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A thesis entitled

AN NMR STUDY OF LANTHANIDE SHIFT REAGENTS

presented by

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David Westwood

SUMMARY

The work in this thesis describes a detailed study of the nature of the interactions occurring in solution between lanthanide shift reagents and organic substrates. The determination of intrinsic parameters, such as equilibrium binding constants and limiting incremental shift values feature prominently in this work.

Chapter I gives a brief outline of the historical background and the chemistry of lanthanide shift reagents, namely, chelates of rare-earth ions and selected β-diketones.

In the first part of Chapter II, an account is presented of the experimental conditions that need to be considered for the determination of reliable intrinsic parameters. An account of simple data treatment methods used to determine intrinsic parameters is also presented. The results of these determinations are discussed in terms of assumed 1:1 and 2:1 stoichiometries.

The second part of Chapter II describes a rigorous data treatment method for determining intrinsic parameters. A discussion of the results obtained for a series of alcohols, ketones, ethers and nitrogen containing substrates complexed with the shift reagent $Eu(fod)_3$, is also presented. Selected results of the rigorous data treatment method are then compared with results from simple data treatment methods.

Chapter III describes several independent, but simple data treatment methods which may be used as alternatives to the data treatment method presented in Chapter II. These methods employ shift reagent resonance frequencies and also competition experiments involving two competing substrates. In view of the expected complexity of the solution equilibria for a 2:1 stoichiometry, only those systems exhibiting a predominantly 1:1 stoichiometry have been considered.

In Chapter IV an account of the contact and pseudocontact lanthanide induced shift mechanisms is presented. Various methods for separating the contact shift contribution from the total paramagnetic induced shift are then described. Contact shifts for several substituted pyridine-Eu(fod)$_3$ complexes are determined and the results discussed in terms the symmetry of the shift reagent-substrate complex.

CONTENTS

1.1 Historical Background

Nuclear magnetic resonance (nmr) spectroscopy is one of the most powerful structural tools available to chemists. In addition to this more advances have probably been made in nmr spectroscopy than in any other spectroscopic field. Amongst the more recent advances, concerned with the interpretation of highly complex spectra has been the development of superconducting magnets for producing large magnetic field strengths. The need for higher operating field strengths arises mainly from the increased separation brought about in the chemically shifted signals in the nmr spectrum. This increased separation, relative to the spin-spin coupling constants, frequently simplifies the spectral interpretation. Other instrumental developments (incorporating fourier transform techniques coupled with digital computer use) have also tremendously increased the scope of the nmr experiment. Fourier transform methods can be of considerable value in the enhancement of signal to noise. Also the time required to obtain a spectrum of a low sensitive nucleus, example C^{13} has been greatly reduced. Relaxation times, also studied by fourier transform techniques can be much more easily and accurately determined. Unfortunately, the advantages offered by these significant and highly successful techniques are somewhat offset by the exorbitant initial expenses. In view of this, perhaps one of the most dramatic developments in recent years was that reported by Hinckley (1) which related to the applications of lanthanide shift reagents in nmr spectroscopy.

Hinckley's report that certain lanthanide β-diketonate chelates (Lewis acids) can be easily used to facilitate considerable nmr spectral simplifications of a large number of substrate species (Lewis bases), has stimulated a great deal of response concerning the theoretical aspects and the applications of these so called lanthanide shift reagents. In the field of nmr spectroscopy the spectral changes brought about by the presence of paramagnetic species is not a new phenomenon (2) . Prior to Hinckley's discovery it had long been known that the large magnetic moment of an unpaired electron in a paramagnetic transition metal ion could cause changes to occur in the resonance frequencies of nuclei in an attached ligand. In many cases the paramagnetic induced shift arising from time dependant magnetic field fluctuations experienced by the nuclear spin system within the ligand led to simplified and readily interpretable spectra. The general applicability of these transition metal complexes was however extremely limited. Usually the transition metal complexes exhibited shifts which were much smaller than the induced shifts caused by the lanthanide shift reagents. Also the line widths of the lanthanide induced shifts are generally much narrower than those displayed by the transition metal complexes (3,4).

1.2 Lanthanide Shift Reagents

Shift reagents are Lewis acids which when added to solutions of Lewis base type substrates often afford immense spectral simplifications. This is achieved by the shifting to various extents the resonance positions of the nuclei present. Quite often a complex spectrum is transformed into a spectrum that is amenable to first-order analysis. In 1969, Hinckley reported that the bis-pyridine adduct of tris

(2,2,6,6-tetramethyl-3,5-heptanedianato) europium (III), $Eu(dpm)_3(py)_2$, caused large downfield induced shifts in the proton nmr spectrum of cholesterol monohydrate in carbon tetrachloride (1). He also reported that the observed paramagnetic induced shifts were the direct consequence of bonding between the lanthanide metal chelate and the cholesterol monohydrate. From a graph of the observed proton shifts versus the cube of the reciprocal distance between the lanthanide metal and the protons studied, it was shown that the shifts were produced by a pseudocontact mechanism and not via a contact interaction.

It was subsequently pointed out (5) that the pyridine free complex $Eu(dpm)_3$, would be a superior shift reagent. Indeed this has been found (6) and now many shift reagents, produced by using different lanthanide metal ions and/or β-diketones have since been used with varying degrees of success (7,8).

It has also been shown (9) that praseodymium chelates (e.g. $Pr(dpm)_3$) induce shifts in the proton nmr spectra that are opposite in direction, and are much greater in magnitude than those shifts reported with the europium chelates.

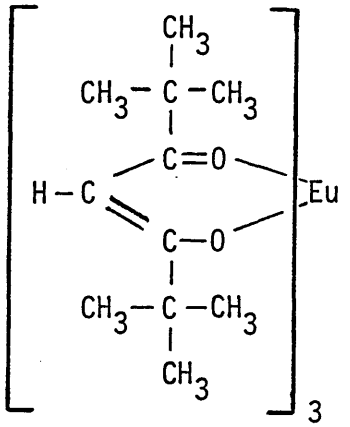
The effectiveness of $Eu(dpm)_3$ as a shift reagent is somewhat reduced when used with weak Lewis bases. More over the solubility of this chelate is relatively low in non-alcoholic solution (10). Improvements with respect to solubility and Lewis acidity derived from the use of partially fluorinated ligands were shown by Rondeau and Sievers (11). The shift reagent used was tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethy-4,6-octanedianato) europium (III), $Eu(fod)_3$. The authors state that the partially fluorinated ligand increases the solubility of the metal chelate and also that the electron withdrawing properties of the

fluorine atoms present increases the residual acidity of the cation making a better coordination site for weak Lewis base donors. Other fluorinated side chain derivatives have since been used (12).

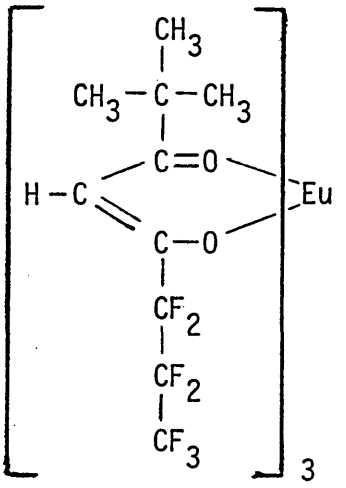
Paramagnetic lanthanide chelates contain unpaired electrons. These unpaired electrons may cause rapid relaxation of nearby magnetic nuclei. Consequently the induced shifts are then accompanied by severe signal broadening. In some cases the line broadening is so severe that the resonances are not observed. When the electron spin lattice relaxation time, T_{1e}, is sufficiently short, the spin lattice relaxation times of the neighbouring magnetic nuclei are barely affected and little signal broadening occurs (13). Horrocks (14) has reported line broadening characteristics of a series of lanthanide shift reagents and has concluded that europium and praseodymium chelates are amongst the lanthanides which produce very little signal broadening.

The chemical shift of diastereotopic protons should be non-equivalent. In general this non-equivalency may be too small to be resolved. The use of chiral shift reagents to enhance the non-equivalent chemical shifts of diastereotopic protons has been shown by Whitesides (15). Tris (tert-butylhydroxy-methylene-d-camphorato) europium (III) was used to determine the purity of enantiomeric amines dissolved in achiral solvents. Fluorinated chiral shift reagents have also been introduced which afford better resolution (16). Small induced shifts and appreciable line widths however make these types of shift reagents unsuitable for normal use. In addition the resonance frequencies of the ligand protons occasionally obscure the substrate proton signals under investigation. To date, $Eu(fod)_3$ still remains one of the most

popular shift reagents for inducing large downfield pseudocontact shifts. Alternatively, if it is desirable to induce upfield paramagnetic shifts then $Pr(fod)_3$ is generally the shift reagent chosen. The respective two-dimensional structures of $Eu(dpm)_3$ and $Eu(fod)_3$ are shown below.



$Eu(dpm)_3$



$Eu(fod)_3$

It is well established (17) that the total paramagnetic lanthanide induced shift is the sum of contributions from two sources: namely a contact shift mechanism which is a through bond effect and a pseudocontact shift mechanism which is a through space effect. For lanthanide metal chelates the induced shift, resulting from complexation with organic substrates, occurs predominantly via the pseudocontact shift mechanism. This is particularly so when the distance between the metal ion and the nucleus to be studied is quite large. However when nuclei are positioned very close to the lanthanide metal ion, significant contact shift contributions can be detected. Furthermore when organic compounds containing conjugated π electron systems are studied

the ease with which delocalisation of the π electrons occurs helps to facilitate electron spin density transfer. This can produce contact shifts for nuclei that are well separated from the metal ion. For saturated compounds such as alcohols, ketones and ethers the induced shift is considered to be predominantly pseudocontact in nature (18) whereas for aromatic type systems a contribution from both contact and pseudocontact shift mechanisms, is considered present (19). Contact shifts, which are scalar (Fermi) isotropic hyperfine interactions, arise from electron spin delocalisation or spin polarisation of unpaired electrons via the molecular orbitals of the organic substrates. This process may be regarded qualitatively as paramagnetic induced shifts due to transfer of bonding electron density. As a result of this, the unpaired electron spin density is spread over a number of atomic sites within the substrate molecule. For contact shifts the through bond effect, which may involve both π and σ bonds, rapidly decreases as the number of bonds separating the metal ion and the nuclei to be studied increases. Also large contact shift contributions result from the covalent bonding between the metal ion and the organic substrate molecule.

Pseudocontact shifts are caused by dipolar interactions between the lanthanide metal unpaired electron(s) and the nuclei of interest. This type of interaction may cause anisotropic changes in the magnetic field strength at the point in space where the nuclei are located. The magnetic field of the electron magnetic dipole interacts directly with that of the nuclear magnetic dipole. The interaction is often referred to as a "through space" effect since the number and nature of the chemical bonds separating the dipole centres plays no part in

the interaction. Hinckley (20) originally stated that the induced shift was related to g-tensor anisotropy. This theory had previously been considered by McConnell and Robertson (21) for the transition metal complexes. Recently however it has been shown by La Mar, Horrocks and Allen (22, 23) and by Bleaney (24) that the lanthanide induced pseudocontact shift can be evaluated in terms of magnetic susceptibility anisotropy data. Surprisingly one significant feature serves to distinguish between the theory of Horrocks et al with that of Bleaney. Horrocks et al relate the induced shift to the reciprocal temperature (T^{-1}) whereas the theory postulated by Bleaney suggests a squared reciprocal temperature dependancy (T^{-2}). Both theories, although using different parameters, appear successfully to account for the variation of the pseudocontact shifts produced by a series of different lanthanide shift reagents (25, 26).

At room temperature only one resonance signal is observed for a particular proton ofa substrate in the presence of a lanthanide shift reagent. This is indicative of a rapid chemical exchange process taking place between the free substrate molecules, the metal chelate and the bonded substrate-chelate complex (27). The chemical shift position of this single resonance signal (δ_{obs}) represents a weighted average of the chemical shift of the free substrate (δ_o) measured in the absence of any shift reagent and the chemical shift of the totally complexed substrate (δ_∞). A diagramatic representation of this is shown in Figure 1.1

The relative difference between the chemical shift of a proton in the free substrate and the chemical shift of the same proton in an equilibrium mixture of the substrate in the presence of a small

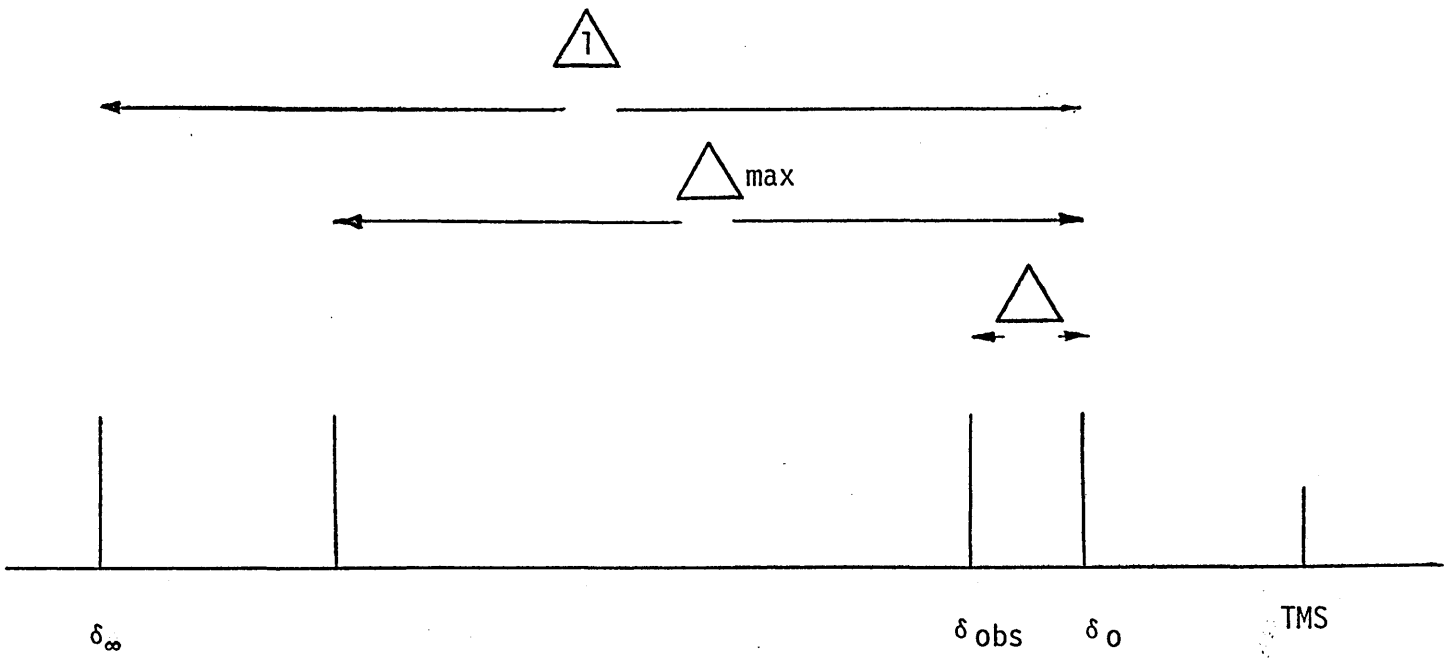
Figure 1.1

Chemical Shift Positions

δ_0 chemical shift of free substrate.

δ_{obs} chemical shift of substrate in an equilibrium mixture of substrate and small amount of shift reagent.

δ_∞ chemical shift of the totally complexed substrate, i.e. the chemical shift of the 1:1 adduct.


1
max
δ_∞
δ obs
δ o
TMS

amount of shift reagent is referred to as the lanthanide induced shift, Δ. Also the chemical shift of the totally complexed substrate, (in this case the chemical shift of the stoichiometric 1:1 substrate-shift reagent adduct) relative to the chemical shift of the free substrate is referred to as the limiting incremental shift, Δ_1. Alternatively this is sometimes referred to as delta max. 1. Usually at any given substrate concentration the value of the induced shift increases as the shift reagent concentration increases. However, the maximum observable value of the induced shift, $\Delta_{max.}$ is rarely consistent with the limiting incremental shift value just defined. The maximum observable induced shift is rather a limiting shift due to solubility limitations and the possible effects of multiple equilibria. The corresponding limiting incremental shift value for a 2:1 substrate-shift reagent complex is Δ_2 and is commonly referred to as delta max. 2.

As pointed out, the observed shift is a weighted average of the chemical shift of the free substrate and the chemical shift of the totally complexed substrate; consequently it can be shown that for a 1:1 stoichiometry the lanthanide induced shift, Δ is given by equation 1.1 (28).

$$\Delta = \frac{[ES]\Delta_1}{[S_T]} \qquad \ldots 1.1$$

where $[S_T]$ is the total substrate concentration and $[ES]$ is the equilibrium concentration of the substrate-shift reagent complex.

Δ and Δ_1 are as defined.

The corresponding equation for a 2:1 substrate-shift reagent stoichiometry is

$$\triangle = \frac{[ES]\triangle 1}{[S_T]} + \frac{2[ES_2]\triangle 2}{[S_T]} \quad \ldots 1.2$$

$[ES_2]$ is the equilibrium concentration of the 2:1 substrate-shift reagent adduct and the other symbols have their usual meaning. These two equations have been used extensively in the studies of lanthanide shift reagents (29).

The enormous number of publications that have appeared over the last few years covering the applications of lanthanide shift reagents is a tribute to the tremendous potential of the lanthanide metal chelates. The ease with which detailed information, relating to a vast number of substrates, can readily be obtained AND interpreted has helped greatly to popularise these shift reagents. As a result of the immense spectral simpliciation often afforded by these lanthanide metal chelates, analyses hitherto extremely difficult to perform are carried out with ease. Furthermore for many applications the approach is simple and unambiguous and the expanded near first order spectra can frequently be used without reference to any assumptions concerning concentrations, shift mechanisms and adduct stoichiometries. The reviews of Mayo (30) and Cockerill et al (31) cover a large range of these simple applications.

The study of the exact nature of the substrate-shift reagent interaction has however received only limited attention and in order to gain a better understanding of the mechanism of the shift reagent interaction more comprehensive studies are required. The demanding

experimental conditions necessary for these comprehensive studies and also the apparent success of the earlier and much simpler methods reported have possibly led to the absence or neglect of such detailed studies. The work reported in this thesis covers a range of controlled experiments in which great care has been exercised when interpreting the shift results.

1.3 Proposed Study

The application of shift reagents to the determination of molecular geometry has aroused a great deal of interest. The methods used in these determinations have however not been adequately evaluated and the work published so far has generally not been based on accurately determined data. Also in several cases, the interpretations put forward have been assessed on incorrect and unproven assumptions. Initially the aim of the work reported in this thesis was directed towards the determination of molecular structures. Specifically involved in this was the development of a reliable and accurate method for the determination of limiting incremental shift values, equilibrium binding constants and the adduct stoichiometry. Careful considerations were given to practical and theoretical factors and the results obtained interpreted accordingly. The molecular systems studied were those likely to exhibit induced shifts caused solely by the pseudocontact mechanism. Finally, it was envisaged that contact shift interactions might, for certain compounds, play an important part in the interpretation of their lanthanide induced shift data. Consequently an attempt was made to develop a method for determining the individual contributions from the pseudocontact and contact shift mechanisms.

2.1 Introduction

Many donor - acceptor associations, of which the substrate-shift reagent interactions can be included, are described in terms of Lewis base - Lewis acid interactions. In solution, rapid chemical exchange exists between the interacting species present.

Lanthanide Shift Reagent + Substrate $\rightleftharpoons$ Lanthanide Shift Reagent - Substrate Complex

(Acceptor) (Donor)

Soon after Hinckley's initial report it was realised that the lanthanide induced shift caused by these interactions, coupled with concentration studies, offered a means of determining the intrinsic parameters, namely the equilibrium binding constants and the limiting incremental shift values.

The first and simplest quantitative treatment of these interactions was based on the assumption of a 1:1 stoichiometry (32). Narrow limits were imposed on the substrate and shift reagent concentrations and the calculations for determining the equilibrium binding constant and limiting incremental shift values were shown to be straight forward. The popularity of this method lay in its simplicity and the ease and speed with which the calculations of the intrinsic parameters could be carried out.

More rigorous studies however, encompassing a comprehensive range of substrate and shift reagent concentrations later indicated the inadequacy of the assumption of the 1:1 stoichiometry and a 2:1 substrate-shift reagent interaction was proposed (33). The problems of

determining the intrinsic parameters of a 2:1 stoichiometry are amplified by the need to calculate two equilibrium binding constants. Also the 1:1 and 2:1 substrate - shift reagent adducts which in solution may be present simultaneously, possess their own limiting incremental shift values. Consequently the observed shift is no longer directly related to a single limiting incremental shift but is a weighted average of two such quantities. These limiting incremental shift values must in principle be considered different, unless proved otherwise. The analysis of results based upon an assumption of a 2:1 stoichiometry is therefore much more complicated and generally more time consuming.

The choice of method used to determine equilibrium binding constants and in particular the limiting incremental shift values is generally made on the basis of the information sought and the use to be made of that information. It must be stressed however that any method used must be carried out under strictly controlled experimental conditions.

2.2 Experimental Factors

The experimental conditions required for reliable intrinsic parameter determinations have generally been recognised as being of great importance. Despite this however work has yet to be reported that has been based on completely adequate experimental conditions.

In 1971, Ernst and Mannschreck (34) reported that the purity of the shift reagent played an important part in the study of lanthanide shift reagent - amine systems. The authors presented evidence showing that up to 30% discrepancies occurred in the induced shift values

when different commercial samples of $Eu(dpm)_3$ were used. Desreux et al (35) and Pfeffer et al (36) have also arrived at similar conclusions regarding shift reagent purity.

Experiments carried out in this laboratory using differential scanning calorimetry, showed that freshly sublimed $Eu(fod)_3$ contained traces of moisture. A sample of $Eu(fod)_3$ that had been allowed to stand uncovered in the atmosphere produced an almost idential thermogram to the one which was produced by a freshly sublimed $Eu(fod)_3$ sample.

A comparison of the results reported by Armitage et al in 1971 (38) and 1972 (39) clearly shows how the presence of water and other impurities in the substrate or solvent affect the values of the intrinsic parameters determined. Substantial differences arise in the equilibrium binding constants and limiting incremental shift values when the presence of competing substrates is not totally eliminated. These huge differences, evident from the work of Armitage are shown in Table 2.1. A similar comparison carried out on the work of Shapiro et al (40, 41) also highlights the variation of the intrinsic parameter values which can occur in the results when extreme experimental precautions are not taken. Consequently shift reagent, substrate and solvent species must be purified to the highest possible degree. It would seem, as indeed was pointed out by Huber (42), that the presence of small amounts of impurities in the substrate - solvent systems leads to apparently smaller equilibrium binding constants and larger limiting incremental shift values.

The choice of solvent is also another important experimental factor. Solvent molecules possessing their own functional groups will coordinate with the shift reagent and effectively reduce the metal chelate

Table 2.1

Intrinsic parameters obtained by Armitage et al where competing substrates are not eliminated (a)

a) Equilibrium binding constants and limiting incremental shift values for $Eu(dpm)_3$ - complexes in $CDCl_3$.
b) Units of $dm^3.mol^{-1}$.
c) Units of Hz.

Substrate	K (b)	Δ^1 (c)	Reference
$CH_3CH_2\underline{CH_2}NH_2$	12.3	2322	38
n-propylamine	32.1	768	39
$(CH_3)_3C\underline{CH_2}OH$	6.2	1422	38
2,2-dimethylpropanol	9.7	1182	39

concentration. This results in smaller induced shifts which in turn affects the intrinsic parameters determined. Other factors influencing the choice of solvent include possible substrate - solvent intermolecular interactions and substrate - shift reagent solubility limitations (43).

It is now well established (44) that the presence of paramagnetic ions in solution affects the bulk magnetic susceptibility of the sample. Consequently as the shift reagent concentration increases then so will the changes occuring in the magnetic subsceptibility. Herz et al (45) have reported that the internal standard used in their studies did not compensate for the magnetic changes occuring in solution when the shift reagent concentration was increased. With respect to the position of the internal standard non-selective upfield shifts resulted as a consequence of the magnetic changes produced by the presence of the lanthanide shift reagent. These shifts although small (in the order of 60 - 90 Hz) are highly significant when considering protons which are at a distance from the site of association. The interpretation of the limiting incremental shifts for these protons can cause large errors in subsequent geometrical studies determined from the lanthanide induced shift data.

Reports have recently appeared which highlight the possible occurrence of shift reagent dimerisation. Dimeric forms of $Eu(dpm)_3$ (46) and $Pr(dpm)_3$ (47) are known to exist in the crystalline state and extensive evidence has now shown that shift reagent dimers occur in solution. Desreux (35) has shown by vapour phase osmometry data that shift reagent aggregates occur in solution, the concentration of which increases along the solvent series chloroform, carbontetrachloride,

n-hexane. Springer et al (48) have reported that the shift reagent $Pr(fod)_3$ is highly associated in carbontetrachloride at concentrations near 0.01 mol. dm^{-3}. Under the same conditions the chelates of the larger lanthanide ions are more highly associated than those of the smaller lanthanide ions. For example, $Pr(fod)_3$ dimerises more readily in benzene than does $Yb(fod)_3$ (49). It is also clear however that a decrease in the state of hydration of a shift reagent results in an increase in the extent of shift reagent dimerisation (50). Consequently in solutions of extreme purity which are those necessary for reliable intrinsic parameter determinations, there is a greater tendency for the shift reagent to dimerise than say in solutions which contain trace amounts of moisture. These experimental factors play an important part in the methods developed for reliable intrinsic parameter determinations. The effect of neglecting to remove all traces of water and other impurities has been shown and the attention to detail, ensuring the use of truly anhydrous conditions cannot be over emphasised.

2.3 Simple Data Treatment Methods

2.3.1 Initial Developments

In 1971 the first (and simplest) method for determining intrinsic parameters was described by Armitage et al (32). It was assumed that the reaction stoichiometry was 1:1 and the substrate - shift reagent interaction considered was:-

$$E + S \rightleftharpoons ES \qquad \ldots 2.1$$

E is the shift reagent used in the study

S is the substrate and

ES is the adduct formed by the interaction

The equilibrium binding constant K, defined for the reaction was given by :

$$K = \frac{[ES]}{([E_T]-[ES])([S_T]-[ES])} \quad \ldots 2.2$$

which on rearrangement gives

$$[ES]^2 - [ES]\left([E_T]+[S_T]+\frac{1}{K}\right)+[E_T][S_T] = 0 \quad \ldots 2.3$$

$[E_T]$ is the total shift reagent concentration and the other symbols are as defined previously.

Armitage et al pointed out that when the shift reagent concentration was small $[ES]^2$ may be neglected, whereupon equation 2.3 can be rearranged to give:

$$[E_T][S_T] - [ES]\left([E_T]+[S_T]+\frac{1}{K}\right) = 0 \quad \ldots 2.4$$

As was pointed out in Chapter I the lanthanide induced shift is a weighted average of the chemical shift of the free substrate and the chemical shift of the 1:1 complex; consequently substitution of $[ES]$ of equation 1.1 into equation 2.4 gives, by rearrangement equation 2.5.

$$[S_T] = \frac{[E_T]\triangle 1}{\triangle} - \left(\frac{1}{K}+[E_T]\right) \quad \ldots 2.5$$

This was the equation derived by Armitage following a series of experiments where the induced shift of a substrate proton was measured as a function of the substrate concentration whilst the shift reagent concentration was kept constant. Thus Armitage et al showed that at a small but constant shift reagent concentration a plot of the reciprocal induced shift versus the substrate concentra-

tion would yield equilibrium binding constants and limiting incremental shift values from the intercept and slope measurements respectively. Such a plot is shown in Figure 2.1.

Confidence in this method resulted from the good agreement that was found for the equilibrium binding constants that were calculated from the measured intercept values for different protons on the same substrate. These values, reported for a series of $Eu(dpm)_3$ - complexes in deuterochloroform are shown in Table 2.2 (38).

A slightly different approach described by Kelsey (51) used another simple approximation. Kelsey argued that if the shift reagent concentration used in the experiments was kept constant at a value which was much smaller than the concentration of the substrate i.e. $[S_T] \gg [E_T]$ then an approximation $[S_T] = \left([S_T] - [ES]\right)$ can be made and equation 2.3 rearranged to give:

$$[S_T] = \frac{[E_T]\,\Delta 1}{\Delta} - \frac{1}{K} \qquad \ldots 2.6$$

Again, a plot of the substrate concentration versus the reciprocal induced shift enables equilibrium binding constants and limiting incremental shift values to be calculated from intercept and gradient values respectively. The interpretation of results obtained from both methods give identical values of limiting incremental shifts but the agreement between the equilibrium binding constants is very poor, especially when high K values are expected. Table 2.3 compares the large differences that occur when equilibrium binding constants are calculated from intercept measurements using equations 2.5 and 2.6 derived by Armitage et al and by Kelsey respectively. Although the approximations made in both methods are small the results from Table 2.3 demonstrate the significant differences that may occur

Figure 2.1

A plot of equation 2.5

$$\left[S_T\right] = \frac{\left[E_T\right]\triangle 1}{\triangle} - \left(\frac{1}{K} + \left[E_T\right]\right) \qquad \ldots \qquad 2.5$$

Separate lines represent different protons on the same substrate molecule.

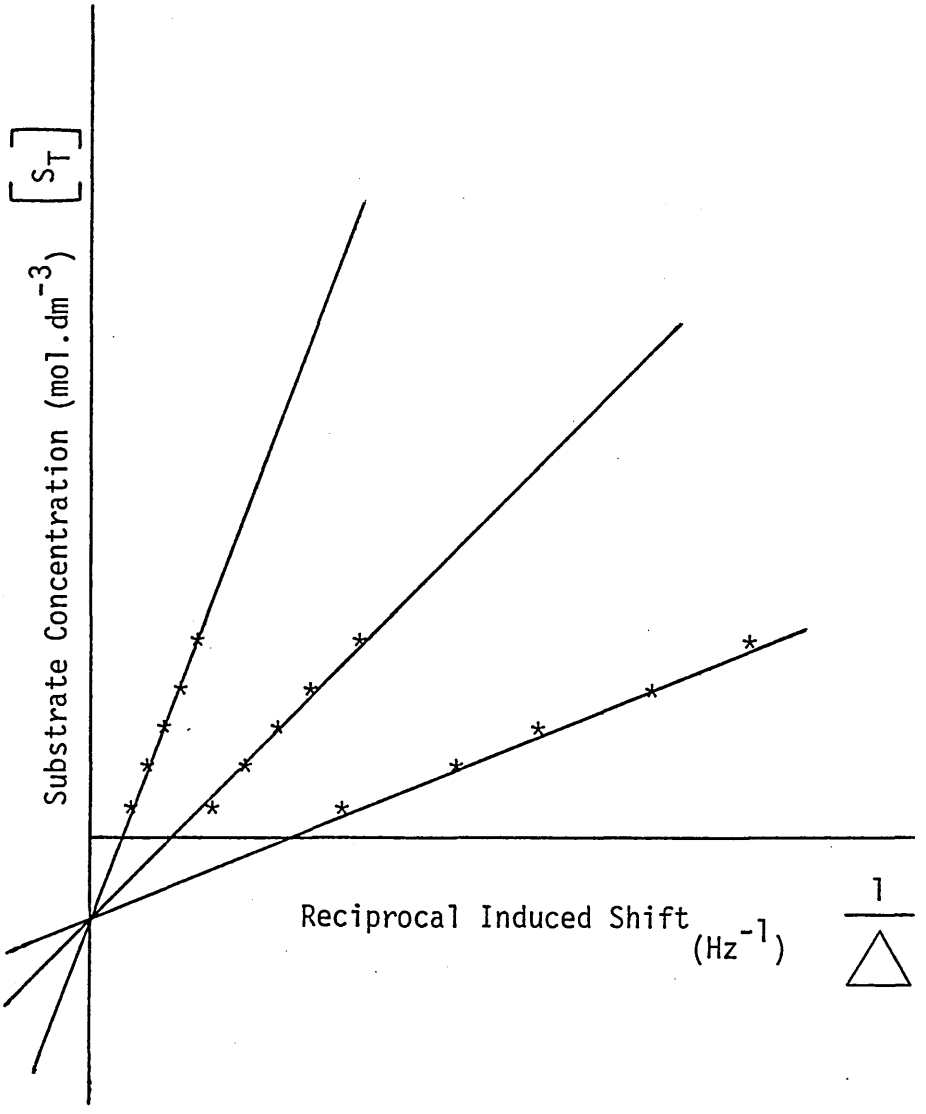

Substrate Concentration (mol.dm^{-3}) $[S_T]$
Reciprocal Induced Shift (Hz^{-1})
$\frac{1}{\Delta}$

Table 2.2

Equilibrium binding constants obtained from equation 2.5 [a]

a) Complexes of $Eu(dpm)_3$ in $CDCl_3$ reported by Armitage et al (38).
b) Units of $dm^3.mol^{-1}$.

Substrate	Equilibrium Binding Constant [b]
$CH_3CH_2\underline{CH_2}NH_2$	12.3
$CH_3\underline{CH_2}CH_2NH_2$	11.5
$\underline{CH_3}CH_2CH_2NH_2$	12.9
$(CH_3)_3C\underline{CH_2}OH$	6.2
$(\underline{CH_3})_3CCH_2OH$	6.5

Table 2.3

A comparison of the equilibrium constants obtained from intercept measurements of equations 2.5 and 2.6

a) Units of $dm^3.mol^{-1}$

b) Shift reagent concentration = 0.008 $mol.dm^{-3}$.

Equation 2.5 intercept = $\left(\frac{1}{K} + [E_T]\right)$

Equation 2.6 intercept = $\frac{1}{K}$

Measured Intercept	Equilibrium Binding Constants [a]	
	Equation 2.5 (Armitage)[b]	Equation 2.6 (Kelsey)
-0.01	500	100
-0.02	84	50
-0.03	46	33
-0.05	24	20
-0.10	11	10

when reliable intrinsic parameter values are sought.

In both experiments a constant shift reagent concentration is used and the induced shift is measured as a function of the substrate concentration. In the method described by Armitage et al, the constant shift reagent concentration must be small, whereas in Kelsey's method, provided the substrate concentration is always far greater than the shift reagent concentration then no limits, other than those imposed by association and solubility effects are placed on the shift reagent concentration. Although the method described by Armitage et al is probably more accurate, the approach of Kelsey is perhaps more widely adopted.

Many workers (52, 53, 54) have advocated the use of the graph of the lanthanide induced shift versus the mole ratio for determining limiting incremental shift values. The mole ratio is the ratio of the total shift reagent concentration relative to the total substrate concentration. Indeed it has now become customary (55, 56) to plot this graph as a means of gaining information regarding the proton induced shifts of the substrate molecule under investigation.

It can be shown that if a 1:1 stoichiometry is assumed and the experiments are performed in the region where $[S_T] \gg [E_T]$ then rearrangement of equation 2.6 gives

$$\triangle = \frac{K[E_T]\triangle 1}{1 + K[S_T]} \qquad \ldots 2.7$$

Considering the limit where $K[S_T] \gg 1$ strong association and/or large substrate concentration, then equation 2.7 rearranges to

$$\triangle = \frac{[E_T]\triangle 1}{[S_T]} \qquad \ldots 2.8$$

Consequently a plot of the lanthanide induced shift versus the mole ratio as shown in Figure 2.2 enables the limiting incremental shift values to be calculated from the slope measurements. A zero or near zero intercept value is expected. An additional feature to this method is that the substrate concentration can be kept constant whilst the shift reagent concentration is changed. Small amounts of solid shift reagent can then be added successively to the substrate solution. This is the easiest and consequently the most widely adopted procedure in shift reagent work. One disadvantage of this method is that equilibrium binding constants cannot be calculated.

However, if the other limit is considered, i.e. $1 \gg K[S_T]$ weak association and/or small substrate concentrations, then equation 2.6 now rearranges to

$$\Delta = K[S_T]\Delta_1\left(\frac{[E_T]}{[S_T]}\right) \qquad \ldots 2.9$$

Under these conditions a graph of the lanthanide induced shift versus the mole ratio cannot be used to determine the limiting incremental shift values since

i) K is seldom known and

ii) the gradient of the line is now dependent upon the absolute substrate concentration at which the experiments were performed (57).

Consequently if several experiments are carried out at constant substrate concentrations which are different for different experiments and the induced shift is measured as a function of the shift reagent concentration, then a graph resembling the one shown in Figure 2.3 can be obtained. From the graph it can be seen that greater slope

Figure 2.2

A plot of equation 2.8 (a)

The lines represent different protons in the same substrate

a) $$\triangle = \frac{\triangle 1 \left[E_T\right]}{\left[S_T\right]} \quad \ldots \; 2.8$$

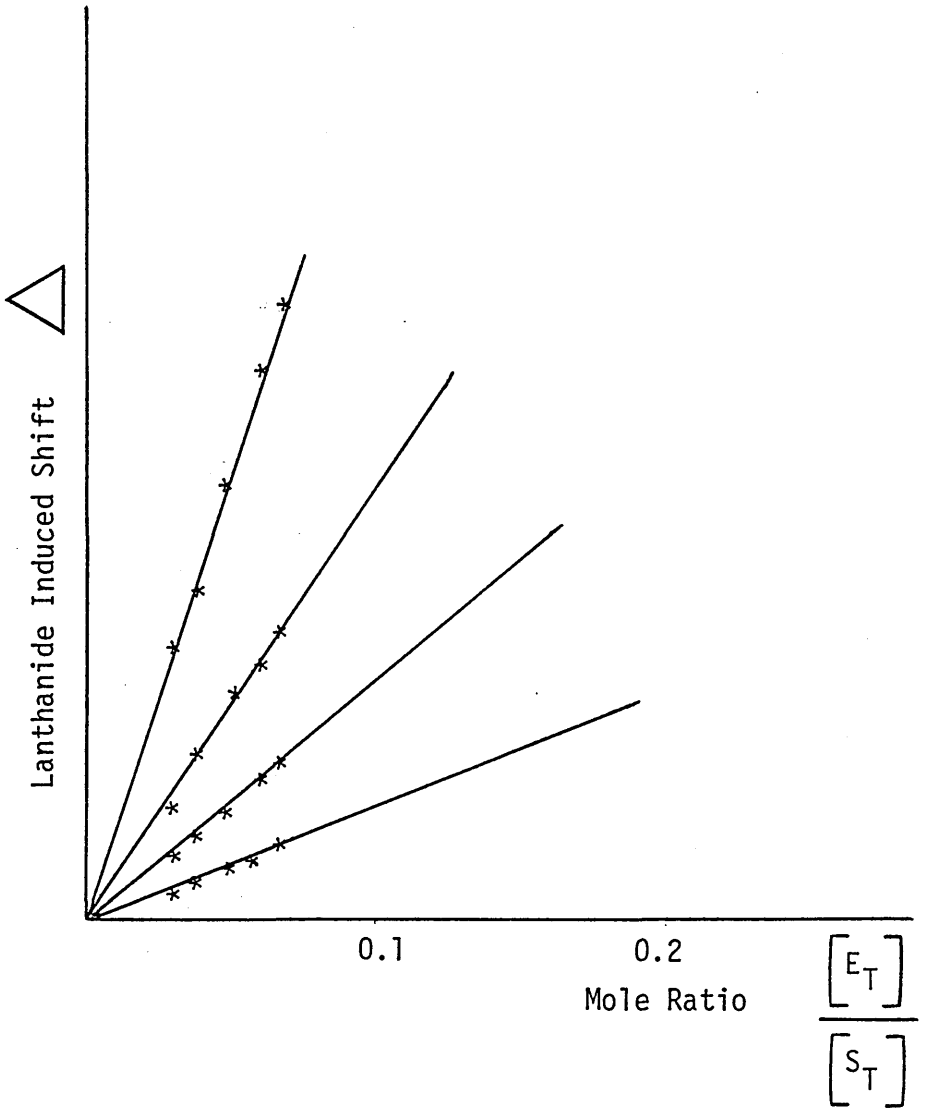
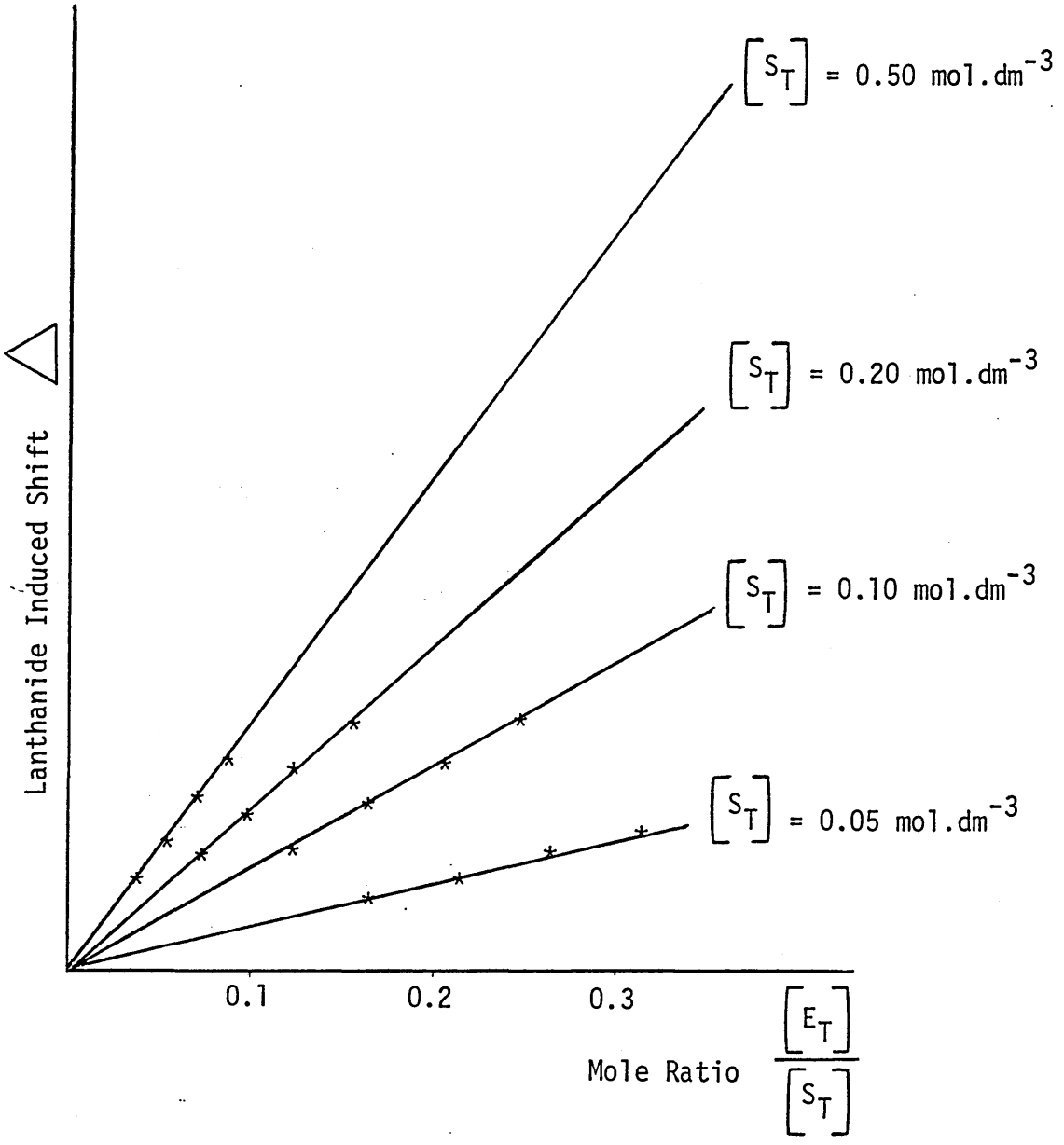

Lanthanide Induced Shift
Δ
0.1
0.2
Mole Ratio
[E_T] / [S_T]

Figure 2.3

A plot of the induced shift versus the mole ratio, illustrating substrate concentration dependency.

All the lines represent the same proton for which the shift reagent concentration was varied whilst the substrate concentration was kept constant at the value listed for that line (32).

$$\triangle = \frac{K\left[S_T\right]\triangle 1}{\left(\frac{\left[E_T\right]}{\left[S_T\right]}\right)} \qquad \ldots \quad 2.9$$


Lanthanide Induced Shift Δ
[S_T] = 0.50 mol.dm^-3
[S_T] = 0.20 mol.dm^-3
[S_T] = 0.10 mol.dm^-3
[S_T] = 0.05 mol.dm^-3
0.1
0.2
0.3
Mole Ratio [E_T]/[S_T]

values are obtained at the higher substrate concentrations. It is also found that at these higher substrate concentrations larger limiting incremental shift values are determined (57).

In view of the uncertainty of which limit applies, or indeed if some contribution from both limits is to be used, this method is not recommended for the determination of absolute limiting incremental shift values.

Numerous other simple methods have also been described for determining intrinsic parameters (27, 58, 59, 60). Generally however the stoichiometry of the substrate-shift reagent interaction has been assumed to be 1:1 and inherent in the methods have been some simple approximations whereby the concentration range has been restricted to the region where $[S_T] \gg [E_T]$. In addition to these studies Bouquant and Chuche (61) have carried out solvent studies and have shown that the equilibrium binding constant of a particular substrate - shift reagent complex increases in magnitude along the solvent series deuterochloroform, benzene, carbon disulphide, carbontetrachloride and n-hexane. The same authors have also reported that the limiting incremental shift of a proton in a substrate molecule remains approximately constant throughout the solvent series. The authors point out that this is to be expected if the same geometrical substrate - shift reagent structure is present in all solvents. Distance and angle measurements of the structure will be approximately equal which in turn will lead to similar limiting incremental shift values.

Faced with the apparent success of these simple methods and considering the experimental precautions that are necessary for reliable intrinsic parameter determinations, a series of experiments were performed, the

object of which was to obtain accurate and reliable intrinsic parameter values.

2.3.2 Results based on a 1:1 stoichiometry

The correct interpretation of the chemical shifts induced by lanthanide shift reagents in the spectra of organic substrates requires substantial knowledge of the nature of the adduct species present in solution. This knowledge can only be obtained from a detailed study of intrinsic parameters. The methods presented by Armitage et al and by Kelsey appeared satisfactory in the way equilibrium binding constants and limiting incremental shifts were obtained. Consequently the method derived by Kelsey was chosen as a means of determining reliable intrinsic parameters. The experimental factors outlined earlier were considered and extreme experimental precautions taken to ensure the accuracy of the results obtained.

Suitable model compounds that possessed monofunctional groups and that were most likely to exhibit pseudocontact induced shifts were studied. Generally these compounds were stable at room temperature and easily purified. Also the chemical shifts of the free substrates measured in the absence of shift reagent, were clear and well defined. It has been suggested (62) that chemical shifts of the free substrate can be obtained from a plot of the lanthanide observed shift versus the mole ratio. Interpolation to zero shift reagent concentration then gives the value of the chemical shift of the free substrate. This method is particularly useful when the chemical shifts of the free substrate are hidden or obscured by other resonance frequencies. However it has also been reported (63) that the chemical shifts of

the free substrate obtained in this way can vary quite significantly with the actual chemical shift values of the free substrate. Since reliable chemical shift positions are necessary for accurate intrinsic parameter determinations it was considered that compounds containing proton chemical shifts that were clear and well defined were best suited for such determinations.

Wherever possible substrates possessing proton groups capable of spin-spin coupling were not used. At low substrate concentrations spin-spin coupling between the proton groups present in the substrate cause rapid diminution of the signal intensity resulting in uncertainty in the precise chemical shift positions.

In view of solubility effects, toxicity, cost and ease of handling, deuterochloroform was the solvent used throughout these studies and the rigorous experimental conditions employed for the work in this thesis are given in Chapter V.

Suitable compounds were then studied and the induced shift measured as a function of increasing substrate concentration whilst the shift reagent concentration was kept constant.

From the substrate and shift reagent concentrations, and the induced shift measurements, a graph was plotted according to the equation derived by Kelsey (equation 2.6). A method of least squares was used to obtain the best straight line and a linear correlation coefficient was determined. The gradient and the intercept values were then calculated by a least squares analysis.

The exact nature of the stoichiometry at room temperature between shift reagent and substrate has yet to be determined. Consequently, in the methods used for the determination of equilibrium binding

constants and limiting incremental shifts there has generally been an assumption made regarding the reaction stoichiometry. Inherent in Kelsey's method was an assumption of a 1:1 stoichiometry. Strictly speaking in the calculations used, activities and not molar concentrations should be used to determine equilibrium binding constants. However, it has been usual to incorporate an assumption where the activity coefficients of the species present in solution approximate to unity. This assumption is common to most methods used in investigations of complex formations as most measurements have involved dilute solutions of substrate and shift reagent in some inert solvent. In this thesis, the molarity equilibrium constant is calculated, as this has been the value most widely determined.

In all the systems studied, when the shift reagent concentration was kept constant, the lanthanide induced shift increased as the substrate concentration decreased. This is in total agreement with the induced shift prediction based on equation 1.1 (page 9). Also the pseudocontact shift mechanism seemed predominant. The nuclei positioned close to the central metal ion suffered much larger induced shifts than those nuclei more distant from the lanthanide ion.

Using Kelsey's equation,

$$[S_T] = \frac{[E_T]\,\Delta 1}{\Delta} - \frac{1}{K} \qquad \ldots 2.6$$

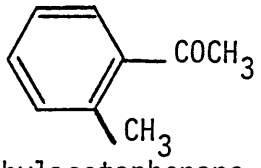
a plot of the substrate concentration versus the reciprocal induced shift was used to calculate equilibrium binding constants and limiting incremental shift values. Table 2.4 highlights some typical results

Table 2.4

Equilibrium Binding Constants obtained from a plot of equation 2.6[a]

a) $Eu(fod)_3$ - substrate complexes in $CDCl_3$.
b) Units of $dm^3.mol^{-1}$.
c) Linear correlation coefficient.

$$\left[S_T\right] = \frac{\left[E_T\right]\triangle 1}{\triangle} - \frac{1}{K} \qquad \ldots \quad 2.6$$

Substrate	Proton Signal	K (b)	r (c)
$CH_3COCH_2CH_2CH_3$ n-propylmethylketone	$C\underline{H}_3CO$ $COC\underline{H}_2$ $C\underline{H}_2CH_3$ $CH_2C\underline{H}_3$	79 164 76 121	0.9996 0.9998 0.9996 0.9980
 2-methylacetophenone	$C\underline{H}_3CO$ 2 $C\underline{H}_3$ 6 $\underline{H}$	140 133 142	1.0000 1.0000 0.9996
$(CH_3)_3CC(CH_3)_2OH$ 2,3,3-Trimethylbutan-2-ol	$(C\underline{H}_3)_3$ $(C\underline{H}_3)_2$	3624 3510	1.0000 0.9999
$((CH_3)_2CH)_2CHOH$ 2,4-Dimethylpentan-3-ol	$C\underline{H}_3$(i) $C\underline{H}_3$(ii) $(CH_3)_2C\underline{H}$ $(CH_3)_2CHC\underline{H}$ $O\underline{H}$	131 107 101 81 111	0.9998 1.0000 0.9996 0.9996 0.9999
$(CH_3)_2CHCH_2OH$ isobutanol	$(C\underline{H}_3)_2CH$ $(CH_3)_2C\underline{H}$ $C\underline{H}_2OH$ $O\underline{H}$	220 95 3584 204	1.0000 1.0000 0.9995 0.9999
$(CH_3)_2CHCH(CH_3)OH$ 3-methylbutan-2-ol	$(C\underline{H}_3)_2CH$ $(CH_3)_2C\underline{H}$ $CH_3C\underline{H}$ $C\underline{H}_3CH$ $O\underline{H}$	161 180 698 524 123	1.0000 0.9999 1.0000 0.9998 0.9999

obtained from the graphs plotted.

Excellent straight lines are obtained as are shown by the very good linear correlation coefficients. Shapiro and Johnston (33) have reported that the non-linearity of straight lines of this type is an excellent way in which the presence of small amounts of impurities can be shown. Consequently, if good linear correlation coefficients were not produced then the results obtained were not used.

Despite these good linear correlation coefficients however the agreement between the equilibrium binding constants calculated for different protons in the same substrate molecule is surprisingly poor. Although some exceptions are shown in Table 2.4, even when very large K values are determined, the inconsistencies are generally common. This contrasts directly with those results reported by Armitage et al (38). The equilibrium binding constants calculated by Armitage are very small compared with the values shown in Table 2.4 and consequently the inconsistencies could result from the extremely small differences arising in the measured intercept values of the different protons which are extremely critical when large K values are calculated. As a result of these inconsistencies in the determined values the method was considered too insensitive for accurate equilibrium binding constant determinations, especially when K values greater than 200 are involved.

Even so, the results in Table 2.4 do show that the general order of stability of the complexes formed in solution between lanthanide shift reagents and organic substrates agree very well with published data (64,65). The observation that 2,3,3-trimethylbutan-2-ol possesses such a large equilibrium binding constant at first appears outstanding.

However since this compound is extremely hygroscopic and forms stable hydrates (66) perhaps this result is not too unexpected. In view of the very large equilibrium binding constant it would seem that electronic considerations of this substrate far outweigh any steric hindrance effect which may arise due to the presence of the substituted methyl groups. Also, from Table 2.4 it would appear that primary alcohols generally coordinate better with lanthanide shift reagents than do secondary alcohols, which in turn coordinate better than ketones.

The effect of steric hindrance is shown by 2,4-dimethylpentan-3-ol. A relatively much smaller equilibrium binding constant is observed compared with those of the other alcohols studied. The presence of the isopropyl groups so close to the coordinating site probably inhibits the approach of the shift reagent. Consequently the coordinating ability of the substrate is reduced, resulting in a subsequent decrease in the equilibrium binding constant.

Limiting incremental shift values also obtained from a plot of equation 2.6 are shown in Table 2.5 together with linear correlation coefficients. From the table it is evident that there exists certain similarities between the limiting incremental shift values for substrates possessing similar structural features. The similarities observed would support the presence of the pseudocontact shift mechanism since induced shifts resulting from this interaction are dependent on the adduct structures. The fact that slight differences are observed for substrates with similar structural features could also result from some steric hindrance effect due to the different substituent groups present near the coordinating site. The variation

Table 2.5

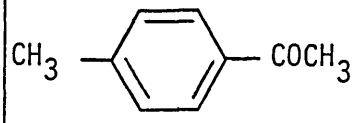
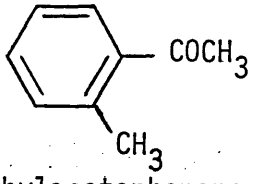
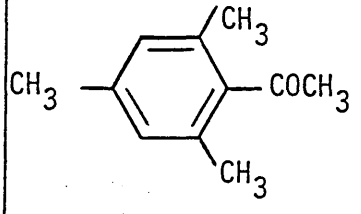
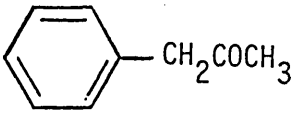
Limiting incremental shifts obtained via a plot of equation 2.6 (a)

a) Eu(fod)3 - substrate complexes in $CDCl_3$.
b) Units of Hz.
c) Linear correlation coefficient.

$$\left[S_T\right] = \frac{\left[E_T\right]\Delta 1}{\Delta} - \frac{1}{K} \qquad \ldots 2.6$$

Substrate	Proton Signal	$\Delta\nu_{1/2}$ (b)	r(c)
$CH_3COCH_2CH_2CH_3$ n-propylmethylketone	$\underline{CH_3}CO$ $CO\underline{CH_2}$ $\underline{CH_2}CH_3$ $CH_2\underline{CH_3}$	1033 962 748 347	0.9996 0.9998 0.9996 0.9980
$CH_3COCH(CH_3)_2$ isopropylmethylketone	$\underline{CH_3}CO$ $CO\underline{CH}$ $(\underline{CH_3})_2$	1034 924 602	0.9999 0.9998 1.0000
$CH_3COCH=C(CH_3)_2$ 4-methylpentan-3-ene-2-one	$\underline{CH_3}CO$ $CO\underline{CH}$ $(\underline{CH_3})$(i) $(\underline{CH_3})$(ii)	1010 820 703 303	0.9995 0.9870 0.9995 0.9999
$(CH_3)_2CHCH_2OH$ isobutanol	$O\underline{H}$ $CH_2O\underline{H}$ $(CH_3)_2C\underline{H}$ $(\underline{CH_3})_2CH$	7302 1585 1149 667	0.9999 0.9995 1.0000 1.0000
$CH_3CH=CHCH_2OH$ but-2-ene-1-ol	$O\underline{H}$ $CH_2O\underline{H}$ $\underline{CH_3}$	6932 1531 290	1.0000 0.9999 0.9992
$(CH_3)_3COH$ tert. butanol	$(\underline{CH_3})_3$	1022	0.9914
$(CH_3)_2CHCH(CH_3)OH$ 3-methylbutan-2-ol	$(CH_3)_2C\underline{H}$ $\underline{CH_3}CH$ $(\underline{CH_3})_2CH$	1133 1044 742	0.9999 0.9998 1.0000

..... contd.

Substrate	Proton Signal	$\Delta\nu$ (b)	r(c)
 4-methylacetophenone	$\underline{CH_3}CO$ 2-6 $\underline{H}$ 3-5 $\underline{H}$ 4 $\underline{CH_3}$	1007 763 156 154	0.9998 1.0000 0.9996 1.0000
 2-methylacetophenone	$\underline{CH_3}CO$ 6 $\underline{H}$ 2 $\underline{CH_3}$	932 596 743	1.0000 0.9996 0.9999
 2,4,6-trimethylacetophenone	$\underline{CH_3}CO$ 2-6 $\underline{CH_3}$ 3-5 $\underline{H}$ 4 $\underline{CH_3}$	727 372 213 241	0.9996 1.0000 0.9998 1.0000
 benzylmethylketone	$\underline{CH_3}CO$ $\underline{CH_2}$	953 940	0.9998 0.9999

in the limiting incremental shift values for a series of substituted aromatic ketones can also be seen in Table 2.5. It appears that the effect of steric hindrance causes a drastic lowering of some limiting incremental shifts and yet at the same time causes other groups to exhibit larger limiting incremental shift values. This may be explained by the fact that steric hindrance due to the substituent groups present possibly alters the approach of the shift reagent towards the coordination site. Consequently the difference in the limiting incremental shift values results from the change brought about in the proton-lanthanide metal angle and proton-lanthanide metal distance within the adduct structure as the shift reagent coordinates with the substrate in a different position. This explanation also supports the predominance of the pseudocontact shift mechanism. However, in view of the disagreement between the calculated K values for protons in the same substrate, a different approach was adopted in order to find a suitable alternative method for determining the intrinsic parameters.

2.3.3 Results based on a 2:1 stoichiometry

A one-step 2:1 reaction mechanism

Armitage (39) has pointed out that if a one-step 2:1 substrate-shift reagent interaction of the type

$$E + 2S \rightleftharpoons ES_2 \qquad \ldots\ 2.10$$

is considered, then an equilibrium binding constant K^* for this reaction can be given by

$$K^* = \frac{\left[ES_2\right]}{\left(\left[E_T\right] - \left[ES_2\right]\right)\left(\left[S_T\right] - 2\left[ES_2\right]\right)^2} \qquad \ldots\ 2.11$$

Since the fast exchange condition still applies the observed shift will be a weighted average of the chemical shift of the free substrate and the chemical shift of the stoichiometric 2:1 complex.

Hence

$$\triangle = \frac{2[ES_2]\,\triangle_2}{[S_T]} \qquad \ldots 2.12$$

where the symbols used have their usual meaning.

If experiments are restricted to the concentration range where

$$[S_T] >> [E_T] \quad \text{and assuming} \quad \left([S_T] - 2[ES_2]\right) = [S_T]$$

substitution of $[ES_2]$ from equation 2.12 into equation 2.11 gives by rearrangement equation 2.13 (39).

$$[S_T]^2 = \frac{2[E_T]\,\triangle_2\,[S_T]}{\triangle} - \frac{1}{K^*} \qquad \ldots 2.13$$

This equation corrects the reported equation which did not account for the stoichiometry factor 2, as is given in equation 2.12.

Using the experimental data obtained in the previous method, in which the induced shift was measured as a function of the substrate concentration whilst the shift reagent concentration was kept constant, plots of equation 2.13 were made. A least squares analysis was used to calculate the limiting incremental shift values and equilibrium binding constants which are obtainable from the slope and intercept measurements respectively. Linear correlation coefficients were also determined.

As before excellent straight lines are produced as are shown from some typical results presented in Table 2.6.

From a comparison of these results and those shown in Table 2.4 it would appear that the assumption of a one-step 2:1 reaction mechanism instead of a 1:1 stoichiometry leads to the determination of much larger equilibrium binding constants. Again however, little correlation exists between the equilibrium constants calculated for different protons in the same molecule. Perhaps in view of the accuracy required to measure the extremely small intercept values this is not too surprising. Although the equilibrium binding constants for the two mechanisms differ, the general order of stability of the complexes is approximately the same as before.

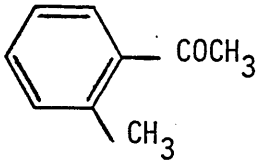
A comparison of the limiting incremental shift values calculated from the two equations (equations 2.6 and 2.13) shows that the delta max.1 values obtained when a 1:1 stoichiometry is assumed are approximately twice (2.01 - 2.07) those delta max. 2 values reported when a one-step 2:1 interaction is assumed. This is probably a result of the stoichiometry factor used in equation 2.10 to determine the induced shift of the 2:1 adduct. Alternatively, one may intuitively expect that a 1:1 adduct will possess a larger limiting incremental shift value than a 2:1 adduct. The possibility of steric crowding of two substrate molecules around the central metal ion results in a greater separation between the lanthanide metal and the coordinating sites, resulting in a smaller limiting incremental shift value being observed. Armitage and co-workers (67) have recently reported similar findings in that much larger equilibrium binding constants were calculated for an assumed one-step 2:1 stoichiometry while an assumed 1:1

Table 2.6

Intrinsic parameters obtained from a plot of equation 2.13 [a]

a) $Eu(fod)_3$ - substrate complexes in $CDCl_3$.
b) Units of $dm^6.mol^{-2}$.
c) Units of Hz.
d) Linear correlation coefficient.

$$\left[S_T\right]^2 = \frac{2\left[E_T\right]\triangle 2\left[S_T\right]}{\triangle} - \frac{1}{K^*} \quad \ldots \quad 2.13$$

Substrate	Proton Signal	K*(b)	$\triangle_2$ (c)	r(d)
$CH_3COCH_2CH_2CH_3$ n-propylmethylketone	$\underline{CH_3}CO$	1011	501	0.9999
	$CO\underline{CH_2}$	1475	476	0.9999
	$\underline{CH_2}CH_3$	793	364	0.9998
	$CH_2\underline{CH_3}$	814	172	0.9991
 2-methylacetophenone	$\underline{CH_3}CO$	3020	452	1.0000
	2 $\underline{CH_3}$	2723	359	1.0000
	6 $\underline{H}$	2621	290	0.9999
$(CH_3)_3CC(CH_3)_2OH$ 2,3,3-Trimethylbutan-2-ol	$(\underline{CH_3})_3$	61937	303	1.0000
	$(\underline{CH_3})_2$	23046	541	1.0000
$((CH_3)_2CH)_2CHOH$ 2,4-Dimethylpentan-3-ol	$\underline{CH_3}$(i)	2573	272	1.0000
	$\underline{CH_3}$(ii)	2383	356	1.0000
	$(CH_3)_2\underline{CH}$	1944	509	0.9999
	$(CH_3)_2CH\underline{CH}$	1597	964	0.9999
	$O\underline{H}$	2288	2974	1.0000
$(CH_3)_2CHCH_2OH$ isobutanol	$(\underline{CH_3})_2$	5968	325	1.0000
	$C\underline{H}$	-	541	1.0000
	$C\underline{H_2}$	-	790	0.9999
	$O\underline{H}$	5905	3544	1.0000
$(CH_3)_2CHCH(CH_3)OH$ 3-methylbutan-2-ol	$(\underline{CH_3})_2CH$	3540	362	1.0000
	$(CH_3)_2\underline{CH}$	3668	555	1.0000
	$CH_3\underline{CH}$	-	901	1.0000
	$\underline{CH_3}CH$	-	517	1.0000
	$O\underline{H}$	2821	3847	1.0000

stoichiometry led to the determination of smaller K values. Slightly different results regarding the limiting incremental shift values were however reported. The delta max. 1 values reported by Armitage for the assumed 1:1 stoichiometry were approximately 2.4 times the delta max. 2 values for the 2:1 stoichiometry.

Again however in view of the disagreement found for the equilibrium binding constants calculated for different protons in the same substrate molecule, another approach was tried.

A two-step 2:1 reaction mechanism

When a two-step 2:1 reaction mechanism of the type

$$\begin{aligned} E + S &\rightleftharpoons ES \\ ES + S &\rightleftharpoons ES_2 \end{aligned} \qquad \ldots \ 2.14$$

is considered, then two equilibrium binding constants need to be determined. These constants K_1 and K_2 are given by

$$K_1 = \frac{\left[ES\right]}{\left(\left[E_T\right] - \left[ES\right] - \left[ES_2\right]\right)\left(\left[S_T\right] - \left[ES\right] - 2\left[ES_2\right]\right)} \qquad \ldots \ 2.15$$

and

$$K_2 = \frac{\left[ES_2\right]}{\left[ES\right]\left(\left[S_T\right] - \left[ES\right] - 2\left[ES_2\right]\right)} \qquad \ldots \ 2.16$$

If the fast exchange condition applies there will only be one resonance signal observed for any particular substrate proton. Also since both 1:1 and 2:1 complexes may be present in solution simultaneously, the resonance frequency observed will be in a position which is a weighted average of the chemical shift of the free substrate, the chemical shift of the stoichiometric 1:1 complex and also the

chemical shift of the 2:1 complex. As already stated the limiting incremental shifts of the 1:1 and 2:1 adducts must in principle be considered different. The arrangement of two substrate molecules around the lanthanide metal ion will probably differ from that of one substrate molecule. Consequently whether any relationship exists between the two shift values must first be shown and not assumed. Hence the lanthanide induced shift for the 2:1 mechanism can be shown as

$$\triangle = \frac{[ES]\triangle_1}{[S_T]} + \frac{2[ES_2]\triangle_2}{[S_T]} \qquad \ldots 1.2$$

If, as before, the experimental conditions are restricted to the range where $[S_T] >> [E_T]$ then the approximation

$[S_T] = \left([S_T] - [ES] - 2[ES_2]\right)$ can be made and equations 2.15 and 2.16 can be rearranged to give equations 2.17 and 2.18 respectively.

$$K_1 = \frac{[ES]}{[S_T]\left([E_T] - [ES] - [ES_2]\right)} \qquad \ldots 2.17$$

$$[ES] = \frac{[ES_2]}{K_2[S_T]} \qquad \ldots 2.18$$

If $[ES]$ from equation 2.18 is substituted into equation 2.17 then equation 2.19 can be obtained.

$$[ES_2] = \frac{K_2[E_T][S_T]}{1 + K_2[S_T] + \frac{1}{K_1[S_T]}} \qquad \ldots 2.19$$

Furthermore, under these experimental conditions, i.e. where there is a great excess of substrate over shift reagent then $2[ES_2] \gg [ES]$ and the fast exchange condition reduces to

$$\triangle = \frac{2[ES_2]\,\triangle_2}{[S_T]} \qquad \ldots \ 2.20$$

Consequently substitution of $[ES_2]$ from equation 2.19 into equation 2.20 gives

$$\triangle = \frac{2\,K_2\,[E_T]\,\triangle_2}{1 + K_2[S_T] + \dfrac{1}{K_1[S_T]}} \qquad \ldots \ 2.21$$

In a situation where strong association prevails then the limit $1 + K_2[S_T] \gg {}^{1}\!/_{K_1[S_T]}$ can be considered. Using this limit, equation 2.21 reduces to

$$\triangle = \frac{2\,K_2[E_T]\,\triangle_2}{1 + K_2[S_T]} \qquad \ldots \ 2.22$$

which on rearrangement gives

$$[S_T] = \frac{2[E_T]\,\triangle_2}{\triangle} - \frac{1}{K_2} \qquad \ldots \ 2.23$$

Thus using experimental conditions where $[S_T] >> [E_T]$ and assuming a two-step 2:1 stoichiometry, a plot of $[S_T]$ versus ${}^{1}\!/\triangle$ enables delta max. 2 and K_2 to be calculated from the slope and intercept values respectively.

Equation 2.23 is however synonymous with equation 2.6 which was derived by Kelsey when a 1:1 $[S_T]$ versus $1/\Delta$, assumed. If a 1:1 stoichiometry is assumed de[illegible] can in principle be obtained from a plot of $[S_T]$ versus $1/\Delta$, whereas if a two-step 2:1 stoichiometry is assumed the values obtained from the slope and intercept measurements now give delta max. 2 and K_2. Least squares analysis result in identical linear correlation coefficients and the only difference regarding the slope and intercept values lay in their interpretation. Since the use of equation 2.6 was considered too insensitive for the determination of equilibrium binding constants, no advantage is offered by equation 2.23 for determining the intrinsic parameters.

If an alternative limit for equation 2.21 is chosen, say $K_2[S_T] + 1/K_1[S_T] \gg 1$ in which strong association still holds true, then equation 2.21 reduces to

$$\Delta = \frac{2\,K_2[E_T]\,\Delta_2}{K_2[S_T] + \dfrac{1}{K_1[S_T]}} \qquad \ldots\ 2.24$$

which rearranges to

$$[S_T]^2 = \frac{[S_T]\,2\,[E_T]\,\Delta_2}{\Delta} - \frac{1}{K_1\,K_2} \qquad \ldots\ 2.25$$

A plot of this equation $[S_T]^2$ versus $[S_T]/\Delta$ thus enables delta max. 2 and the product of the two equilibrium binding constants to be calculated from the slope and intercept values respectively. This equation however is very similar to the one derived by Armitage

and co-workers when a one-step 2:1 stoichiometry was assumed, i.e. equation 2.13. Although the slope values in both equations enable delta max. 2 to be calculated, the interpretation of the intercept values depends upon the stoichiometry assumed. As already stated if a one-step 2:1 stoichiometry is assumed, then in principle K^* is obtained whereas if a two-step 2:1 stoichiometry is assumed the product of K_1 and K_2 is determined.

When Armitage and co-workers obtained their equation for determining the intrinsic parameters of a one-step 2:1 stoichiometry, they postulated a stoichiometry dependency on the solvent used (67). According to Armitage et al., in the norcamphor - $Eu(fod)_3$ system the substrate-shift reagent stoichiometry changes from 1:1 to 2:1 when the solvent used changes from carbon tetrachloride to deuterochloroform. This conclusion was reached when plots of equation 2.6 and 2.13, obtained from the same data, were compared. When carbontetrachloride was used a plot of $[S_T]$ versus $1/\Delta$ gave a much better straight line fit than did a plot of $[S_T]^2$ versus $[S_T]/\Delta$. This was interpreted as indicating a 1:1 stoichiometry. In the case of deuterochloroform however the reverse was found, i.e. a plot of $[S_T]^2$ versus $[S_T]/\Delta$ gave a better straight line fit than a plot of $[S_T]$ versus $1/\Delta$ suggesting a one-step 2:1 stoichiometry. The derivation of equations 2.23 and 2.25 however discredit this theory since plots of $[S_T]$ versus $1/\Delta$ and $[S_T]^2$ versus $[S_T]/\Delta$ both give straight line fits which are suggestive of a two-step 2:1 stoichiometry.

In earlier experiments performed in this laboratory involving alcohol-

$Eu(fod)_3$ systems in deuterochloroform, plots of $[S_T]^2$ versus $[S_T]/\Delta$ gave better straight line fits than did plots of $[S_T]$ versus $1/\Delta$. However the solutions used in these early experiments were thought to contain traces of moisture and/or other impurities. Only when rigorous purification measures were carried out were similar straight line fits obtained for plots of both equations. Consequently the results shown in Table 2.4, instead of representing equilibrium constants for a 1:1 stoichiometry, could represent the K_2 values of a two-step 2:1 mechanism. Similarly the limiting incremental shift values shown in Table 2.5 (divided by two) could be the corresponding delta max. 2 values. Also the results in Table 2.6 can now be interpreted according to equation 2.25, the large equilibrium binding constants reported, representing the product (K_1K_2) of a two-step 2:1 stoichiometry and not K*of a one-step 2:1 stoichiometry.

In theory, since both the above mentioned limits used in the derivation of equations 2.23 and 2.25 are applicable for systems where large equilibrium binding constants are expected i.e. strong association, then it would seem possible that by combining the use of plots of equations 2.23 and 2.25, values of delta max. 2, K_2 and K_1 can be determined. Indeed this was attempted and the results for the substrate, 2,4-dimethylpentan-3-ol are shown in Table 2.7.

As is shown the delta max. 2 values obtained from the slope values of plots of both equations agree very well with each other and are generally to within 2%. This is in total agreement with the predicted behaviour based on the assumption of a two-step 2:1 stoichiometry and is much better than the agreement found by Armitage et al. (67), where a figure of about 9% was obtained. Also when the product of

Table 2.7

Intrinsic parameters obtained from plots of equations 2.23 and 2.25 [a]

a) 2,4-dimethylpentan-3-ol - $Eu(fod)_3$ complex in $CDCl_3$
b) Units of Hz
c) Units of $dm^6.mol^{-2}$.
d) Units of $dm^3.mol^{-1}$.

$$[S_T] = \frac{2[E_T]\triangle^2}{\triangle} - \frac{1}{K_2} \quad \ldots 2.23$$

$$[S_T]^2 = \frac{2[E_T]\triangle^2[S_T]}{\triangle} - \frac{1}{K_1K_2} \quad \ldots 2.25$$

$((CH_3)_2CH)_2CHOH$

Proton Signal	$\triangle_2$ (b)			K_2 (d)	(K_1K_2) (c)	$\frac{(K_1K_2)}{K_2} = K_1$ (d)
	Equation 2.23	Equation 2.25	Mean	Equation 2.23	Equation 2.25	
$\underline{OH}$	3077	2974	3026 ± 51 (1.7%)	111	2288	21
$\underline{CH}OH$	1006	964	985 ± 21 (2.1%)	81	1597	20
$(CH_3)_2\underline{CH}$	524	509	516 ± 8 (1.5%)	101	1944	19
$\underline{CH}_3$(i)	369	356	362 ± 6 (1.7%)	107	2383	22
$\underline{CH}_3$(ii)	280	272	276 ± 4 (1.4%)	131	2573	20

the equilibrium binding constants (K_1K_2) obtained from equation 2.25 is divided by the corresponding K_2 value obtained from equation 2.23, a value representing K_1 is given. Again good agreement was found as is shown by the values reported in Table 2.7.

Initially this procedure appeared to be quite promising. From a series of 17 substrate - $Eu(fod)_3$ systems in deuterochloroform incorporating over 60 proton signals, the slope values, and hence limiting incremental shift values, obtained from a plot of equation 2.23 gave results very much in agreement with those values obtained from equation 2.25. At worst the agreement was never more than $\pm$ 4%. However when the equilibrium binding constants were calculated the K_1 values determined always gave results which ranged between 16 - 24 $dm^3.mol^{-1}$. It thus appeared that every substrate studied possessed a K_1 value of about 20 $dm^3.mol^{-1}$. Intuitively this observation must result from the small but significant differences arising from the simple approximations made in deriving the equations. Although both approximations are based on strong association between the substrate and shift reagent, the small but finite differences between them significantly affects any combined use of the resulting equations derived. Consequently, although equations 2.23 and 2.25 can be used to obtain limiting incremental shift values, they could not be used to determine reliable equilibrium binding constants.

2.3.4 Conclusions

Several simple data treatment methods have been used to analyse the experimental shift obtained with lanthanide shift reagents. These methods have given rise to various interpretations. When a 1:1 stoichiometry is assumed a plot of $[S_T]$ versus $1/\Delta$ will

permit K and delta max. 1 to be determined. However if a two-step 2:1 stoichiometry is assumed the same plot enables values of K_2 and delta max. 2 to be calculated. In addition if it is assumed that the reaction mechanism has a one-step 2:1 stoichiometry then a plot of $[S_T]^2$ versus $[S_T]/\Delta$ will give K^* and delta max. 2 values. However the use of this plot to calculate the intrinsic parameters of a two-step 2:1 stoichiometry enables the product of K_1 and K_2 and a value of delta max. 2 to be determined. Table 2.8 summarises the equations and the intrinsic parameters obtainable when certain reaction mechanisms are assumed.

From the theory presented, the same limiting incremental shift values are expected from the slope values of the plots of $[S_T]$ versus $1/\Delta$ and of $[S_T]^2$ versus $[S_T]/\Delta$. At worst, the limiting incremental shifts found by this way in this laboratory differed by no more than $\pm$ 4% and in the majority of cases was much better. This compares with an average 9% reported by Armitage et al. (67). Also their postulate of a solvent-stoichiometry dependency, based on the use of these equations is shown to be incorrect. The results obtained in this laboratory so far show that accurate limiting incremental shifts can be determined from easily accessible data provided that rigorous experimental purification procedures are taken. The results also show however that very little agreement is found for the equilibrium binding constants calculated for different protons in the same substrate molecule. This is in spite of the expected behaviour predicted by theory and also the rigorous experimental precautions taken. One explanation is that the equilibrium binding

Table 2.8

A summary of the reaction mechanisms and equations

Reaction Mechanism	Reference	Equation	Parameters obtainable Slope	Intercept
$E + S \rightleftharpoons ES$ 1:1 stoichiometry	51	$[S_T] = \frac{[E_T]\triangle 1}{\triangle} - \frac{1}{K}$ ... 2.6	$\triangle 1$	K
$E + 2S \rightleftharpoons ES_2$ one-step 2:1 stoichiometry	39	$[S_T]^2 = \frac{2[E_T]\triangle 2[S_T]}{\triangle} - \frac{1}{K^*}$ ... 2.13	$\triangle 2$	K^*
$E + S \rightleftharpoons ES$ $ES + S \rightleftharpoons ES_2$ two-step 2:1 stoichiometry		$[S_T] = \frac{2[E_T]\triangle 2}{\triangle} - \frac{1}{K_2}$ ... 2.23 $[S_T]^2 = \frac{2[E_T]\triangle 2[S_T]}{\triangle} - \frac{1}{K_1K_2}$ ... 2.25	$\triangle 2$ $\triangle 2$	K_2 (K_1K_2)

constants are much too large to be calculated accurately by these "insensitive" methods. Nonetheless, as is shown, some information can be obtained using these simple data treatment methods.

It must be stressed however that these equations derived above can only be applied when the experimental conditions such that $[S_T] >> [E_T]$ are used i.e. in the mole ratio range of less than 0.1. Failure to observe this condition will result in non-linearity of the plots obtained. Also for reliable determinations the need for rigorous purification and experimental precautions cannot be over emphasised.

2.4 Rigorous Data Treatment Methods

2.4.1 Previous Methods

In 1969 Deranleau (68) gave a detailed description of the errors likely to be encountered in the determination of intrinsic parameters. However, the significance of this report was not widely appreciated or recognised until several years later. Deranleau showed that for a 1:1 stoichiometry

$$E + S \rightleftharpoons ES$$

the experiment in which $[ES]$ varies from zero to $[E_T]$ is conveniently described in terms of a saturation fraction of the dilute component. This saturation fraction, s, is defined as

$$s \equiv \frac{[ES]}{[E_T]}$$

$$1 \geq s \geq 0$$

Deranleau also showed that the most accurate values of the equilibrium binding constants are obtained when the saturation fraction lies between 0.2 and 0.8 and that outside this region the determined values become extremely uncertain. In addition to this insufficient data is available to adequately fit a given stoichiometric model. It was shown that approximately 75% of the saturation curve is required to show the correspondence between the equation of the model, i.e. the reaction mechanism, and the equation fitting the data. In other words, the concentration of the complex species present in solution must cover as wide a range as possible i.e. $0 \leqslant [ES] \leqslant [E_T]$

Mackie and Shepherd (69) were the first authors to use a "comprehensive" range of substrate and shift reagent concentrations. The shift reagent concentration was fixed at approximately 0.015 $mol.dm^{-3}$ and the substrate concentration varied between 0.02 and 0.3 $mol.dm^{-3}$. Consequently

$[S_T] \geqslant [E_T]$ and not as was previously used $[S_T] >> [E_T]$

when simple data treatment methods were discussed. The induced shifts of particular substrate protons were then measured as a function of the changing substrate concentration. An <u>exclusive</u> 1:1 stoichiometry was assumed and it was shown that substitution of $[ES]$ from equation 1:1

$$\triangle = \frac{[ES]\triangle_1}{[S_T]} \qquad \ldots \ 1.1$$

into equation 2.2

$$K = \frac{[ES]}{([E_T]-[ES])([S_T]-[ES])} \quad \ldots 2.2$$

leads by rearrangement to equation 2.26

$$K = \left(\frac{1}{\Delta} - \frac{1}{\Delta}\right)\left(\frac{[E_T]}{\Delta} - \frac{[S_T]}{\frac{1}{\Delta}}\right) \quad \ldots 2.26$$

In contrast to the simple data treatment methods equation 2.26 is derived without using any approximations whatever and Figure 2.4 shows the theoretical relationship obtained by Mackie and Shepherd for plots of the substrate concentration versus the reciprocal induced shift, computed on the basis of equation 2.26. Plots of this sort, used earlier in section 2.3 to determine intrinsic parameters were based on simple approximations when equations 2.6 and 2.13 were derived. From Figure 2.4 it can be seen that for large K values slight curvature is observed in the high mole ratio region. This curvature is observed in the simple data treatment methods when the experimental conditions $[S_T] >> [E_T]$ are not upheld and the simple approximations made no longer hold true. In these cases interpolation of the linear part of the curve to the y-axis results in a very small intercept value. It is this value which is used in the simple data treatment methods for determining the equilibrium binding constant, hence the need for great accuracy in this intercept measurement. For the determination of equilibrium binding constants Mackie and Shepherd guessed a trial value of delta max. 1 which was then computed

Figure 2.4

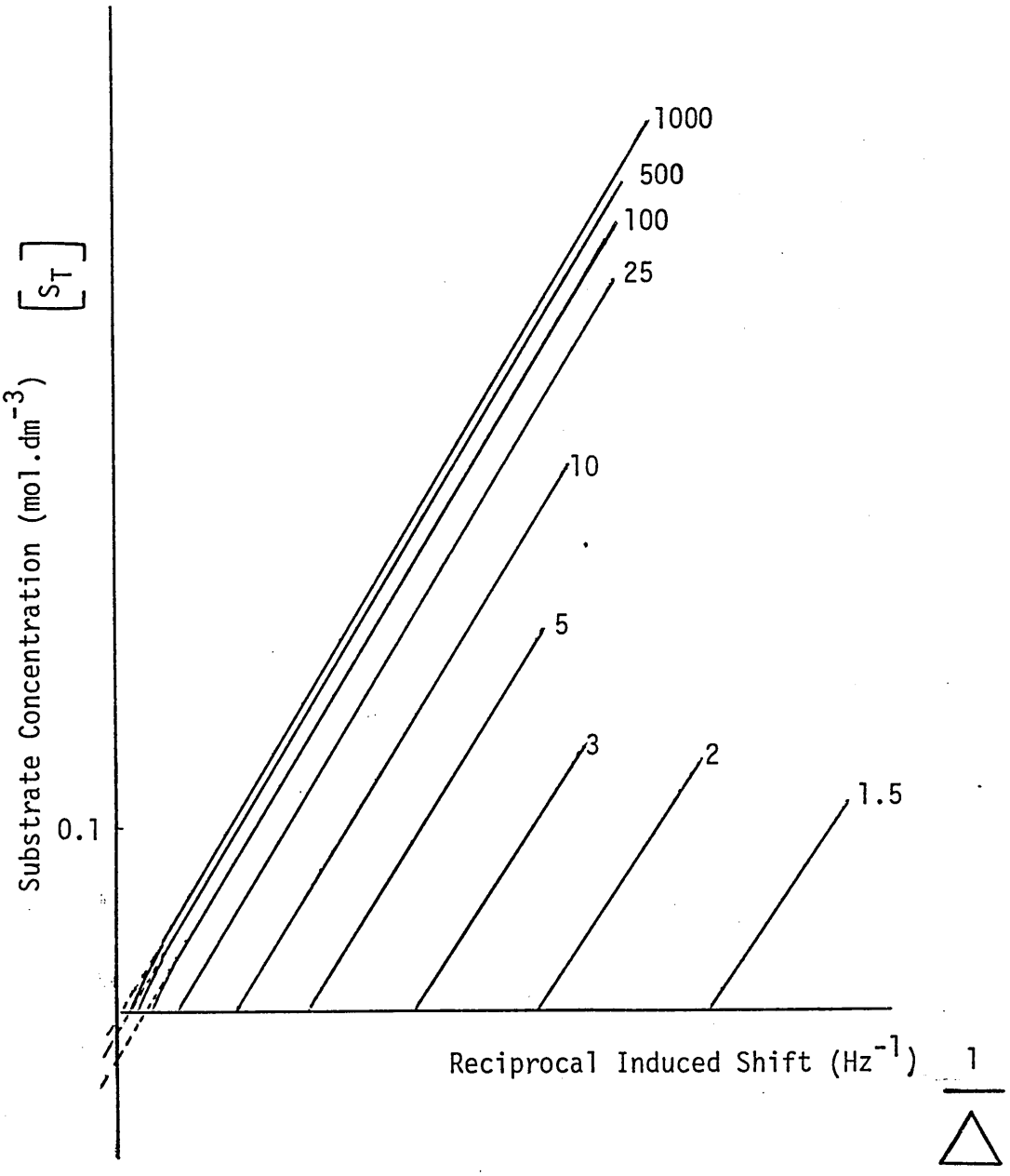
A computed relationship based on equation 2.26

K values are as indicated in units of $dm^3.mol^{-1}$.

$[E_T]$ = 0.015 $mol.dm^{-3}$

Δ_1 = 300 Hz.

$$K = \left(\Delta_1 - \Delta\right)\left(\frac{[E_T]}{\Delta} - \frac{[S_T]}{\Delta_1}\right) \quad \ldots \quad 2.26$$


1000
500
100
25
10
5
3
2
1.5
0.1
Substrate Concentration (mol.dm^{-3}) $[S_T]$
Reciprocal Induced Shift (Hz^{-1}) $\frac{1}{\Delta}$

into equation 2.26 with experimental values of $[E_T]$, $[S_T]$ and $\triangle$. A set of K values was then obtained for the various substrate concentrations used in the experiment and a percentage standard deviation obtained for the K values determined. The procedure was then repeated with other trial values of delta max. 1 until the most consistant set of K values, measured in terms of the percentage standard deviation, was obtained.

However, an examination of the computer program used by Mackie and Shepherd (see Appendix1)revealed that although a comprehensive range of substrate concentrations was used they were not utilised and the method was modified to one in which the conditions $[S_T] >> [E_T]$ prevailed. From the experimental values of the substrate concentrations and the induced shifts obtained by Mackie and Shepherd a plot of $[S_T]$ versus $1/\triangle$ was constructed and a least squares analysis carried out to obtain the best straight line. Consequently any point situated in a region of curvature as shown in Figure 2.4 is made to fit a straight line by a least squares analysis. Shift values from this straight line were then computed with trial values of delta max. 1 in equation 2.26 and equilibrium binding constants calculated as described. However this straight line can be obtained using experimental conditions where $[S_T] >> [E_T]$ as was described in the simple data treatment methods, without resorting to a comprehensive range of substrate concentrations.

Two main disadvantages of this method limit its use for determining reliable intrinsic parameters. Firstly, the exclusive assumption of a 1:1 stoichiometry prohibits any comparison that could be made with other reaction mechanisms. Secondly, when large K values are

expected, the curvature which is observed but fitted to a straight line analysis could result in large errors being introduced into the determination of equilibrium binding constants and to a lesser extent the limiting incremental shift values.

In 1972 Shapiro and Johnston (33) reported a detailed account of the substrate-shift reagent equilibrium. In this report a comprehensive range of concentrations, as recommended by Deranleau, was used to determine the intrinsic parameters of 1:1 and 2:1 reaction mechanisms. It was found that the agreement between the theoretically predicted and the experimentally observed data plots was much better when a 2:1 stoichiometry was assumed than when a 1:1 stoichiometry was assumed. For a 1:1 stoichiometry, values of K and delta max. 1 were obtained by minimising a quantity Q

$$Q = \sum_{i=1}^{i=N} \left(\Delta_{ob_i} - \Delta_{ca_i} \right)^2$$

where N is the number of data points

Δ_{ob_i} is the observed lanthanide induced shift at a particular data point and

Δ_{ca_i} is the corresponding calculated lanthanide induced shift.

Calculated lanthanide induced shift values were obtained by using a trial (guess) value of K, from which values of $[ES]$ were calculated for a series of substrate and shift reagent concentrations. This was done by solving the quadratic equation

$$[ES]^2 - [ES]\left([S_T] + [E_T] + \frac{1}{K}\right) + [E_T][S_T] = 0 \quad \ldots \quad 2.3$$

The best delta max. 1 value corresponding to this trial K value was then determined by minimising Q with respect to delta max. 1

$$\frac{\partial Q}{\partial \Delta 1} = 0 = \frac{\sum_{i=1}^{i=N} \left(\Delta_{ob_i} - \frac{[ES]\,\Delta 1}{[S_T]} \right)^2}{\Delta 1}$$

Solving for delta max. 1 gave

$$\Delta 1 = \frac{\sum_{i=1}^{i=N} \frac{\Delta_{ob_i}\,[ES]}{[S_T]}}{\sum_{i=1}^{i=N} \left(\frac{[ES]}{[S_T]} \right)^2}$$

or using a shorthand notation for the summations

$$\Delta 1 = \frac{S_{\circ}\,\Delta\alpha}{S.\,\alpha^2}$$

Using this best delta max. 1 value the calculated induced shift value was then determined from

$$\Delta_{ca_i} = \frac{[ES]\,\Delta 1}{[S_T]}$$

and then Q calculated. The procedure was then repeated using different K values until a minimum value of Q was obtained. The K and delta max. 1 values resulting in the minimum observable Q value

were chosen as the intrinsic parameters of that system studied.

In a similar way Shapiro and Johnston showed that for a 2:1 stoichiometry

$$Q = \sum_{i=1}^{i=N} \left(\Delta_{ob_i} - \frac{[ES]\,\triangle 1}{[S_T]} - \frac{2[ES_2]\,\triangle 2}{[S_T]} \right)^2$$

and using the shorthand notation as before

$$\triangle 1 = \frac{S.\Delta\alpha \; S.\beta^2 - S.\Delta\beta \; S.\alpha\beta}{S.\alpha^2 \; S.\beta^2 - S.\alpha\beta^2}$$

and

$$\triangle 2 = \frac{S.\Delta\beta \; S.\alpha^2 - S.\Delta\alpha \; S.\alpha\beta}{S.\alpha^2 \; S.\beta^2 - S.\alpha\beta^2}$$

The same iterative procedure used for the 1:1 stoichiometic calculations was used to determine values of K_1 and K_2 and their associated delta max. 1 and delta max. 2 values. The minimum Q values obtained for both 1:1 and 2:1 stoichiometries were then compared to find which reaction machanism was favoured.

This report was the first major one of its kind to attempt a comparison between experimental data plots and theoretically predicted data plots of known intrinsic parameters for 1:1 and 2:1 stoichiometries. The comparison measured in terms of the agreement factor Q outlined above, was the basis by which intrinsic parameters were determined. Although this report is of considerable importance, there are nonetheless slight alterations necessary for the reliable determination

of intrinsic parameters. In view of the possibility of shift reagent dimerisation and the possible changes in the magnetic subsceptibility of the solution as the shift reagent concentration is increased, then the experimental conditions chosen by Shapiro and Johnston are perhaps unsuitable. The authors have used a constant substrate concentration of about 0.15 mol. dm^{-3} and the induced shifts are measured as the shift reagent concentration is allowed to vary between 0 - 0.45 mol. dm^{-3}. At shift reagent concentrations approaching 0.45 mol.dm^{-3} it is possible to envisage extensive shift reagent dimerisation. The effect of this shift reagent dimerisation however can be accounted for by appropriate changes in the equations used in the calculations. The effect on the lanthanide induced shift from the possible magnetic susceptibility changes however cannot be.

Since in the calculations used by Shapiro and Johnston for the case of the 2:1 stoichiometry, the ratio K_1/K_2 was not allowed to vary and was restricted to a value of 3.5,there may be other ratio values, other than this fixed one which could lead to the observation of even smaller Q values. (Justification for using a constant K_1/K_2 ratio was due to the prohibited use of excessive computer time). The intrinsic parameters associated with perhaps smaller agreement factors could reflect a truer value of the equilibrium occurring in solution. Also a percentage standard deviation would probably be better suited in these determinations. A degree of uncertainty exists in the measurement of very large induced shift values. This arises because the signal broadening characteristics of the lanthanide shift reagent prevents the mid point of the signal being accurately determined. For these large induced shifts the difference between the observed

and any calculated induced shift $\left(\triangle_{ob_i} - \triangle_{ca_i}\right)$ may be significant. However for small shift values where accuracy can be maintained in the measurement of the mid point of the resonance signal the difference between observed and calculated induced shifts may in absolute terms be small. In the iterative procedure used by Shapiro and Johnston for the agreement factor Q,

$$Q = \sum_{i=1}^{i=N} \left(\triangle_{ob_i} - \triangle_{ca_i}\right)^2$$

a theoretical curve is fitted to the experimental curve. This, in part, is done by minimising the difference between observed and calculated induced shifts. The difference between the observed and calculated induced shifts for large shift values (where accuracy is uncertain) will be minimised at the expense of the difference between the observed and calculated induced shifts for small shift values (where accuracy can be maintained). Hence the curve will fit closer to the larger but also to the least accurately determined shift values. Consequently the theoretical fit will place undue emphasis upon the least accurately determined data points. A percentage standard deviation will however treat all the data points equally. It is the percentage difference which will be minimised and not the absolute difference between observed and calculated induced shifts.

A report by Reuben (70) also arrived at similar conclusions to those reported by Shapiro and Johnston, i.e. the adducts formed in solution between substrate and shift reagent possessed a 2:1 stoichiometry. Again a comprehensive range of concentrations as recommended by

Deranleau was used to compare theoretical and experimental data plots. Since however in this particular case it appears that no rigorous experimental precautions were taken to ensure truly anhydrous conditions, the intrinsic parameters reported by Reuben must be regarded with scepticism. If experimental data plots are to be compared with theoretical data plots then extreme purification measures must be taken to ensure the reliability of the experimental data.

The agreement factor used by Reuben to compare theoretical and experimental data plots for 1:1 and 2:1 stoichiometries namely:

$$\text{standard deviation} = \left(\frac{\sum_{i=1}^{i=N} \left(\Delta_{ob_i} - \Delta_{ca_i} \right)^2}{N - 1} \right)^{\frac{1}{2}}$$

also possesses the disadvantage, outlined earlier, by giving undue emphasis to the least accurately determined data points. In view of the experimental conditions used, i.e. the substrate concentration was kept constant and the induced shift measured as a function of the changing shift reagent concentration, Reuben considered the possible effect of shift reagent dimerisation and concluded that only a slight difference occurred in the association between the substrate and shift reagent. This may however result from the use of impure solutions, the effect of which outweighed the effect of any shift reagent dimerisation.

The main features arising from the analysis carried out by Reuben were

i) that the lanthanide induced shifts for the ES and ES_2 complexes might be different and

ii) that the relative shift contributions from the 1:1 and 2:1 complexes

to the total induced shift depends upon the relationship between the equilibrium binding constants and the limiting incremental shifts.

Quoting Reuben

> "It thus appears that no general predictions can be made and for each case a complete analysis should be carried out ..... Clearly more examples and careful study are needed before making any generalisations".

In order therefore to make a significant contribution to the understanding of shift reagent equilibria, a detailed study of intrinsic parameter determinations for 1:1 and 2:1 stoichiometries was carried out in this laboratory.

2.4.2 Theoretical and Experimental Considerations

In view of the emphasis placed on the experimental conditions for the reliable determination of accurate intrinsic parameters, the most stringent precautions were taken to ensure anhydrous conditions. Also because of possible shift reagent dimerisation and magnetic susceptibility changes occurring with high shift reagent concentrations, the concentration of the lanthanide metal chelate was kept constant at about 0.006 mol. dm^{-3}. This concentration is sufficient to produce substantial shifts whilst at the same time cause minimum adverse effects. Also, in order that a comprehensive range of substrate concentrations could be studied, the concentration of the substrate was allowed to vary between approximately 0.05 and 0.005 mol. dm^{-3}. These low concentrations whilst helping to preserve solution ideality, also enabled molar concentrations and not activities to be used in the calculations. As a result of these low substrate concentrations rigorous experimental precautions were taken to ensure the purity of the solutions used. No assumptions regarding the stoichiometries were made and the experimental data plots obtained were compared, as will be shown, with

theoretically predicted data plots. These methods must therefore be based on reliable experimental data.

As already shown, the following equation may be derived for a 1:1 stoichiometry

$$[ES]^2 - [ES]\left([S_T] + [E_T] + \frac{1}{K}\right) + [E_T][S_T] = 0 \quad \ldots 2.3$$

A real solution to this quadratic equation is (71)

$$2[ES] = \left([E_T] + [S_T] + \frac{1}{K}\right) - \left(\left([E_T] + [S_T] + \frac{1}{K}\right)^2 - 4[E_T][S_T]\right)^{\frac{1}{2}} \quad \ldots 2.27$$

Consequently, using a trial value of K and substituting the experimental values of $[E_T]$ and $[S_T]$ it is possible to calculate values of $[ES]$. The values of $[ES]$ can then be used with a trial (guess) value of delta max. 1, so that the calculated induced shifts can be determined for a series of substrate concentrations.

$$\Delta_{ca_i} = \frac{[ES]\,\Delta 1}{[S_T]}$$

The calculated induced shift values are then compared with the observed shift values using a percentage standard deviation,

$$\% \text{ S.D.} = \left(\frac{\sum_{i=1}^{i=N} \left(\frac{100\left(\Delta_{ob_i} - \Delta_{ca_i}\right)}{\Delta_{ob_i}} \right)^2}{N - 1} \right)^{\frac{1}{2}}$$

As explained earlier the use of an equation of this type treats all the data points equally and ensures that no undue emphasis is placed on the least accurately determined data points. The procedure is then repeated with different K and delta max. 1 values and those values resulting in the minimum observable percentage standard deviation are chosen as the intrinsic parameters of that 1:1 reaction mechanism.

For the case of a 2:1 stoichiometry

$$K_1 = \frac{\left[ES\right]}{\left(\left[E_T\right] - \left[ES\right] - \left[ES_2\right]\right)\left(\left[S_T\right] - \left[ES\right] - 2\left[ES_2\right]\right)} \quad \ldots 2.15$$

and

$$K_2 = \frac{\left[ES_2\right]}{\left[ES\right]\left(\left[S_T\right] - \left[ES\right] - 2\left[ES_2\right]\right)} \quad \ldots 2.16$$

From equation 2.16

$$\left[ES_2\right] = \frac{K_2\left[ES\right]\left(\left[S_T\right] - \left[ES\right]\right)}{\left(1 + 2K_2\left[ES\right]\right)} \quad \ldots 2.28$$

which when substituted into equation 2.15 gives, by rearrangement,

$$A\left[ES\right]^3 + B\left[ES\right]^2 + C\left[ES\right] + D = 0 \quad \ldots 2.29$$

$$A = \frac{K_2\left(K_1 - 4K_2\right)}{K_1}$$

$$B = 1 - 2K_2\left[E_T\right] - \frac{4K_2}{K_1}$$

$$C = 2K_2[E_T][S_T] - [E_T] - [S_T] - \frac{1}{K_1} - [S_T]^2 K_2$$

$$D = [E_T][S_T]$$

It can be shown that when $K_2 = 0$, equation 2.29 reduces to equation 2.3, which is the equation for a 1:1 stoichiometry. Experimental values of $[S_T]$ and $[E_T]$ and trail values of K_1 and K_2 are computed into equations 2.28 and 2.29 and values of $[ES]$ and $[ES_2]$ calculated. These values are then used with trial (guess) values of delta max. 1 and delta max. 2 so that the calculated induced shifts can be determined.

$$\Delta_{ca_i} = \frac{[ES]\Delta 1}{[S_T]} + \frac{2[ES_2]\Delta 2}{[S_T]}$$

The calculated and experimental induced shift values for a series of substrate concentrations are then compared using the percentage standard deviation equation given above. Again the procedure is repeated with different intrinsic parameter values until a minimum percentage standard deviation is obtained. This minimum value is then compared with the minimum value obtained for a 1:1 stoichiometry. A listing of the computer program used to facilitate the enormous number of calculations is shown in the Appendix. Using Taylor's

expansion series, Newton's approximation to a root of an equation (72) was used to determine values of $[ES]$ in the quadratic and cubic equations given. In contrast with the work of Shapiro and Johnston, no restrictions were placed on the values of K_1 and K_2. Similarly with the trial values of delta max. 1 and delta max. 2. In any one determination approximately 200,000 combinations of K_1, K_2, delta max. 1 and delta max. 2 were tried in order to find a minimum percentage standard deviation.

Initially several theoretical curves were drawn in order that experience could be obtained in the type and shape of the data plots produced with known intrinsic parameters. These data plots were represented by graphs of the induced shift versus the mole ratio. The mole ratio being defined as the ratio of the total shift reagent concentration to the total substrate concentration. It has previously been shown in section 2.3.1 that for a 1:1 stoichiometry the initial slope of the plot of the induced shift versus the mole ratio can be used as a measure of the limiting incremental shift. This method, although NOT recommended for experiments in which the substrate concentration is kept constant whilst the shift reagent concentration is varied is however useful in these cases where a knowledge of the equilibrium binding constants is available and where suitable experimental conditions are used.

If the experimental conditions are performed so that mole ratio values remain less than 0.1 i.e. $[S_T] \gg [E_T]$ then for a 2:1 stoichiometry it can be shown that the following equations may be obtained

$$K_1 = \frac{[ES]}{[S_T]\left([E_T] - [ES] - [ES_2]\right)} \qquad \ldots \quad 2.17$$

$$\left[ES\right] = \frac{\left[ES_2\right]}{K_2\left[S_T\right]} \qquad \ldots \quad 2.18$$

Substitution of $\left[ES\right]$ from equation 2.18 into equation 2.17 gives by rearrangement,

$$\left[ES_2\right] = \frac{K_2\left[S_T\right]\left[E_T\right]}{1 + K_2\left[S_T\right] + \frac{1}{K_1\left[S_T\right]}}$$

Since the fast exchange condition still applies, i.e. only one resonance signal is observed, then the induced shift is given by

$$\triangle = \frac{\left[ES\right]\triangle_1}{\left[S_T\right]} + \frac{2\left[ES_2\right]\triangle_2}{\left[S_T\right]}$$

Substitution of $\left[ES\right]$ and $\left[ES_2\right]$ into this equation gives by rearrangement.

$$\triangle = \frac{\left[E_T\right]}{\left[S_T\right]}\left(\frac{\triangle_1 + 2\triangle_2 K_2\left[S_T\right]}{1 + K_2\left[S_T\right] + \frac{1}{K_1\left[S_T\right]}}\right) \qquad \ldots \quad 2.30$$

Three conditions may now apply. Firstly, when strong association takes place the approximation $K_2\left[S_T\right] \gg 1 + \frac{1}{K_1\left[S_T\right]}$ can be made and equation 2.30 rearranged to

$$\triangle = \frac{\left[E_T\right]}{\left[S_T\right]}\left(\frac{\triangle_1}{K_2\left[S_T\right]} + 2\triangle_2\right)$$

which reduces to

$$\triangle = \frac{[E_T]\ 2\ \triangle_2}{[S_T]} \qquad \ldots\ 2.31$$

Hence for strongly coordinated substrates a plot of the induced shift versus the mole ratio has an initial slope value equal to twice delta max. 2.

Secondly, if a weakly associated system prevails (or indeed a 1:1 stoichiometry exists) then an approximation $1 \gg K_2[S_T] + \frac{1}{K_1[S_T]}$ can be made, whereupon equation 2.30 rearranges to

$$\triangle = \frac{[E_T]\left(\triangle_1 + 2\triangle_2 K_2 [S_T]\right)}{[S_T]}$$

This equation then reduces to

$$\triangle = \frac{[E_T]\ \triangle_1}{[S_T]} \qquad \ldots\ 2.32$$

Consequently the same plot of the induced shift versus the mole ratio for weakly coordinated substrates will possess an initial slope value of delta max. 1.

Finally the induced shift versus mole ratio plot of a system containing intermediate coordinating substrates will consist of an initial slope value possessing unknown and indeterminate contributions of delta max. 1 and delta max. 2.

Figure 2.5 shows several theoretical data plots for a 1:1 stoichiometry. From these plots two useful factors can be of help in the analysis of

Figure 2.5

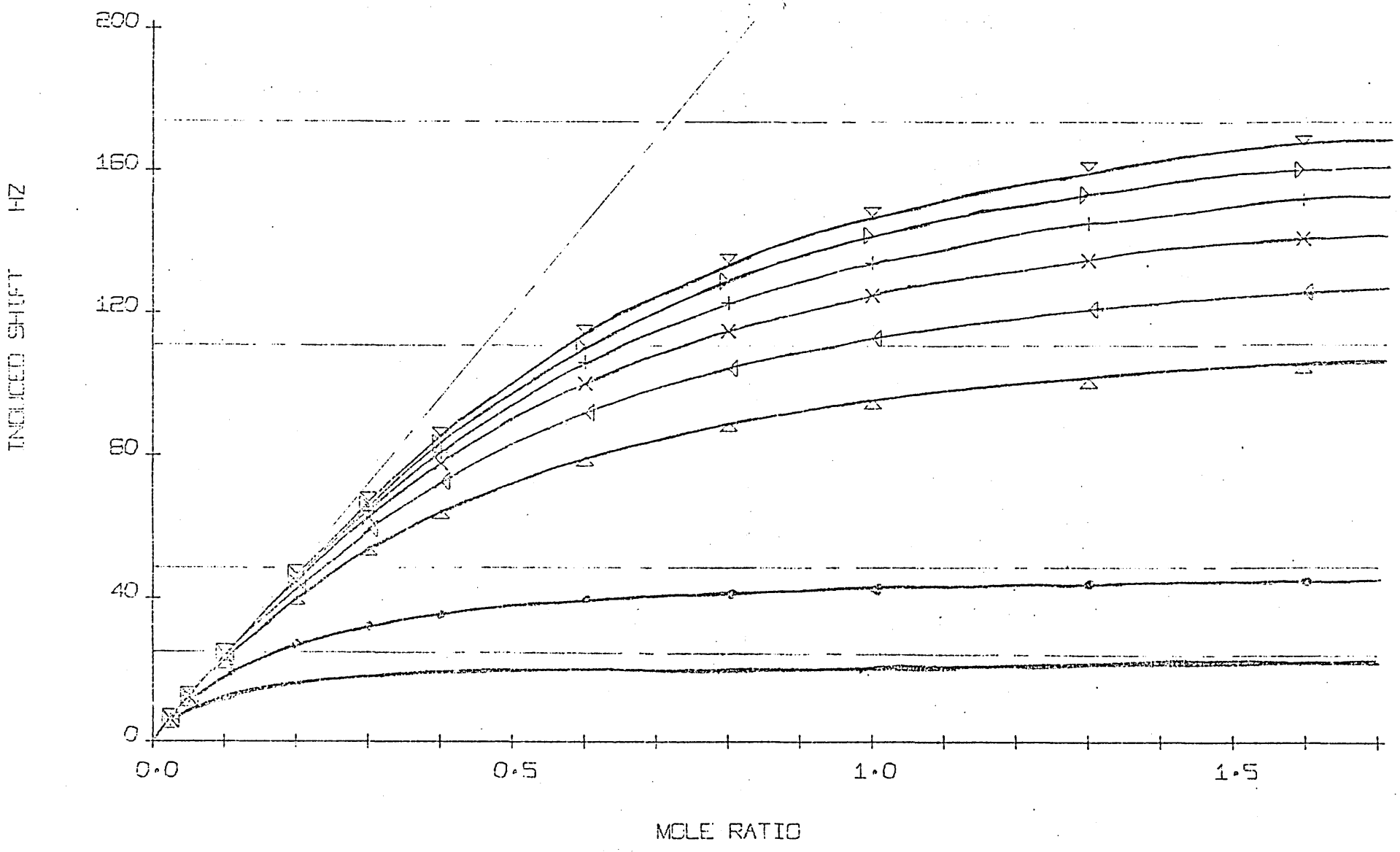
Theoretical data plots for a 1:1 stoichiometry

$[E_T]$ = 0.005 mol.dm^{-3}

$\triangle_1$ = 250 Hz.

Curve	K_1 dm^3.mol^{-1}.	Calculated Initial Slope Value $\triangle_1$ Hz.	Approximate Measurement of Maximum observed induced shift $\triangle$ max. Hz.
▽	700	248	175
▷	600	248	a
+	500	247	a
×	400	247	a
◁	300	246	a
△	200	244	110
•	50	227	50
—	20	199	25

a For clarity these values are not shown.


200
160
120
80
40
0
0·0
0·5
1·0
1·5
INDUCED SHIFT HZ
MOLE RATIO

the curve,

i) the initial slope value (obtained in the region where mole ratio values are less than 0.1) and

ii) the maximum observed induced shift value Δ_{max}, (obtained in the region where mole ratio values are greater than 1.0)

As is shown the initial slopes, calculated when K values are large, are in excellent agreement with the theoretical limiting incremental shift values used. However when the value of K becomes much smaller the initial slope values decrease rapidly. Also the region where the initial slope remains linear depends upon the value of the equilibrium binding constant. If K is large then the initial slope will be linear over a mole ratio range of between 0 - 0.2. However when K is small the linear region of the curve only extends over a mole ratio range of about 0 - 0.05, i.e. where $[S_T] >> [E_T]$

For a 1:1 stoichiometry the lanthanide induced shift produced by complexation increases monotonically until a maximum observed induced shift value is reached. This maximum observed induced shift value Δ_{max} is rarely consistent with the limiting incremental shift value Δ_1, and should therefore never be used as a measure of the limiting incremental shift. ApSimon et al. (74) have reported that the maximum observed induced shift gives a better approximation for the limiting incremental shift than does the initial slope value. From the results of the theoretical curves plotted however this would seem not to be the case. The predicted maximum observed induced shifts, although depending upon solubility limitations etc., increase as the corresponding equilibrium binding constants increase. Only when very large K values are expected does the maximum observed

induced shift approach the value of the limiting incremental shift. Figure 2.6 shows the theoretical data plot obtained for a 2:1 stoichiometry. The same intrinsic parameters used for the 1:1 stoichiometries are used in the first step of the association for these 2:1 stoichiometric cases. Initially the curves may appear very similar, however certain differences do exist between them. The effect of a second strong association step taking place is illustrated by a change in the initial slope value determined and since K_2 is large it is delta max. 2 that is calculated. Again excellent agreement is found for the slope values calculated and the limiting incremental shift values used. The value of K_1 appears to have very little effect on the slope values calculated, but this is expected since in this region where $\left[S_T\right] >> \left[E_T\right]$ the effect of the second association step predominates. For a 2:1 stoichiometry two cases arise,

i) $K_1 > K_2$ and

ii) $K_2 > K_1$

When $K_1 > K_2$ the lanthanide induced shift increases as the mole ratio values increase. This is similar to the 1:1 model. Again however the maximum observed induced shift does not represent a value of the limiting incremental shift. The maximum observed induced shifts measured for the examples where $K_1 > K_2$ are very similar to those obtained in the 1:1 stoichiometric cases reflecting identical K_1 and delta max. 1 values used in both stoichiometries.

When $K_2 > K_1$ the induced shift increases, reaches a maximum and then begins to decrease as the mole ratio values increase. This contrasts directly with the 1:1 model where the induced shift increased as the mole ratio values increased. As before however the maximum

Figure 2.6

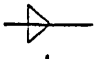
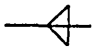
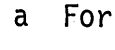
Theoretical data plots for a 2:1 stoichiometry

$[E_T]$ = 0.005 mol.dm^{-3}

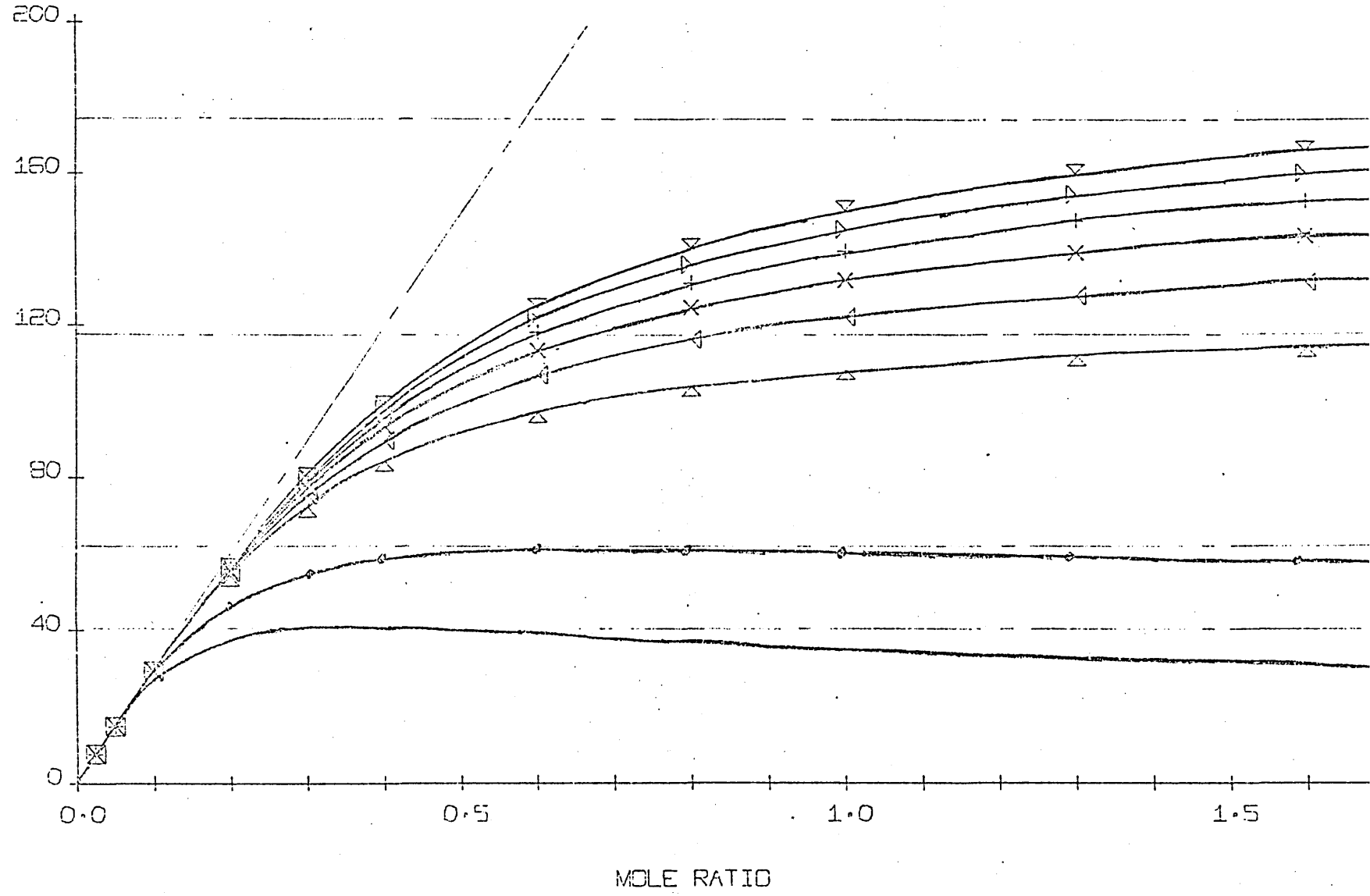
Δ_1 = 250 Hz.

Δ_2 = 150 Hz.

K_2 = 200 dm^3.mol^{-1}.

Curve	K_1 dm^3.mol^{-1}.	Calculated Initial Slope Value Δ_2 Hz.	Approximate Measurement of Maximum observed induced shift Δ max. Hz.
	700	149	175
	600	149	a
	500	149	a
	400	149	a
	300	149	a
	200	149	115
	50	149	65
	20	148	40

a For clarity these values are not shown.


200
160
120
80
40
0
INDUCED SHIFT HZ
0·0
0·5
1·0
1·5
MOLE RATIO

observed induced shift gives no indication to the limiting incremental shift value.

Figure 2.7 also shows the theoretical data plots obtained for a 2:1 stoichiometry. Again the initial slope values calculated from the data are in excellent agreement with the delta max. 2 value used and reflect the large K_2 values used. Also the maximum observed induced shift values indicate a very large K_1 value, but again do not give a value of the limiting incremental shift used. Furthermore, in the region of the entire curve but in particular where small mole ratio values exist, very little difference exists between the curves plotted even though K_2 varies between 100 - 600 $dm^3 . mol^{-1}$. This observation would therefore support Deranleau's argument that if reliable intrinsic parameters are to be determined then experimental data must be obtained over a comprehensive range of concentrations and not over the limited region where $[S_T] >> [E_T]$. Clearly in this limited region insufficient data is obtained to adequately fit a given stoichiometry to the experimental data. This could explain the inconsistent results obtained using the simple data treatment methods outlined in section 2.3.3 even when rigorous experimental precautions were taken.

2.4.3 Results with the shift reagent $Eu(fod)_3$

Amongst the first substrates to be studied were pentan-2-one and 4-methylacetophenone. The equilibrium binding constants and limiting incremental shift values for these substrates, obtained by the comparison of theoretically predicted and experimentally observed data plots, are shown in Table 2.9. The results represent findings for both 1:1 and 2:1 reaction mechanisms. From a comparison of the minimum agree-

Figure 2.7

Theoretical data plots for a 2:1 stoichiometry

$[E_T]$ = 0.005 $mol.dm^{-3}$

K_1 = 2000 $dm^3.mol^{-1}$.

Δ_1 = 250 Hz.

Δ_2 = 150 Hz.

Curve	K_2 $dm^3.mol^{-1}$	Calculated Initial Slope Value Δ_2 Hz.	Approximate Measurement of Maximum observed induced shift Δ max. Hz.
	600	150	195
	500	150	a
	400	150	a
	300	150	a
	200	149	a
	100	149	210

a For clarity these values are not shown.

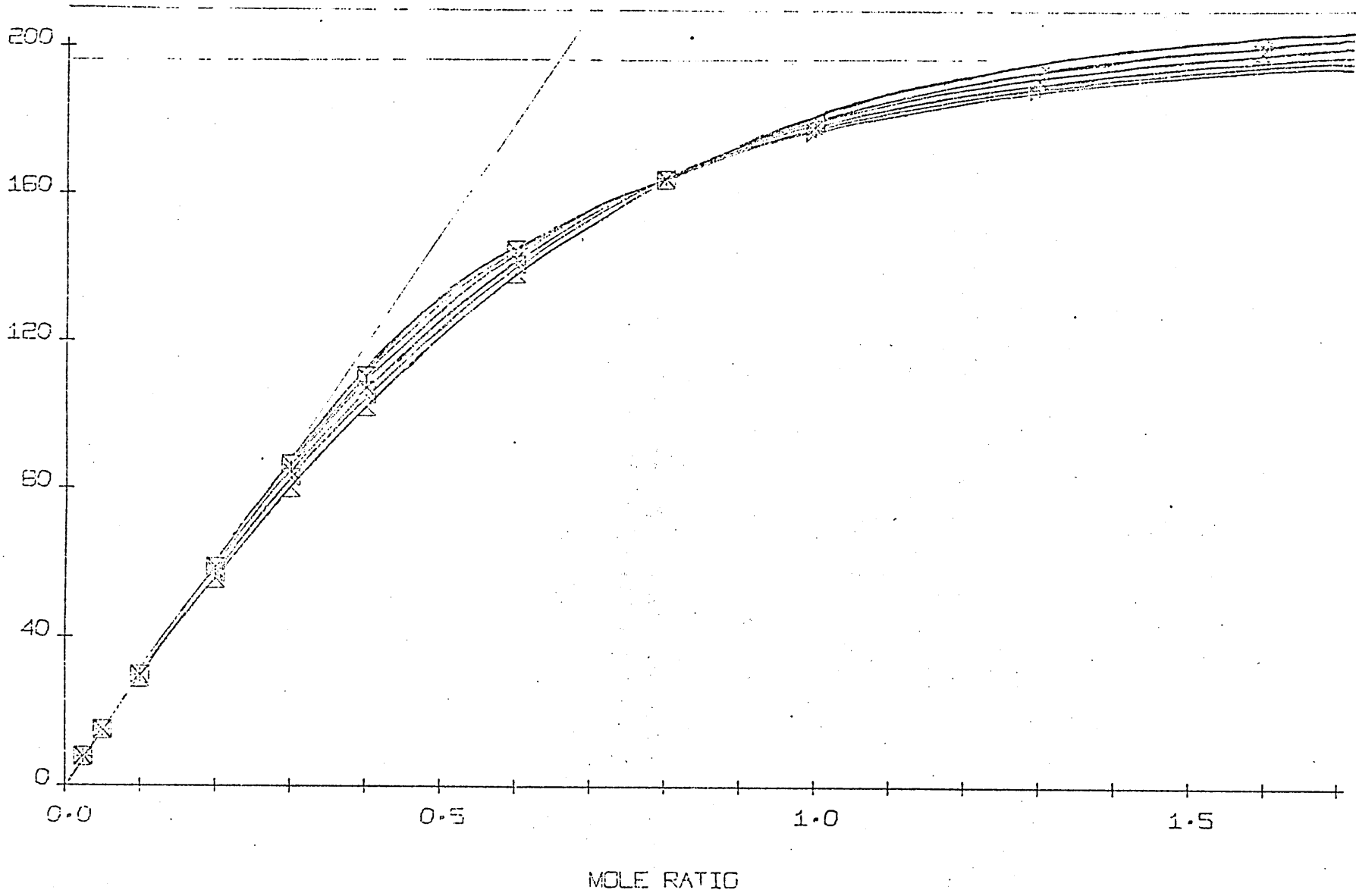
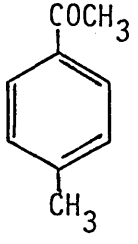

200
160
120
80
40
0
0·0
0·5
1·0
1·5
INDUCED SHIFT HZ
MOLE RATIO

Table 2.9

Intrinsic parameters for pentan-2-one and 4-methylacetophenone[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$.
b) Units of $dm^3.mol^{-1}$.
c) Units of Hz.
d) Percentage standard deviation.

Substrate	Proton Signal	1:1 Stoichiometry			2:1 Stoichiometry					
		$K^{(b)}$	$\triangle_1$ (c)	%SD(d)	K_1(b)	K_2(b)	$\triangle_1$ (c)	$\triangle_2$ (c)	$\triangle_1/\triangle_2$	%SD(d)
$CH_3COCH_2CH_2CH_3$ pentan-2-one	$\underline{CH_3}CO$	254	1005	2.48	1630	430	650	420	1.55	0.95
	$CO\underline{CH_2}$	254	1015	2.59	1690	410	650	425	1.53	0.94
	$CH_2\underline{CH_3}$	264	352	1.97	1600	400	230	150	1.53	1.06
 4-methyl - acetophenone	$\underline{CH_3}CO$	470	936	2.70	5440	480	530	440	1.20	1.38
	2-6 $\underline{H}$	494	741	2.24	5440	420	430	355	1.21	1.29
	3-5 $\underline{H}$	530	195	3.21	5440	410	116	95	1.22	2.07
	4-$\underline{CH_3}$	496	126	1.96	5400	400	75	60	1.25	1.38

ment factors obtained (i.e. the percentage standard deviations) it is clearly shown that a 2:1 stoichiometry is favoured. Also, an outstanding feature of these results is the excellent agreement found for the equilibrium binding constants determined for different protons within the same molecule. This agreement contrasts completely with the results reported by the simple data treatment methods outlined previously and probably results as a direct consequence of the comprehensive concentration ranges used in the experiments. Results reported in later tables show similar agreement between the equilibrium binding constants calculated for different protons in the same molecule, but for convenience, are reported as an average of the values obtained for the number of proton signals studied. Consequently, in view of this excellent agreement, even for cases involving very large equilibrium binding constants, greater confidence must result in the use of this method.

The remaining results presented in this section are given in order of functional group behaviour and concern several ketones, alcohols, ethers and nitrogen containing substrates complexed with the shift reagent $Eu(fod)_3$ in deuterochloroform. The results for both 1:1 and 2:1 stoichiometries show that the 2:1 stoichiometry is favoured in most cases. Even though the effects of steric hindrance and substrate basicity are extremely difficult to assess it will be seen that the values of the intrinsic parameters determined are influenced by steric hindrance and substrate basicity.

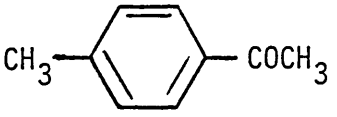
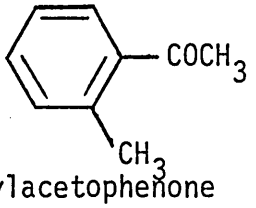
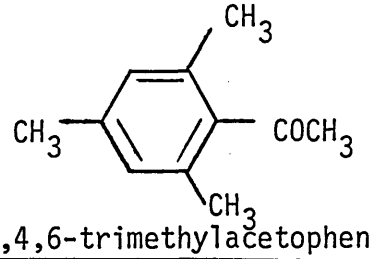
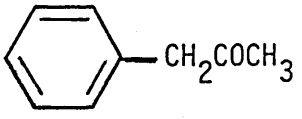
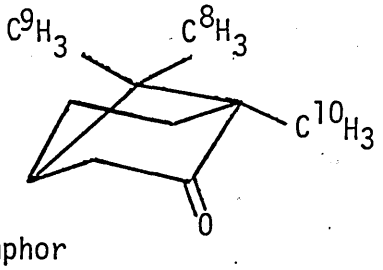
Ketones

The equilibrium binding constants determined for a series of ketones are shown in Table 2.10. The value of the equilibrium binding constant

Table 2.10

Equilibrium binding constants for a series of ketones[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$.
Units of $dm^3.mol^{-1}$.
The corresponding percentage standard deviations are shown in Table 2.11.

SUBSTRATE	1:1 Stoichiometry	2:1 Stoichiometry	
	K	K_1	K_2
$CH_3COCH_2CH_2CH_3$ pentan-2-one	259	1640	420
$CH_3COCH(CH_3)_2$ 3-methylbutan-2-one	390	5140	430
$CH_3COC(CH_3)_3$ 3,3-dimethylbutan-2-one	440	2720	240
$CH_3COCH=C(CH_3)_2$ 4-methylpentan-3-ene-2-one	1457	8000	450
 4-methylacetophenone	495	5440	420
 2-methylacetophenone	173	1100	220
 2,4,6-trimethylacetophenone	117	170	85
 benzylmethylketone	108	1080	140
 camphor	310	1040	100

indicates the stability of the complex formed in solution. The greater the equilibrium constant the more stable the complex. Consequently, any effect which results in an increase in the amount of electron lone pair availability will increase the degree of association between the substrate and the shift reagent. In the case of 4-methylpentan-3-ene-2-one (mesityl oxide), the presence of the conjugated double bond probably leads to some degree of electron delocalisation. If the electron delocalisation is toward the eletronegative carbonyl double bond then the delocalisation will assist the coordination between the substrate and shift reagent by placing more electron density on the donor oxygen atom. This is reflected by the very large equilibrium binding constant determined for this system. In contrast to this, steric hindrance has a less dramatic effect on the values of the equilibrium binding constants determined. This is seen particularly from a series of substituted acetophenones, as methyl groups progressively replace the 2 and 6 hydrogen atom positions. Intuitively it can be expected that the steric hindrance will prevent the large shift reagent molecule from approaching the lone pair of electrons on the donor oxygen atoms. This in turn may lead to an increase in the oxygen-lanthanide metal bond distance resulting in a corresponding decrease in the stability of the complex. Indeed this is reflected in the decreasing K values obtained when an increase in methyl substitution occurs.

One unusual, although perhaps coincidental feature regarding the equilibrium binding constants determined for the 2:1 adducts is that where no steric hindrance effects are apparently noticeable, then the value of K_2 determined appears to be fairly constant. This is shown

by the values 420 - 450 $dm^3.mol^{-1}$ which are reported for several ketones in Table 2.10.

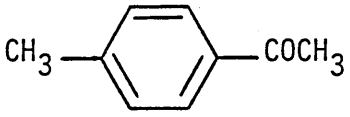
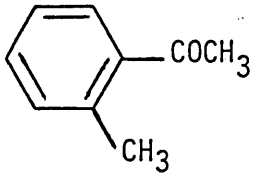
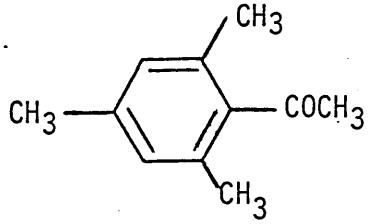
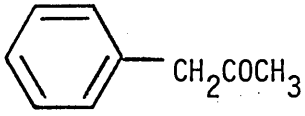
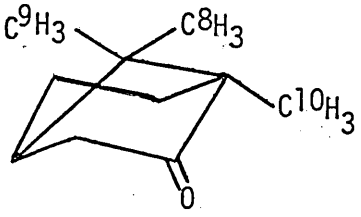
The limiting incremental shift values associated with these ketones are shown in Table 2.11, plus the corresponding minimum agreement factors obtained. The effects of steric hindrance are also noticeable from the limiting incremental shift values determined. Generally for an assumption of a 1:1 stoichiometry, the methyl groups adjacent to a coordinating carbonyl group, $COCH_3$, possess a limiting incremental shift value of between 940 - 1000 Hz. This indicates that the "point of attachment" between the substrate molecule and the shift reagent is similar in all cases. A comparison of the limiting incremental shift values for pentan-2-one and benzylmethylketone also indicates that this attachment point lies, on average, approximately midway between the carbonyl bond axis. Two exceptions to the values of 940 - 1000 Hz are apparent. In one case, 2,4,6-trimethylacetophenone, the difference may be explained by the presence of steric hindrance. If the limiting incremental shift values of 2,4,6-trimethylacetophenone are compared with those of (say) 4-methylacetophenone, then not only is the oxygen-lanthanide metal bond distance presumably increased, but also a molecular reorientation must take place between the substrate and shift reagent. This molecular reorientation process may explain why the limiting incremental shift values in the 4 and 3-5 positions of 2,4,6-trimethylacetophenone increase while the limiting incremental shift value of the α-methyl group decreases when compared with the corresponding limiting incremental shift values of 4-methylacetophenone. The small limiting incremental shift value observed for the 2-6 methyl group of 2,4,6-trimethylacetophenone when compared with the limiting

Table 2.11

Limiting incremental shift values for several ketones[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$. Units of Hz.

b) Percentage standard deviation.

SUBSTRATE	PROTON SIGNAL	1:1 Stoichiometry		2:1 Stoichiometry			
		1	%SD(b)	1	2	1/2	%SD(b)
$CH_3COCH_2CH_2CH_3$	CH_3CO	1005	2.48	650	420	1.55	0.95
	$COCH_2$	1015	2.59	650	425	1.53	0.94
pentan-2-one	CH_2CH_3	352	1.97	230	150	1.53	1.06
$CH_3COCH(CH_3)_2$	CH_3CO	956	2.68	520	450	1.16	1.07
3-methylbutan-2-one	$(CH_3)_2$	606	2.52	340	285	1.19	1.01
$CH_3COC(CH_3)_3$	CH_3CO	996	1.19	640	485	1.32	1.13
3,3-dimethylbutan-2-one	$(CH_3)_3$	615	1.66	420	295	1.42	0.71
$CH_3COCH=C(CH_3)_2$	CH_3CO	832	1.82	660	410	1.61	1.57
	(CH_3)	666	1.98	540	325	1.66	1.62
4-methypentan-3-ene-2-one	$COCH$	564	2.26	470	280	1.68	2.28
	(CH_3)	324	1.65	260	160	1.63	1.56
	CH_3CO	936	2.70	530	440	1.20	1.38
	2-6H	741	2.24	430	355	1.21	1.29
	3-5H	195	3.21	116	95	1.22	2.07
4-methylacetophenone	4-CH_3	126	1.96	75	60	1.25	1.38
	CH_3CO	938	3.52	440	425	1.04	1.69
	2-CH_3	748	3.77	370	335	1.10	1.55
2-methylacetophenone							
	CH_3CO	820	2.52	580	360	1.61	1.18
	2-6CH_3	482	2.49	340	210	1.62	1.03
	3-5H	242	2.77	200	105	1.90	1.24
	4-CH_3	170	1.43	120	85	1.41	1.49
2,4,6-trimethylacetophenone							
	CH_3CO	998	4.04	340	450	0.76	0.74
benzylmethylketone	$COCH_2$	1062	4.52	370	480	0.77	1.35
	$C^{10}H_3$	672	2.08	530	330	1.61	1.45
	C^8H_3	450	1.96	360	220	1.64	1.44
	C^9H_3	308	1.96	220	150	1.47	1.92
camphor							

incremental shift value of the 2-methyl group of 2-methylacetophenone, also supports the molecular reorientation process. In the second case, 4-methylpentan-3-ene-2-one, the cause is perhaps less certain. It may be that the electron delocalisation discussed earlier, causes a preferred arrangement to take place decreasing the α-methyl limiting incremental shift value.

Other similarities associated with an assumption of a 1:1 stoichiometry are the limiting incremental shift values obtained for the methyl groups of the isopropyl (606 Hz) and the tert. butyl (615 Hz) groups of 3-methylbutan-2-one and 3,3-dimethylbutan-2-one respectively. This would indicate that any steric hindrance that may have been anticipated due to the presence of the tert. butyl group is not reflected in the limiting incremental shift values. Furthermore the equilibrium binding constants for these substrates also support this.

The limiting incremental shift values of the α-methylene groups $CH_3COC\underline{H}_2$ of pentan-2-one and benzylmethylketone also suggest similar structural features between complexes of ketones with $Eu(fod)_3$. The effect of steric hindrance is best shown by a comparison of the limiting incremental shift values obtained for 4-methylacetophenone, 2-methylacetophenone and 2,4,6-trimethyacetophenone. These results indicate that an increase in the oxygen-lanthanide metal bond distance is not the only feature arising from steric hindrance. If this were so then it might be expected that the limiting incremental shift values of the substrates affected would all decrease gradually throughout the series as steric hindrance became progressively more pronounced. As is shown, this does not occur, suggesting that some kind of intramolecular reorientation or rearrangement must be taking place between the

sterically hinded substrates and the shift reagent. This would then affect proton-oxygen-lanthanide metal bond angles and distances, resulting in changes in the limiting incremental shift values determined. When a 2:1 stoichiometry is assumed the presence of the second substrate molecule is not in a "mirror image" position of the first substrate. This is reflected by the difference in delta max. 1 and delta max. 2 values reported for the 2:1 stoichiometries. Again certain similarities are observed for several substrates but generally these follow the remarks outlined for the 1:1 stoichiometry. For most of the ketones studied it is shown that the delta max. 2 values of the methyl group adjacent to the carbonyl group - $COCH_3$, are approximately 450 Hz. This would suggest that the positioning of the second substrate molecule is probably similar in all these cases. The major exception to this is the α-methyl group of 2,4,6-trimethylacetophenone which is probably a result of steric hindrance as explained earlier, but now further complicated by the presence of a second substrate molecule. The effects of steric hindrance are more noticeable in the delta max. 1 values reported than in the delta max. 2 values. This is perhaps reasonable, since the first substrate molecule is expected to be closer to the lanthanide metal ion and is thus more easily affected by slight changes in its environment, i.e. bond angles and distances. Where no steric hindrance affects are apparent, the limiting incremental shift values of the α-methyl group approaches about 650 Hz. However, the dangers of assuming the presence of steric hindrance are extremely great since no indication can be given towards the value of the limiting incremental shifts expected. In some cases, the limiting incremental shift values obtained might be greater than expected whilst in other

cases they will be less than those anticipated. This arises because the induced shifts, which depend upon the positioning of the substrate around the central metal ion, can be significantly affected by bond angles and nuclear distances, which may act in different directions. An example of this is the limiting incremental shift value of benzylmethylketone where the value of delta max. 1 falls below the value of delta max. 2. Normally, it might be expected that the second substrate molecule will be further away from the lanthanide metal ion, thus experiencing a ~~deshielding~~ decreased effect. This would then result in a small limiting incremental shift value for the 2:1 adduct than for the 1:1 adduct. In the case where the reverse happens, i.e. delta max. 1 is less than delta max. 2, the cause is probably due to the reorientation process discussed earlier caused by steric hindrance.

Another salient feature of these results is the variation of the limiting incremental shift ratios $\left(\triangle 1_i / \triangle 2_i \right)$ obtained for the 2:1 stoichiometry of the various ketones. Whilst several of these ratios appear fairly constant for a given substrate molecule, other molecules show significant differences. These ratio values will however be discussed in more detail later.

From these results it would seem that, even though certain similarities exist, generally no predictions can be made regarding the intrinsic parameters of ketones complexed with lanthanide shift reagents. This would support the suggestion put forward by Reuben (70) and is particularly so regarding the interpretation of results with reference to the effects from steric hindrance and substrate basicity.

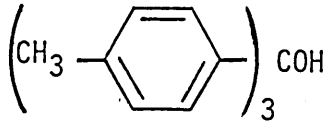
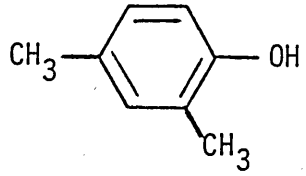
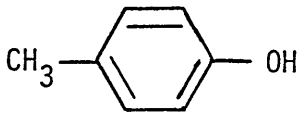
Alcohols

The intrinsic parameters of a series of alcohols are shown in Tables 2.12 and 2.13. Again results are expressed for both 1:1 and 2:1 stoichiometries. Also in all the systems studied a better theoretical-experimental fit was observed when a 2:1 stoichiometry was assumed. It is generally found that the equilibrium binding constants determined for the alcohol series are larger than those values determined for the series of ketones. It seems therefore that alcohols coordinate more effectively with $Eu(fod)_3$ in deuterochloroform than do ketones. The results obtained by this rigorous treatment indicate that there is no general order of stability between the lanthanide complexes of primary, secondary and tertiary alcohols. It appears that substrate basicity and steric hindrance are both major contributors to the induced chemical shifts and each alcohol must therefore be treated individually and not as part of a series. The results for tri(4-methylphenyl) carbinol, 2,4-dimethylphenol and 4-methylphenol were not computed since there were no induced shifts observed when these alcohols were examined in the presence of $Eu(fod)_3$ in deuterochloroform. This would indicate that no coordination takes place between these substrates and the shift reagent $Eu(fod)_3$ in deuterochloroform. A comparison of the equilibrium binding constants determined for tert. butanol and 2,3,3-trimethylbutan-2-ol with the results of tri(4-methylphenyl) carbinol would seem to indicate that steric hindrance effects are the probable cause for the lack of coordination between tri(4-methylphenyl) carbinol and $Eu(fod)_3$. Steric crowding of the substituent phenyl groups in tri(4-methylphenyl) carbinol must be far greater than the steric crowding caused by the methyl groups in tert. butanol and 2,3,3-

Table 2.12

Equilibrium binding constants for several alcohols[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$.
Units of $dm^3.mol^{-1}$.
The corresponding percentage standard deviations are shown in Table 2.13.

SUBSTRATE	1:1 Stoichiometry	2:1 Stoichiometry	
	K	K_1	K_2
$(CH_3)_3COH$ tert.butanol	674	8000	640
$(CH_3)_2CHCH_2OH$ isobutanol	506	5480	340
$CH_3CH{=}CHCH_2OH$ but-2-ene-1-ol	264	3640	200
$((CH_3)_2CH)_2CHOH$ 2,4-dimethylpentan-3-ol	644	5470	250
$(CH_3)_2CHCH(CH_3)OH$ 3-methylbutan-2-ol	551	5360	205
$(CH_3)_3CC(CH_3)_2OH$ 2,3,3-trimethylbutan-2-ol	674	5520	920
 tri(4-methylphenyl)carbinol	0	0	0
 2,4-dimethylphenol	0	0	0
 4-methylphenol	0	0	0

trimethylbutan-2-ol. It is reasonable to expect that this crowding prevents the shift reagent from approaching the oxygen donor atom to such an extent that effective complexation is prohibited. The absence of any induced shifts observed for 2,4-dimethylphenol and 4-methylphenol is probably however a result of electronic factors and not steric hindrance. These phenols, the oxygen lone pair of electrons of which, may be delocalised within the benzene nucleus, will have their coordinating ability severely reduced. An alternative explanation may be that at the small substrate concentrations used in these studies, intermolecular substrate-solvent interactions take place which preclude the substrate-shift reagent coordination. Under certain conditions, phenols will hydrogen bond with chloroform (75). Deuterochloroform may therefore compete with the shift reagent for the available substrate and in the absence of any induced shifts, perhaps the formation of a phenol-deuterochloroform complex is more favourable than a phenol-shift reagent complex. In experiments where large substrate concentrations have been used, small induced shifts have been reported for several phenols complexing with $Eu(fod)_3$ in deuterochloroform (76). This may however reflect an equilibrium mixture where phenol-deuterochloroform and phenol-shift reagent complexesexist together in solution. As explained in section 2.3.2, 2,3,3-trimethylbutan-2-ol is extremely hygroscopic and forms stable hydrates; consequently this compound might be expected to form complexes quite readily. This probably explains the larger than average equilibrium binding constants reported for this substrate.

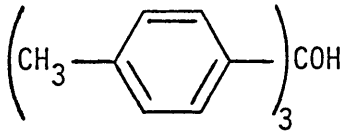
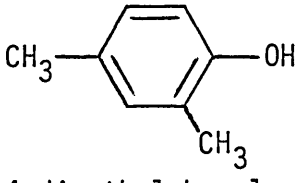
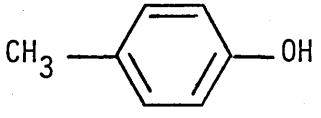
The limiting incremental shift values reported in Table 2.13 for several alcohols show certain similarities for similarly positioned

Table 2.13

Limiting incremental shift values for several alcohols[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$. Units of Hz.

b) Percentage standard deviation.

SUBSTRATE	PROTON SIGNAL	1:1 Stoichiometry		2:1 Stoichiometry			
		Δ_1	%SD[b]	Δ_1	Δ_2	Δ_1/Δ_2	%SD[b]
$(CH_3)_3COH$ tert. butanol	$(C\underline{H}_3)_3$	988	2.87	680	475	1.43	1.60
$(CH_3)_2CHCH_2OH$ isobutanol	$(C\underline{H}_3)_2$	642	2.93	400	310	1.29	1.74
	$C\underline{H}_2$	1602	1.76	1280	805	1.59	1.72
$CH_3CH=CHCH_2OH$	$C\underline{H}_2$	1608	1.04	-	-	-	-
but-2-ene-1-ol	$C\underline{H}_3$	262	2.21	130	125	1.04	1.44
$((CH_3)_2CH)_2CHOH$	$(C\underline{H}_3)$	502	0.47	340	250	1.36	0.45
2,4-dimethylpentan-3-ol	$(C\underline{H}_3)$	648	2.43	410	325	1.26	2.38
$(CH_3)_2CHCH(CH_3)OH$	$(C\underline{H}_3)_2$	672	1.80	420	340	1.24	1.47
3-methylbutan-2-ol	$C\underline{H}_3$	1004	1.66	650	500	1.30	1.42
$(CH_3)_3CC(CH_3)_2OH$	$(C\underline{H}_3)_3$	633	2.72	390	300	1.30	1.19
2,3,3-trimethylbutan-2-ol	$(C\underline{H}_3)_2$	1140	3.05	690	540	1.28	0.99
 tri(4-methylphenyl)carbinol		NO INDUCED SHIFT OBSERVED					
 2,4-dimethylphenol		NO INDUCED SHIFT OBSERVED					
 4-methylphenol		NO INDUCED SHIFT OBSERVED					

proton groups. When a methyl group is situated adjacent or α to the hydroxyl group i.e. $\underline{CH}_3-\overset{|}{C}-OH$, as in tert. butanol, 3-methylbutan-2-ol and 2,3,3-trimethylbutan-2-ol, then it is found that these methyl groups possess very similar limiting incremental shift values. This is also observed for isobutanol, 2,4-dimethylpentan-3-ol, 3-methylbutan-2-ol and 2,3,3-trimethylbutan-2-ol, where a methyl group is situated β to the hydroxyl group, i.e. $\underline{CH}_3-\overset{|}{C}-\overset{|}{C}-OH$. The methylene protons - $\underline{CH}_2OH$ of the primary alcohols isobutanol and but-2-ene-1-ol also show close agreement for the limiting incremental shift values. The similarities in the limiting incremental shift values may reflect similar structural features within the lanthanide metal complexes and also the predominance of the pseudocontact shift mechanism. Consequently, it would appear that in the absence of any apparent steric hindrance effects, certain shift similarities will exist for closely related alcohol substrates.

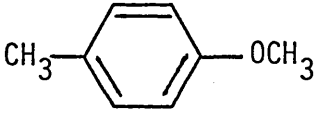
Ethers

The intrinsic parameters of several ethers are shown in Table 2.14. The much smaller equilibrium binding constants obtained for this series of substrates indicates that ethers are much less susceptible to coordination with lanthanide shift reagents than are either alcohols or ketones. This probably reflects the lower substrate basicity of ethers when compared with alcohols and ketones. In view of the smaller nature of the equilibrium binding constants and the absence of any induced shifts in two cases, these compounds seem to be severely affected by steric hindrance and electronic factors. No induced shifts were observed for 4-methylanisole in the presence of $Eu(fod)_3$ in deuterochloroform. This is probably a result of electron delocali-

Table 2.14

Intrinsic parameters for several ethers[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$.
b) Units of $dm^3.mol^{-1}$.
c) Units of Hz.

SUBSTRATE	PROTON SIGNAL	1:1 Stoichiometry			2:1 Stoichiometry					
		$K^{(b)}$	$\triangle_1^{(c)}$	%SD	$K_1^{(b)}$	$K_2^{(b)}$	$\triangle_1^{(c)}$	$\triangle_2^{(c)}$	$\triangle_1/\triangle_2$	%SD
$(CH_3)_2CHCOCH_3$	$OC\underline{H}_3$	30	1902	1.86	61	19	970	805	1.20	1.11
isopropylmethylether	$(C\underline{H}_3)_2$		1100	1.18			610	550	1.11	1.17
$(CH_3CH_2CH_2)_2O$	$C\underline{H}_3$		360	2.72			170	160	1.06	3.20
	$CH_3C\underline{H}_2$	17	956	3.98	38	14	430	390	1.10	3.14
dipropylether	$C\underline{H}_2CH_2$		1678	2.73			790	810	0.98	2.55
$((CH_3)_2CH)_2O$	$(C\underline{H}_3)_2$	NO INDUCED SHIFTS OBSERVED								
diisopropylether										
	$C\underline{H}_3$	NO INDUCED SHIFTS OBSERVED								
4-methylanisole	CH_3O									

sation of the oxygen lone pair of electrons away from the oxygen into the benzene nucleus. This electron delocalisation would then reduce the availability of the electron lone pair and hence prevent coordination taking place. The absence of any induced shifts for diisopropylether in the presence of $Eu(fod)_3$ is probably however, a result of steric hindrance. The presence of two adjacent isopropyl groups possibly prevents the approach of the lanthanide shift reagent molecule from reaching the potential oxygen coordination site. When only one isopropyl group is present, as in isopropylmethylether, coordination does occur, albeit only to a small extent. It is probable that steric hindrance also affects the limiting incremental shift values of the ethers studied. This would explain the slight differences observed in the limiting incremental shift values of similarly positioned proton groups. From these results it would seem, as in the ketones, no general predictions can be made regarding the intrinsic parameters obtained.

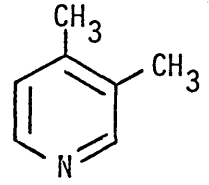
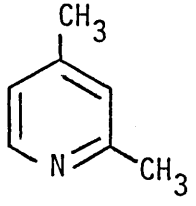
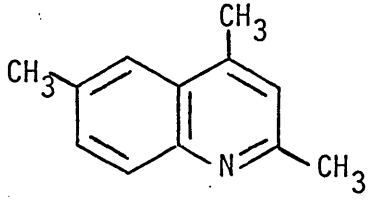
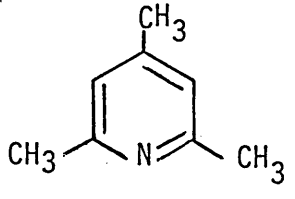
Nitrogen containing substrates

The intrinsic parameters of several substituted pyridines are shown in Table 2.15. Only those proton groups that gave absorption peaks with clear and well defined chemical shift positions were used in the calculations to determine the intrinsic parameters. Since very large chemical induced shifts are severely affected by signal broadening and signal/noise limitations, both of which influence the precise measurement of the induced shift, those proton groups situated very close to the coordinating centre were not used. For similar reasons, those proton groups situated at a distance from the coordination site which possess only very small chemical induced shifts, were also not

Table 2.15

Intrinsic parameters for several substituted pyridines[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$.
b) Units of $dm^3.mol^{-1}$.
c) Units of Hz.

SUBSTRATE	PROTON SIGNAL	1:1 Stoichiometry			2:1 Stoichiometry					
		$K^{(b)}$	$\triangle_1^{(c)}$	%SD	$K_1{}^{(b)}$	$K_2{}^{(b)}$	$\triangle_1^{(c)}$	$\triangle_2^{(c)}$	$\triangle_1/\triangle_2$	%SD
 3,4-dimethylpyridine	3-CH_3	938	530	3.09	8280	90	380	290	1.31	1.06
	4-CH_3	1305	347	3.20	8680	80	270	190	1.42	1.10
 2,4-dimethylpyridine	4-CH_3	1294	334	2.37	8720	80	270	180	1.50	1.46
 2,4,6-trimethylquinoline	4-CH_3	42	266	4.01	75	65	140	100	1.40	2.46
 2,4,6-trimethylpyridine	4-CH_3	76	156	5.26	35	35	257	47	5.47	1.30

used.

A comparison of the results for 1:1 and 2:1 stoichiometries indicate that the 2:1 reaction mechanism is the one most favoured. Also, since electronic factors of those substituted pyridines might be expected to be very similar, any differences in the intrinsic parameters determined for similarly positioned proton groups, e.g. the 4-methyl group, can be attributed to the presence of steric hindrance effects.

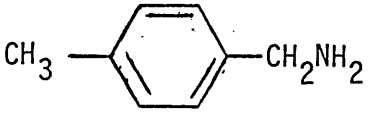
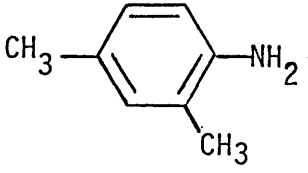
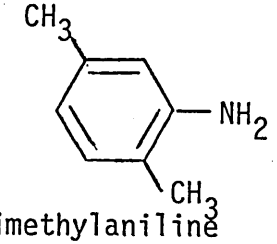
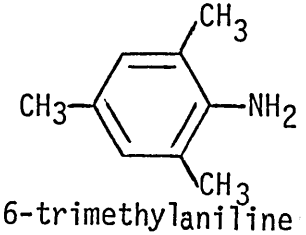
From the results of the 4-methyl proton groups of 3,4-dimethylpyridine and 2,4-dimethylpyridine, it would seem that substitution in the 2 (or 6) position of the pyridine nucleus causes little, if any, steric hindrance. However, the results of 2,4,6-trimethylpyridine and 2,4,6-trimethylquinoline indicate that substitution in both the 2 and 6 positions of the pyridine nucleus has a very large effect on the values of the intrinsic parameters. The steric crowding caused by the presence of a second large substrate molecule is reflected in the results of the 2:1 complexes of the substituted pyridines studied. The greater the steric hindrance effect then the smaller the intrinsic parameters that are determined. However, the results of the 2:1 stoichiometry for the complexes of 3,4-dimethylpyridine, 2,4-dimethylpyridine and 2,4,6-trimethylpyridine show that steric hindrance effects, although affecting the equilibrium binding constants, do not greatly affect the limiting incremental shift values determined. This may indicate that small differences in the lanthanide metal-nitrogen bond distances greatly affect the stability of the complexes formed in solution, but not the positioning of the substrate in the lanthanide metal complex.

The results for several amines are shown in Table 2.16. The values obtained indicate that a 2:1 stoichiometry is clearly the one most

Table 2.16

Intrinsic parameters of several amines[a]

a) Complexed with $Eu(fod)_3$ in $CDCl_3$.
b) Units of $dm^3.mol^{-1}$.
c) Units of Hz.

SUBSTRATE	PROTON SIGNAL	1:1 Stoichiometry			2:1 Stoichiometry					
		K_1 (b)	$\triangle_1$ (c)	%SD	K_1 (b)	K_2 (b)	$\triangle_1$ (c)	$\triangle_2$ (c)	$\triangle_1/\triangle_2$	%SD
$(CH_3)_2CHCH_2NH_2$ isobutylamine	$(C\underline{H}_3)_2$	374	642	5.23	2520	920	400	295	1.36	1.14
 4-methylbenzylamine	$C\underline{H}_3$	259	172	8.10	5280	1340	75	75	1.00	1.52
 2,4-dimethylaniline	2-$C\underline{H}_3$	112	720	2.37	150	50	570	320	1.78	1.78
	4-$C\underline{H}_3$	42	158	4.44	170	10	60	126	0.48	2.30
 2,5-dimethylaniline	2-$C\underline{H}_3$	84	722	2.45	150	70	450	315	1.43	0.87
	5-$C\underline{H}_3$	36	299	1.60	70	20	170	140	1.21	2.06
 2,4,6-trimethylaniline	2-6 $C\underline{H}_3$	83	541	2.19	120	30	440	425	1.04	1.72
	3-5 $\underline{H}$	83	253	2.20	170	40	160	120	1.33	1.94
	4-$C\underline{H}_3$	152	345	5.90	90	10	45	6	7.50	3.66

favoured and that the large K_2 values obtained for aliphatic amines reflect the large basicity of amines in relation to alcohols, ketones and ethers. Since there is now substantial evidence (77, 78) to show that lanthanide induced shifts in aromatic systems occur via pseudo-contact and contact shift mechanisms, the assumption of a predominant pseudocontact induced shift, used throughout these studies, no longer seems valid. This could explain the inconsistant results obtained for the equilibrium binding constants of the aromatic amines studied. Consequently only those proton groups that are well removed from the nitrogen coordinating centre can be regarded as being relatively free from contact shift mechanisms and hence representing a more correct account of the intrinsic parameters determined. The absence of contact induced shifts for proton groups that are well removed from the coordinating centre in aliphatic amines may be shown by comparing the limiting incremental shift values of the methyl group in isobutylamine (Table 2.16) with the corresponding limiting incremental shift values obtained for isobutanol (Table 2.13) where it is regarded that alcohols contain no contact induced shift contributions when complexed with lanthanide shift reagents. The excellent agreement found for these limiting incremental shift values could also reflect similar structures which isobutanol and isobutylamine might be expected to show. The limiting incremental shift values obtained for the 4-methyl proton group of 2,4,6-trimethylaniline possibly highlights the situation where a functional group is capable of, but at the same time is severely restricted from, coordinating with the shift reagent. The steric hindrance, probably arising from the presence of the second substrate molecule, is shown by the much smaller delta max. 2 value compared

with the value of delta max. 1, 6 and 45 Hz respectively. This may also be seen from the limiting incremental shift values of the 4-methyl group of 2,4,6-trimethylpyridine, 47 and 257 Hz for the delta max. 2 and delta max. 1. values respectively.

It would seem that in general, substituted anilines coordinate with lanthanide shift reagents only to a limited extent. This may result from possible electron delocalisation within the benzene nucleus which reduces the availability of the nitrogen lone pair of electrons.

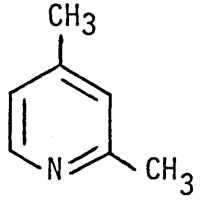
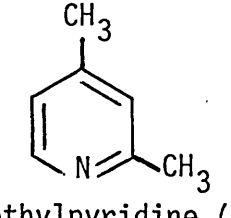
2.4.4 Results with other shift reagents

The intrinsic parameters determined for several substrates complexed with the shift reagents $Pr(fod)_3$ and $Yb(fod)_3$ as shown in Table 2.17. The induced chemical shifts caused by complexation with $Pr(fod)_3$ were upfield in direction whilst those of $Yb(fod)_3$ were downfield. Also, the magnitude of the induced shifts caused by $Pr(fod)_3$ and $Yb(fod)_3$ were much larger than the corresponding $Eu(fod)_3$ induced shifts. In general the results obtained for the substrate - $Pr(fod)_3$ complexes, although lower in magnitude follow the same relative order of stability found for the corresponding $Eu(fod)_3$ systems. Hence alcohols coordinate with $Pr(fod)_3$ more effectively than do ketones or ethers.
The stability of the complexes formed in solution between organic substrates and lanthanide metal chelates depends upon the ionic radius of the lanthanide metal ion (79). As the ionic radius decreases, the stability of the complex, and hence the value of the equilibrium binding constant increases. Consequently, since the ion radius decreases as

Table 2.17

Intrinsic parameters of several substrates complexed with $Pr(fod)_3$ and $Yb(fod)_3$

a) Units of $dm^3.mol^{-1}$.
b) Units of Hz.
c) Complexed with $Pr(fod)_3$ in $CDCl_3$.
d) Complexed with $Yb(fod)_3$ in $CDCl_3$.

SUBSTRATE	PROTON SIGNAL	1:1 Stoichiometry			2:1 Stoichiometry					
		K_1 (a)	Δ_1 (b)	%SD	K_1 (a)	K_2 (a)	Δ_1 (b)	Δ_2 (b)	Δ_1/Δ_2	%SD
$(CH_3)_3CC(CH_3)_2OH$	$(C\underline{H}_3)_3$	78	-1202	2.30	290	70	-560	-535	1.05	0.69
2,3,3-trimethylbutan-2-ol (c)	$(C\underline{H}_3)_2$		-1972	2.34			-770	-895	0.86	0.50
$(CH_3)_3CCOCH_3$	$(C\underline{H}_3)_3$	53	-1290	1.28	60	2	-1220	-960	1.27	1.45
3,3-dimethyl-butan-2-one (c)	$COC\underline{H}_3$		-1994	1.36			-1730	-1400	1.24	1.63
$(CH_3)_2CHOCH_3$	$(C\underline{H}_3)_2$	5	-1920	3.91	-	-	-	-	-	-
isopropylmethylether (c)	$OC\underline{H}_3$		-2992	4.62						
 2,4-dimethylpyridine (c)	4-$C\underline{H}_3$	112	-696	2.52	390	60	-370	-340	1.09	2.22
 2,4-dimethylpyridine (d)	4-$C\underline{H}_3$	11540	816	2.87	15300	18	810	415	1.95	2.83

the atomic number increases, complexes of $Yb(fod)_3$ are expected to be more stable than the corresponding $Eu(fod)_3$ complexes, which in turn will be more stable than the corresponding $Pr(fod)_3$ complexes. The equilibrium binding constants obtained for the 2,4-dimethylpyridine complexes of $Pr(fod)_3$, $Eu(fod)_3$, and $Yb(fod)_3$ (Tables 2.15 and 2.17) agree excellently with this theory. This contrasts markedly with those results reported by Armitage et al (57) where the neo-pentanol complexes of $Pr(dpm)_3$, $Eu(dpm)_3$ and $Tm(dpm)_3$ in deuterochloroform are reported to possess identical equilibrium binding constants. Their reason put forward for the similarity in values was in accordance with similar chemical properties across the lanthanide series. The work in this thesis has now shown however that the simple data treatment methods, similar to those used by Armitage et al, are not appropriate to the determination of reliable equilibrium binding constants. From the results in this thesis it is also shown that the value of K_2 steadily decreases when the atomic number of the lanthanide metal ion is increased. This effect is also reported by Evans and Wyatt (94). Another feature of the results obtained for complexes of different shift reagents is the value reported for the limiting incremental shift ratio, $\Delta_1 i / \Delta_2 i$ obtained for the 2:1 stoichiometry. When the ionic radius of the lanthanide metal ion is large, it may be possible for the shift reagent to accommodate two substrate molecules more effectively than when the ionic radius is small. Consequently, the position of the second substrate molecule may be nearer to the metal ion, resulting in a relative increase in the limiting incremental shift values observed for the 2:1 adduct compared with a 1:1 adduct. Thus the limiting incremental shift

ratio will decease for metal ions with large ionic radii. The limiting incremental shift ratio for proton groups in a 2:1 substrate - $Pr(fod)_3$ complex will therefore be smaller than the corresponding shift ratios of $Eu(fod)_3$ complexes, since the praseodymium metal ion has a larger ionic radius than europium. The limiting incremental shift ratios observed for $Yb(fod)_3$ complexes will consequently be the largest of all the shift ratios obtained for the shift reagents studied here. From a comparison of the limiting incremental shift ratios shown in Table 2.17 with the corresponding $Eu(fod)_3$ values, shown in Tables 2.11, 2.13 and 2.15, it is clear that this behaviour is observed.

2.4.5 The analysis of induced shift data

It has been shown in the previous sections how a rigorous data treatment method can be used to determine reliable intrinsic parameters. It is now therefore possible to compare the results of the analysis presented with the results obtained from any simple data treatment method which might be used in the analysis of induced shift data. Consequently, this comparison may help to demonstrate what reliable information, if any, may be obtained by various simple data treatment methods. Certain shortcomings of attempting to derive reliable information regarding equilibrium binding constants, limiting incremental shift values and the reaction stoichiometry will also be highlighted from this comparison.

2.4.5.1 Induced shifts versus mole ratio plots

The results of the 4-parameter data treatment method are used in order to demonstrate what information can be obtained by a simple inspection of induced shift data. Consequently, the shape of several induced

shifts versus mole ratio plots are analysed in detail. The use of this simple inspection may prove useful when substrates need to be analysed but where a 4-parameter data treatment method cannot be employed, say where extensive computer facilities are not available. Figures 2.8 - 2.12 show the theoretical and experimental data plots obtained when the induced shift is measured as a function of the substrate concentration whilst the shift reagent concentration is kept constant. Also shown are the predicted induced shift contributions of the 1:1 and 2:1 complexes present in solution for a 2:1 stoichiometry. For clarity the data plots of the 1:1 reaction mechanism are not shown since invariably these results lead to poorer agreement factors. It will be shown that the curves are quite characteristic and can therefore be used as a useful guide to the analysis of the experimental data obtained. As already stated, two useful factors help towards the analysis of the data plot, namely the initial slope value, obtained at very small mole ratio values, and the maximum observed induced shift value, obtained at large mole ratio values. Another useful guide to the analysis is the extent and degree of curvature shown by such a plot. Curvature, observed in the lower mole ratio region (say 0 - 0.5) indicates a small equilibrium binding constant, whereas a more linear plot suggests a strong association between substrate and shift reagent (73).

Figure 2.8 shows the data plot obtained for the 4-methyl proton group of the 2,4-dimethylpyridine-Yb(fod)$_3$ complex in deuterochloroform. There is very little curvature observed and this is indicative of a very large equilibrium binding constant. Also, the initial slope value compares favourably with the maximum observed induced shift

Figure 2.8

A plot of the induced shift versus mole ratio[(a)]

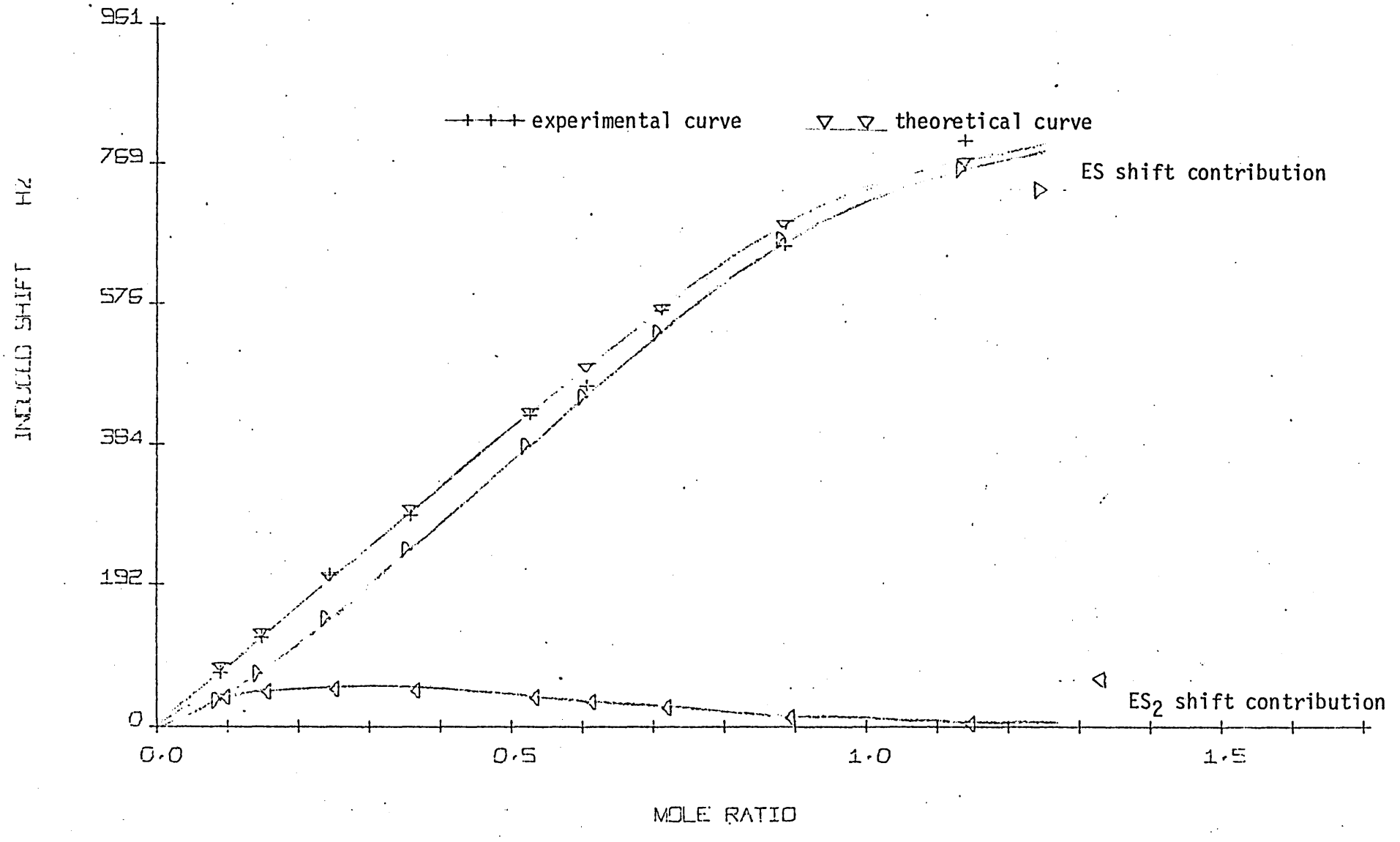
a) 4-methyl signal of 2,4-dimethylpyridine - $Yb(fod)_3$ complex in $CDCl_3$.

the intrinsic parameters obtained by the full 4-parameter data treatment are -

K_1	=	15300	$dm^3.mol^{-1}$
K_2	=	18	$dm^3.mol^{-1}$
Δ_1	=	810	Hz
Δ_2	=	415	Hz
%SD	=	2.83	

The calculated initial slope value gives Δ_1 = 814 Hz

and the maximum observed induced shift Δ_{max} = 801 Hz


951
769
576
384
192
0
INDUCED SHIFT Hz
experimental curve
theoretical curve
ES shift contribution
ES2 shift contribution
0.0
0.5
1.0
1.5
MOLE RATIO

value, 814 and 801 Hz respectively, suggesting a strong association process. As was shown in section 2.4.2 the significance of the initial slope should be made in the light of the magnitude of the equilibrium binding constant. If a 1:1 reaction mechanism exists or indeed if the value of K_2 for a 2:1 stoichiometry is very small, then the initial slope value approximates to delta max. 1. Using this criterion, excellent agreement is found between the initial slope value and the best delta max. 1 value predicted by theory for the substrate-shift reagent interaction, i.e. 814 and 810 Hz respectively. In this case however, no value can be obtained from the curve to represent delta max. 2. Hence a qualitative analysis of the curve reveals a very large equilibrium binding constant for a 1:1 reaction mechanism or alternatively, a very large K_1 value with a very small K_2 value plus a delta max. 1 value of 814 Hz for a 2:1 stoichiometry. Indeed the best predicted analysis of the experimental-theoretical fit, produces K_1 and K_2 values of 15300 and 18 $dm^3.mol^{-1}$ respectively and delta max. 1 and delta max. 2 values of 810 and 415 Hz respectively. Figure 2.9 shows the data plot obtained for the isopropyl $\underline{CH}_3$ protons of the isopropylmethylether-Eu(fod)$_3$ complex in deuterochloroform. The large degree of curvature shown in the lower mole ratio region between 0 - 0.3 suggests a very weak association between the substrate and shift reagent, as indeed is indicated by the large difference between the initial slope value and the maximum observed induced shift, 741 and 111 Hz respectively. In a case such as this, an analysis of the induced shift versus mole ratio plot can only be performed with a prior knowledge of the intrinsic parameters obtained from the full 4-parameter data treatment method. This is because the initial slope

Figure 2.9

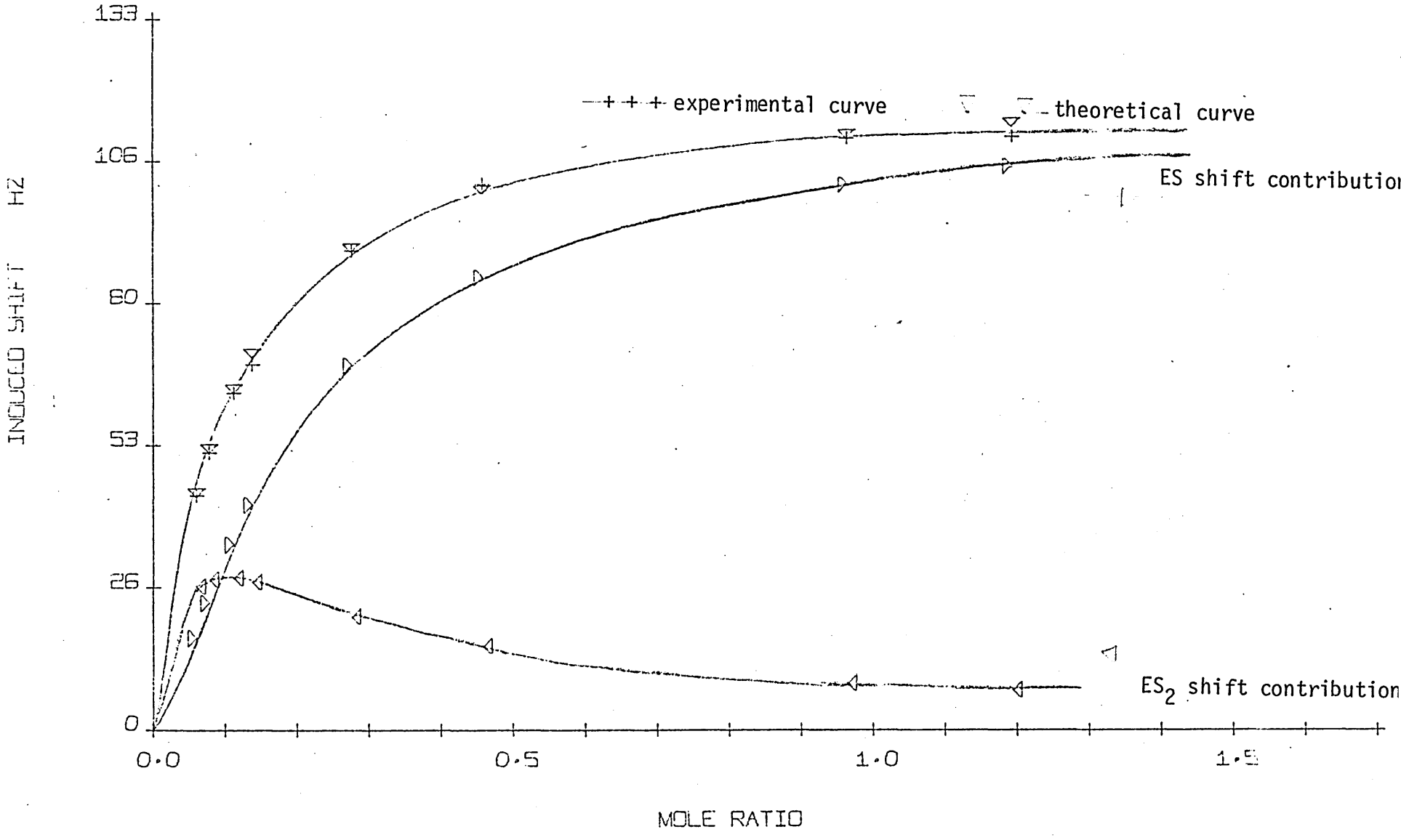
A plot of the induced shift versus mole ratio[a]

a) isopropyl - CH_3 proton signal of isopropylmethylether - $Eu(fod)_3$ complex in $CDCl_3$.

The intrinsic parameters obtained by the full 4-parameter data treatment are -

K_1	=	61	$dm^3.mol^{-1}$
K_2	=	19	$dm^3.mol^{-1}$
Δ_1	=	610	Hz
Δ_2	=	550	Hz
%SD	=	1.17	

The calculated initial slope value = 741 Hz and the maximum observed induced shift Δ_{max} = 111 Hz


133
106
80
53
26
0
INDUCED SHIFT HZ
0·0
0·5
1·0
1·5
MOLE RATIO
experimental curve
theoretical curve
ES shift contribution
ES2 shift contribution

value contains substantial contributions from both delta max. 1 and delta max. 2 which are unknown and indeterminate. The equilibrium binding constants predicted by theory, K_1 and K_2 values of 61 and 19 $dm^3.mol^{-1}$ respectively, agree very well with the suggestion of a weak association. Also the poor comparisons between the predicted delta max. 1 and delta max. 2 values (610 and 550 Hz respectively) and the initial slope value, 741 Hz, shows that the initial slope cannot be used as a limiting incremental shift value. Furthermore, when the limiting incremental shift ratios, $\Delta_{1_i} / \Delta_{2_i}$ vary for different protons within the same molecule and since the initial slope contains unknown contributions from both delta max.1 and delta max.2, then the initial slope of the induced shift versus mole ratio plot cannot be used to calculate accurate relative induced shift values, which are often used in shift reagent work. The relative induced shift is defined as the ratio of the induced shift of say proton i (regarded as standard) to the induced shift of proton j, i.e. Δ_i / Δ_j The induced shift contributions predicted for the ES and ES_2 complexes show that, at small mole ratio values between 0 - 0.2, both complexes contribute significantly to the total induced shift.

Figure 2.10 shows the data plot obtained for the C_8-methyl protons of the camphor-$Eu(fod)_3$ complex in deuterochloroform. The fairly close agreement between the maximum observed induced shift and the initial slope value, 171 and 300 Hz respectively, indicates a fairly strong association between the substrate and shift reagent. This is also indicated by the linearity of the plot in the lower mole ratio region between 0-0.2 although generally, the curvature observed over the entire range of mole ratio values is fairly significant. The best

Figure 2.10

A plot of the induced shift versus mole ratio[a]

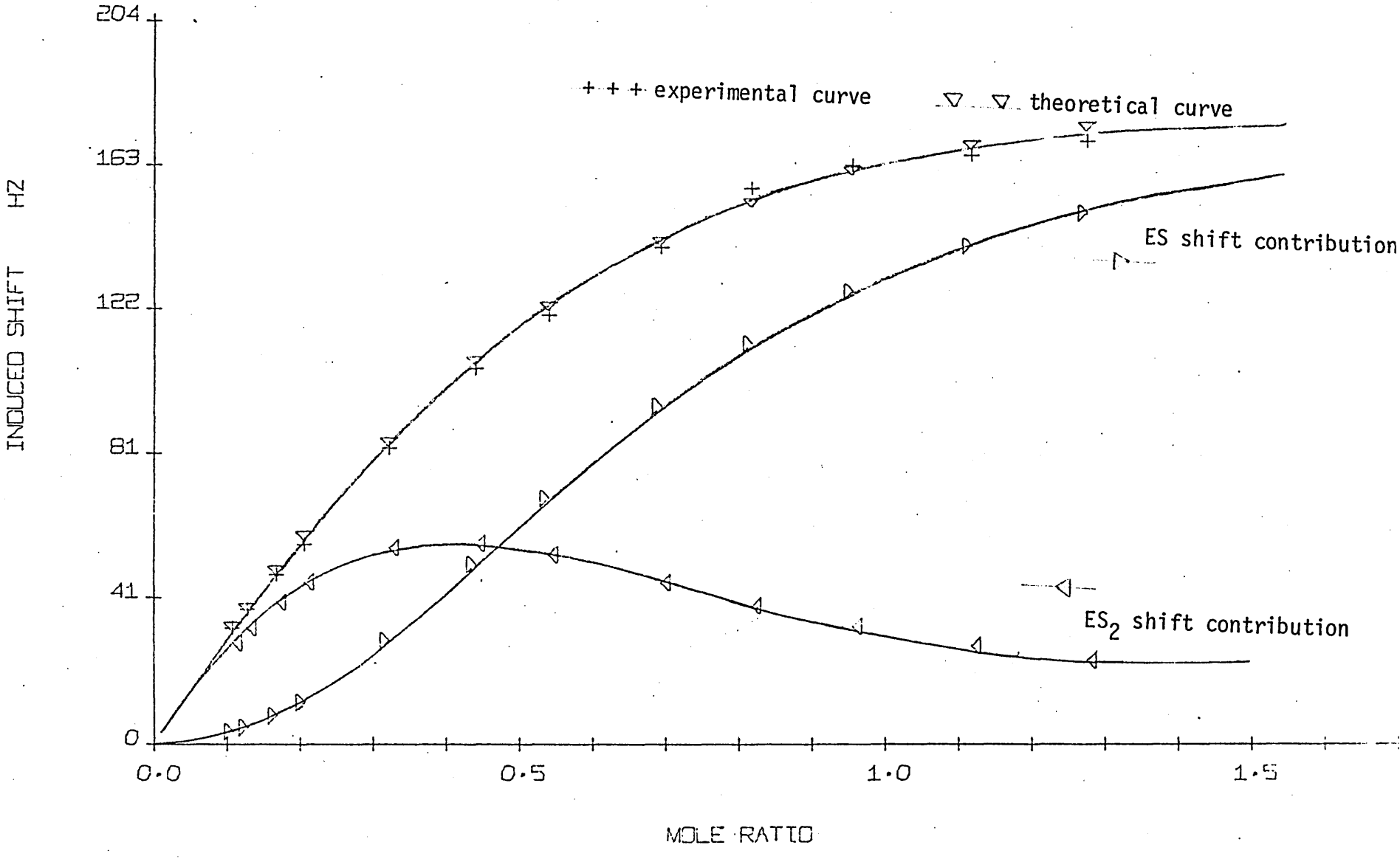
a) C_8-methyl signal of the camphor-Eu(fod)$_3$ complex in $CDCl_3$.

The intrinsic parameters obtained by the full 4-parameter data treatment are -

K_1	=	1040	$dm^3.mol^{-1}$
K_2	=	100	$dm^3.mol^{-1}$
Δ_1	=	220	Hz
Δ_2	=	150	Hz
%SD	=	1.92	

The calculated initial slope value gives Δ_2 = 150 Hz

and the maximum observed induced shift is Δ_{max} = 171 Hz


204
163
122
81
41
0
INDUCED SHIFT HZ
+ + + experimental curve
▽ ▽ theoretical curve
ES shift contribution
ES2 shift contribution
0·0
0·5
1·0
1·5
MOLE RATIO

predicted K_1 and K_2 values 1040 and 100 $dm^3.mol^{-1}$ respectively, support a strong association. Since, as already stated, the magnitude of K_2 determines the significance of the initial slope value, then if as in this case, K_2 is large, the initial slope obtained gives a delta max.2 value which is in excellent agreement with the best predicted delta max. 2 obtained from the full 4-parameter data treatment method, both values being 150 Hz respectively. Also shown in Figure 2.10 are the predicted induced shift contributions for the ES and ES_2 complexes. At small mole ratio values between 0 - 0.2, the induced shift contribution from ES_2 predominates whilst at larger mole ratio values, say above 0.6, the shift contribution from the 1:1 complex is much greater. These shift contributions contrast markedly with those shown in Figures 2.8 and 2.9. In Figure 2.8, $K_1 >>> K_2$ and also K_1 is very large, consequently the shift contribution for the 1:1 complex predominates over most of the mole ratio values. In Figure 2.9, K_1 and K_2 are fairly comparable and small, and although the shift contribution for the 1:1 complex is greater at large mole ratio values, the contribution for both complexes in the lower mole ratio region between 0 - 0.2 are of equal importance. The shift contribution shown in Figure 2.10 however, reflects the increasing value of K_2 and consequently,the shift contribution for the 2:1 complex predominates at lower mole ratio values between 0 - 0.2. Since $K_1 >> K_2$ however, the shift contribution for the 1:1 complex increases and becomes more important at larger mole ratio values.

A comparison of the induced shift versus mole ratio plots shown in Figures 2.10 and 2.11 reveals the dangers that may be encountered in the qualitative analysis of such plots. From the full 4-parameter

Figure 2.11

A plot of the induced shift versus mole ratio[a]

a) Methyl proton signal of the benzylmethylketone-Eu(fod)$_3$ complex in $CDCl_3$.

The intrinsic parameters obtained by the full 4-parameter data treatment are -

K_1 = 1080 $dm^3.mol^{-1}$

K_2 = 140 $dm^3.mol^{-1}$

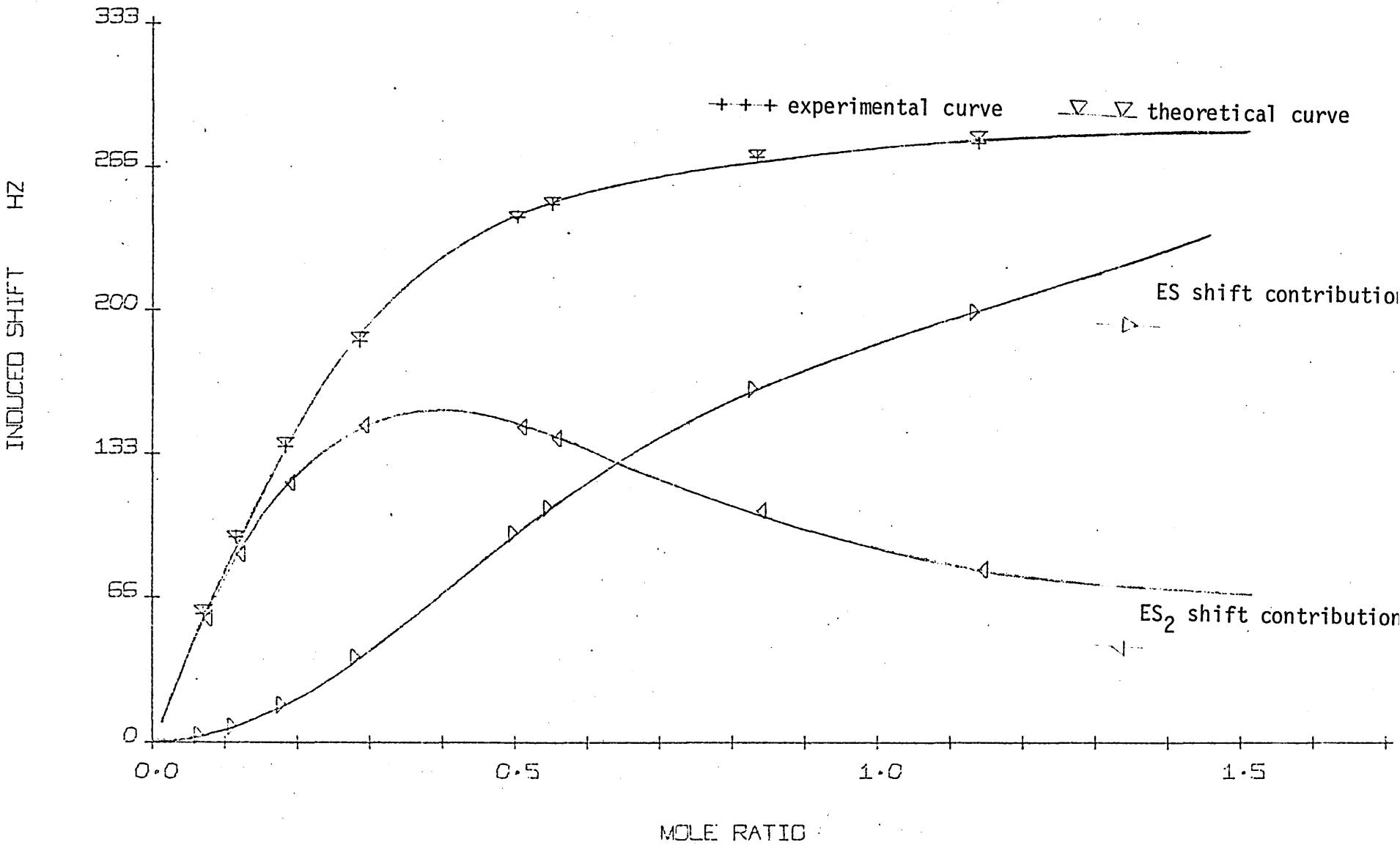
Δ_1 = 340 Hz

Δ_2 = 450 Hz

%SD = 0.74

The calculated initial slope value gives Δ_2 = 442 Hz

and the maximum observed induced shift is Δ_{max} = 278 Hz


333
266
200
133
65
0
INDUCED SHIFT HZ
0·0
0·5
1·0
1·5
MOLE RATIO
+ + + experimental curve
∇ ∇ theoretical curve
ES shift contribution
ES2 shift contribution

data treatment method, it has been shown that the equilibrium binding constants associated with both data plots are approximately the same, and for Figure 2.10 are shown to be indicative of a fairly substantial association process. The linearity of the data plot observed in Figure 2.11 for the lower mole ratio region between 0 - 0.3 also suggests a fairly strong association. Furthermore when K_2 is large, as indeed has been shown, then the limiting incremental shift, delta max. 2, obtained from the initial slope gives a value which is in excellent agreement with the delta max. 2 value predicted from the theoretical-experimental fit, i.e. 442 and 450 Hz respectively. On the other hand, the poor comparison shown in Figure 2.11 between the initial slope and the maximum observed induced shift, 884 and 278 Hz respectively, is indicative of a fairly weak association process. A possible explanation for the conflicting indications of strong and weak associations, lies in the limiting incremental shift values predicted from the 4-parameter data treatment method. In this latter example shown in Figure 2.11, delta max. 1 is found to be smaller than delta max. 2. This is reflected in a smaller value of the maximum observed induced shift value than that which might otherwise have been expected; this in turn leads to an incorrect indication of weak association. The fact that delta max. 1 is smaller than delta max. 2 can only be shown by the comprehensive data treatment method and not by a simple inspection of the experimental data plot. In addition, the comparison between the maximum observed induced shift and the predicted delta max. 1 value 278 and 340 Hz respectively, shows the normally expected agreement found for a fairly strong association process. This supports the evidence of strong association indicated by the linearity of the

data plot observed in the lower mole ratio regions between 0 - 0.3. Finally, Figure 2.12 shows the induced shift versus mole ratio plot of the methyl proton group of the 4-methylbenzylamine-Eu(fod)$_3$ complex in deuterochloroform. The linearity of the plot in the lower mole ratio region between 0 - 0.4 indicates a very strong association, as indeed is suggested by the close comparison between the maximum observed induced shift and the initial slope value. There is also excellent agreement between the predicted delta max. 2 and the delta max. 2 value obtained from the initial slope, 75 and 73 Hz respectively. The induced shift contribution from the 2:1 complex is significant over the entire mole ratio range 0 - 1.0 and reflects the very large value of K_2 for this system. Consequently as expected, a comparison of Figure 2.12 with Figures 2.8 - 11 shows how the contribution from the 2:1 complex dramatically increases as the magnitude of K_2 increases.

Whilst the measurement of a spectroscopic signal is very simple, reliable quantitative analysis and interpretation are extremely difficult. The limiting incremental shift values, delta max. 1 and delta max. 2, are of considerable interest when geometrical structures are to be investigated, since as already stated, the limiting incremental shift is dependent upon the conformational structure of the complex formed in solution between shift reagent and substrate. The limiting incremental shift ratio $\Delta 1_i / \Delta 2_i$ is therefore a most important parameter when dealing with structural determinations. Limiting incremental shift ratios however can only be obtained from a full 4-parameter data treatment method and not by any simple data treatment method.

Figure 2.12

A plot of the induced shift versus mole ratio[a]

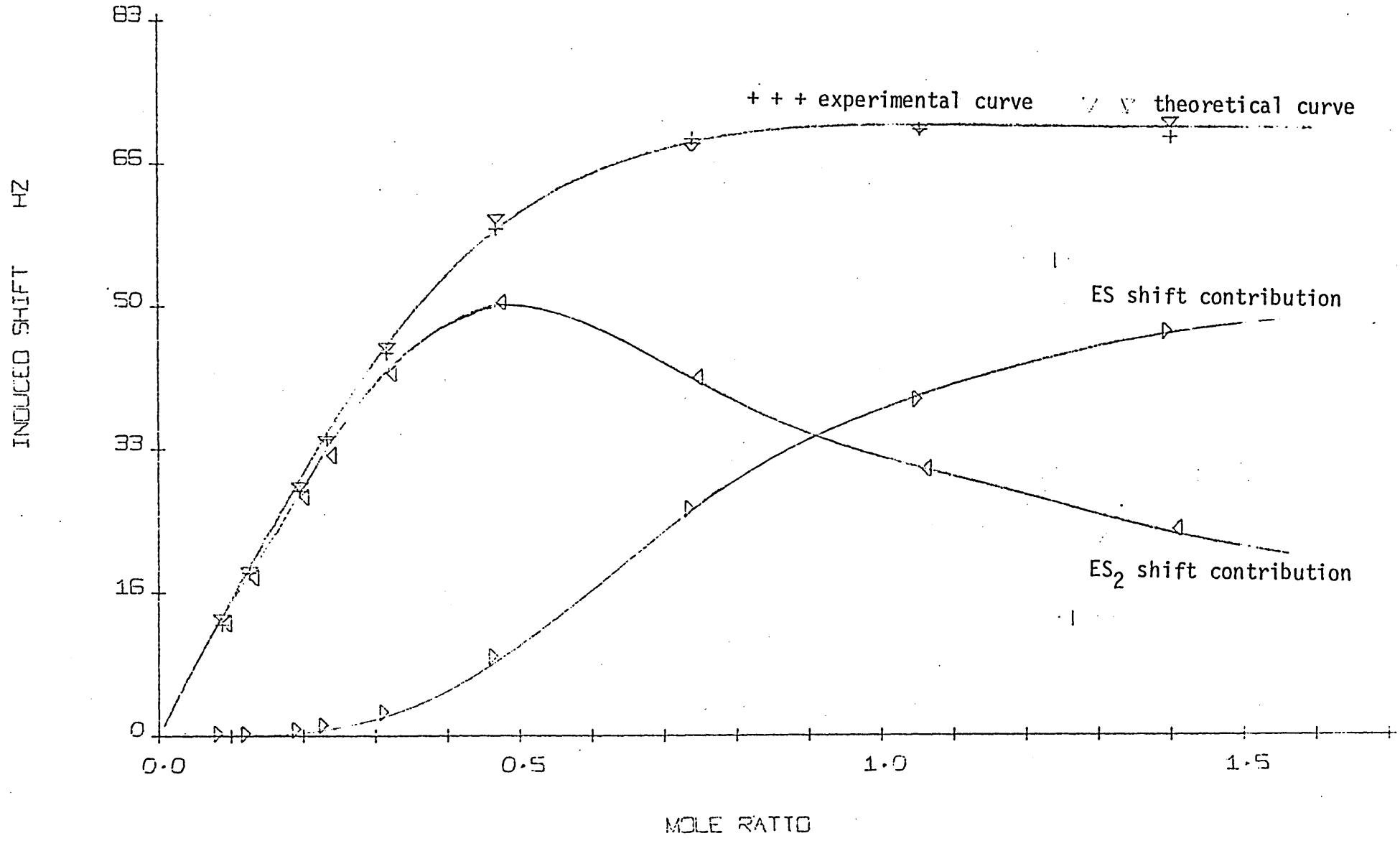
a) Methyl proton signal of the 4-methylbenzylamine-Eu(fod)$_3$ complex in $CDCl_3$.

The intrinsic parameters obtained by the full 4-parameter data treatment are -

K_1	=	5280	$dm^3.mol^{-1}$
K_2	=	1340	$dm^3.mol^{-1}$
Δ_1	=	75	Hz
Δ_2	=	75	Hz
%SD	=	1.52	

The calculated initial slope value gives Δ_2 = 73 Hz

and the maximum observed induced shift is Δ_{max} = 70 Hz


83
66
50
33
16
0
INDUCED SHIFT HZ
+ + + experimental curve
theoretical curve
ES shift contribution
ES2 shift contribution
0·0
0·5
1·0
1·5
MOLE RATIO

In any structural investigation it has to be decided which limiting incremental shift value, delta max. 1 or delta max. 2, represents the best parameter that enables a comparison between the experimentally determined limiting incremental shifts and the theoretically predicted limiting incremental shifts produced from known structures. In many structural determinations, limiting incremental shift values have sometimes been scaled, relative to a given proton (90, 91). If the limiting incremental shift ratios for different protons within the same substrate are approximately constant then either value of the limiting incremental shift can be used. In this case little or no difference will be observed in the structural determination carried out using delta max. 1 or delta max. 2. The results presented here for several substrates have however, shown that the limiting incremental shift ratios can vary for different protons within the same molecule. In these cases, the choice of the limiting incremental shift, delta max. 1 or delta max. 2, is critical since substantial differences may arise in the structural determinations carried out. This will be discussed in more detail in chapter IV. The majority of structural investigations carried out (92, 93) have normally employed plots of the induced shift versus mole ratio similar to those shown in Figures 2.8 - 2.12 for determining the limiting incremental shifts. It has been shown however that the amount of information obtainable from such plots depends upon the type of curve observed. In cases of a 1:1 stoichiometry, or where for a 2:1 stoichiometry, K_1 is extremely large, then delta max. 1 is the limiting incremental shift value obtained. A value of delta max. 2 cannot be determined and consequently

limiting incremental shift ratios cannot be calculated. In examples where K_2 becomes very large, then delta max. 2 is the limiting incremental shift value determined. Again however limiting incremental shift ratios cannot be obtained since delta max. 1 is now unobtainable. In a system where the values of K_1 and K_2 are small, then no reliable information can be obtained from the plot of the induced shift versus mole ratio, and for substrates in this class successful structural investigations are improbable unless a full 4-parameter data treatment fit has been made.

2.4.5.2 Relative induced shift data

The reaction mechanism of any shift reagent-substrate interaction is extremely difficult to determine and consequently certain assumptions regarding a given stoichiometry have been made in the 4-parameter data treatment method presented. For the majority of substrates shown in this thesis, it has been found that the two-step 2:1 reaction mechanism gives better results, when measured in terms of the agreement factor outlined, than the results of a 1:1 reaction mechanism. Again, a comparison of the results obtained from the 4-parameter data treatment method with the results obtained by simple means, may afford information concerning the true nature of the equilibrium occurring in solution. Hence an analysis of the relative induced shift ratio was attempted, where this ratio is defined as the ratio of the induced shift of one proton relative to that of another proton.

If both ES and ES_2 complexes exist in solution, then the lanthanide induced shift is given by

$$\triangle = \frac{[ES]\triangle_1}{[S_T]} + \frac{2[ES_2]\triangle_2}{[S_T]} \quad \ldots 1.2$$

The relative induced shift ratio for protons i and j within the same substrate molecule will therefore be given by

$$\frac{\Delta_i}{\Delta_j} = \frac{x\,\Delta^1_i + y\,\Delta^2_i}{x\,\Delta^1_j + y\,\Delta^2_j} \qquad \ldots \ 2.33$$

where

$$x = \frac{[ES]}{[S_T]} \qquad \text{and} \qquad y = \frac{2[ES_2]}{[S_T]}$$

In a 1:1 stoichiometry, y = o and the relative induced shift reduces to

$$\frac{\Delta_i}{\Delta_j} = \frac{\Delta^1_i}{\Delta^1_j} \qquad \ldots \ 2.34$$

This ratio will therefore be independent of the substrate and shift reagent concentrations and will be constant throughout the experiment. Alternatively, if for a 2:1 stoichiometry, delta max. 1 is the same as delta max. 2, or indeed if a simple mathematical relationship exists between delta max. 1 and delta max. 2, i.e. a constant limiting incremental shift ratio exists, then equation 2.33 reduces to

$$\frac{\Delta_i}{\Delta_j} = \frac{\Delta^1_i}{\Delta^1_j} = \frac{\Delta^2_i}{\Delta^2_j} \qquad \ldots \ 2.35$$

This ratio will again be constant throughout the experiment.

On the other hand the observation of varying relative induced shift ratios is a definite indication of a 2:1 stoichiometry; intuitively it can be expected that the presence of induced shifts resulting from the contact shift mechanism may also lead to different relative induced shift ratios for different protons in the same substrate. However, with substrates that are considered to be free from contact shifts, e.g. saturated ketones, the observation of varying relative induced shift ratios not only indicates a 2:1 reaction mechanism between shift reagent and substrate but also that there is no simple mathematical relationship between the values of delta max. 1 and delta max. 2.

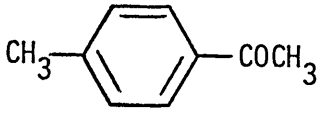
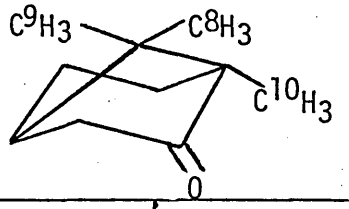
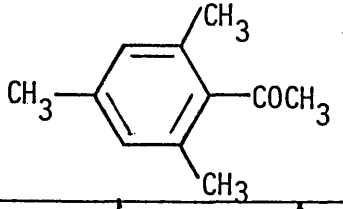
Table 2.18 lists some of the relative induced shift ratios calculated for several substrate ketone molecules, obtained from the experimental induced shifts measured as a function of the substrate concentration whilst the shift reagent concentration is kept constant. In the case of pentan-2-one, the relative induced shift ratios obtained are approximately constant throughout the experiment. These results could indicate a 1:1 stoichiometry, but in view of the results of the 4-parameter data treatment method are more likely to represent a 2:1 stoichiometry with a simple mathematical relationship between the values of delta max. 1 and delta max. 2, i.e. a constant limiting incremental shift ratio. This is shown to be so from the constant limiting incremental shift ratios obtained for pentan-2-one and shown in Table 2.11.

The varying relative induced shift ratios obtained for the other substrates in Table 2.18 indicates the presence of a 2:1 stoichiometry and that no simple relationship exists between delta max. 1 and delta max. 2. Also since the adduct geometry of the substrate-shift reagent

Table 2.18

Relative induced shift ratios [a]

a) Complexes of $Eu(fod)_3$ in $CDCl_3$ obtained at constant shift reagent concentrations.

pentan-2-one		4-methylacetophenone			camphor		2,4,6-trimethylacetophenone		
$CH_3COCH_2CH_2CH_3$									
$\triangle^{CH_3CO}_{COCH_2}$	$\triangle^{CH_3CO}_{CH_2CH_3}$	$\triangle^{CH_3CO}_{2\text{-}6\ H}$	$\triangle^{CH_3CO}_{3\text{-}5\ H}$	$\triangle^{CH_3CO}_{4\text{-}CH_3}$	$\triangle^{C^{10}H_3}_{C^8H_3}$	$\triangle^{C^{10}H_3}_{C^9H_3}$	$\triangle^{CH_3CO}_{2\text{-}6CH_3}$	$\triangle^{CH_3CO}_{3\text{-}5\ H}$	$\triangle^{CH_3CO}_{4\text{-}CH_3}$
1.00	2.83	4.62	1.24	7.29	1.52	2.45	1.69	3.33	5.74
0.98	2.82	4.63	1.24	7.22	1.52	2.44	1.69	3.31	5.71
0.99	2.83	4.74	1.24	7.45	1.52	2.44	1.67	3.36	5.70
1.01	2.85	4.60	1.26	7.45	1.52	2.43	1.69	3.35	5.66
0.99	2.83	4.67	1.26	7.38	1.51	2.40	1.69	3.29	5.46
0.99	2.83	4.60	1.26	7.41	1.51	2.35	1.70	3.29	5.50
0.99	2.83	4.75	1.25	7.45	1.50	2.32	1.69	3.30	5.46
0.99	2.83	4.78	1.25	7.25	1.49	2.26	1.68	3.34	5.30
		4.78	1.26	7.48	1.50	2.16	1.70	3.34	5.10
					1.48	2.14	1.71	3.34	4.85

complex changes when the stoichiometry changes, then the relative induced shift ratios will change as the substrate concentration changes whilst the shift reagent concentration is kept constant. At low substrate concentrations, where the 1:1 adduct predominates, the relative induced shift ratio will approach the value of the corresponding ratio of the delta max. 1 values obtained for the protons under investigation. Similarly, when the substrate concentration increases, favouring 2:1 complex formation, then the relative induced shift ratio will approach the value of the ratio obtained from the corresponding delta max. 2 values. Highlighting this behaviour are the relative induced shift values obtained for the $C^{10}H_3$ and $C^{9}H_3$ proton groups of the camphor-$Eu(fod)_3$ complex which range between 2.45 - 2.14 as the substrate concentration is increased. These values are very close to the ratio values $\left(\frac{\triangle_1 C^{10}H_3}{\triangle_1 C^{9}H_3} \text{ and } \frac{\triangle_2 C^{10}H_3}{\triangle_2 C^{9}H_3} \right)$ of 2.41 and 2.20 respectively obtained from the corresponding limiting incremental shift values shown in Table 2.11.

2.4.6 Conclusions

A method has been outlined which enables intrinsic parameters to be determined. This method involves a comparison between experimental data and theoretically predicted data obtained from known intrinsic parameters. The results for both 1:1 and 2:1 stoichiometries have been recorded, but it is shown that for the majority of substrates studied, the 2:1 stoichiometry gives better results, when measured in terms of a percentage standard deviation, than the results of a 1:1 stoichiometry. Also, results obtained but not shown, for a one-

step 2:1 reaction mechanism were very much inferior to those results obtained for a two-step 2:1 reaction mechanism and indeed even for a 1:1 stoichiometric reaction.

In general, alcohols coordinate more effectively with the shift reagent $Eu(fod)_3$ than either ketones or ethers, but less effectively than nitrogen containing substrates, e.g. amines and substituted pyridines. The effects of steric hindrance and substrate basicity however, prohibit any general predictions regarding the intrinsic parameters obtained, even though certain similarities are found for chemically related substrates.

The results for substrates complexed with other shift reagents show that the relative order of stability for complexes of $Pr(fod)_3$ follow the same order as the corresponding $Eu(fod)_3$ complexes. The stability of the complexes however increases as the lanthanide ionic radius decreases.

A rigorous analysis of the induced shift versus mole ratio plot can only be attempted with a knowledge of the associated equilibrium binding constants. In certain cases where there is a very strong association between the substrate and shift reagent, then a very good estimate of one of the limiting incremental shift values can be obtained from this simple mole ratio plot. However for weakly coordinated systems, little or no information can be obtained in this way.

For structural investigations accurate limiting incremental shift values must generally be used. When, within a substrate molecule there exists a constant limiting incremental shift ratio $\left(\Delta_{1_i} / \Delta_{2_i}\right)$ then it is immaterial which limiting incremental shift value is used since scaling factors normally used will result in little or no

differences being observed in the resulting structural determinations. This may not be the case however if the limiting incremental shift ratios vary for different protons within a molecule, since the choice of the correct limiting incremental shift value is of the utmost importance. The observation of varying relative induced shift ratios $\left(\frac{\Delta_i}{\Delta_j}\right)$ obtained for several proton groups within a substrate, indicates a 2:1 stoichiometry and also the lack of a simple mathematical relationship between delta max. 1 and delta max. 2.

The theoretical equations derived for the simple data treatment methods outlined in section 2.3 are correct only when the limits appropriate to each equation hold true. When a strong association exists then it has been shown that several equations (2.23, 2.25 and 2.31) can be used to determine a limiting incremental shift value. Table 2.19 shows the limiting incremental shift value, namely delta max. 2, obtained by these simple data treatment methods and compares the values with the theoretically predicted delta max. 2 values, obtained by the full 4-parameter data treatment method. The good agreement obtained illustrates that limiting incremental shift values can be determined from easily accessible data but that a strong association process is a prerequisite. The above agreement is not found for weakly coordinated systems and it must be emphasised that only one of the limiting incremental shift values can be determined by these simple methods.

As pointed out, the full 4-parameter data treatment method is based on a comparison between theoretically predicted and experimentally observed data. Consequently, rigorous experimental precautions must be taken to ensure the reliability of the experimental data obtained.

Table 2.19

A comparison of limiting incremental shift values[a]

a) Obtained for $Eu(fod)_3$-substrate complexes in $CDCl_3$ possessing very large equilibrium binding constants.

b) Units of Hz.

c) Full 4-parameter data treatment method.

Figures in brackets represent the corresponding linear correlation coefficients.

SUBSTRATE	PROTON SIGNAL	$\triangle_2$ (b)			
		(c)	Equation 2.23	Equation 2.25	Equation 2.31
$(CH_3)_3CC(CH_3)_2OH$ 2,3,3-trimethylbutan-2-ol	$(\underline{CH}_3)_3$	300	304 (1.0000)	303 (1.0000)	300 (0.9999)
$(CH_3)_2CHCH_2OH$ isobutanol	$(\underline{CH}_3)_2$	310	333 (1.0000)	325 (1.0000)	296 (1.0000)
$(CH_3)_2CHCH_2NH_2$ isobutylamine	$(\underline{CH}_3)_2$	295	313 (0.9997)	303 (0.9999)	294 (0.9999)

CHAPTER III SUPPLEMENTARY METHODS FOR DETERMINING INTRINSIC PARAMETERS

3.1 Introduction

In this chapter, alternative methods of determining intrinsic parameters are examined with a view to

i) finding simpler, but yet adequate means of obtaining intrinsic parameters and

ii) of determining intrinsic parameters independently in order that a check can be made on the accuracy and reliability of the results obtained by the 4-parameter data treatment method.

To date all published data concerning shift reagent-substrate equilibria have attempted to calculate intrinsic parameters based only on the analysis of the substrate resonance frequencies. The ideal system however for nmr studies of molecular complexes should possess several, if not all, of the following features.

i) Both donor (substrate) and acceptor (shift reagent) molecules should contain protons (or other suitable magnetic nuclei) preferably giving single, sharp, absorption peaks.

ii) The concentrations of both substrate and shift reagent should be able to be made large with respect of each other, i.e. a comprehensive range of concentrations ought to be used.

iii) The nmr absorptions should not overlap.

If these three conditions apply, then it should be possible to determine the limiting incremental shift values of the substrate protons, the limiting incremental shift values of the shift reagent protons and also two independently derived values of the equilibrium binding constant, which in theory, should be identical. The analysis of

shift reagent chemical shift frequencies therefore offers an alternative way for determining intrinsic parameters.

Competition experiments offer another independent way in which intrinsic parameters may be determined. If a substrate, possessing known intrinsic parameters, is complexed with a shift reagent, then the equilibrium occuring in solution can be characterised. In the presence of a second substrate however, the original equilibrium will be altered. The change in this equilibrium position will reflect the intrinsic parameters of the second substrate. Consequently, the difference observed between the theoretically expected induced shift values of the first substrate, measured in the absence of a second substrate, and the experimentally produced induced shift values of the first substrate measured in the presence of the second substrate, will, together with the concentrations of the second substrate, offer a means of calculating the intrinsic parameters of the second substrate. Alternatively, a comparison of the induced shift values observed for both competing substrates will enable the intrinsic parameters of the second substrate to be calculated. In view of the complex nature of the interactions involved when two competing two-step 2:1 reaction mechanisms are being considered, it was anticipated that the assumption of two competing 1:1 reaction machanisms would lead to much simpler calculations. Consequently, in the supplementary methods used for determining intrinsic parameters, only those substrates for which the assumption of a 1:1 stoichiometry was a good approximation were used.

3.2 A Shift Reagent Data Treatment Method

When the following equilibrium is considered

$$E + S \rightleftharpoons ES$$

and when $[S_T] >> [E_T]$ then it can be shown (80) that the fractional population of the complex formed in an ideal solution is given by

$$\frac{[ES]}{[E_T]} = \frac{K[S_T]}{1 + K[S_T]} \qquad \ldots 3.1$$

This equation is obtained by rearrangement of

$$K = \frac{[ES]}{\left([E_T] - [ES]\right)[S_T]}$$

where the approximation $[S_T] = \left([S_T] - [ES]\right)$ has been made because $[S_T] >> [E_T]$

Since the fast exchange condition applies and only one resonance signal is observed which is in a weighted position of the chemical shift of the free shift reagent and the chemical shift of the shift reagent in the 1:1 adduct, then as was shown for the induced shift of the substrate protons, the lanthanide induced shift of the shift reagent is given by

$$\triangle_{SR} = \frac{[ES]\,\triangle_{SR}}{[E_T]} \qquad \ldots 3.2$$

where $\triangle_{SR}$ is the induced shift of the lanthanide metal chelate and $\triangle_{SR}$ is the corresponding limiting incremental shift value of the 1:1 complex. Substitution of $[ES]$ from equation 3.2 into equation 3.1 gives a rearranged form of the Benesi-Hildebrand equation (81), namely

$$\frac{1}{\Delta_{SR}} = \frac{1}{K\left[S_T\right]\Delta_{SR}} + \frac{1}{\Delta_{SR}} \quad \ldots \quad 3.3$$

Equation 3.3 shows that the chemical shift of the lanthanide metal chelate does not depend upon the shift reagent concentration, provided that $\left[S_T\right] \gg \left[E_T\right]$. Rearrangement of equation 3.3 gives

$$\frac{\Delta_{SR}}{\left[S_T\right]} = K\Delta_{SR} - K\Delta_{SR} \quad \ldots \quad 3.4$$

which is a form of the Scatchard equation used by several authors (82, 83) for the determination of the composition and formation constants of weak molecular complexes. Consequently if experiments are carried out where the induced shift of the lanthanide shift reagent is measured as a function of the substrate concentration whilst the shift reagent concentration is kept constant, then a plot of $\Delta_{SR}/\left[S_T\right]$ versus Δ_{SR} will enable the equilibrium binding constant and the limiting incremental shift value of the shift reagent to be determined from the slope and intercept measurements respectively.

Figure 3.1 and Table 3.1 illustrates the results obtained when isopropylmethylether and di-n-propylether are each complexed separately in the presence of the shift reagent $Eu(fod)_3$ in deuterochloroform. The induced shift of the tert. butyl proton group of $Eu(fod)_3$ was measured as the concentration of the substrate was varied and the shift reagent concentration held constant at about 0.005 $mol.dm^{-3}$. Whilst the proton induced shifts of the substrate complexed with $Eu(fod)_3$ are downfield in direction, the induced shifts of the shift

Figure 3.1

Graphs obtained from the Scatchard equation[a]

Curve ⊣⊣⊣ represents the isopropylmethylether-Eu(fod)$_3$ complex in $CDCl_3$.

Curve ⊗⊗⊗ represents the di-n-propylether-Eu(fod)$_3$ complex in $CDCl_3$.

a) $$\frac{\triangle_{SR}}{[S_T]} = K\triangle_{SR} - K\triangle_{SR} \quad \ldots 3.4$$

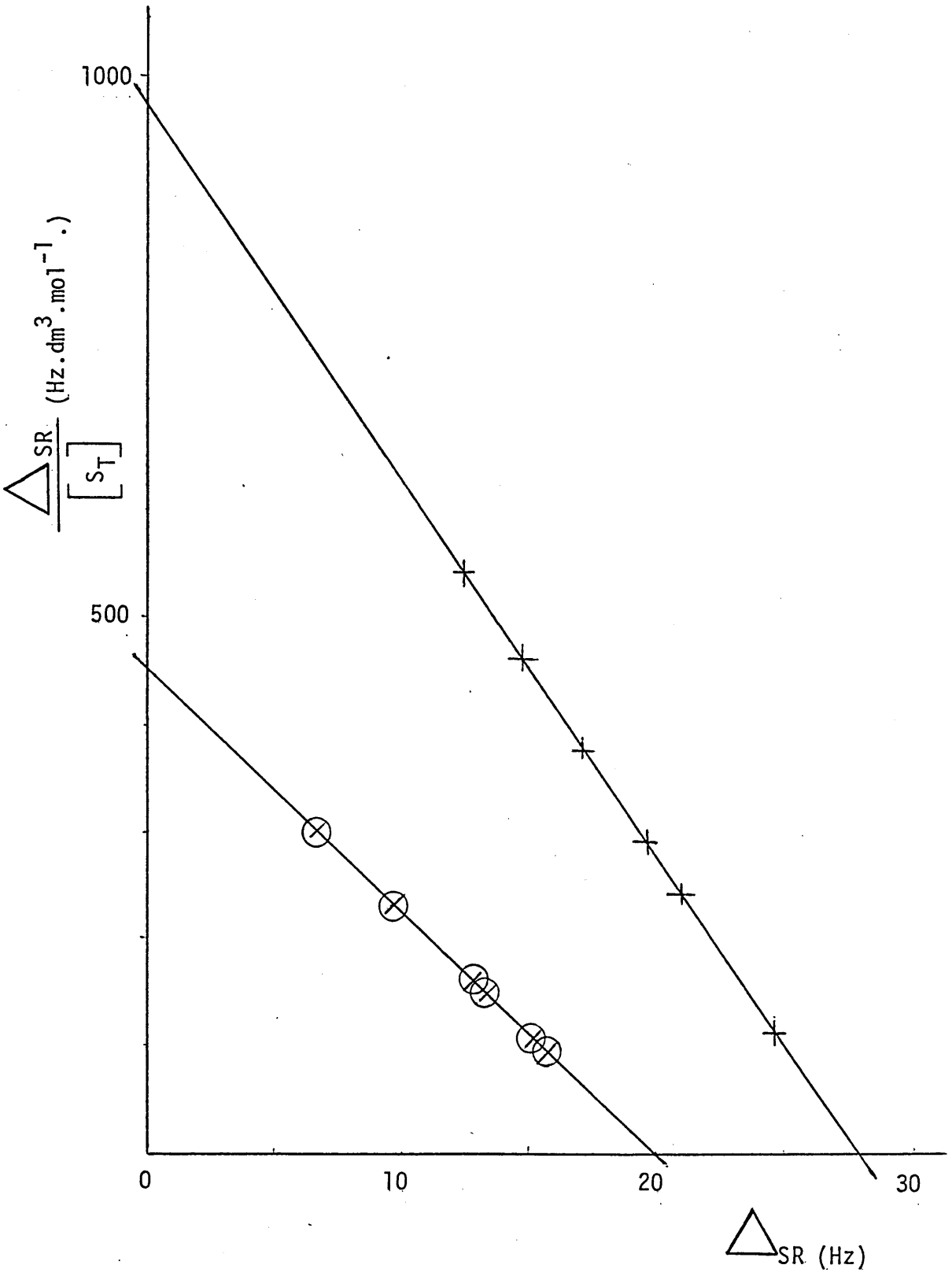

1000
500
0
10
20
30
$\frac{\Delta SR}{[S_T]}$ (Hz.dm^3.mol^{-1}.)
ΔSR (Hz)

Table 3.1

Intrinsic parameters obtained from shift reagent resonance frequencies[a]

a) Substrates complexed with $Eu(fod)_3$ in $CDCl_3$.

b) Equilibrium binding constants determined by the full 4-parameter data treatment method. Units of $dm^3.mol^{-1}$.

c) Equilibrium binding constants obtained from a plot of the Scatchard equation. Units of $dm^3.mol^{-1}$.

d) Linear correlation coefficients.

e) Limiting incremental shift value of the tert. butyl protons in $Eu(fod)_3$. Units of Hz.

SUBSTRATE	$K^{(b)}$	$K^{(c)}$	$r^{(d)}$	$\triangle_{SR}^{(e)}$
$(CH_3)_2CHOCH_3$ isopropylmethylether	30	35.3	-0.9887	27.8
$(CH_3CH_2CH_2)_2O$ di-n-propylether	17	22.2	-1.0000	20.1

reagent are upfield. It is shown that good straight lines are obtained as is reflected by the linear correlation coefficients obtained by a least squares analysis. Also, the limiting incremental shift values of the tert. butyl proton groups of the shift reagent are small when compared with the limiting incremental shift values of the substrate protons shown in Table 2.14. This probably reflects the distance between the tert. butyl protons and the coordinating centre, and also the internuclear angles between the lanthanide metal, the oxygen donor atom and the protons concerned. A comparison of the equilibrium binding constants obtained by this method shows fairly good agreement with the corresponding equilibrium binding constants obtained from the full 4-parameter data treatment method. The reason why better agreement has not been obtained is possibly attributed to several factors. Firstly, the assumption of an exclusive 1:1 stoichiometry may be unjustified. The results from the full 4-parameter data treatment method shows that although only small differences exist between the minimum agreement factors obtained for both reaction mechanisms, the results of the 2:1 stoichiometry are more favourable. Secondly, it has been shown in the previous chapter that for accurate intrinsic parameter determinations, a comprehensive range of substrate-shift reagent concentrations must be studied. The experimental conditions outlined for this simple data treatment method are too restricted to adequately fit the experimental data to a given stoichiometry. This is particularly so when $[S_T] >> [E_T]$ and the induced shifts of the substrate ether protons are measured as a function of the substrate concentration whilst the shift reagent concentration is kept constant.

Finally, the induced shifts of the tert. butyl protons of the shift reagent $Eu(fod)_3$ are only small in magnitude. This could result in the introduction of errors greater than those normally tolerated or experienced in chemical shift measurements. Despite this, the agreement found between the 4-parameter data treatment method and the supplementary method is nevertheless encouraging.

An analysis of the shift reagent induced shifts of other $Eu(fod)_3$-substrate complexes revealed that very poor linear correlation coefficients were obtained from plots of the Scatchard equation. This may arise because the method is only applicable to weak molecular complexes while for strongly coordinated systems the nature of the shift reagent induced shift may become too difficult to quantify. This in part, agrees with Williams et al (6) who stated that the behaviour of the tert. butyl resonances $\left[\text{ of } Eu(dpm)_3 \right]$ is too complex to be able to be used in the determination of equilibrium binding constants.

Certainly, in experiments presented in this thesis involving strongly coordinated systems, no correlation could be obtained between the Scatchard equation and the magnitude of the shift reagent induced shifts. The only noteworthy feature was that shift reagent induced shifts were always opposite in direction to the substrate induced shifts.

A linear plot of the Scatchard equation is indicative of a 1:1 stoichiometry or of a 2:1 stoichiometry where a special case exists, namely

$$K_1 = 4K_2 \quad \text{and} \quad \triangle_1 = 2\triangle_2$$

This latter condition is however specialised and refers to a condition of equivalent and independent binding sites. For a step wise equilibrium process, as in the case of a two-step 2:1 reaction mechanism, the formation of ES and ES_2 are neither independent nor equivalent. However, approximate linearity can be observed by 2:1 reaction mechanisms under certain circumstances (80).

3.3 Competition Experiments

3.3.1 A concentration data treatment method

If 1:1 stoichiometries are considered between a shift reagent and two competing substrates, Sa and Sb, then the following equilibria will be established.

$$E + Sa \rightleftharpoons ESa \qquad 3.5a$$

$$E + Sb \rightleftharpoons ESb \qquad 3.5b$$

where ESa and ESb are the corresponding 1:1 complexes formed in solution. The equilibrium binding constants Ka and Kb respectively are given by

$$Ka = \frac{[ESa]}{[E_F]([Sa] - [ESa])} \qquad \ldots 3.6$$

$$Kb = \frac{[ESb]}{[E_F]([Sb] - [ESb])} \qquad \ldots 3.7$$

where $[Sa]$ and $[Sb]$ are the total concentrations of substrates Sa and Sb respectively and $[ESa]$ and $[ESb]$ are the equilibrium concentrations of the ESa and ESb complexes respectively.

$[E_F]$ is the equilibrium concentration of the free or uncomplexed shift reagent and is given by

$$[E_F] = [E_T] - [ESa] - [ESb]$$

Substitution of $[E_F]$ from equation 3.6 into equation 3.7 leads by rearrangement to

$$[ESb] = \frac{[Sb][ESa]}{\frac{Ka\left([Sa] - [ESa]\right)}{Kb} + [ESa]} \quad \ldots 3.8$$

which when substituted into equation 3.6 rearranges to

$$Kb = \frac{\left(\frac{Ka\left([Sa] - [ESa]\right)[E_T]}{[ESa]} - 1 - Ka\left([Sa] - [ESa]\right)\right)}{\left([Sb] - [E_T] + [ESa] + \frac{[ESa]}{Ka\left([Sa] - [ESa]\right)}\right)} \quad \ldots 3.9$$

Alternatively, equation 3.9 can be rearranged to give

$$[Sb] = G\left(\frac{[E_T]}{[ESa]} - 1 - \frac{1}{Ka([Sa] - [ESa])}\right) \quad \ldots \quad 3.10$$

$$\text{where} \quad G = \left(\frac{Ka([Sa] - [ESa])}{Kb} + [ESa]\right)$$

Consequently in the presence of a standard substrate, Sa, the intrinsic parameters of which are known it is possible, by the addition of known amounts of a competing substrate Sb, to be able to calculate the equilibrium binding constant of that competing substrate. Alternatively, if the equilibrium binding constant of the competing substrate is known, it is possible to determine the concentration of the competing substrate. It can be envisaged that the effect of considering the possibility of shift reagent dimerisation will cause a further competition and hence complicate matters more (84, 85). However as already discussed in Chapter Two, if very small shift reagent concentrations are used in the experiments then the effects resulting from possible shift reagent dimerisation will be negligible. Furthermore, if a 2:1 stoichiometry is assumed between the competing substrates and the shift reagent then the following equilibria will have to be considered.

$$E + Sa \rightleftharpoons ESa$$

$$ESa + Sa \rightleftharpoons ES_2a$$

and

$$E + Sb \rightleftharpoons ESb$$

$$ESb + Sb \rightleftharpoons ES_2b$$

Further equilibria may involve

$$ESa + Sb \rightleftharpoons ESaSb$$

and $$ESb + Sa \rightleftharpoons ESbSa$$

The appropriate expressions for the equilibrium binding constants are given by

$$K_1a = \frac{[ESa]}{[E_F]\left([Sa] - [ESa] - 2[ES_2a] - [ESaSb] - [ESbSa]\right)}$$

$$K_2a = \frac{[ES_2a]}{[ESa]\left([Sb] - [ESa] - 2[ES_2a] - [ESaSb] - [ESbSa]\right)}$$

$$K_1b = \frac{[ESb]}{[E_F]\left([Sb] - [ESb] - 2[ES_2b] - [ESbSa] - [ESaSb]\right)}$$

$$K_2b = \frac{[ES_2b]}{[ESb]\left([Sb] - [ESb] - 2[ES_2b] - [ESbSa] - [ESaSb]\right)}$$

$$Kab = \frac{[ESaSb]}{[ESa]\left([Sb] - [ESb] - 2[ES_2b] - [ESbSa] - [ESaSb]\right)}$$

$$\text{and } Kba = \frac{[ESbSa]}{[ESb]\left([Sa] - [ESa] - 2[ES_2a] - [ESaSb] - [ESbSa]\right)}$$

$$\text{where } [E_F] = \left([E_T] - [ESa] - [ES_2a] - [ESb] - [ES_2b] - [ESbSa] - [ESaSb]\right)$$

Consequently, solutions to these equations, which can be derived in a similar way to those derived for the 1:1 stoichiometric cases, will be almost impossible to solve. In view of this complexity, only the 1:1 stoichiometry was considered.

In view of the results obtained from the 4-parameter data treatment method, then if appropriate substrates are chosen so that a 1:1 stoichiometry is approached, say where $K_1 >> K_2$, then the choice of these substrates will help in the analysis of the experimental data obtained from these competition experiments. Also, substrate-substrate interactions can be minimised by choosing substrates of a similar nature which show no tendency to self-associate e.g. ketones. Small substrate concentrations may also be used.

Initially, for a 1:1 stoichiometry with known values of Ka, $[Sa]$ and $[E_T]$ it is possible to predict a value of $[ESa]$. This is achieved by solving the quadratic equation (similar to equation 2.3) resulting from the rearrangement of equation 3.6. Since the limiting incremental shift value Δ_a is also known, it is possible to calculate the lanthanide induced shift Δ_{ao} expected from the substrate Sa, in the absence of competing substrate Sb.

$$\Delta_{ao} = \frac{[ESa]\,\Delta_a}{[S_T]} \qquad \ldots \; 3.11$$

In the presence of a competing substrate Sb, the effective concentration of the shift reagent towards substrate Sa will decrease, leading

to a reduction in the observed induced shift of substrate Sa. The difference between the observed induced shift in the presence of the competing substrate Sb and the predicted induced shift expected in the absence of substrate Sb reflects the association between the shift reagent and substrate Sb. The induced shift of substrate Sa observed in the presence of the competing substrate Sb can therefore be used to calculate the new equilibrium concentration of ESa

$$[ESa] = \frac{\Delta_{a'}[Sa]}{\Delta_{a}}$$

$\Delta_{a'}$ is the induced shift of substrate Sa in the presence of competing substrate Sb. Substitution of this new equilibrium concentration into equation 3.9 then enables the equilibrium binding constant of substrate Sb to be calculated. If the induced shift of substrate Sa, the concentration of which is kept constant, is measured as a function of the concentration of the competing substrate Sb, then a series of Kb values can be calculated and a standard deviation obtained. Alternatively if it is assumed that a value of Kb is known, then a graphical comparison can be obtained from the experimental concentrations of Sb and the theoretically predicted concentrations of Sb based on equation 3.10.

In view of the emphasis already placed on the experimental conditions for the determination of reliable intrinsic parameters, extreme experimental precautions were again taken to ensure the removal of all traces of moisture and other impurities which act as competitors for the available shift reagent. Consequently only anhydrous conditions were employed. The shift reagent and standard substrate concentrations

were kept constant at about 0.005 and 0.01 mol.dm^{-3} respectively. These concentrations were adequate for obtaining substantial induced shifts whilst at the same time cause minimum adverse effects such as possible shift reagent dimerisation and solution non-ideality. The competing substrate concentration was allowed to vary between 0.004 and 0.04 mol.dm^{-3} and was sufficient to successfully compete with the standard substrate so that the observed induced shifts of the standard substrate varied over as wide a range of values as possible. The results obtained from several experiments,where the induced shifts of the standard substrate were measured as a function of the competing substrate concentration are shown in Table 3.2. The shift reagent $Eu(fod)_3$ in deuterochloroform was used with several selected ketones. The intrinsic parameters determined by the full 4-parameter data treatment method were used as the standard values and the ketones chosen were selected for several reasons:

i) there was less probability of the substrates associating in solution and affecting the assumed 1:1 stoichiometric equilibrium, i.e. substrate-substrate interactions were minimised.

ii) The intrinsic parameters determined previously were large and consequently substantial induced shifts were expected enabling accurate and reliable chemical shift measurements to be made.

iii) Since the values of the equilibrium binding constants determined for the 2:1 stoichiometry were such that $K_1 >> K_2$ then the reaction would approximate to that of a 1:1 stoichiometry.

A comparison of the equilibrium binding constants shown in Table 3.2 reveals fairly good agreement between the values determined from the full 4-parameter data treatment method and those determined by the

Table 3.2

Equilibrium binding constants Kb determined from competition experiments[a]

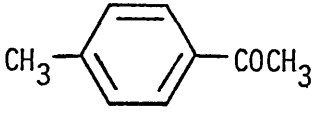
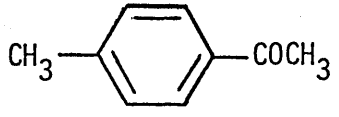
a) Substrate-Eu(fod)$_3$ complexes in $CDCl_3$.

b) Units of $dm^3.mol^{-1}$.

c) Units of Hz.

d) Figures in brackets represent standard deviation values.

e) Determined by the full 4-parameter data treatment method.

COMPETING SUBSTRATE Sb	STANDARD SUBSTRATE Sa	PROTON RESONANCE SIGNAL Sa	STANDARD INTRINSIC PARAMETERS USED		EQUILIBRIUM BINDING CONSTANTS Kb[b]	
			Δ_a[c]	Ka[b]	(d)	(e)
$CH_3COCH=C(CH_3)_2$ 4-methylpentan-3-ene-2-one	$CH_3CH_2CH_2COCH_3$ pentan-2-one	$\underline{CH_3}CH_2$	352	259	1387 (85.2)	1457
		$CO\underline{CH_3}$	1005	259	1217 (71.8)	1457
 4-methylacetophenone	$CH_3CH_2CH_2COCH_3$ pentan-2-one	$\underline{CH_3}CH_2$	352	259	377 (30.7)	495
		$CO\underline{CH_3}$	1005	259	394 (25.8)	495
$CH_3COCH=C(CH_3)_2$ 4-methylpentan-3-ene-2-one	 4-methylacetophenone	$4\text{-}\underline{CH_3}$	126	495	1248 (123.6)	1457
		$CO\underline{CH_3}$	936	495	1415 (57.1)	1457

competition experiments.This agreement is encouraging since all the experiments were carried out at different times and consequently may involve slight temperature, concentration and purity variations. The results between 4-methylacetophenone and pentan-2-one show the poorest agreement, but since the equilibrium binding constants of these substrate-Eu(fod)$_3$ complexes are both relatively small then perhaps the assumption that a 1:1 stoichiometry is approached is not totally applicable. Certainly the results obtained by the full 4-parameter data treatment method indicates that the 2:1 stoichiometry is more favourable. The variation between the equilibrium binding constants calculated for different protons within the same competing substrate molecule, e.g. the values of 4-C$\underline{H}_3$ and COC$\underline{H}_3$ proton resonances of 4-methylacetophenone, may not only reflect the incorrect assumption of a 1:1 stoichiometry, but may also result from the fact that the induced shift is a weighted average of two limiting incremental shift values, $\triangle_1$ and $\triangle_2$, and as shown, limiting incremental shift ratios may differ for different protons within the same molecule.

An alternative way of expressing the results of the competition experiments is to compare the experimental competing substrate concentrations with those concentrations predicted by theory from known values of $\left[E_T\right]$, Ka, $\left[Sa\right]$ and Kb, as based upon equation 3.10. A graphic comparison can then be carried out and also a percentage standard deviation calculated between the experimental and theoretical curves. In this way the results of the COC$\underline{H}_3$ proton resonance signal of 4-methylacetophenone complexed with Eu(fod)$_3$ in deuterochloroform in the presence of a competing substrate, 4-methylpentan-3-ene-2-one, are shown in Figure 3.2. The excellent agreement shown between the

Figure 3.2

A comparison of theoretical and experimental competing substrate concentrations

Standard substrate 4-methylacetophenone with

Ka = 495 $dm^3.mol^{-1}$.

Δl_a = 936 Hz. ($COCH_3$ resonance signal)

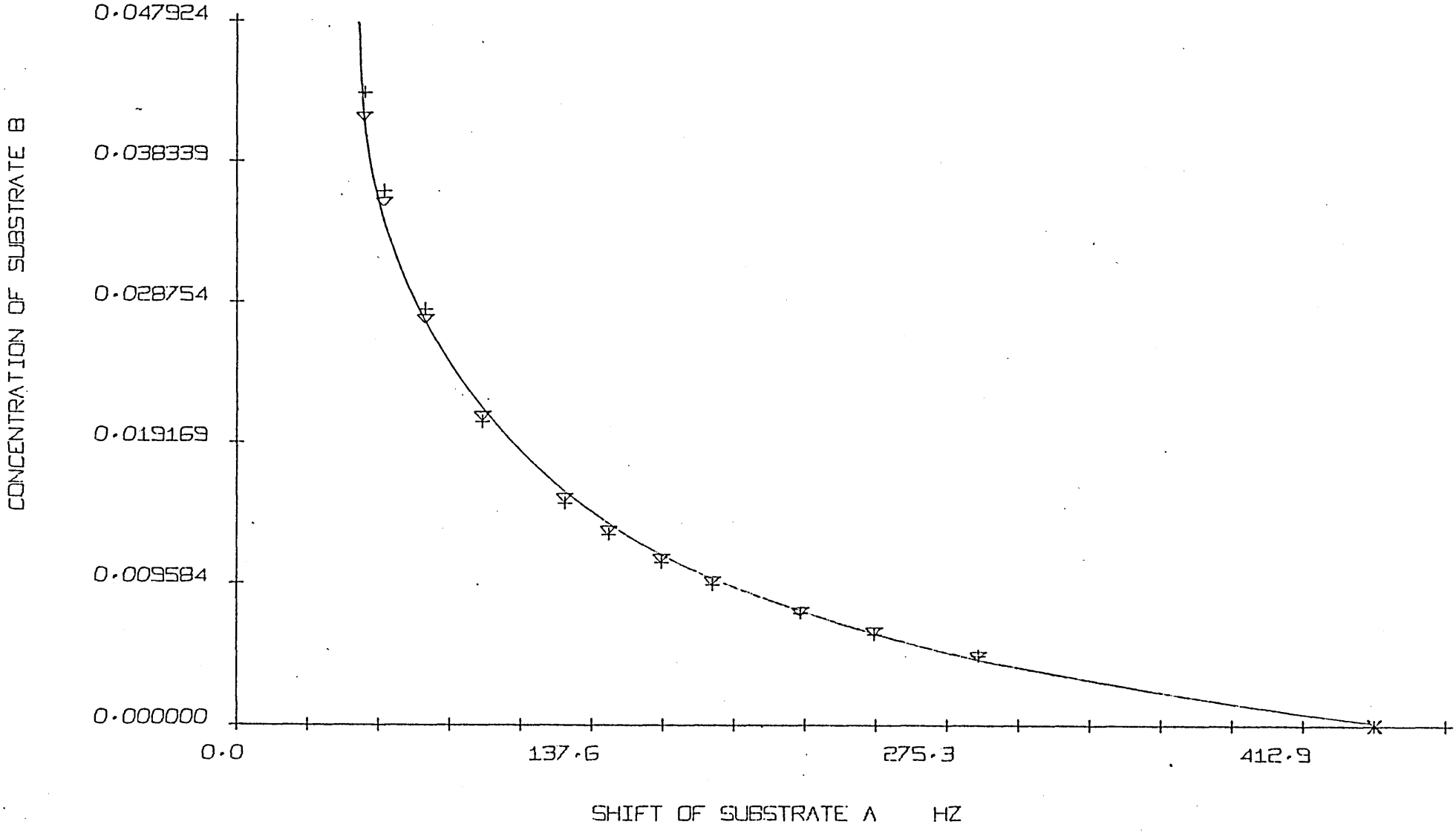
Competing substrate 4-methylpentan-3-ene-2-one with

Kb = 1457 $dm^3.mol^{-1}$.

+ + + + experimental points.

▽ ▽ ▽ ▽ theoretical curve.

%SD = 2.86


0.047924
0.038339
0.028754
0.019169
0.009584
0.000000
CONCENTRATION OF SUBSTRATE B
0.0
137.6
275.3
412.9
SHIFT OF SUBSTRATE A HZ

experimental and theoretical curves, measured in terms of the percentage standard deviation (as outlined in Chapter Two) reflects the accuracy and reliability of the intrinsic parameters determined independently by the 4-parameter data treatment method and used as standard values in the competition experiments.

Provided the intrinsic parameters of all the competing substrates present in a solution are known, then a possible application of this method lies in the quantitative analysis of mixtures of substrates. Previous quantitative analyses carried out with lanthanide shift reagents lies in the separation of resonance frequencies followed by integration of the signal intensities (86, 87). Since the above technique relies only on the measurement of the chemical shift positions of the resonance signals, the method obviates the necessity of determining absorption peak areas.

Two major disadvantages of comparing the intrinsic parameters determined by these competition experiments with those of the full 4-parameter data treatment method are

i) only equilibrium binding constants can be calculated and

ii) traces of moisture and other impurities must be removed.

The following method however describes how both equilibrium binding constants and limiting incremental shift values can be calculated, even in the presence of trace amounts of moisture or other impurities.

3.3.2 An induced shift data treatment method

Where any equilibrium involves the presence of trace amounts of moisture, or other impurities, the effect of the presence of these impurities on the equilibrium of other species present in solution must be considered. If therefore a situation is considered in which

the equilibrium may be represented as

$$E + Sa \rightleftharpoons ESa$$
$$E + Sb \rightleftharpoons ESb$$
$$E + W \rightleftharpoons EW$$

where Sa, Sb and W represent substrates Sa and Sb and traces of moisture or impurities respectively and Esa, ESb and EW the corresponding complexes formed in solution.

It can be shown the the corresponding equilibrium binding constant

$$Ka = \frac{[ESa]}{[E_F]([Sa] - [ESa])}$$

can be rearranged to give

$$\frac{1}{[E_F]} = Ka\left(\frac{[Sa]}{[ESa]} - 1\right) \quad \ldots \quad 3.12$$

Since the fast exchange condition applies and only one resonance is observed then

$$\frac{\triangle_a}{\triangle_{1a}} = \frac{[ESa]}{[S_T]}$$

which on substitution into equation 3.12 gives

$$\frac{1}{[E_F]} = Ka\left(\frac{\triangle_{1a}}{\triangle_a} - 1\right) \quad \ldots \quad 3.13$$

Equations of this sort can similarly be derived for Kb and Kw. Consequently the induced shift of a particular substrate in the presence of other competing substrates, will depend upon the concentration of the free or uncomplexed shift reagent (88, 89). From this it can then be shown that

$$Ka\left(\frac{\Delta_a}{\Delta_a} - 1\right) = Kb\left(\frac{\Delta_b}{\Delta_b} - 1\right) \quad \ldots \; 3.14$$

rearranges to give

$$\frac{1}{\Delta_b} = \frac{1}{\Delta_a}\left(\frac{Ka\,\Delta_a}{Kb\,\Delta_b}\right) + \frac{Kb - Ka}{Kb\,\Delta_b} \quad \ldots \; 3.15$$

Hence a plot of the reciprocal lanthanide induced shifts of two competing substrates enables intrinsic parameters to be determined from slope and intercept values. Table 3.3 shows the results of several experiments where the observed lanthanide induced shifts of two competing substrates are measured in the presence of $Eu(fod)_3$ in deuterochloroform. The intrinsic parameters of one of the substrates are regarded as standard and the values for the other substrate are calculated. A least squares analysis was carried out and the linear correlation coefficients obtained varied between 0.9978 and 0.9999. A comparison between the intrinsic parameters obtained by this method and the intrinsic parameters obtained by the 4-parameter data treatment method, also shown in Table 3.3, generally reveals a fairly good agreement

Table 3.3

Intrinsic parameters obtained from a plot of equation 3.15[a]

a) Substrate-Eu(fod)$_3$ complexes in $CDCl_3$.

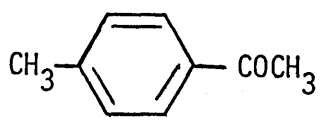
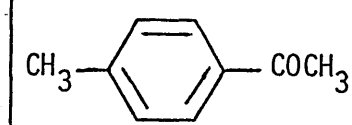
b) Units of $dm^3.mol^{-1}$.

c) Units of Hz.

d) Induced shift data treatment method. The figures shown are average values of the two substrate Sa proton resonance values. Linear correlation coefficients observed varied between 0.9978 - 0.9999.

e) 4-parameter data treatment method.

$$\frac{1}{\Delta_b} = \frac{1}{\Delta_a}\left(\frac{Ka\,\Delta_a}{Kb\,\Delta_b}\right) + \frac{Kb - Ka}{Kb\,\Delta_b} \quad \ldots \quad 3.15$$

COMPETING SUBSTRATE Sb	STANDARD SUBSTRATE Sa	PROTON RESONANCE SIGNAL Sa	STANDARD INTRINSIC PARAMETERS USED		INTRINSIC PARAMETERS DETERMINED (d)		(e)		PROTON RESONANC SIGNAL Sb
			K_a (b)	$\Delta\nu_a$ (c)	K_b (b)	$\Delta\nu_b$ (c)	K_b (b)	$\Delta\nu_b$ (c)	
$(CH_3)_2C{=}CHCOCH_3$	$CH_3CH_2CH_2COCH_3$	CH_3CH_2	259	352	1399	806	1457	832	CH_3CO
						666		666	CH_3(i)
4-methylpentan-3-ene-2-one	pentan-2-one	$COCH_3$	259	1005		308		324	CH_3(ii)
	$CH_3CH_2CH_2COCH_3$	CH_3CH_2	259	352	392	127	495	126	4-CH_3
4-methylacetophenone	pentan-2-one	$COCH_3$	259	1005		1007		936	$COCH_3$
$(CH_3)_2C{=}CHCOCH_3$		4-CH_3	495	126	1358	844	1457	832	CH_3CO
						712		666	CH_3(i)
4-methylpentan-3-ene-2-one	4-methylacetophenone	$COCH_3$	495	936		339		324	CH_3(ii)

between the two methods.

Since the assumption of an exclusive 1:1 stoichiometry is probably incorrect, perhaps a better agreement between the two methods is not forthcoming. It has already been pointed out that for these substrates the results of the 4-parameter data treatment method favour a 2:1 stoichiometry as indeed do the results of the relative induced shift ratios discussed in section 2.4.5. Varying limiting incremental shift ratios observed for different protons within the same substrate will also account for the lack of total agreement between the two methods since the induced shift will be in a weighted average position of twolimiting incremental shift values and not one as used. Furthermore, in the presence of a mixed substrate-$Eu(fod)_3$ complex, ESaSb or ESbSa, as defined in section 3.3.1, the effect of the resulting lanthanide induced shift in a competing substrate mixture cannot be predicted since the limiting incremental shift values of these mixed complexes are not known. To incorporate the above features in any analysis would be to severely limit the use of competition experiments of this sort since too many variables would be introduced. Bearing in mind the limitations of the method, the results obtained from these competition experiments are nevertheless in a satisfactory agreement with the results of the 4-parameter data treatment method. This would indicate that competition experiments can be performed say in cases where expensive and small quantities of substrates have to be analysed by a shift reagent technique.

3.4 Conclusions

An attempt has been made to determine intrinsic parameters reliably by the use of simple, but at the same time, alternative and independent data treatment methods. In view of the expected complexity of the nature of the solution equilibria, a 1:1 stoichiometric reaction was the only example considered.

It is shown that fairly good agreement is obtained between equilibrium binding constants, determined for several $Eu(fod)_3$-ether complexes using shift reagent resonance frequencies when compared with the corresponding values obtained by the 4-parameter data treatment method. Good agreement is also found between the intrinsic parameters determined by the 4-parameter data treatment method compared with those values obtained from a series of competition experiments involving two competing ketone substrates in the presence of the shift reagent $Eu(fod)_3$. Despite obvious limitations to the use of these simple data treatment methods, the satisfactory agreement found between the various methods, shows that a certain amount of information can be obtained from easily accessible data. The agreement also demonstrates that the methods presented complement one another and in some way support the accuracy of the results obtained by the 4-parameter data treatment method.

4.1 Lanthanide induced shift mechanisms

It is generally believed that the predominant induced shift mechanism resulting from the substrate-shift reagent coordination is the pseudo-contact shift mechanism (95). Indeed in the determination of intrinsic parameters carried out in the previous chapters a pseudocontact shift was taken to be the sole mechanism. This assumption allows a comparison to be made between the equilibrium binding constants determined for each proton group situated within the same molecule. Identical equilibrium binding constants are expected to be calculated from the data of each proton group present.

This was shown, in Chapter Two, to be the case for several aliphatic saturated organic substrates such as alcohols, ketones and ethers.

If, however the contact induced shift mechanism is also involved, little or no correlation is expected for the equilibrium binding constants calculated for each proton group. This is because the induced shift resulting from this mechanism depends on the nature and number of chemical bonds separating the lanthanide metal ion and the various proton groups within the substrate, and will, very probably, differ for each proton group within the substrate. Consequently, unless shift contributions from both mechanisms can be determined individually, no useful information will be obtained from the induced shift data.

Recent evidence (96, 97) now suggests that the contact induced shift mechanism is likely to contribute significantly to the total lanthanide induced shift, especially for coordinated europium-aromatic systems.

If this is so, then the induced shift must be discussed in terms of two different mechanisms; namely contact and pseudocontact shifts. The Fermi-contact induced shift, which is a through bond effect, arises from isotropic hyperfine coupling between a substrate nucleus and a lanthanide metal electron. This interaction enables the unpaired spin density present at the resonating nucleus to be determined. On the other hand, the pseudocontact or dipolar shift, which is a through-space effect, arises from dipolar interactions between the lanthanide metal electronic magnetic moment and the substrate nuclear spin moment. This interaction provides information about the molecular structure of the substrate-shift reagent complex formed in solution.

Several expressions for the Fermi-contact induced shift have appeared in the literature (98,99,100) and for the lanthanide series maybe given by (101)

$$\triangle_{con} = \frac{-2\pi\beta\nu AJ(J+1)\, g_1\, (g_1 - 1)}{2kT\gamma} \qquad \ldots 4.1$$

where $A = \frac{f_s\, A_s}{2S}$

and ν and γ are the resultant Larmor frequency and the magnetogyric ratio, β is the Bohr magneton, J is the resultant electronic spin angular momentum and g_1 is the Landé g-factor: T is the absolute temperature and k is the Botzmann constant and A is the scalar coupling constant in Hz: A_s is the isotropic coupling constant due to one unpaired electron in an s orbital and 2S is the number of unpaired electrons on the lanthanide ion. The value f_s is the fractional spin occupancy.

Similar theoretical developments for the pseudocontact shift mechanism have also been reported (102). Two popular, although slightly different theories for the pseudocontact shift have been developed by Bleaney (24) and by LaMar, Horrocks and Allen (22,23). Both theories appear to account successfully for the pseudocontact induced shifts produced by a series of lanthanide shift reagents. Golding (103) has refined Bleaney's theory for the pseudocontact induced shift, but at present no essential improvement to this theory is achieved. The theories of Bleaney and of Horrocks et al both suggest that the pseudocontact shift arises from the anisotropy in the magnetic susceptibility of the lanthanide ion and is given by

$$\triangle_{pseudo} = \frac{-D' (3\cos^2 \theta - 1)}{r^3} + \frac{D'' (\sin^2 \theta \cos 2\phi)}{r^3} \quad \ldots 4.2$$

where r is the vector distance between the lanthanide metal ion and a given nucleus, θ is the angle formed between the given nucleus and the principal magnetic axis and ϕ is as defined in Figure 4.1. This expression for the pseudocontact induced shift is the point dipole field perturbation expression. The exact nature of the constants D' and D" depends upon the system studied and their formulation has been subject to continuous modification arising from detailed theoretical studies of particular systems (104). If the substrate-shift reagent complex is axially symmetric or if effective axial symmetry arises, then equation 4.2 reduces to the more popular form attributed to McConnell and Robertson (21)

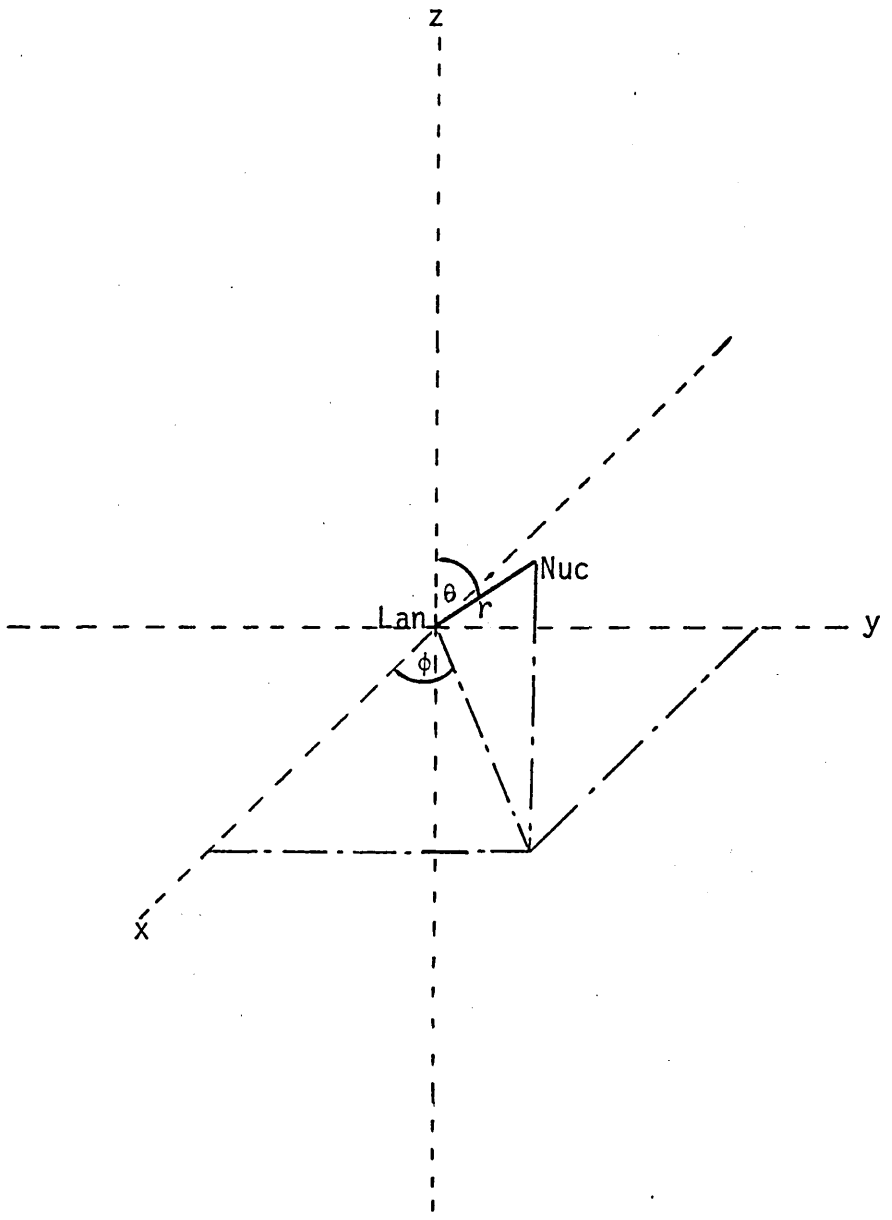
$$\triangle_{pseudo} = \frac{K C (3\cos^2 \theta - 1)}{r^3} \quad \ldots 4.3$$

Figure 4.1

The coordinate system for the pseudocontact shift as based on equation 4.2

r is the vector distance between the lanthanide metal ion Lan, and the nucleus under investigation, Nuc.

θ is the angle between this vector distance and the principal magnetic axis, which is assumed to be colinear with the +z direction.


z
Nuc
θ
r
Lan
y
ϕI
x

K is assumed constant for a particular complex and is independent of the lanthanide metal ion, whilst C contains lanthanide metal dependent parameters. The major significant differences between the two theories presented by Horrocks et al and by Bleaney arise in the derivations of K and C. In these derivations, Horrocks et al relate the pseudocontact shift to the reciprocal temperature (T^{-1}) such that

$$\Delta_{pseudo} = -\left(\frac{\nu\beta^2 J(J+1)}{9kT}\right)\left(g_z^2 - \frac{g_x^2}{2} - \frac{g_y^2}{2}\right)\left(\frac{3\cos^2\theta - 1}{r^3}\right) \quad \ldots 4.4$$

The g-tensor components g_x, g_y and g_z are related to the magnetic susceptibilities χ_x, χ_y and χ_z along the principal axes of the complex by (23).

$$\chi_i = \left(\frac{\beta^2 J(J+1)}{3kT}\right) g_i^2 \quad \ldots 4.5$$

and the other symbols are as previously described.

On the other hand, Bleaney relates the pseudocontact shift with a squared reciprocal temperature dependency (T^{-2}) such that

$$\Delta_{pseudo} = \left(\frac{\beta^2}{60\,(kT)^2}\right)\left(\frac{3\cos^2\theta - 1}{r^3}\right)\left(2A_2^0 \left\langle r^2 \right\rangle\right)$$

$$\left(g^2 J(J+1)\,(2J-1)\,(2J+3)\left\langle J \| \alpha \| J \right\rangle\right) \quad \ldots 4.6$$

The first term includes the temperature dependency and the second term shows the angular and distance dependencies, which are the same as those found for the anisotropic g-tensors calculated by Horrocks et al. The third term indicates an energy or crystal field coefficient which is assumed to be constant for a given substrate complexed throughout the lanthanide series. The fourth term shows a numerical coefficient which is purported to be proportional to the variation in the induced shift which should occur if the crystal field coefficient and the configuration of the molecule were completely independent of the lanthanide ion present (24). Confidence in both theories has been demonstrated by several independent groups of workers (105, 106, 107). It has recently been suggested that a third contribution to the total paramagnetic induced shift may be involved (108, 109). This "complex formation induced shift", as this contribution is often referred to, is attributed in part, to changes in the substrate electron density caused by the presence of a <u>diamagnetic</u> lanthanide metal chelate and can be measured by using lanthanum (La) or lutetium (Lu) compounds. This shift is then deducted from the total paramagnetic induced shift observed with lanthanide shift reagents. It has been shown, by experiments carried out in this laboratory, that when a series of organic substrates are complexed with the lanthanide metal chelate $La(fod)_3$, then no induced shifts are observed for the substrates studied. Consequently, in the following discussion, this contribution to the induced shift will be ignored.

The total paramagnetic induced shift resulting from coordination between substrate and shift reagent can therefore be given by (110)

$$\triangle_{total} = \triangle_{con} + \triangle_{pseudo}$$

which in turn can be expressed as

$$\Delta_{total} = F_i \langle S_z \rangle_j + G_i C_j \quad \ldots 4.7$$

where the contact shift is proportional to a term F_i whose value depends upon the nucleus being observed and is independent of the lanthanide ion, and also to a term $\langle S_z \rangle_j$, the lanthanide electron spin component in the z direction, whose value is independent of the substrate nucleus and depends only on the lanthanide ion. The pseudocontact shift is proportional to a term G_i which depends upon the location of the nucleus under investigation and hence the geometrical structure of the substrate-shift reagent complex and also to a term C_j which depends only on the lanthanide cation. From these theoretical treatments presented, values of $\langle S_z \rangle_j$ and C_j have been tabulated for the lanthanide series (111, 112). Some of these values will later be used to establish the accuracy of the experimental data obtained and presented in this thesis. Hence with a knowledge of the total paramegnetic induced shift, it is possible to calculate the contact shift contribution, provided that the contribution from the pseudocontact shift mechanism is known, and vice versa.

4.2 Considerations of pseudocontact shift contributions

In most of the methods used for the analysis of lanthanide induced shift data, it has generally been assumed that the lanthanide metal ion occupies a unique position in space with respect to the substrate molecule. This position is found by systemmatically varying the Cartesian coordinates of the lanthanide metal ion and then assessing, by simple comparison, the agreement found between the experimentally observed shifts and

theoretically predicted ones. In practice this means comparing the experimental shifts with those shifts predicted for known substrate structures. Generally the methods differ mainly in the manner used to define the best fit between experimental and theoretical data. Common to most methods however, has been the use of three basic assumptions:

i) The substrate-shift reagent complex possesses axial symmetry.

ii) the interpretation of the paramagnetic induced shift must be considered solely on the basis of a pseudocontact shift, i.e. contact shift contributions are absent.

iii) The substrate-shift reagent interaction must be described in terms of a 1:1 stoichiometry.

These assumptions are dealt with in turn.

4.2.1 The assumption of axial symmetry

Horrocks (26) has reported that the second term in equation 4.2 relating to non-axial symmetry, contributes up to 15% of the total paramagnetic induced shift observed for $Ln(dpm)_3$ - 4-methylpyridine systems. The author warned however that this might not be a general result. Also, Cramer et al (113) has shown that this term may contribute from 29 to 80% of the total shift observed for $Eu(dpm)_3$-pyridine complexes. Further evidence reported by Newman (37) shows that the induced shifts are considerably better explained if the axially symmetric McConnell-Robertson equation is extended to include the term for non-axial symmetry. Shift contributions between 1 and 40% of the total shift are claimed for the non-axial term for $Ln(fod)_3$-aliphatic ketone complexes. In addition, evidence from solid state crystalographic studies (114, 115, 116) have shown that substrate-shift reagent complexes are not

axially symmetric. Magnetic susceptibility studies (117, 118) have also supported this result. Furthermore, recent low temperature work (119, 120) on substituted pyridines dissolved in carbon disulphide in the presence of $Eu(dpm)_3$ have resulted in the observation of two ortho and two meta resonance frequencies, again indicating the assymmetry of the subtrate-shift reagent complex. All these studies point to serious limitations in the use of the McConnell-Robertson equation for the interpretation of induced shift data. Even so, a great deal of success has been achieved using equation 4.3 (121-124).

4.2.2 Contact Shifts

For the determination of intrinsic parameters shown in Chapter Two the presence of any contact shift contributions has been ignored and assumed negligible. This is reasonable since the similarity of the equilibrium binding constants calculated for the proton groups situated within the same substrate molecule, has indicated the absence of contact induced shifts, particularly for aliphatic substrates. In contrast, the results for aromatic amines show that contact shifts are likely to be present in these systems.

An alternative and simpler way in which the absence of contact shifts can be inferred, is by comparing the relative induced shift ratios $\left(\Delta_i / \Delta_j\right)$ obtained for complexes of a series of lanthanide shift reagents. The relative induced shift ratio is defined as the ratio of the limiting incremental shift value of say proton i, relative to the corresponding shift value of proton j situated in the same molecule. From equation 4.3 it can be shown that the relative induced shift ratio is given by

$$\frac{\Delta_i}{\Delta_j} = \frac{K_i\,(3\cos^2\theta - 1)\,r_i^3}{K_j\,(3\cos^2\theta - 1)\,r_i^3} = R_{ij} \quad \ldots \quad 4.8$$

Consequently, if the corresponding shift ratio values obtained for a particular substrate-shift reagent complex are compared with those values obtained with other lanthanide shift reagents in the same series, and if these values are independent of the lanthanide cation, then the induced shifts have their origin from a pseudocontact shift mechanism and the complex possesses effective axial symmetry (125). In addition it has been inferred (126) that identical relative induced shift ratios observed for different lanthanide shift reagents indicates that substantially the same type of complex is being formed with each metal chelate and that the shifts are again predominantly pseudocontact. Furthermore, Barry et al (127) suggests that since the lanthanide ions have a very similar solution chemistry, it is possible to make isomorphous replacement of one lanthanide ion with another and obtain comparable results. However, as was shown in Chapter Two, although the lanthanides may possess similar solution chemical properties, this does not preclude differences arising in their behaviour as lanthanide shift reagents. Also, Smid et al (128) have suggested that it is not always justified to convert proton shifts obtained for one complex into those of another lanthanide complex simply by applying a constant shift ratio factor. Furthermore, since the constants D' and D" in equation 4.2 arise from the mixing of different excited energy levels with the ground state, and as each lanthanide cation in a given crystal field has a different set of excited states then D' cannot be proportioned

to D" throughout the lanthanide series. Consequently an equation similar to equation 4.8 cannot be obtained by simplifying equation 4.2. It would seem therefore that the absence of differences in the relative induced shift ratios observed for complexes of different shift reagents signify reasonable evidence for the absence of contact shifts. On the other hand, the presence of such differences in the shift ratios cannot itself be used as conclusive evidence of contact induced shifts, since these differences may arise from binding geometry and stoichiometric changes (129, 130, 131) and also from the use of the more correct form of the pseudocontact shift equation, i.e. involving non-axial symmetry.

4.2.3. The assumption of a 1:1 stoichiometry

Inherent in the use of equations 4.2 and 4.3 for the interpretation of lanthanide induced shift data is the assumption that only a 1:1 complex exists in solution. Substantial evidence now suggests this may not be the case. Indeed all the results reported in Chapter Two, show that the formation of a 2:1 stoichiometry is more favourable than the formation of a 1:1 stoichiometry. Furthermore, it is shown that, in terms of the ratio K_1/K_2 praseodymium shift reagents form 2:1 complexes more favourably than do ytterbium shift reagents. As the atomic number of the lanthanide ions increase, the ionic radius of the cation decreases. Consequently, the formation of 2:1 complexes may be affected by the change in the lanthanide ionic radius. In addition, the results of the limiting incremental shift values shown in Chapter Two, illustrate that as the ionic radius decreases, the limiting incremental shift ratios $\left(\Delta_{1_i}/\Delta_{2_i}\right)$ observed for the various shift reagents increases. Consequently, as well as reflecting a 2:1 stoichiometry, the results also suggest that geometrical differences

arise between the complexes of different shift reagents. The decrease in ionic size must therefore inhibit the approach of the substrate molecules towards the central metal ion. These results suggest that it should never be assumed that the geometries of the complexes of different shift reagents formed in solution are identical, or indeed even that the same shift mechanism operates (132). The results recorded here are in total agreement with the results recently reported by Ernst et al (133) and by Sherry et al (134).

It should also be emphasised, that the induced shift data contains information only on the weighted average substrate-shift reagent geometry and that the substrate conformation of the complex may well differ from the conformation in the free substrate (135, 136). Again ionic size and the resulting steric hindrance effects may effect shift differences that are observed between various lanthanide shift reagents, even though only a 1:1 complex may be present in solution. These results indicate that the assumption of a 1:1 stoichiometry need not necessarily be correct and that experimental confirmation is required in order that the assumption can be justified.

In view of the preceding discussion, the use of the McConnell-Robertson equation for obtaining structural information seems very susceptible and it is probably that no simple interpretation is possible. For conformational conclusions to be placed on a firm basis it is essential that the observed shift be shown to arise from a pseudocontact shift mechanism. It is not surprising therefore that several authors (137, 138) have recently warned of the dangers that may be encountered in obtaining structural information and that great care should be exercised in the interpretation of such results. It has been shown that although

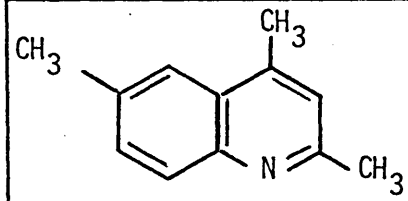
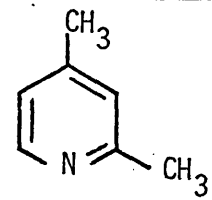
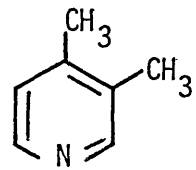
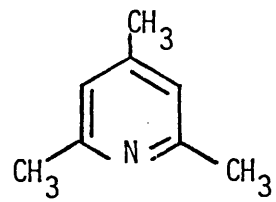
shift differences observed with complexes of various shift reagents can help in the analysis of lanthanide induced shift data, a detailed interpretation regarding these differences is essential.

4.3. Results with substituted pyridine complexes

Since the recognition that contact induced shifts possibly contribute significantly to the total paramagnetic induced shift, there have been several attempts to separate and evaluate both contact and pseudocontact shift contributions (139, 140). The work reported in this thesis involves several substituted pyridine compounds complexed with various lanthanide shift reagents. Substituted pyridines were chosen as model compounds because they are more likely to exhibit contact induced shifts and because the proton groups within the substrate molecules possess clear and well defined chemical shift resonance frequencies. Also, substituted pyridines possess very simple geometrical structures. The molecules are planar and hence, accurate internal atomic coordinates can easily be calculated from known bond lengths and internuclear angles (141). As already pointed out, in the majority of methods used in the interpretation of lanthanide induced shift data, the observed shifts are compared with theoretically predicted shifts based on molecules possessing known structures. The internal coordinates of these subtrates, used in the calculations of the geometric factor $(3\cos^2\theta - 1) \ / \ r^3$ is consequently very important when determining the best fit between experimental and theoretical shift values. The atomic coordinates for the substituted pyridine molecules, used in subsequent calculations are shown in Table 4.1. Severe signal broadening restricted in many cases the analysis of the induced shift data by the 4-parameter data treatment method, Also, when contact shifts are likely to be present, accurate

Table 4.1

Cartesian coordinates of several substituted pyridines

SUBSTRATE	COORDINATES	PROTON GROUP POSITIONS							
		N	2	3	4	5	6	7	8
 2,4,6-Trimethylquinoline	x	0	0	0	0	0	0	0	0
	y	0	2.835	2.148	0	-2.407	-5.260	-4.538	-2.390
	z	0	0.267	-2.620	-4.673	-3.860	-3.027	-0.140	1.100
 2,4-Dimethylpyridine	x	0	0	0	0	0	0		
	y	0	2.835	2.148	0	-2.148	-2.130		
	z	0	0.267	-2.620	-4.673	-2.620	-0.140		
 3,4-Dimethylpyridine	x	0	0	0	0	0	0		
	y	0	2.130	2.852	0	-2.148	-2.130		
	z	0	-0.140	-3.027	-4.673	-2.620	-0.140		
 2,4,6-Trimethylpyridine	x	0	0	0	0	0	0		
	y	0	2.835	2.148	0	-2.148	-2.835		
	z	0	0.267	-2.620	-4.673	-2.620	0.267		

intrinsic parameters will not be determined by this method since pseudocontact and contact shifts cannot be identified and separated. However, as was shown in Chapter Two, when strong association exists between substrate and shift reagent, the initial slope of a plot of the induced shift versus mole ratio gives a very good estimate for the limiting incremental shift value. Consequently, in subsequent calculations, the slope values obtained from these plots, were used to represent the total paramagnetic induced shift. The slope values obtained for the $Pr(fod)_3$, $Eu(fod)_3$ and $Yb(fod)_3$ shift reagent complexes are shown in Table 4.2. No shifts were observed when the diamagnetic lanthanide chelate $La(fod)_3$, was used. In order to facilitate a comparison of the results for different shift reagents, the limiting incremental shift values have been scaled relative to the 4-methyl proton signal of each complex. This proton group was chosen because it is the furthest removed from the lanthanide metal ion and is considered free from the interference of contact induced shifts. Indeed, Horrocks (14) has reported the absence of contact shifts for the 4-methyl proton group of 4-methylpyridine when complexed with the shift reagent $Gd(dpm)_3$. The relative induced shift ratio values calculated for the complexes are shown in Table 4.3. It can be seen that certain similarities exist, particularly between $Pr(fod)_3$ and $Yb(fod)_3$ complexes. This agrees with theoretical predictions that praseodymium and ytterbium shift reagent complexes are likely to exhibit only small contact shift contributions (143). In contrast, certain $Eu(fod)_3$ shift ratios show marked differences. This follows theoretical predictions that the $Eu(fod)_3$ shift reagent complexes are likely to exhibit substantial contact induced shift contributions (144). Overall however, the

Table 4.2

Limiting incremental shift values[a]

a) Units of Hz.

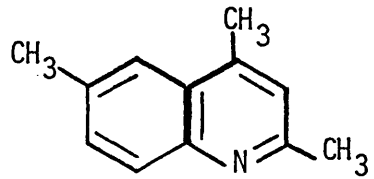
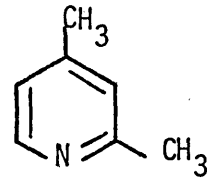
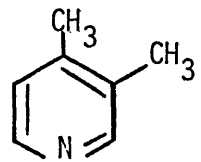
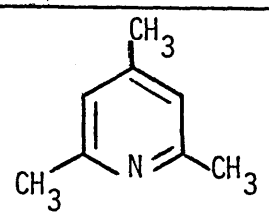
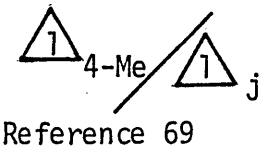
SUBSTRATE	SHIFT REAGENT	LIMITING INCREMENTAL SHIFT VALUES						
		2	3	4	5	6	7	8
 2,4,6-Trimethylquinoline	$Eu(fod)_3$	821	281	168	104	38	81	612
 2,4-Dimethylpyridine	$Pr(fod)_3$	-2465	-1173	-543	-849	-3075		
	$Eu(fod)_3$	1134	497	313	328	1114		
	$Yb(fod)_3$	3104	1869	835	1316	3418		
 3,4-Dimethylpyridine	$Pr(fod)_3$	-3949	-802	-802	-1356	-3949		
	$Eu(fod)_3$	1630	515	361	545	1635		
	$Yb(fod)_3$	4923	992	992	1750	4923		
 2,4,6-Trimethylpyridine	$Eu(fod)_3$	553	184	138	184	553		

Table 4.3

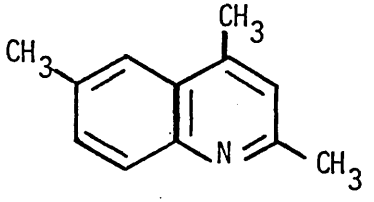
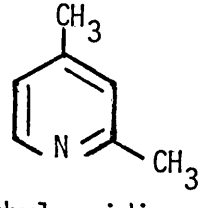
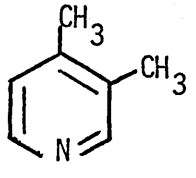
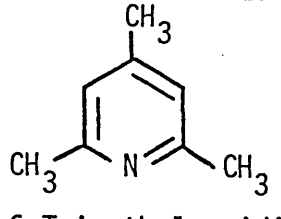
Experimentally determined

Relative induced shift ratios[a]

a)  based on the values in Table 4.2

b) Reference 69

c) Reference 142

SUBSTRATE	SHIFT REAGENT	PROTON GROUP POSITIONS						
		2	3	4	5	6	7	8
 2,4,6-Trimethylquinoline	$Eu(fod)_3$	0.20	0.60	1.00	1.61	4.39	2.06	0.27
 2,4-Dimethylpyridine	$Pr(fod)_3$	0.22	0.46	1.00	0.64	0.18		
	$Eu(fod)_3$	0.28	0.63	1.00	0.95	0.28		
	$Yb(fod)_3$	0.27	0.45	1.00	0.63	0.24		
 3,4-Dimethylpyridine	$Pr(fod)_3$	0.20	1.00	1.00	0.59	0.20		
	$Eu(fod)_3$	0.22	0.70	1.00	0.66	0.22		
	$Yb(fod)_3$	0.20	1.00	1.00	0.56	0.20		
 2,4,6-Trimethylpyridine	$Eu(fod)_3$	0.25	0.75	1.00	0.75	0.25		
	$Eu(dpm)_3$ (b)	0.25	0.64	1.00	0.64	0.25		
	$Yb(dpm)_3$ (c)	0.24	0.57	1.00	0.57	0.24		

different shift ratios observed for the shift reagents used, may, as already pointed out, arise from

i) different contact shift contributions from the different shift reagents and

ii) different adduct geometries, which may arise for different shift reagent substrate complexes as the ionic radius of the lanthanide cation changes. For the purpose of this analysis, i.e. the evaluation of contact shifts the geometrices of the various shift reagent-substrate complexes are, by necessity, assumed constant throughout the lanthanide series. In practice however, and from the results obtained in Chapter Two, this assumption may not be totally correct.

In earlier shift reagent research (145), both for reasons of simplicity and rapidity in carrying out calculations, the angular dependency shown in equation 4.3 is ignored and the lanthanide induced shift analysed in terms of a distance dependency only. Hence equation 4.3 becomes

$$\Delta_{pseudo} = k / r^3 \qquad \ldots 4.9$$

which upon rearrangement in log form gives

$$\log \Delta = \log k - n \log r \qquad \ldots 4.10$$

A plot of $\log \Delta$ ts the distance dependency.

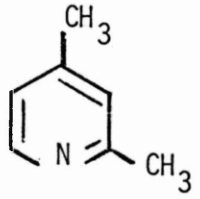
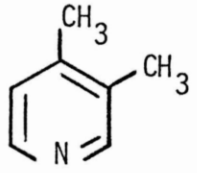
A plot of $\log \Delta$ versus log r should therefore be a straight line with gradient - n and intercept log k. Deviations from this straight line have then been interpreted as resulting from contact shift contributions, the magnitude of which are easily calculated from the plot. Deviations below the line represent contact shifts in the opposite

direction to the pseudocontact shift whilst deviations above the line represent contact shifts which are in the same direction as the pseudo-contact shift. Using the atomic coordinates shown in Table 4.1, values of log r obtained for 2,4-Dimethylpyridine and 3,4-Dimethylpyridine complexes of $Pr(fod)_3$, $Eu(fod)_3$ and $Yb(fod)_3$ were plotted against the corresponding log $\triangle$ values obtained from Table 4.2. Although fairly good straight lines are obtained with slope values of about -2, which are in good agreement with previous published data (146, 147), it is evident that the slope values obtained depend upon the assumed lanthanide-nitrogen bond distance used in the calculations to determine log r, (r is the distance between the lanthanide metal ion and the proton nucleus under investigation). The greater the assumed lanthanide-nitrogen bond length then the greater the numerical value of the gradient. These values are shown in Table 4.4. Similarly, the shift deviations observed from the straight line plot are also dependent upon the assumed lanthanide-nitrogen bond distance. Since there is no way of determining the solution bond distance between the lanthanide ion and the coordinating nitrogen atom, the gradient values and the associated contact shift contributions obtained from these plots become almost meaningless unless the nitrogen-lanthanide bond length is specified. Since it is clear that the omission of the angular term could result in the observation of poor theoretical-experimental comparisons, an alternative method for determining contact induced shifts was attempted. This method was based on theoretical $(3\cos^2\theta - 1) / r^3$ geometrical factors. If, for these simple model substrates, an axially symmetric or effective axially symmetric substrate-shift reagent complex is considered, and the lanthanide-nitrogen bond direction is colinear with the principal magnetic axis, then geometrical

Table 4.4

Slope values obtained from a plot of equation 4.10

$$\log \triangle = \log k - n \log r \qquad \ldots\ldots \quad 4.10$$

SUBSTRATE	ASSUMED LANTHANIDE-NITROGEN BOND DISTANCE, UNITS OF nm	SHIFT REAGENT		
		$Pr(fod)_3$	$Eu(fod)_3$	$Yb(fod)_3$
	0.2	-2.18	-1.73	-1.81
	0.3	-2.36	-1.85	-2.02
2,4-Dimethylpyridine	0.4	-2.55	-2.03	-2.17
	0.2	-2.01	-1.89	-2.03
	0.3	-2.27	-2.15	-2.26
	0.4	-2.56	-2.39	-2.58
3,4-Dimethylpyridine				

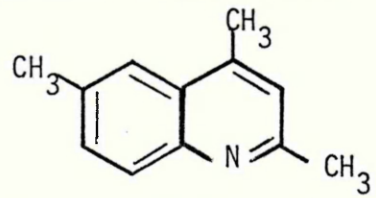
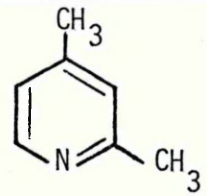
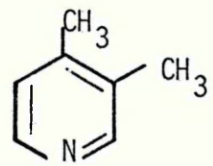
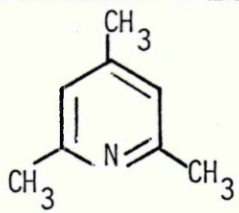
factors based on equation 4.3 can be calculated for known structures and from the internal atomic coordinates of the proton groups within the substrate. These values were calculated and to facilitate comparison, were then scaled relative to the 4-methyl proton group of each substrate. These resulting scaled ratio values are shown in Table 4.5 together with corresponding assumed lanthanide-nitrogen bond distances. From these calculations it is evident that the assumed lanthanide-nitrogen bond length affects several of the theoretical geometrical ratio values calculated for various proton groups within the substrate, whilst for other proton groups remain insensitive to the assumed bond distance. Consequently, the scaled pseudocontact shift expected for various proton groups within a substrate will depend upon the assumed lanthanide - coordinating centre bond distances. Highlighting this behaviour are the 2-Me proton signals of 2,4-Dimethylpyridine and 2,4,6-Trimethyl-pyridine. Relative to the 4-methyl proton group, the theoretical pseudo-contact shift of the 2-Me proton group changes dramatically with the assumed lanthanide-nitrogen bond length. On the other hand however, the pseudocontact shifts of the 5-H proton signals of 2,4-Dimethyl-pyridine and 3,4-Dimethylpyridine change very little with respect to the 4-methyl proton group, when the assumed lanthanide-nitrogen bond length changes. To some extent, the shift-distance dependency discussed above can account for the varying relative induced shift ratios $\left(\Delta_{4\text{-Me}} / \Delta_{j}\right)$ observed for the different shift reagents and not, as is often interpreted, as resulting from contact shift contributions. For nuclei positioned very close to the coordinating centre, it is commonly reported that the discrepancies which arise in the shift ratio values observed for different shift reagents, are generally attributed

Table 4.5

Theoretically determined relative geometrical values[a]

a) These values are based purely on the pseudocontact shift equation of McConnell and Robertson and are scaled relative to the 4-Methyl proton group. The coordinates shown in Table 4.1 are used.

b) The assumed lanthanide-nitrogen bond distance in units of nm.

SUBSTRATE	Ln - N(b)	THEORETICAL GEOMETRICAL RATIO VALUES						
		2	3	4	5	6	7	8
 2,4,6-Trimethylquinoline	0.2	-1.34	0.61	1.00	1.09	6.00	-1.87	-0.18
	0.3	0.61	0.60	1.00	1.02	3.22	-26.23	0.78
	0.4	0.35	0.60	1.00	0.98	2.25	1.96	0.21
 2,4-Dimethylpyridine	0.2	-1.34	0.61	1.00	0.61	0.37		
	0.3	0.61	0.60	1.00	0.60	0.23		
	0.4	0.35	0.60	1.00	0.60	0.23		
 3,4-Dimethylpyridine	0.2	0.37	1.02	1.00	0.61	0.37		
	0.3	0.23	0.90	1.00	0.60	0.23		
	0.4	0.23	0.85	1.00	0.60	0.23		
 2,4,6-Trimethylpyridine	0.2	-1.34	0.61	1.00	0.61	-1.34		
	0.3	0.61	0.60	1.00	0.60	0.61		
	0.4	0.35	0.60	1.00	0.60	0.35		

to an assumption regarding contact shifts. From the above discussion, this need not necessarily be the case and may reflect the different lanthanide-coordinating centre bond distances associated with changes in the lanthanide ionic radii. A comparison of the theoretical ratios shown in Table 4.5 with the experimental ratios observed in Table 4.3 does reveal however, that certain anomalies do exist which cannot be explained by the dependency of the geometrical values on the lanthanide nitrogen bond lengths. In these cases, a situation other than the presence of a purely pseudocontact shift mechanism must be involved. The relative induced shift ratios observed for the 3-H atoms of the 2,4-Dimethylpyridine complexes of $Pr(fod)_3$ and $Yb(fod)_3$ are very similar, as indeed are those values for the 5-H groups, (approximately 0.45 and 0.63 respectively). This similarity could reflect the theoretical expectations of these shift reagent complexes in that little or no contact shifts are observed with these shift reagents. However, theoretical geometrical ratio values, based on the simple model of axial symmetry predict a ratio value from the known structure for both the 3-H and 5-H proton groups to be about 0.6. The differences observed between the experimental and theoretical values could therefore represent, either the presence of contact shifts induced by $Pr(fod)_3$ and $Yb(fod)_3$ or an incorrect assumption regarding axial symmetry. If the theoretical expectations of praseodymium and ytterbium shift reagents are correct, and assuming that the induced shifts are purely pseudocontact in nature, then by comparison, the observed shift ratios reported for the $Eu(fod)_3$ complex must indicate the presence of contact induced shifts experienced by those nuclei. Relative to the 4-methyl proton group, the 3-H and 5-H atoms therefore exhibit contact shifts of -191 and -165 Hz respectively

or 27.8% and 33.5% of the expected $Eu(fod)_3$-induced pseudocontact shifts. The contact shifts calculated are in the opposite direction to the pseudocontact shift. Similar reasoning shows that the $Eu(fod)_3$ induced contact shifts of the 3-H and 5-H proton groups of 2,4,6-Trimethylpyridine are approximately -45 Hz or 20.0% of the expected pseudocontact shift. Again contact and pseudocontact shifts are in opposite directions; pseudocontact shifts being downfield whilst contact shifts are upfield. The relative shift ratio values observed for the 2,4,6-Trimethylpyridine-$Eu(fod)_3$ complex shown in Table 4.3 are compared with the ratio values reported by Mackie and Shepherd (69) and by Wolkowski et al (142) for the corresponding $Eu(dpm)_3$ and $Yb(dpm)_3$ complexes respectively. These latter values are quite close to the theoretically expected pseudocontact shift ratio values shown in Table 4.5 and possibly reflect that only small contact shifts are induced in this substrate with the shift reagents $Eu(dpm)_3$ and $Yb(dpm)_3$.

It can be seen that the shift ratios observed for the 2-Me and 6-H proton groups in 2,4-Dimethylpyridine differ slightly for all three $Ln(fod)_3$ complexes. It is extremely difficult however to interpret these differences in terms of contact induced shifts, since slight differences in the ratio values can arise from the scaled pseudocontact shifts resulting from the various assumed lanthanide-nitrogen bond distances, as discussed earlier. Consequently, no attempt has been made to calculate contact shift contributions in these positions.

In the $Ln(fod)_3$ - 3,4-Dimethylpyridine complexes however, the similarity of the relative induced shift ratio values observed for the 2-H and 6-H proton groups of all three shift reagent complexes, suggests that no contact shifts are exhibited by these proton groups, even though they

are positioned very close to the coordinating site. The ratio values observed are also very close to the theoretical geometrical values expected from a purely pseudocontact shift mechanism. In contrast, the ratio values observed with $Eu(fod)_3$ for the 3-Me and 5-H proton groups, differ significantly from the $Pr(fod)_3$ and $Yb(fod)_3$ shift ratios, and also from the theoretically expected pseudocontact shift values. If the presence of contact shifts is assumed to be responsible for these differences, then the 5-H atom experiences a $Eu(fod)_3$-induced contact shift of approximately -83Hz or 13.2% of the expected pseudocontact shift. Again the contact and pseudocontact shifts are in opposite directions. The results for the 3-Me proton group are significantly different however. The $Eu(fod)_3$-induced contact shift experienced by this proton group amounts to 154 Hz or 42.7% of the expected pseudocontact shift. Furthermore in this case, the contact and pseudocontact shifts are in the same direction, i.e. both are downfield. From the theoretical ratio values shown in Table 4.5, it is expected that the 4-Me and 5-H proton groups in 2,4,6-Trimethylquinoline should possess similar pseudocontact shifts. The relative values are quite insensitive to the assumed lanthanide-nitrogen bond lengths. The experimental ratio values however, observed for the $Eu(fod)_3$ complex, suggests that relative to the 4-methyl proton-group, a contact shift of -64 Hz or 38.1% of the expected pseudocontact shift is experienced by the 5-H atom. The induced shifts are again in opposite directions. With the exceptions of the 3-H and 5-H atoms, the theoretical shift ratio values of the other proton groups present in the quinoline substrate, vary significantly with the assumed lanthanide-nitrogen bond distance. Consequently, it is very difficult to interpret

the observed experimental and theoretical shift ratio differences in terms of a contact shift contribution. Indeed, it could be reasoned that, with the exception of the 5-H atom, the experimental ratio values are approximately those which might be expected from a purely pseudo-contact shift mechanism. In the case of the 3-H atom, the theoretically expected ratio value and the experimentally observed value are in very good agreement. This suggests the absence of contact induced shifts in this position, despite the fact that the 3-H atom is closer to the lanthanide metal ion than the 5-H atom.

In view of the uncertainties regarding the interpretation of results with respect to assumed lanthanide-nitrogen bond lengths and effective axial symmetry etc., the contact shift values calculated for the above examples can only be regarded as tentative values. The results do confirm however, that significant contact shift contributions are exhibited by these compounds and show that approximate estimates of their contributions can be determined.

It has been commonly regarded that nuclei positioned closest to the coordinating site suffer the greatest contact induced shift (148). Indeed, as already pointed out, this has often been assumed to be the cause of large discrepancies which are apparent in conformational studies. It has been reported however, both with experimental observations (149) and with theoretical justifications (150, 151) that contact shifts depend on the spatial arrangement of the substrate-shift reagent complex. Contact induced shifts, particularly through saturated bonds, occur most strongly when the resonating nucleus, the lanthanide cation and the intervening atoms, are in a plane, forming a zig-zag pattern. Whether this phenomenom occurs through aromatic systems has not yet been reported. The $Eu(fod)_3$ contact shifts determined for the

3-H and 5-H atoms of 2,4-Dimethylpyridine, the 3-Me and 5-H proton groups of 3,4-Dimethylpyridine, the 5-H atom of 2,4,6-Trimethylquinoline and also the 3-H and 5-H atoms of 2,4,6 - Trimethylpyridine, all indicate that the spatial dependency of contact shifts may be appropriate to substituted pyridines. Since these molecules are planar and since effective axial symmetry has been assumed, the europium cation will be in the same plane as the aromatic molecule. The rigidity of the molecule ensures that only a zig-zag pattern can be followed between the lanthanide ion and the resonating nuclei mentioned above. Consequently, significant contact shifts are experienced by proton groups that are four or five bond lengths away from the europium cation whereas nuclei positioned closer to the lanthanide metal appear not to be affected by contact induced shifts.

From the theoretical treatments outlined in Section 4.1 it was shown - that

$$\Delta_{total} = F_i \langle S_z \rangle_j + G_i C_j \qquad \ldots 4.7$$

Values of $\langle S_z \rangle_j$ and C_j have been tabulated by several authors (25, 112) and are shown in Table 4.6. If F_i and G_i are assumed dependent only on the nuclei being observed then

$$\frac{\Delta_{total}}{\langle S_z \rangle_j} = F_i + G_i \frac{C_j}{\langle S_z \rangle_j} \qquad \ldots 4.11$$

and a plot of $\Delta_{total} / \langle S_z \rangle_j$ versus $C_j / \langle S_z \rangle_j$ should be linear provided F_i and G_i are constant along the lanthanide series (138 and refs 26 and 27 therein). Using the values shown in

Table 4.6

Theoretical values of $\langle S_z \rangle_j$ and C_j[a]

a) These relative values are taken from Golding (112) and Bleaney (25) respectively.

LANTHANIDE	PSEUDOCONTACT C_j VALUES	CONTACT $\langle S_z \rangle_j$ VALUES
La	0.0	0.0
Ce	-6.3	0.970
Pr	-11.0	2.972
Nd	-4.2	4.487
Pm	2.0	4.014
Sm	-0.7	-0.063
Eu	4.0	-10.682
Gd	0.0	-31.500
Tb	-86.0	-31.818
Dy	-100.0	-28.545
Ho	-39.0	-22.629
Er	33.0	-15.374
Tm	53.0	-8.208
Yb	22.0	-2.587
Lu	0.0	0.0

Table 4.6 for praseodymium, europium and ytterbium and the corresponding experimentally observed induced shifts shown in Table 4.2 for the various proton groups in 2,4-Dimethylpyridine and 3,4-Dimethylpyridine, graphs of the above equation were plotted. Since three points on a line are not representative of the above equation, the approximately linear lines obtained are not conclusive proof of the accuracy of the experimental data used. Any deviation from the expected straight line could well signify that the values of F_i and G_i are not constant throughout the lanthanide series. Indeed, this has been suggested both in this thesis and elsewhere (152).

An alternative approach which was attempted, using the theoretical values of $\langle S_z \rangle_j$ and C_j, is to calculate a set of F_i and G_i values for each complex.

From the equation

$$\Delta_{total} = F_i \langle S_z \rangle_j + G_i C_j \qquad \ldots 4.7$$

experimental shift data for the proton groups in several shift reagent complexes are used to solve resulting simultaneous equations and values of F_i and G_i calculated. These G_i values now represent the theoretical pseudocontact shifts expected from the experimental shift data, assuming of course, the values of $\langle S_z \rangle_j$ and C_j are correct. A computer program which generates theoretical pseudocontact shifts for substrates with known molecular structures, was then used to compare the pseudo-contact shifts obtained from both methods. If the experimental data used to calculate the values of G_i (and hence the corresponding pseudo-contact shifts) is reliable, then excellent agreement should be found from the computer treatment. This will result in the observation of

very small percentage minimum agreement factors. The program was also used to compare the theoretical pseudocontact shifts, based on known structures, with the actual experimental shifts, to see what agreement if any, is found. If the experimental shifts are predominantly pseudocontact, then again good agreement should be expected and small agreement factors observed.

Hence, using the experimental shifts shown in Table 4.2 for $Pr(fod)_3$ and $Yb(fod)_3$ and the corresponding $\langle S_z \rangle_j$ and C_j values in Table 4.6, the simultaneous equations obtained for all the proton groups in 2,4-Dimethylpyridine and 3,4-Dimethylpyridine were solved and values of F_i and G_i determined. These values are shown in Table 4.7. To facilitate a comparison with the other values shown in Tables 4.3 and 4.5, the G_i values were scaled relative to the 4-methyl proton group of each complex. These values are also shown in Table 4.7.

The computer program PDIGM used in these studies was made available by Willcott and Davies (153). In their program, the following assumptions were made regarding the determination of pseudocontact shifts based on known molecular structures.

i) A single set of Cartesian coordinates is used to describe the substrate-shift reagent complex.

ii) The complex possesses axial symmetry enabling the use of the McConnell-Robertson equation.

iii) The principal magnetic axis of the lanthanide complex passes through the site of coordination.

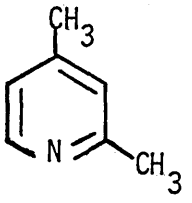
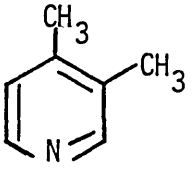
iv) An agreement factor R, is used to affect the comparison between theoretical and experimetally observed shifts (154) such that

Table 4.7

Calculated F_i and G_i values[a]

a) Based on theoretical $\langle S_z \rangle_j$ and C_j values (Table 4.6) for $Pr(fod)_3$ and $Yb(fod)_3$ and experimental shifts (Table 4.2)

b) G_i values scaled relative to the 4-Methyl proton group i.e. G_i 4-Me / $G_i(j)$. Compare Tables 4.3 and 4.5.

SUBSTRATE	PROTON GROUP	F_i	G_i	G_i RATIOS[b]
 2,4-Dimethylpyridine	2-CH_3	-544	77.1	0.38
	3-H	-142	68.2	0.43
	4-CH_3	-75	29.2	1.00
	5-H	-114	46.4	0.63
	6-H	-814	59.7	0.49
 3,4-Dimethylpyridine	2-H	-886	119.6	0.20
	3-CH_3	-182	23.7	1.00
	4-CH_3	-182	23.7	1.00
	5-H	-278	48.3	0.49
	6-H	-886	119.6	0.20

$$R = \left(\frac{\sum \left(\Delta_{obs} - \Delta_{calc} \right)^2}{\left(\sum \Delta_{obs} \right)^2} \right)^{\frac{1}{2}} \qquad \ldots 4.12$$

Hence the program allows pseudocontact shifts to be calculated from known molecular structures based on an assumed axially symmetric substrate-shift reagent geometry. As well as recording the minimum agreement factors R, obtained between the theoretical and experimental shifts, the location of the lanthanide metal ion, resulting in this minimum agreement factor, is also reported. The results determined from this computer treatment are shown in Table 4.8. In the examples used, the coordinating nitrogen atom is positioned at the centre of the Cartesian coordinate system and the location of the lanthanide ion is defined in Figure 4.2.

The very small agreement factors, 0.91 and 1.42, observed for the 3,4-Dimethylpyridine complexes of $Yb(fod)_3$ and $Pr(fod)_3$ respectively, indicates the good agreement between the theoretical pseudocontact shifts (based on known molecular structures) and the experimentally observed induced shifts. This illustrates that the experimental shifts for these shift reagent complexes are predominantly pseudocontact in nature, as described by the assumed axially symmetric model. This agrees very well with the theoretical expectations of the ytterbium and praseodymium cations regarding the absence of contact induced shifts. The agreement factor, 2.44, shown for the corresponding $Eu(fod)_3$ complex, although slightly larger than those reported for $Yb(fod)_3$ and $Pr(fod)_3$, does not reflect however the very large contact shift contributions which

Table 4.8

Results obtained using the computer program PDIGM

a) Minimum agreement factors as percentage.
b) Corresponding lanthanide-nitrogen bond length in nm.
c) See Figure 4.2.
d) Based on the theoretical values shown in Table 4.7.

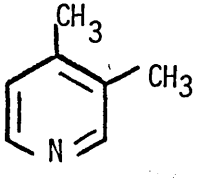
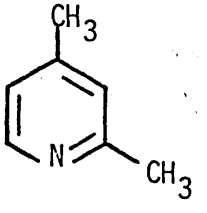
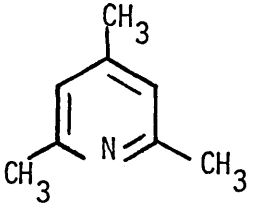
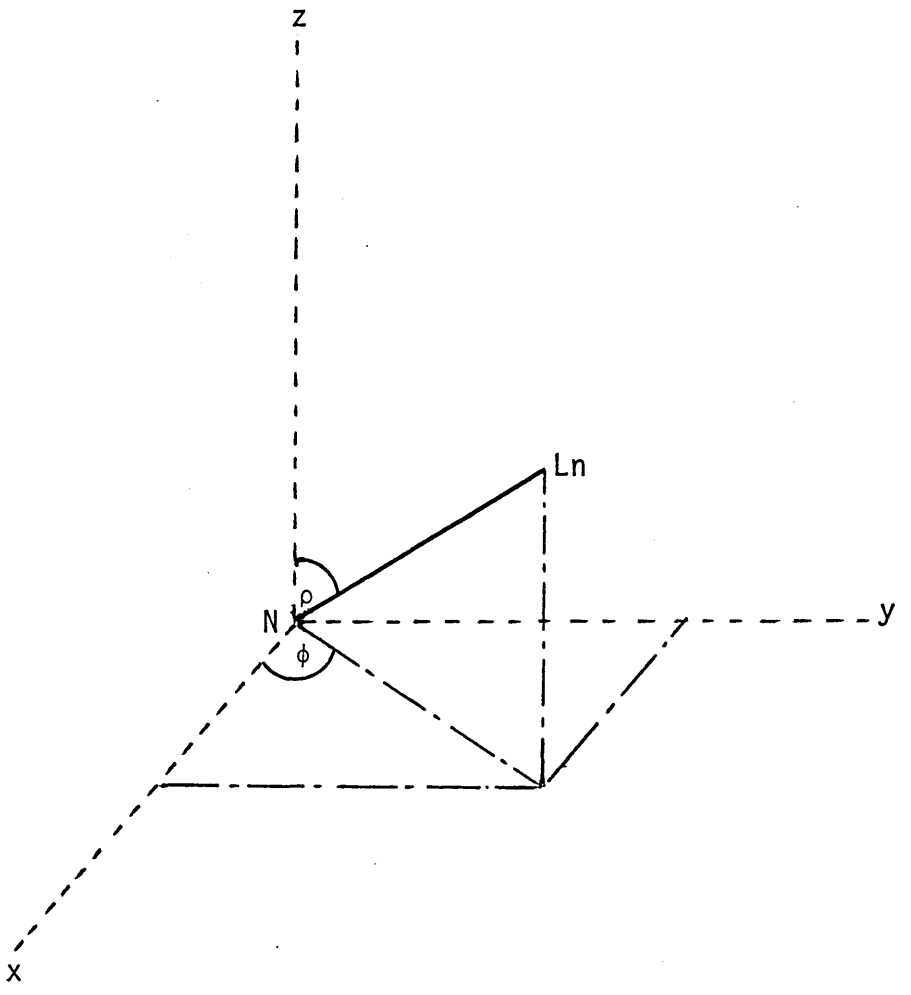
SUBSTRATE	SHIFT REAGENT	R[a]	Ln - N[b]	ρ[c]	ϕ[c]
 3,4-Dimethylpyridine	$Yb(fod)_3$	0.91	0.24	50	360
	$Pr(fod)_3$	1.42	0.26	50	354
	$Eu(fod)_3$	2.44	0.26	40	328
	G_i values[d]	0.87	0.22	50	360
 2,4-Dimethylpyridine	$Yb(fod)_3$	13.34	0.24	60	205
	$Pr(fod)_3$	16.72	0.26	60	210
	$Eu(fod)_3$	18.00	0.24	60	205
	G_i values[d]	5.49	0.24	30	250
 2,4,6-Trimethypyridine	$Eu(fod)_3$	21.80	0.34	90	180

Figure 4.2

The coordinate system used in the computer program PDIGM

N is the coordinating nitrogen atom situated at the centre of the system and

Ln represents the position of the lanthanide metal ion at a distance Ln - N from the coordinating centre.


z
Ln
ρ
N
y
ϕ
x

were earlier determined for this $Eu(fod)_3$-complex. Very good agreement (minimum agreement factor 0.87) is also found between the theoretically predicted pseudocontact shifts (based on known molecular structures) and the pseudocontact shifts based on the G_i values calculated from the theoretical values of Bleaney (25) and Golding (112) and from the experimental shift data. This agreement is expected to be good if the experimental shift data is sufficiently accurate.

In a further example, 2,4-Dimethylpyridine, considerable variation (13.34, 16.72 and 18.00) in the minimum agreement factors is obtained for the ytterbium, praseodymium and europium shift reagent complexes. It could therefore be reasoned that significant contact shift contributions occur within these systems. However, as already stated, and shown by the similarity of the relative induced shift ratios observed for $Pr(fod)_3$ and $Yb(fod)_3$, significant contact shifts are not apparent for these complexes. The observation of large agreement factors must therefore be a consequence of the incorrect use of the axially symmetric model used in the computer program. In this case, non axial symmetry must be due to steric hindrance effects caused by the presence of the 2-methyl proton group.

It can also be seen however, that although significant contact shifts have been determined for the $Eu(fod)_3$ complex, the location of the lanthanide metal ion is not too different from the locations predicted for the praseodymium and ytterbium metal ions. Consequently, the effect of non-axial symmetry far outweighs the effects caused by significant contact shift contributions. Furthermore, the agreement found between theoretical pseudocontact shifts, based on G_i values calculated from experimental and theoretical data, and the pseudocontact

shifts, based on known structures within the axially symmetric model, is also poor, 5.49. Since however, the accuracy of the experimental data and the probable absence of contact induced shifts have been shown, the poor agreement found between these theoretically derived pseudo-contact shifts again indicates the incorrect use of the assumption regarding axial symmetry. The results obtained with the $Eu(fod)_3$ - 2,4,6-Trimethylpyridine complex also suggests this incorrect assumption. When geometrical ratios, based on the results shown in Table 4.5 and also on a graphical model proposed by Wing and Early (155), were used in this computer treatment method, excellent agreement was found. However, these hypothetical values only serve to emphasise the incorrect use of axial symmetry. Although two similar substrates, namely 2,4- and 3,4-Dimethylpyridine, have been studied, the results suggest that steric hindrance dramatically affects the assumption regarding axial or effective axial symmetry. Where steric hindrance effects appear negligible, as with the 3,4-Dimethylpyridine complexes, very good correlation is found between the experimental shifts and the pseudocontact shifts based on the assumption of axial symmetry. However, when steric hindrance effects may be envisaged, as with 2,4-Dimethylpyridine and 2,4,6-Trimethylpyridine, very poor correlation is found between the experimental and theoretical shifts based on axial symmetry.

In part, these results support the views of Horrocks (26), Cramer et al (118) and Newman (37) and suggests the use of the more correct pseudo-contact shift equation which includes a term for describing non-axial symmetry behaviour. The results also show that the errors, measured in terms of the minimum agreement factor R, resulting from the presence of substantial contact shift contributions, are insignificant, when

compared to the errors resulting from the incorrect use of the McConnell-Robertson equation and hence the assumption of axial symmetry. For several substituted pyridines, it appears that both contact and pseudocontact shifts occur. However, owing to the unpredictable effect of axial or non-axial symmetry, the two shift effects cannot, at times, be distinguished and the overall effect determined. The results presented in this chapter show that tentative estimates of contact shifts contributions can be determined, but that finite conclusions regarding their validity must be reserved.

4.4. Conclusions

Various methods for the determination of contact shift contributions have been described and contact shifts determined for several substituted pyridine - $Eu(fod)_3$ complexes. For examples of this type, it appears that the contact shift contributions depend upon the spatial arrangement of the resonating nucleus, the lanthanide cation and the intervening atoms. The determination of contact shifts on nuclei positioned very close to the coordinating nitrogen atom, has not been attempted. This is due mainly to the difficulties experienced in the interpretation of the induced shift data. Variation in relative induced shift values observed for the complexes of several shift reagents, possibly reflects the presence of contact induced shifts. However, the same variation in relative shift values may also indicate pseudocontact shift differences within these complexes. These differences arise from the varying lanthanide-nitrogen bond lengths expected for the different lanthanide shift reagents used as the ionic radius of the lanthanide cation changes throughout the series. Furthermore, the effects caused by the incorrect assumption regarding axial symmetry probably far outweigh the effects cause by the presence of substantial contact shift contributions.

A ~~JOEL~~ JEOL C-60 HL nmr spectrometer was used for the work described in this thesis. All ^1H spectra were recorded at 60 MHz and at room temperature. The instrument was housed in a temperature controlled laboratory (20° C).

The shift reagent $Eu(fod)_3$ was initially prepared by the method of Sievers et al (156) and later purchased from the Ryvan Chemical Company Ltd., Southampton. Prior to use, the shift reagent was vacuum heated at 120° C / 0.5 mm Hg for 2-3 hours and then vacuum sublimed at approximately 160°C.

The solvent, deuterochloroform, was distilled and then stored over type 4A molecular sieves for at least 48 hours prior to use. These sieves had previously been heated at 120°C for 24 hours.

All substrates used in this study were dried and purified by a variety of techniques including fractional distillation, vacuum distillation and vacuum sublimation. The substrates were then, where appropriate, stored over type 4A molecular sieves. In several cases infrared (ir) and nmr spectroscopic techniques were used to test for the absence of small amounts of water and other impurities. When necessary, purification procedures were repeated to ensure the use of anhydrous conditions.

Solution preparation of all samples was controlled and carried out in a nitrogen filled dry-box in which phosphorus pentoxide was used as a dessicant with frequent renewal. All glassware used was dried at 120°C for 24 hours prior to use.

Sample solutions were prepared in the following manner: A weighed amount of the substrate under investigation was dissolved in deuterochloroform and made up to volume in a tared 25 cm^3 volumetric flask. The mass of the substrate and deuterochloroform was then recorded. A stock solution of the shift reagent was also prepared in a similar way. Varying amounts of the substrate stock solution were weighed in to 5 cm^3 volumetric flasks. Into each of these flasks was pipetted 2 cm^3 aliquots of the shift reagent stock solution. Each 5 cm^3 flask was then made up to volume with deuterochloroform containing approximately 1% tetramethylsilane (TMS) as internal standard. Approximately 0.5 cm^3 of the resulting solution was then transferred to a clean, dry nmr tube which was capped and subsequently sealed with parafilm. This seal prevented the entry of moisture etc. whilst the nmr tubes were removed from the dry-box and inserted in the nmr spectrometer. Typical substrate concentrations varied between 0.004 and 0.2 $mol.dm^{-3}$ and the shift reagent concentration fixed at about 0.006 $mol.dm^{-3}$.
Sample solutions used in the competition experiments were prepared in a similar way.

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Appendix One

Computer program used by Mackie and Shepherd (69) to calculate the intrinsic parameters of a 1:1 stoichiometry.

```
      DIMENSION A(20),B(20),SHIFT(20),S(20),D(100),X1(20),X2(20),X3(20),
     1 CONST(20),PDEVN(100),DIF(100), DIFF(100),ITIT(20)
      READ(5,10)NRUN
10    FORMAT(I2)
      DO 999 I=1,NRUN
      READ(5,20) ITIT
20    FORMAT(20A4)
      READ(5,30)N
30    FORMAT(I2)
      READ(5,40)(A(J),B(J),SHIFT(J),J=1,N)
40    FORMAT(3F10.0)
      DO 50 J=1,N
50    S(J)=1.0/SHIFT(J)
      SUMX=0.0
      SUMY=0.0
      SUMXX=0.0
      SUMXY=0.0
      DO 60 J=1,N
      SUMX=SUMX+B(J)
      SUMY=SUMY+S(J)
      SUMXX=SUMXX+B(J)**2
60    SUMXY=SUMXY+S(J)*B(J)
      G=N
      DENOM=SUMX**2-G*SUMXX
      SLOPE=(SUMX*SUMY-G*SUMXY)/DENOM
      Z=(SUMX*SUMXY-SUMY*SUMXX)/DENOM
      DO 70 J=1,N
70    S(J)=1.0/(SLOPE*B(J) +Z)
      DO 67 J=1,N
67    CONTINUE
      X=1.0
      K=1
      D(K)=15.0
      GO TO 100
80    K=K+1
      D(K)=D(K-1) +X
100   DO 105 J=1,N
      X1(J)=D(K)-S(J)
      X2(J)=A(J)/S(J)
```

```
      X3(J)=B(J)/D(K)
      CONST (J)=1.0/(X1(J)*(X2(J)-X3(J)))
  105 CONTINUE
      SUM=0.0
      DO 110 J=1,N
  110 SUM=SUM+CONST(J)
      G=N
      AV=SUM/G
      DO 120 J=1,N
      DIF(J)=CONST(J) -AV
  120 DIFF(J)=DIF(J)**2
      SUM=0.0
      DO 130 J=1,N
  130 SUM=SUM+DIFF(J)
      DEVN=(SUM/(G-1.0))**0.5
      PDEVN(K)=ABS(DEVN/AV)*100.0
      IF(K.EQ.1)GO TO 80
      IF (K.EQ.50) GO TO 150
      IF (PDEVN(K)-PDEVN(K-1))80, 140, 140
  140 X=-X/2.0
      GO TO 80
  150 CONTINUE
      XX=D(50)
      WRITE(6,160)ITIT
  160 FORMAT(1H1,20X,20A4)
      WRITE(6,170)XX
  170 FORMAT(1HO,25X,'INFINITE SHIFT = ',F10.4)
      WRITE(6,180)AV
  180 FORMAT(1HO,25X,'ASSOCIATION CONSTANT = ',F10.4)
      WRITE(6,190)PDEVN(K)
  190 FORMAT(1HO,25X,'PERCENTAGE DEVIATION = ',F10.4)
      WRITE(6,200)
  200 FORMAT(1HO,5X,'LN.'5X,'LIGAND',5X,'OBS. SHIFT',5X,'CORR.SHIFT',5X
     1,'ASSOC. CONSTANT')
      DO 220 J=1,N
      WRITE(6,210)A(J),B(J),SHIFT(J),S(J),CONST(J)
  210 FORMAT(1HO,2X,F8.6,2X,F8.6,7X,F5.2,7X,F5.2,10X,F10.2)
  220 CONTINUE
  999 CONTINUE
      STOP
      END
```

Computer program of the full 4-parameter data treatment method presented in this thesis.

```
      DIMENSION SHIFT(20), SHCAL(20), ITIT(40), DIFF(20), RHO(20),
     1RHO1(20), RHO2(20),SH1(20), SH2(20),PES(20), PES2(20), DOFF(20)
      COMMON SUBT(20), ES(20), ES2(20)
C SHIFT INPUT IN HZ DELTA MAX. INPUT IN HZ, K VALUE INPUT IN DM3.MOL-1
      READ(1,910) NRUN
  910 FORMAT(I2)
      DO 999 JJ = 1,NRUN
      READ(1,911) ITIT
  911 FORMAT(40A2)
      READ(1,912) NPTS
  912 FORMAT(I2)
      READ(1,913) (SUBT(J), SHIFT(J), J=1,NPTS)
  913 FORMAT(2F10.0)
      READ(1,914) REAGT
  914 FORMAT(F10.0)
      READ(1,915) NUMK, STEPK, DCN1
  915 FORMAT(I3,F10.0,F20.0)
      READ(1,916) NUMKK, STPKK, DCN2
  916 FORMAT(I3,F10.0,F20.0)
      READ(1,917) NUMI, STEP1, DTA1
  917 FORMAT(I3,2F10.0)
      READ(1,918) NUMII, STPII. DTA2
  918 FORMAT(I3,2F10.0)
      WRITE (5,800) ITIT
  800 FORMAT(1H1,3X,40A2)
      WRITE (5,1007)
 1007 FORMAT(1HO,20X,'E + S = = = = ES ')
      WRITE (5,1077)
 1077 FORMAT(1HO,20X,'ES + S = = = = ES2 ')
      ANUMK  =  NUMK
      ANMKK  =  NUMKK
      ANUMI  =  NUMI
      ANMII  =  NUMII
      STNMK  =  STEPK*(ANUMK-1.0)
      STMKK  =  STPKK*(ANMKK-1.0)
      STNMI  =  STEP1*(ANUMI-1.0)
      STMII  =  STPII*(ANMII-1.0)
      DCNII  =  DCN1 + STNMK
      DCN22  =  DCN2 + STMKK
      DTA11  =  DTA1 + STNMI
      DTA22  =  DTA2 + STMII
      WRITE (5,801) NUMK, STEPK, DCN1, DCN11
  801 FORMAT(1HO,5X,'NO. OF STEPS =',I3,', STEP =',F9.5,', RANGE OF EQ
     1UI1.  K1  VALUES =  ',F10.4,' TO  ',F10.4)
      WRITE (5,802) NUMKK, STPKK, DCN2, DCN22
  802 FORMAT(1HO,5X,'NO. OF STEPS = ',I3',  STEP = ',F9.5', RANGE OF EQ
```

```
   1UIL.  K2  VALUES =  ',F10.4,' TO  ',F10.4)
    WRITE (5,803) NUMI, STEP1, DTA1, DTA11
803 FORMAT(1HO,5X,'NO. OF STEPS =',I3,',  STEP =',F9.5,', RANGE OF DE
   ILTA MAX.1 VALUES =  ',F10.4, ' TO ',F10.4)
    WRITE (5,804) NUMII, STPII, DTA2, DTA22
804 FORMAT(1HO,5X,'NO. OF STEPS =',I3,',  STEP =',F9.5,', RANGE OF DE
   ILTA MAX.2 VALUES =  ',F10.4,' TO  ',F10.4)
    DO 555 J=1, NPTS
    RHO(J) = REAGT / SUBT(J)
555 CONTINUE
    CN = FLOAT(NPTS-1)
    STD  = 100.0
    JK   = 0
    JKK  = 0
    JI   = 0
    JII  = 0
    DEQUI = DCNI
    DEQU2 = DCN2
    DLMX1 = DTA1
    DLMX2 = DTA2
    DO 707 M=1,2,3
    DO 600  K=1,NUMK
    DEQUI = DCNI + (STEPK*(K-1.0))
    DO 601 KK=1,NUMKK
    DEQU2 = DCN2 + (STPKK*(KK-1.0))
    DO 500 J=1,NPTS
    IF(FLOAT(J)-1.9) 195,195,191
191 ESFRE = ES(J-1)
    GO TO 196
195 CONTINUE
    ESFRE = REAGT/2.0
196 CONTINUE
    CALL ESES2(REAGT, SUBT(J), DEQU1, DEQU2, ESFRE, ES(J), ES2(J))
500 CONTINUE
    DO 602  I=1,NUMI
    DLMX1 = DTA1 + (STEP1*(I-1.0))
    DO 603 II=1,NUMII
    DLMX2 = DTA2 + (STPII*(II-1.0))
    STAND = 0.0
    DO 550 J=1,NPTS
    SHCAL(J) = ((ES(J)*DLMX1)/SUBT(J)) + ((2.0*ES2(J)*DLMX2)/SUBT(J))
    DEVN = ( 1.0 - (SHCAL(J)/SHIFT(J)))*100.0
    STAND = STAND + (DEVN**2)
550 CONTINUE
    SD = SQRT(STAND/CN)
    IF(SD-STD) 83,85,85
 83 STD = SD
    JK  = K
    JKK = KK
    JI  = I
    JII = II
 85 CONTINUE
603 CONTINUE
```

```
 602 CONTINUE
 601 CONTINUE
 600 CONTINUE
     DEQUI = DCN1 + (STEPK*(JK-1.0))
     DEQU2 = DCN2 + (STPKK*(JKK-1.0))
     DLMX1 =   DTA1 + (STEP1*(JI-1.0))
     DLMX2 = DTA2 + (STPII*(JII-1.0))
     STK = STEPK
     STKK = STPKK
     STI = STEPI
     STII = STPII
     AJK = JK
     IF( AJK -  7.5 ) 6211, 6211, 6202
6202 DCN1 = DCN1 + (STEPK*(ANUMK-2.0))
     GO TO 201
6211 CONTINUE
     IF( AJK -1.5) 6220, 6220, 211
6220 DCN1 = DCN1 - ((STEPK)*(ANUMK-2.0))
     GO TO 201
 211 CONTINUE
     DCN1 = DCN1 + (STEPK*(AJK-2.0))
     STEPK = STEPK / 2.0
 201 CONTINUE
     JK = AJK
     AJKK = JKK
     IF( AJKK -  7.5 ) 7211, 7211, 7220
7220 DCN2 = DCN2 + (STPKK*(ANMKK-2.0))
     GO TO 7201
7211 CONTINUE
     IF(AJKK-1.5) 7202, 7202, 2211
7202 DCN2 = DCN2 - ((STPKK)*(ANMKK-2.0))
     GO TO 7201
2211 CONTINUE
     DCN2 = DCN2 + (STPKK*(AJKK-2.0))
     STPKK = STPKK / 2.0
7201 CONTINUE
     JKK = AJKK
     AJI = JI
     IF( AJI -  7.5 ) 7311, 7311, 7322
7322 DTA1 = DTA1 + (STEP1*(ANUMI-2.0))
     GO TO 203
7311 CONTINUE
     IF(AJI-1.5) 7302, 7302, 223
7302 DTA1 = DTA1 - (STEP1*(ANUMI-2.0))
     GO TO 203
 223 CONTINUE
     DTA1 = DTA1 + (STEP1*(AJI-2.0))
     STEP1 = STEP1 / 2.0
 203 CONTINUE
     JI = AJI
     AJII = JII
     IF( AJII -  7.5 ) 7411, 7411, 7422
```

```
7422 DTA2 = DTA2 + (STPII*(ANMII-2.0))
     GO TO 204
7411 CONTINUE
     IF(AJII-1.5) 7402, 7402, 224
7402 DTA2 = DTA2 - (STPII*ANMII-2.0))
     GO TO 204
 224 CONTINUE
     DTA2 = DTA2 + (STPII*(AJII-2.0))
     STPII = STPII / 2.0
 204 CONTINUE
     JII = AJII
 707 CONTINUE
     CONK = 1.0 / DEQUI
     CONKK = 1.0 / DEQU2
     DELMI = DLMXI / 60.0
     DELM2 = DLMX2 /60.0
     WRITE (5,400) DEQUI, CONK
 400 FORMAT(1HO,17X,'EQUILIBRIUM CONSTANT  K1     = ',F10.4,' (L/MOLE)
    1',5X,F12.6,'MOLE/L')
     WRITE (5,402) DEQU2, CONKK
 402 FORMAT(1HO,17X,'EQUILIBRIUM CONSTANT  K2     = ',F10.4,' (L/MOLE)
    1',5X,F12,6,' MOLE/L'
     WRITE (5,404) DELM1 , DLMX1
 404 FORMAT(1HO,17X,'DELTA MAX. 1                 = ',F10.4,' (PPM)',5
    1X,F9.4,' HZ')
     WRITE (5,406) DELM2, DLMX2
 406 FORMAT(1HO,17X,'DELTA MAX. 2                 = ',F10.4,' (PPM)',5
    1X,F9.4,' HZ')
     WRITE (5,408) STD
 408 FORMAT(1HO,17X,'RELATIVE STANDARD DEVN.      = ',F10.4)
     WRITE (5,441) JK, STK, NUMK
 441 FORMAT(1HO,5X,'JK  = ',I3,',  STEP = ',F9.5,', NO. OF STEPS FOR K
    11 = ',I3)
     WRITE (5,442) JKK, STKK, NUMKK
 442 FORMAT (1HO,5X,'JKK = ',I3,',  STEP = ',F9.5,',NO. OF STEPS FOR K
    12 = ',I3)
     WRITE (5,443) JI, STI, NUMI
 443 FORMAT(1HO,5X,'JI = ',I3,',  STEP = ',F9.5,',  NO. OF STEPS FOR D
    IELTA MAX. 1 = ',I3)
     WRITE (5,444) JII, STII, NUMII
 444 FORMAT(HO,5X,'JII = ',I3,',  STEP = 'F9.5,',   NO. OF STEPS FOR D
    IELTA MAX. 2 = ',I3)
     WRITE (5,481)
 481 FORMAT(1HO,5X,'SUBT',5X,'REAGT',5X,'REAGT/SUBT',5X,'SHIFT (EXP)',5
    1X,'SHIFT (CAL)',5X,'DIFF'
     IF(STD-99.0) 491,495,495
 491 CONTINUE
     ST = 0.0
     DO 726 J=1,NPTS
     IF(FLOAT(J)-1.9) 175,175,174
 174 ESFRE = ES(J-1)
     GO TO 176
 175 CONTINUE
     ESFRE = REAGT/2.0
```

```
176 CONTINUE
    CALL ESES2(REACT,  SUBT(J), DEQU1, DEQU2, ESFRE, ES(J), ES2(J))
    SHCAL(J) = ((ES(J)*DLMX1)/SUBT(J)) + ((2.0*ES2(J)*DLMX2)/SUBT(J))
    DIFF(J) = ( 1.0 - (SHCAL(J)/SHIFT(J)))*100.0
    ST = ST  + (DIFF(J)**2)
    DOFF(J) = SHIFT(J) - SHCAL(J)
    RHO1(J) = ES(J) / SUBT(J)
    SH1(J) = RHO1(J)*DLMX1
    RHO2(J) = ES2(J) / SUBT(J)
    SH2(J) = RHO2(J)*2.0*DLMX2
    PES(J) = (100.0*ES(J)) / REAGT
    PES2(J) = (100.0*ES2(J)) / REAGT
    WRITE (5,453) SUBT(J), REAGT, RHO(J), SHIFT(J), SHCAL(J)
   1DOFF(J)
453 FORMAT(1H ,2X,F8.6,2X,F8.6,3X,F11.6,5X,F10.4,6X,F10.4,3X,F8.4)
726 CONTINUE
    TSD = SQRT(ST/CN)
    WRITE (5,666)
666 FORMAT(1HO,10X,'STD',5X,'ES',5X,'ES / SUBT',5X,'SHIFT (ES)',5X,'ES
   12',5X,'ES2 / SUBT',5X,'SHIFT (ES2)',5X,'PERCENT ES',5X,'PERCENT ES
   22')
    DO 665 J=1,NPTS
          WRITE (5,664) TSD, ES(J), RHO1(J), SH1(J), ES2(J), RHO2(J),
   1SH2(J), PES(J), PES2(J)
664 FORMAT(1H ,5X,F9.6,1X,F8.6,1X,F10.6,3X,F11.6,1X,F9.6,3X,F11.6,3X,F
   112.6,4X,F11.6,4X,F12.6)
665 CONTINUE
    WRITE (5,37)
 37 FORMAT(1HO,97X,'OF TOTAL REAGENT')
    GO TO 566
495 CONTINUE
    DO 727 J=1, NPTS
    WRITE (5,455) SUBT(J), REAGT, RHO(J), SHIFT(J)
455 FORMAT(1H ,2X,F8.6,2X,F8.6,3X,F11.6,5X,F10.4)
727 CONTINUE
566 CONTINUE
999 CONTINUE
    CALL EXIT
    END


    SUBROUTINE CUBIC(ESINT, A, B, C, D, ESI)
    ESI = ESINT
    DO 10 J=1,50
    X = ESI
    Q = (A*X**3)  +  (B*X**2)  +  (C*X)  +  D
    QP = (3.0*A*X**2)  +  (2.0*B*X)  +  C
    QT = Q  /  QP
    ESI = X - QT
    QR = ABS(QT)
    CHECK = 0.0001*ABS(ESI)
    IF(QR-CHECK) 20,20,10
 10 CONTINUE
```

```
RETURN
END


SUBROUTINE ESES2(REAGT, SUBT, DEQU1, DEQU2, ESINT, ES, ES2)
A = DEQU2 - ((4.0*DEQU2*DEQU2)/DEQU1)
B = 1.0 - (2.0*REAGT*DEQU2)-((4.0*DEQU2)/DEQU1)
C=(2.0*REAGT*SUBT*DEQU2)-REAGT-SUBT-(1.0/DEQU1)-(SUBT*SUBT*DEQU2)
D = REAGT*SUBT
CALL CUBIC (ESINT, A, B, C, D, ES)
ES2 = (ES*DEQU2*(SUBT-ES))/(1.0+(2.0*DEQU2*ES))
RETURN
END
```

Appendix Two

Post-graduate courses of study

The following post-graduate lectures were attended.

1. At the University of Sheffield.
   a) Principles of nuclear megnatic resonance spectroscopy (12 lectures)

2. At Sheffield City Polytechnic.
   a) M.Sc. course:- Instrumental Analysis - nuclear magnetic resonance spectroscopy (10 lectures)
   b) Introduction to computer programming (6 lectures)

3. Royal Institute of Chemistry, London.
   a) Two one-day courses involving nuclear magnetic resonance and the application of lanthanide shift reagents.