CATALYTIC TRITIATION AND ^{3}H NMR SPECTROSCOPY OF COMPLEX ORGANIC MIXTURES - APPLICATION TO OIL SHALE PROCESS WATERS

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SUMMARY

Several catalytic tritium exchange procedures have been investigated in order to accomplish uniform and random incorporation of tritium into the diverse organic solutes present in an oil shale process water. Comparative proton and tritium nuclear magnetic resonance (NMR) spectroscopy revealed that a Raney nickel/THO system resulted in a reproducible procedure wherein the individual components are tritiated randomly in stable positions in proportion to their concentration. This approach to the labelling of complex organic mixtures opens new avenues for radiotracer studies in environmentally important areas.

Key Words: isotopic hydrogen exchange, catalytic tritiation, ^{3}H NMR spectroscopy, oil shale process water.

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OVERVIEW

Radiotracer methodology, so widely employed over the past thirty years to support biological, environmental, chemical, and physical investigations of organic compounds, has evolved around the preparation and use of single, radiolabelled compounds or relatively simple mixtures. There are, however, numerous areas wherein the organic material of interest exists as a very complex mixture containing hundreds or thousands of individual compounds. Examples of these are fossil energy products and by-products, and industrial process and waste streams. Information is needed on the composite or differential fate, interaction, partitioning, and general biodynamics and chemodynamics of such complex mixtures, especially for environmental and process control studies. The attributes of the radiotracer methodology for the study of single compounds would appear equally valid for the study of complex mixtures. We have, therefore, undertaken a long-range project to develop methods for the preparation of radiolabelled organic mixtures and to apply radiotracer techniques in the investigation of such mixtures. This paper illustrates the general approach to the problem of labelling complex organic material using the dissolved organic fraction of an in situ-produced oil shale process water as an example.

INTRODUCTION

The development of oil shale retorting and extraction technologies to produce shale oil as an alternate fossil fuel to augment dwindling petroleum reserves is being actively pursued, both in the United States and elsewhere. Both surface and underground (in situ) oil shale processes are accompanied by the coproduction of process waters. As a minimum, the amount of oil shale process water coproduced equals the amount of shale oil produced (1). These waters are typically yellow to brown in color, odiferous, have an alkaline pH, and contain high levels of inorganic and organic constituents (2). The primary organic constituents include both polar and non-polar materials with carboxylic acids, heterocyclic compounds, and aliphatic compounds being noteworthy organic contributors (3,4).

A significant portion of oil shale environmental research is being directed at the characterization, transport, interaction, biological effects, treatment, and utilization of process waters (1). Because of the complex nature and abundance of solutes in these waters, environmental studies based on specific indicator compound approaches cannot begin to cover the number of solutes present and provide relatively little information about the solutes acting together in a matrix. Clearly, the availability of a "uniformly labelled fraction" representing the dissolved organic solutes would open new radiotracer approaches for studying the environmental parameters of the organic material present in these waters. In view of the chemical diversity and multiplicity of compound types in the dissolved organic component of oil shale process waters, it is neither practical nor experimentally feasible to reconstitute a "labelled fraction" by admixture of specific labelled indicator compounds. This paper describes research resulting in a procedure for preparing a uniformly labelled sample of dissolved organics derived from an in situ oil shale process water referred to as "Omega-9" process water (see Experimental).

Incorporation of a radioactive isotope was chosen in preference to a stable isotope, such as ^{2}H or ^{13}C, on the basis of greater analytical sensitivity, ease of detection, and absence of naturally occurring interference. The use of a ^{14}C incorporation procedure was excluded for two reasons. First, it would be impossible to impart a "uniform" incorporation of ^{14}C into the diverse compound types present since this approach is reaction-dependent. More importantly, the incorporation of ^{14}C would inherently alter the nature of the material to be labelled as a result of the chemical reaction(s) needed to be employed. A tritium exchange procedure is the only alternative where the objective is uniform incorporation of the radioisotope. Tritium incorporation has the advantages of lower costs and attainment of higher specific activities. Possible complications resulting from radiation decomposition of tritiated compounds (autoradiolysis) and uncertainty in the stability and position of labelling are potential disadvantages requiring experimental resolution. Recent developments in tritium nuclear magnetic resonance spectroscopy (^3H NMR) (8) with its ability to delineate the pattern of labelling and to monitor the stability of the label support the attraction of tritium as a tracer for this type study.

Ideally, the labelled product sought in this study should conform to the following criteria: (a) it should be representative of the total dissolved organic material originally present in the sample; (b) it should be uniformly labelled in stable positions for all components present; (c) it should be of high specific activity, (d) it should be minimally contaminated with artifacts induced by isolation procedures or autoradiolysis; and (e) it should be reproducibly preparable.

EXPERIMENTAL

OMEGA-9 Sample. The oil shale process water used in this investigation was obtained from the Rock Springs, Wyoming, Site 9 true in situ oil shale combustion experiment conducted by the Laramie Energy Technology Center in 1976, and carries the internal designation "Omega-9" process water. The experimental details of the collection, homogenization, filtration through a nominal 0.4-micron filter, and subsequent 4°C storage of this sample were previously described (5). Detailed characterization studies have been reported (2-7). Chemical parameters indicative of the composition of this sample are: NH_4^+ 3,470 mg/L, $S_2O_3^=$ 2,740 mg/L, HCO_3^- 15,930 mg/L, $SO_4^=$ 1,990 mg/L, pH 8.7, total organic carbon 1,020 mg/L, and total dissolved salts 30,000 mg/L. The complexity and diversity of organic compound types present in the water were indicated by reverse phase, gradient elution high-performance liquid chromatographic characterization whereby over 200 multi-component peaks were detected (3). Nitrogen bases (including quinolines, pyridines, and nitriles), as well as carboxylic acids, carbonyls, alcohols, branched-chain aliphatics, and heteroaromatics are known components of the sample (3,7). It is estimated that there are several hundred dissolved organic omponents in the sample with relatively high concentration and several thousand components at extremely low concentrations. The sample was maintained at 4°C throughout its history, including shipment.

Isolation of Dissolved Organics. A 4 L quantity of Omega-9 process water was freeze-dried to produce 100 g of powdery residue. The residue was suspended in 500 ml of acetonitrile and stirred at room temperature for 20 minutes. The solution was filtered and the filtrate evaporated to dryness by rotary evaporation. The filtered material was extracted twice again in the same manner using the recovered acetonitile. On average, 3.6 g of organic material was isolated from the Omega-9 water in this way for a calculated minimum recovery of 88% based on the total dissolved carbon value of 1,020 mg/L.

Initially, a number of different kinds of solvents varying from polar (acetonitrile, chloroform) to aromatic (benzene), aliphatic (n-hexane), non-polar (carbon tetrachloride) and aprotic (dimethyl sulphoxide) were tried. For material containing such a wide range of constituents at low concentrations, a small discriminatory factor would seem likely and this turned out to be the case, there being less than a factor of five difference between the best (acetonitrile) and least successful solvents, the order being $CH_3CN > CHCl_3 > C_6H_6 \sim CCl_4 >> n\text{-}C_6H_{14}$ Dimethyl sulphoxide could not be used because of its tendency to dissolve or interact with some of the inorganic material present.

PtO_2 Isotope Exchange Procedure. The material to be labelled (50-100 mg) together with a known amount of catalyst (50-100 mg) that had been freshly reduced with $NaBH_4$ and 10 μl of HTO (50 Ci/ml) were placed in a narrow tube which was then frozen (liquid N_2) and evacuated. The tube was sealed and the contents kept at a known temperature for a pre-determined time. After being cooled, the tube was opened and the contents mixed with 20 ml of ether; the catalyst was filtered off and the solution twice washed with 1-ml quantities of water in order to remove any labile tritium. After drying over anhydrous sodium sulphate, the ether was freeze-dried and the remaining material taken up in 100 μl of CD_3CN to which had been added a trace of tetramethylsilane.

Raney Nickel Isotope Exchange Procedure. The catalyst, which was prepared according to the method of Dominquez and Lopez (9), was transferred as a slurry in ethanol (0.05-0.5 ml) to a small tube. Excess ethanol was removed before adding the material to be labelled (50-100 mg) and HTO (10 μl, 50 Ci/ml). The contents were cooled with liquid nitrogen, the tube evacuated, and then sealed. After heating at a known temperature (usually 100-160°C) for a given time (2-6 days) the tube was cooled, opened, and the contents taken up in 20 ml of ether before working up as described above.

T_2 Gas Exchange Experiments. In this work, a standard vacuum manifold apparatus was used allowing the material to be labelled, together with a catalyst and, if need be, a solvent, to be stirred with a known amount (usually 2.5 ml) of a T_2:H_2 mixture (in the ratio of 1:10). On completion of the reaction, any unused gas was removed, the catalyst filtered off, the solvent removed by rotary evaporation, and any labile tritium removed by carrying out three successive evaporations with a hydroxylic solvent (MeOH).

^3H NMR Analysis. The solutions, which usually contained 30-100 mCi of radioactivity, were separately loaded by syringe into 3-mm-diameter combination tubes (Wilmad, SK 1374A) which were then sealed and mounted in spinner adapters for a 3-mm microprobe. The ^3H NMR spectra were obtained (mostly with ^1H noise decoupling at 25°C) with a Bruker WH-90 pulse spectrometer operating at 96 MHz for ^3H and with quadrature detection. The flip angle was usually 30°, the repetition interval 1.6 sec., and, depending on the radioactivity present, between 10^4 and 10^5 transients were acquired. The data were stored in 4K channels. Each FID was zero-filled to 8K and Fourier transformed to provide spectral display widths of up to 13 ppm. Referencing was to a ghost reference generated from the ^1H resonance frequency of the internal standard (measured at 90 MHz) by multiplying by the Larmor ratio 1.06663974.

Stability of the Label. The successful use of the product depends on the label being present in stable positions, or at least that the rate of loss of label under specified conditions be known. ^3H NMR spectroscopy enables this information to be obtained, and in the present study the stability of the label was assessed by making up separate solutions in CH_3OD/OCH_3^- (0.1 M) and CH_3COOD/D_2O (0.1 M) and running ^3H NMR spectra at monthly intervals.

RESULTS AND DISCUSSION

The first criteria for the desired labelled product is that it be as representative as possible of the original dissolved organic compound composition. If this and the other criteria are achieved, then the labelled product may be reintroduced to the inactive solution with the surety of imparting a specific activity uniformly throughout the organic matrix. It is, therefore, critical to the success of this approach to arrive at an efficient isolation procedure. Consideration and experimentation in fractional distillation and solvent extraction eliminated these two approaches because of precipitation phenomena upon pH adjustment, thermally induced artifacts, or insufficient recovery of organic material. Independent experiments (7,10) revealed a low concentration ($< 2\%$) of volatile components but experienced recovery difficulties using a number of solid sorbents (10). A series of hot and cold extractions of the residue obtained from freeze-dried Omega-9 process water with a variety of solvents resulted in the selection of acetonitrile as an efficient, nondiscriminatory extraction solvent. This method was selected for the isolation of dissolved organic material.

The organic material isolated by the freeze drying/acetonitrile extraction procedure is a dark colored, viscous gum. Characteristic features of its ^1H NMR spectrum (Figure 1) are strong absorptions at δ 0.90, 0.97, 1.06, 1.14, 1.22, 1.38, 1.57, 1.78, 1.9, 2.25, and 2.61, reflecting a strongly aliphatic composition. The signals in the range δ 1.96-2.01 arise mainly from small amounts of hydrogen in the CD_3CN solvents. Terminal and branched methyl hydrogens (δ < 1), methylene hydrogens in straight chain or branched hydrocarbons (δ 1.2-1.3), and cyclic methylene hydrogens (δ 1.1-1.8) are among the most important contributions. Although there is little evidence of alkene hydrogens (δ 5.3-5.4), in some spectra these may be obscured by the signal arising from the trace amounts of water that are difficult to remove - its chemical shift varies greatly from sample to sample, presumably reflecting slight changes in pH. The resonances at δ 6.8-8.0 suggest that the aromatics contribute somewhat less than ten percent to the mixture of compounds.

The next important criterion for the desired labelled product is that it be uniformly tritiated in stable positions throughout the extensive array of compound types present. Uniform labelling is defined as when the two conditions of (a) random and nonspecific exchange in terms of the labelling pattern and (b) equally proportional incorporation in terms of the tritium population of each exchange site have been simultaneously met. The degree to which the chemical shift tracing of a ^{3}H NMR spectrum may be superimposed on that from the corresponding ^{1}H NMR spectrum of a tritiated sample was established as a measure for the first condition. Likewise, the degree of correspondence between the integration signals was used as an independent measure for the second condition.

An extensive experimental survey of hydrogen isotope exchange procedures was conducted as summarized in Table 1. This work eventually pointed towards metal-catalyzed exchange as being most efficient and resulted in selection of a Raney nickel procedure as optimum.

Both acid- and base-catalyzed procedures were ineffective. With the Lewis acid BBr_3 there was some evidence of a side reaction. Tris(triphenylphosphine)ruthenium dichloride, which has proved to be a very effective catalyst for labelling aliphatic aldehydes and alcohols (11,12), was also unsuccessful. The heterogeneous metal-catalyzed exchange reaction using prereduced PtO_2 or Raney nickel turned out to be the most effective and for these catalysts a number of variables were altered in order to optimize the labelling conditions.

Table 1. Various Kinds of Hydrogen Isotope Exchange Reactions

Catalyst	General Comments	Refs	Comments on Labelling of Dissolved Organics in Omega-9 Process Water
Base	Choice depends on the acidity of the organic compound and the basicity required.	13	Unsuccessful - components are too weakly acidic
Acid	Electrophilic substitution is frequently used to label aromatics; combination of high temperature and low acid concentration offers new opportunities.	14	Unsuccessful - components are not easily protonated
	'Super'-acids may cause other reactions as well as exchange; fluorinated acids which are also good solvents are attractive.	15	
	Lewis acids such as BBr_3, $EtAlCl_2$ usually under heterogeneous conditions have met with wide success.	16	Both catalysts proved ineffective
Metal (homogeneous)	Attractive in principle but catalyst choice is limited, potassium tetrachloroplatinate being the most successful to date.	17	Unsuccessful
Metal (heterogeneous)	A wide range of metallic salts have been used and of these, activated palladium, pre-reduced PtO_2 and Raney nickel have met with most success; HTO is usually the source of the label but T_2 gas can also be used; activation of the latter by a microwave discharge can greatly improve the specific activity of the product.	18 19	Pre-reduced PtO_2 and Raney nickel were the most successful

Table 2. Results of Some Catalytic Tritiations*

Catalyst	Volume of Raney Nickel Slurry (ml)	Weight of PtO_2 Catalyst (mg)	Weight of Material to be Tritiated (mg)	Heating Time (days)	Activity (mCi/100 µl of NMR solvent)
Raney Nickel	0.05 (+ 0.15g of activated charcoal)		50	4	10
	0.50		100	4	30
	0.50		50	4	35
	0.50		50	6	27
	0.50		50	18	74
PtO_2		50	100	3.0	30
		50	100	5.5	66

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*Using 10 µl of HTO (50 Ci/ml) and a temperature of 120°C for Raney nickel and 40 µl of HTO of the same specific activity for PtO_2, also at 120°C.

Initially, poor incorporation was observed even in the metal-catalyzed exchange reactions. The deep coloration of the organic material suggested that catalyst poisoning could be limiting the metal-induced exchange. This problem has been approached in two ways. First, the material to be labelled was treated with activated charcoal prior to carrying out the exchange. Although improved incorporation was achieved, this was only at the expense of altering the relative proportions of the components present, thereby nullifying one of the main objectives of the work. The preferred solution was to alter the substrate:catalyst ratio in such a way that the available surface area of the catalyst is greatly increased. Some representative results are presented in Table 2 and the general findings can be summarized as follows:

(a) When tritiations were carried out in the presence of both catalyst and activated charcoal, the specific activity of the product decreased. Clearly, adsorption onto charcoal is a faster process than exchange.

(b) Decreasing the substrate-to-catalyst ratio led to improved tritium incorporation.

(c) When the heating time was increased, so also did the degree of tritium incorporation. On balance, the shorter heating time was favored as the danger of heat-induced artifacts arising from prolonged heating outweighed the benefits.

Of the two catalysts most extensively studied, Raney nickel was the most active and provided a product that met the requirements most closely. This can best be seen in Figures 2 through 4, where different ^{3}H NMR spectra are presented. The results show that for the PtO_2 catalyst (Fig. 2) the main ^{3}H resonances are confined to the δ 2.2-2.6 range and the aromatic region whereas with the Raney nickel catalyst (Fig. 3) the label is more uniformly distributed. Comparison of Figures 4 and 5 reveals that the ^{3}H and ^{1}H NMR spectra of the radioactive product obtained by the Raney nickel catalyzed exchange procedure are nearly superimposable. This demonstrates the randomness and general uniformity of tritium exchange achieved by the Raney nickel

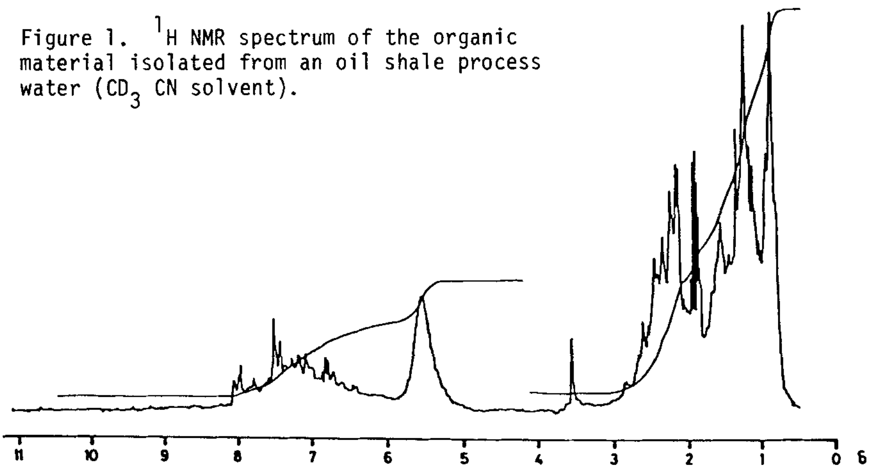

Figure 1. ^{1}H NMR spectrum of the organic material isolated from an oil shale process water (CD_3 CN solvent).

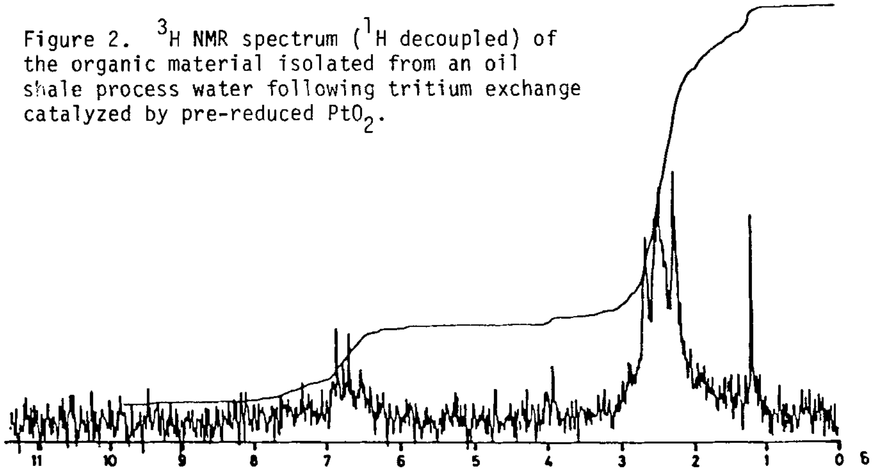

Figure 2. ^{3}H NMR spectrum (^{1}H decoupled) of the organic material isolated from an oil shale process water following tritium exchange catalyzed by pre-reduced PtO_2.

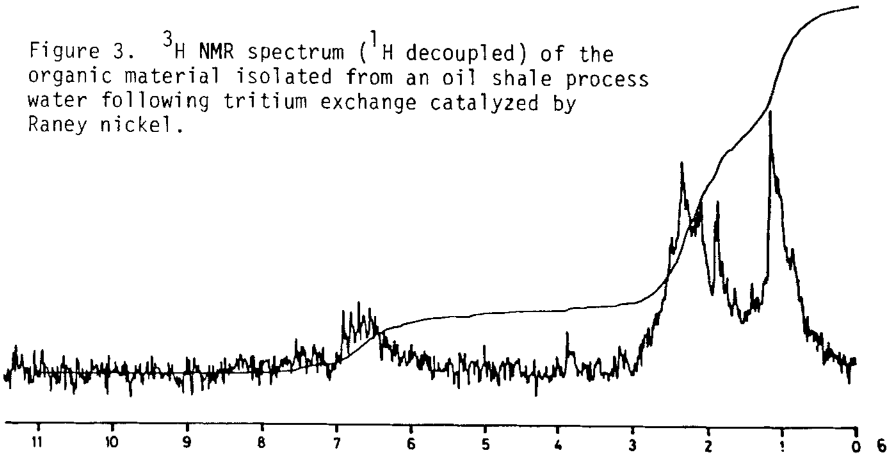

Figure 3. ^{3}H NMR spectrum (^{1}H decoupled) of the organic material isolated from an oil shale process water following tritium exchange catalyzed by Raney nickel.

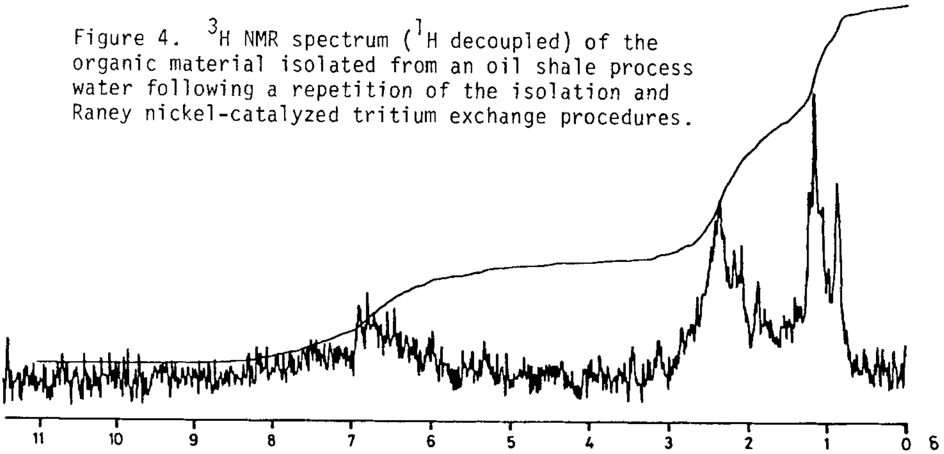

Figure 4. ^{3}H NMR spectrum (^{1}H decoupled) of the organic material isolated from an oil shale process water following a repetition of the isolation and Raney nickel-catalyzed tritium exchange procedures.

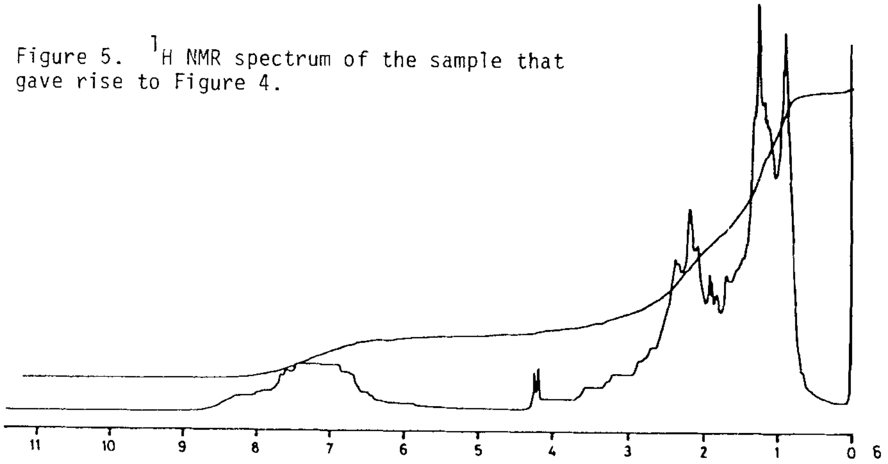

Figure 5. ^{1}H NMR spectrum of the sample that gave rise to Figure 4.

procedure among the diversity of organic compounds present. Although the comparative ^{3}H and ^{1}H NMR analyses does not provide direct proof that all of the organic constituents are labelled, the nearly identical fine structure and integral seen in the two spectra supports the contention that uniform tritium exchange, as defined earlier, has occurred. The reproducibility of the catalytic procedure is good, as can be seen by comparing the results (Figures 3 and 4) from two different experiments.

Substitution of tritium gas for tritiated water in both metal-catalyzed systems resulted in considerably reduced incorporation at low temperatures while the results at higher temperature did not provide the same degree of reproducibility as was obtained using tritiated water.

The stability of the label in the product was assessed by monitoring the ^{3}H NMR spectra of samples kept in 0.1 $\underline{M}$ NaOMe/MeOH or 0.1 $\underline{M}$ HOAc at room temperature over the course of several months. The results showed that no more than five percent of the tritium is released (as HTO) from the first medium over a two month time interval and even less from the acidic medium. As the labelled product is likely to be used under milder experimental conditions and over a much shorter time interval, these findings provide the necessary reassurance that the label has been incorporated into chemically stable positions by the Raney nickel procedure.

CONCLUSION

This work has demonstrated the feasibility of using a Raney nickel/THO system to effect a tritium exchange in a representative sample of dissolved organic material isolated from an oil shale process water. The method meets rigorous criteria of uniform labelling (as attested to by comparative ^{1}H and ^{3}H NMR spectroscopy) throughout the multiplicity of compound types present, high specific activity incorporation, stability of the incorporated label to back exchange, and reproducibility of preparation. The availability of such a sample opens new radiotracer approaches for studying complex organic materials. The principle of the tritiation approach will be expanded in the study of other complex organic materials.

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