INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION
COMMISSION ON ANALYTICAL REACTIONS AND REAGENTS*

ORGANIC ANALYTICAL REAGENTS IN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY†

Prepared for publication by
B. D. FLOCKHART and D. THORBURN BURNS
Department of Pure and Applied Chemistry,
Queen's University of Belfast, Belfast BT9 5AG, N. Ireland, UK

†Series Title: Present and Future Status of Organic Analytical Reagents.

Organic analytical reagents in nuclear magnetic resonance spectroscopy

Abstract – The presence of paramagnetic materials can result in significant chemical shift changes and/or broadening in the NMR spectra of other compounds, both effects resulting from the large magnetic moments of the paramagnetic species. When specific interaction occurs between the paramagnetic additive and the compound under study, much useful information may be obtained. The paramagnetic species of interest are conveniently divided into chemical shift reagents and relaxation reagents. Both classes of reagent are reviewed in this paper, as is also the usefulness of OARs as NMR tagging reagents.

LANTHANIDE SHIFT REAGENTS (LSRs)

Although successful attempts to use paramagnetic complexes as chemical shift reagents had been reported since the early 1960's, a decisive step in the development of this technique was the introduction of lanthanide derivatives of the tris-chelate type in 1969[1]. The term 'shift reagent' is now almost synonymous with the paramagnetic lanthanide chelates, the most commonly used being complexes of europium (III) and praseodymium (III) with β-diketones such as 2,2,6,6-tetramethyl-3,5-heptanedione (abbreviated to thd or tmhd) [also called dipivaloylmethane and abbreviated to dpm] and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione (abbreviated to fod). These complexes are Lewis acids and their action as shift reagents depends entirely on their ability to bind basic molecules (substrates) as labile ligands. Organic molecules that contain heteroatoms with a lone pair of electrons (oxygen, nitrogen, sulphur, phosphorus) are potentially suitable substrates. The exchange rate for the additional ligands is usually rapid on the NMR timescale, so that the observed resonance positions of the substrate nuclei are the weighted averages of those appropriate to the free and complexed states. The position of a given peak is consequently related to the stability of the complex formed, the amount of shift reagent added, and the location of the observed nucleus relative to the lanthanide ion. The mechanisms by which the unpaired spin(s) of the metal affect ligand resonances have been reviewed [2,3].

The most common application of LSRs is that of providing a convenient and economical means by which unresolved resonances may be separated. Signal displacement induced by LSRs results in a marked increase in the chemical shift relative to the nuclear spin-spin coupling constant, often enabling spectra of a (quasi-) first-order nature to be obtained even for complicated spin systems. With such systems the alternatives to the use of LSRs would be either to go to higher external fields, employ multiple resonance techniques, or

attempt a computer-aided analysis of the spectrum. An elegant example of the use of a LSR for spectral simplification is the effect of Eu(thd)$_3$ on the 100-MHz ^{1}H spectrum of cis-4-*tert*-butylcyclohexanol in deuterochloroform [4]. Only two resonances are readily assignable in the absence of a LSR. Sequential addition of Eu(thd)$_3$ improves the resolution, and at a molar ratio of complex to solute of 0.7 the spectrum becomes amenable to first-order analysis, all the signals being readily assigned from the spin-spin splitting pattern. Extrapolation of resonance positions to zero concentration of LSR enables the chemical shift of the uncomplexed substrate to be estimated. Values obtained in this way have been shown [5] to agree to better than 0.05 ppm and often to within $\pm$ 0.01 ppm with values obtained by direct observation where this was possible. In general, coupling constants estimated from a 'shifted spectrum' may be taken as good approximations to the corresponding values for the free substrate [6-8] although some caution should be exercised [9].

LSRs are frequently applied to the elucidation of molecular structures using the qualitative approach: chemical shifts and coupling constants are derived from shifted spectra; structural assignments are then made on the basis of available empirical knowledge. The quantitative approach (outlined below) relies heavily upon the magnitude and directions of the shifts to validate or reject proposed three-dimensional geometries.

A stereochemical problem that still remains unsolved is the question of whether molecular structures determined by X-ray diffraction in a crystal lattice are the same when those compounds are in solution. Strong solvent-solute interactions may produce substantial changes in the spatial arrangement. LSR studies can in principle provide information relating to molecular geometry in solution, and this potential application is being explored with vigour. The problem involved in quantitative structure analysis is essentially three-fold: (i) the shift induced in a substrate on complexation with a LSR is required (the 'limiting shift'); (ii) the dipolar (pseudo-contact) contribution to the limiting shift must be separated from any contact or diamagnetic contribution; (iii) the dipolar shifts must be related to the substrate geometry. Expressions describing the structural dependence of the dipolar shift magnitude include the term $(3 \cos^2\theta -1)\underline{r}^{-3}$, where $\underline{r}$ is the distance between the given ligand nucleus and the lanthanide ion, and θ is the angle between the principal magnetic axis of the complex and the vector from the lanthanide ion to the nucleus under study. Quantitative studies with LSRs are therefore concerned with the search for the structural model for which the geometrical factors ($\underline{r}$ and θ) of the observed nuclei agree best with the experimental values. For such computer searches, a number of computation methods have been reported. Conformational analyses using the LSR method have been described in a number of publications [10-14].

The basic requirement for the application of NMR to the analysis of multi-component mixtures is that a resolved resonance be available for each component to be analyzed. Mixtures of chemically similar compounds often give rise to spectra characterized by overlapping resonances, which has severely limited the general applicability of this form of spectrometry for quantitative analysis. Since LSRs have the ability to separate overlapping signals from the components of mixtures, it is possible to exploit very small differences in this way, an interesting example being the use of $Eu(fod)_3$ in the quantitative analysis of mixtures of cresol isomers [15].

The use of LSRs in the analysis of the micro-structure of polymer chains by ^{1}H and ^{13}C NMR spectroscopy has been reviewed [16]. In a determination of the stereoregularity of poly(methylvinylether), addition of $Eu(fod)_3$ enabled the triad tacticity of the polymer to be determined accurately from the areas of the methoxy triad peaks [17]. The LSR method can provide information on the distribution of monomer units along copolymer chains not obtainable by other methods of physico-chemical analysis [18]. Separation of the resonances of the end-group protons of poly(propylene glycol) from those of the polymeric chain, through the addition of $Eu(thd)_3$, has permitted the determination of molecular weights by direct comparison of signal intensities [19].

Classical methods for the determination of enantiomeric purity are not always satisfactory [20]. Before the introduction of LSRs, NMR procedures were restricted to the use of either chiral solvents or derivatizing agents to convert enantiomers to diastereoisomers. Both procedures have limited usefulness [9]. Chiral LSRs, tris chelates of optically active β-diketonate ligands with lanthanide ions, were introduced in 1970 [21] and since then have been extensively used to determine enantiomeric compositions. Many of the ^{1}H signals for a pair of enantiomers are sufficiently separated by the presenceof a chiral LSR to permit an accurate determination of their ratio by integration. Chiral LSRs have the ability to cause shift differences between protons or groups that are enantiotropic by internal comparison [22], and this has been used to differentiate meso from (+) and (-) diastereoisomers. Three articles specifically reviewing NMR analysis using chiral LSRs have been published [23-25].

A restriction in the use of LSRs is that the substrate must be a sufficiently strong donor to be able to coordinate with the lanthanide ion. An increase in the Lewis acidity of the complexed lanthanide ion should therefore make it a better coordination site for weak donors. Accordingly the lanthanide chelates of 1,1,1,2,2,3,3,7,7,7-decafluoro-4, 6-heptanedione [26] and of 1,1,1,2,2,6,6,7,7,7-decafluoro-3,5-heptanedione [27], being stronger Lewis acids than the fod and thd complexes, can induce substantial shifts in the

spectra of rather weak Lewis bases for which the fod and thd chelates are less effective. None of these mononuclear LSRs, however, induce changes in the chemical shifts of unsubstituted, nonpolar organic compounds. On the other hand, binuclear complexes formed in solution from a lanthanide(III)β-diketonate and silver(I)β-diketonate have been shown to be effective shift reagents for olefins, aromatics, hetereoaromatics, phosphines and some halogenated compounds [28-32]. The silver acts as a bridge between the lanthanide ion and the substrate. The combination of Yb(fod)$_3$ with Ag(fod) and Pr(fod)$_3$ with Ag(tfa), where tfa is the abbreviation for 1,1,1-trifluoro-2,4-pentanedione, presently represent the best selection of downfield and upfield reagents, respectively [31]. Through the proper choice of a chiral lanthanide chelate and silverβ-diketonate it is possible to distinguish certain dextro and levo resonances of enantiomeric unsaturated hydrocarbons [29]. The complexes formed in situ from Eu(fod)$_3$/Ag(fod) or Eu(fod)$_3$/K(fod) are effective shift reagents for ammonium, sulphonium and isothiouronium salts [33,34]. The selection of either Ag(fod) or K(fod) is dependent on the anion of the organic salt, Ag(fod) being more effective with halide salts and K(fod) with tetrafluoroborate salts. The interaction between the organic salt and Ag[Eu(fod)$_4$] or K[Eu(fod)$_4$] probably involves the formation of an ion pair between the organic cation and [Eu(fod)$_4$]$^-$. Shifts in the spectra of these organic salts with [Eu(fod)$_4$]$^-$ are significantly larger than those with Eu(fod)$_3$ [33,34].

Lanthanide complexes with some polycarboxylic acids are useful aqueous shift reagents [35,36]. Lanthanide complexes of the ligand 1,4,7,10-tetraazacyclododecane-N,N',N",N'''-tetraacetate induce only dipolar shifts in ^{23}Na$^+$, ^{35}Cl$^-$ and ^{2}H nuclei in a D$_2$O solution, and largely dipolar shifts in the ^{17}O nuclei of D$_2$O; they are usable to pH 13 [35]. The study of metal ion transport across biological membranes is an important topic in bioinorganic chemistry. The water-soluble anionic complex of Dy(III) with tripolyphosphate allows direct observation of separate resonances from intracellular and extracellular ^{23}Na$^+$ ions in human erythrocytes [37]. Since the shift reagent does not penetrate the cell membrane within the timescale of NMR measurements, only extracellular Na$^+$ ions experience the resonance shift. The same LSR has been used in the measurement of intracellular K$^+$ ion concentrations by NMR [38]. Use of the anionic complex of Tm(III) with triethylenetetraminehexaacetate has permitted the simultaneous determination of intra- and extracellular Na$^+$ ions in suspensions of human erythrocytes [39]. In some membrane transport studies it may be desirable to have the LSR present only inside closed vesicles. This can be accomplished by preparing the vesicles in the presence of the shift reagent anions and then selectively deactivating only the outside shift reagent [40,41]. The deactivation is achieved by titration with the diamagnetic Lu^{3+} ion. The tris complex of anions of 4-hydroxypyridine-2,6-dicarboxylic acid with

Dy(III) acts as an aqueous shift reagent for $^{23}Na^+$, $^{25}Mg^{2+}$, $^{39}K^+$ and $^{87}Rb^+$ NMR peaks, as well as that of the ^{14}N resonance of NH_4^+ [42]. The reagent tris(nitrilotriacetate)Dy(III) produces significant shifts of the $^{43}Ca^{2+}$ and $^{113}Cd^{2+}$ resonances [42]. All these shifts could be useful in future biological studies. As with the monovalent Na^+ and K^+ ions, transmembrane transport of the divalent Mg^{2+} and Ca^{2+} ions is important. Since the ^{43}Ca isotope is rare, many NMR studies of Ca^{2+} biochemistry may be made with $^{113}Cd^{2+}$ as a surrogate [43], and Rb^+ has been used as a surrogate for K^+. The NH_4^+ ion is sometimes encountered in physiological solutions [44].

General works on LSRs in NMR spectroscopy published before 1975 are referenced in an earlier review [9]. A number of general reviews [12,45-55] and two practical guides [56,57] have appeared since then; another practical guide is in press [58]. The thd and fod complexes of praseodymium, europium, gadolinium, dysprosium, holmium and ytterbium are commercially available [59,60]; $Eu(fod)_3$ and $Pr(fod)_3$ can be obtained in tablet as well as powder form [59]. Commercial sources of chiral LSRs are given in ref. 24. Resonances from the shift reagent do not often interfere with the ^1H spectrum of the substrate, but where this is a problem, reagents with deuterated ligands are available [59].

RELAXATION REAGENTS (RRs)

RRs are paramagnetic substances capable of strongly reducing the relaxation times of substrate nuclei without inducing noticeable shifts. Both an electron-nuclear dipolar interaction and an electron-nuclear contact interaction may contribute to the overall relaxation. Most treatments of the influence of paramagnetism on nuclear relaxation times have made use of the Solomon-Bloembergen formulations in which contributions to nuclear relaxation from proton spin-electron spin dipole-dipole interactions and from isotropic proton-electron spin exchange have been considered separately [61].

Tris(acetylacetonato)chromium(III) [$Cr(acac)_3$] is the most widely used RR, although $Fe(acac)_3$ has been claimed to be more efficient [62]. These and other transition metal complexes of acetylacetone are commercially available [59]. If the solubility of $Cr(acac)_3$ in an organic solvent becomes a limiting factor, $Cr(thd)_3$ may be used. Water-soluble RRs are obtained from species such as Cr(III), Gd(III) or Cu(II) bound to a ligand belonging to either the polyamine or the polyaminoacetic acid class.

The change in relaxation times brought about by RRs has been variously exploited. The simplest application is that of assigning signals from nuclei close to the site of complexation, the assignments being based on the extent of line-broadening. Thus, the

selective broadening of the carbon lines of amines complexed with $Cu(acac)_2$ has proved useful for this purpose [63]. In complex spectra with signal overlap, line broadening may be difficult to observe directly. The problem may be overcome by substracting the spectrum of the given compound with added RR from the unperturbed spectrum of the compound. Unchanged signals cancel out so that only those broadened by the RR are observed. Reduction in spin–lattice relaxation times ($\underline{T}_1$) brought about by RRs can remove the effects of nuclear spin–spin coupling. The nuclei near a paramagnetic centre may change spin states so rapidly that neighbouring nuclei experience no coupling to them. To give one example, coordination with $Gd(fod)_3$ leads to selective decoupling in pyridine [64].

The commonest application of RRs is that of shortening ^{13}C relaxation times whereby the time required to obtain spectral data is greatly reduced. The RR most widely used for this purpose is $Cr(acac)_3$ as it shortens $\underline{T}_1$ without broadening the signal. $\underline{T}_1$ values for typical carbons in the absence of a RR are several seconds or more. When ^{13}C Fourier transform NMR is used as a quantitative analytical tool, the delay between successive pulses has to be sufficiently long to ensure essentially complete nuclear relaxation. For $\underline{T}_1$'s of longer than several seconds the spectrometer time required may be unacceptable. Addition of a RR allows for a shortening of delay times between pulses such that practical analysis times are easily realized. As an example, ^{13}C FT NMR has been employed for the quantitative analysis of positional isomers of pentachlorotoluene through the use of $Cr(acac)_3$ [65]. The Fe(III) and Cr(III) complexes of diethylenetriaminepentaacetic acid are good water–soluble RRs for ^{13}C NMR spectrometry [66]. They are effective over the pH range of 1 to 12 and, like $Cr(acac)_3$ in organic solvents, they permit quantitative integration of ^{13}C NMR spectra.

The electron–nuclear dipolar contribution to spin–lattice relaxation is predicted to be $\underline{r}^{-6}$ dependent, where $\underline{r}$ is the distance from the paramagnetic centre to the relevant nucleus [61]. If the contribution of the contact interaction to the nuclear relaxation can be shown to be negligible, $\underline{T}_1$ data can provide quantitative structural information, *viz.*, from the changes in $\underline{T}_1$ at different sites in a molecule the relative distances of the sites from the paramagnetic centre may be calculated. As this geometrical dependence is greatly different from that for dipolar shift (a $\underline{r}^{-3}$ and an angular dependence), the combination of shift and relaxation information should lead to a more precise determination of molecular structures in solution. Structural studies where this combined approach is being used are now being reported [67].

The use of tris(d.d–dicampholylmethanato)Gd(III) as a chiral RR in ^{13}C NMR spectroscopy for the determination of enantiomeric ratios has been demonstrated [68]. The

method is based on the observed difference between the ^{13}C relaxation rates of the diastereomeric association complexes. Whether or not the chiral relaxation method can compete with that using chiral LSRs remains to be established.

The development of NMR imaging systems has provided a method for _in vivo_ research in biological systems using a noninvasive technique. The images that are produced are dependent upon three sample parameters: the proton concentration, $\underline{T}_1$, and the spin-spin relaxation time ($\underline{T}_2$) [69]. Compounds which affect these relaxation times could provide additional contrast for the images. Although the ethylenediaminetetracetate (EDTA) complexes of $FeCl_3$, $GdCl_3$ and $MnCl_2$ are not as effective as the respective salts in reducing $\underline{T}_1$ of water protons at 90 MHz, the diminution in ability of the Mn^{2+}-EDTA complex relative to the Mn^{2+} aquo complex is offset by a significant reduction in toxicity [70].

No review devoted to RRs has hitherto been published.

DERIVATIZING REAGENTS (DRs)

The tagging of a molecule or functional moiety of interest via an appropriate derivative has, like the two previous techniques, greatly increased the utility of NMR as a practical analytical tool. This procedure has been employed, for example, in the determination of enantiomeric composition, _viz._, the conversion of chiral alcohols and amines to diastereomeric esters and amides with an enantiomerically pure chiral acid chloride (commonly α-methoxy-α-trifluoromethylphenylacetyl chloride), followed by NMR analysis [71,72], but the method is limited to these two functionalities. By adding an achiral LSR the shift difference between the diastereomeric esters or amides may be increased. A review of NMR analysis using chiral derivatives has been published [73]; determination of the absolute configuration of carbinols, amines and amino alcohols based on derivatization by α-methoxy-α-trifluoromethylphenylacetyl chloride constitutes part of the article.

A simple but useful assignment technique with ^{13}C spectra is to compare the spectrum of an alcohol with that of the corresponding acetyl derivative. To give one example, acetylation shifts may be used to assign carbons α, β and (sometimes) γ to a hydroxyl group in a steroid molecule [74,75].

Fluorinated derivatives permit the use of ^{19}F NMR and this has potentially two advantages in comparison with ^{1}H NMR: the ^{19}F chemical shift parameter is generally more sensitive to subtle changes in molecular structure, and the virtual absence of fluorine in most analytical samples of interest alleviates the background problem. The potential utility of trifluoroacetyl chloride as a DR to characterize alcohols, phenols,

thiols and amines has been demonstrated [76]. Trifluoroacetic anhydride has been used for characterization of the hydroxyl groups in poly(propylene oxides) by ^{19}F NMR [77]. The use of hexafluoroacetone as a reagent for the ^{19}F NMR spectrometric determination of compounds containing active hydrogen has been reviewed [78]. This reagent has also been utilized in the analysis of mixtures of coal-derived phenols by ^{19}F NMR [79]. 3-Bromo-1,1,1-trifluoropropanone has been shown to be an effective sulfhydryl-specific reagent for proteins and peptides and, as such, is a useful probe in protein NMR studies [80]. The active-site thiol group of papain has been investigated by preparing a derivative of this enzyme utilizing SS-(2,2,2-trifluoro-1,1-dideuteroethyl) O-methyl dithioperoxycarbonate [81]. Sterols and amino acids have been characterized by ^{19}F NMR spectroscopy of their p-fluorobenzoyl derivatives [82]. A variety of different pharmaceutical compounds have been quantitatively analyzed by ^{19}F NMR spectrometry of their pentafluoropropionic anhydride derivatives [83].

The ^{19}F chemical shift range for a great number of p-fluorobenzoyl derivatives is large [84]. Nevertheless, with complex mixtures and large size substrates, spectral overlap can still be troublesome. In a model study of alcohols, phenols and amine derivatives, the problem was overcome by enriching the carbonyl carbon of the p-fluorobenzoyl chloride tagging reagent to a level of 90% with the ^{13}C isotope [85]. Two separate spectral domains (i.e., ^{19}F and ^{13}C spectra) were then available for characterizing the systems. This is the first reported example of a dual tagging reagent for analysis of molecules containing active hydrogen functional groups.

CONCLUDING REMARKS

OARs as LSRs, RRs and DRs have greatly extended the usefulness of the NMR method of analysis. The employment of new and possibly more effective reagents may be expected in the future. Even with the present range, however, a significant growth in the already large area of analytical applications can be confidently predicted within the next decade.

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