humic material, only 9 percent of the recovered activity was free iodide. These results indicate that the loss of iodine from the humic material during passage through sand can be attributed to a non-specifically bound fraction of iodine which is removed at the preparation stage on addition of a reducing agent.

CONCLUSIONS

Humic material has been successfully labelled with ^{14}C using ^{14}C-methylamine. In these experiments the maximum specific activity of ^{14}C labelled humic material obtained was 1.4 MBq mg^{-1} of humic material. The labelled material proved to be stable and suitable for use in mobility studies.

Using a reducing agent to quench the reaction of ^{125}I with humic material reduces the labelling yield to 35 percent of the apparent yield observed when reducing agent is not used. The reducing agent returns any oxidised iodine species to iodide and hence causes the removal of any iodine loosely bound to the humic material via electrostatic attraction. The iodinated product proved to be relatively stable and suitable for use in mobility studies.

Both labelling methods should be applicable to any humic material, allowing the production of site-specific labelled material. The labelled materials produced by these methods may not only be of use in "fate studies" – the effect of humic materials on the migration, degradation and extractibility of metallic and organic contaminants – but they may also provide a useful tool in investigations on the effect of humic-contaminant interactions on, for example, the uptake of contaminants into plants.

^{14}C-labelled humic material has been produced by Jenkinson (1960) by growing plants such as ryegrass in an atmosphere containing $^{14}CO_2$, followed by humification by decomposition in soil. However, this method is very time consuming, taking over a year to produce labelled humic acid, and the material produced is of significantly lower specific activity viz.ca. 400 Bq mg^{-1} humic acid. Labelled humic material produced by this method has been used by Vaughan (1969) to study the effect of humic acid on plant metabolism.

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