Developments and advances concerning the hyperpolarisation technique SABRE

Ryan E. Mewis*

School of Science and the Environment, Division of Chemistry and Environmental Science, Manchester Metropolitan University, John Dalton Building, Chester St., Manchester, M1 5GD, UK

Email: r.mewis@mmu.ac.uk

Abstract

To overcome the inherent sensitivity issue in NMR and MRI, hyperpolarisation techniques are used. SABRE (Signal Amplification By Reversible Exchange) is a hyperpolarisation technique that utilises *para*hydrogen, a molecule which possesses a nuclear singlet state, as the source of polarisation. A metal complex is required to break the singlet order of *para*hydrogen, and by doing so facilitates polarisation transfer to analyte molecules ligated to the same complex through the *J*-coupled network that exists. The increased signal intensities that the analyte molecules possess as a result of this process, have led to investigations whereby their potential as MRI contrast agents has been probed, and to understand the fundamental processes underpinning the polarisation transfer mechanism. As well as discussing literature relevant to both of these areas, the chemical structure of the complex, the physical constraints of the polarisation transfer process and the successes of implementing SABRE at low- and high- magnetic field are discussed.

Keywords: NMR, ^{1}H, ^{13}C, ^{31}P, hyperpolarisation, SABRE

Introduction

Hyperpolarisation techniques are useful in that they help to overcome the inherent sensitivity issue associated with NMR and MRI. At present, the hyperpolarisation techniques that are dynamic nuclear polarisation (DNP),[1] spin exchange optical pumping (SEOP)[2, 3] and *para*hydrogen induced polarisation (PHIP)[4, 5] are making significant inroads in terms of obtaining hyperpolarised MR images to better inform clinical diagnosis. For example, SEOP is used to create hyperpolarised xenon and helium gas to image the lung space[6] in order to visualise more easily disease states such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis, whereas the hyperpolarisation of ^{13}C-labelled pyruvate by DNP, has similarly been shown to be an exciting prospect for the imaging of prostate cancer.[7] PHIP, which utilises the singlet state of *para*hydrogen as the source of polarisation, has been utilised to gain insight into reaction

mechanisms, such as hydroformylation,[8, 9] *via* NMR, although there has still been considerable interest in employing it to produce hyperpolarised agents for use in MRI.[10, 11] All of these techniques have been extensively reviewed,[12-15] but herein the focus is to discuss the advances and developments of the non-hydrogenative form of PHIP, which is more commonly known as SABRE[16] (Signal Amplification By Reversible Exchange). SABRE, unlike PHIP, does not require the molecule of interest to be hydrogenated in order to facilitate polarisation transfer. Instead, magnetisation is propagated through the *J*-coupling that exists between *para*hydrogen derived hydride ligands and those of the ^{1}H nuclei in the substrate at low- (or sometimes high-) magnetic field. A catalytic cycle for the transfer of spin polarisation by SABRE is shown in Figure . Thus, SABRE has wide ranging utility in both NMR and MRI due to the scope of molecules which it can potentially polarise. How SABRE is being utilised in both of these arenas will be highlighted in this review, but to begin, the design of the polarisation transfer catalyst and the optimisation of the physical conditions which affect polarisation transfer will be discussed. The focus of the review will then turn to looking at the use of SABRE in biologically relevant media and in particular, its use in MR imaging at low- and high-magnetic field. For a comprehensive background on the physics of *para*hydrogen, the reader is directed to the review by Green *et al*.[13]

Figure Catalytic cycle in which spin polarisation is transferred to an analyte (pyridine in this instance). Firstly, polarisation is transferred from parahydrogen derived hydride ligands to the ^{1}H nuclei in pyridine (A). The pyridine ligand then dissociates from the complex (B), creating a vacant site for di-hydrogen to bind so that the spent parahydrogen derived hydride ligands (shown in blue) can be replaced. Oxidative addition of the incoming dihydrogen molecule and reductive elimination of the pre-existing hydride ligands (C to D), enables a pyridine ligand to bind so that the process can begin again.

Early developments

SABRE was first reported in 2009 by Duckett *et al*. when it was demonstrated that PHIP could occur without the incorporation of *para*hydrogen into the analyte.[17] It was shown that by using an iridium based catalyst, spin polarisation could be transferred, at low-magnetic field, from *para*hydrogen derived hydride ligands to those of a polarisation acceptor molecule, which in this instance was ^{15}N-labelled pyridine. By polarising the ^{15}N-labelled pyridine, the catalyst operated in a non-conventional way, for it was not chemical change that it facilitated but the

transfer of spin magnetisation. This initial observation was sufficient to demonstrate the applicability of this technique to both NMR and MRI and it led to the phrase SABRE being coined to describe this technique.[16] In this second publication, a range of N-heterocyclic ligands, such as nicotine and 3-fluoropyridine, were polarised by SABRE. Furthermore, the polarisation transfer was evident by inspection of the resulting polarised ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra that were recorded relative to their normal Boltzmann distribution trace (see Figure for examples of chemical structures that were polarised). In terms of signal gain, the enhancement of the ^{1}H spins of pyridine increased by 550-fold following polarisation transfer at 2 x10^{-2} T. In this instance, the spin transfer catalyst $[Ir(H)_2(PCy_3)(pyridine)_3][BF_4]$ was used. In the next section, the structure of the catalyst will be detailed with a view to highlighting how it dictates the efficiency of polarisation transfer.

Figure Chemical structures and names of the some of the molecules polarised by Duckett and co-workers.[16]

1. **Design of the SABRE catalyst**

The SABRE catalyst catalyses the transfer of spin polarisation from *para*hydrogen derived hydride ligands to a substrate of interest. Thus, the ligand architecture surrounding the metal centre is important in that it must facilitate facile analyte ligation to the metal centre. It must have sufficient vacant sites, during the catalytic cycle, to allow both the analyte and *para*hydrogen derived hydride ligands to bind. In addition, both the *para*hydrogen derived hydride ligands and analyte moleculesmust be able to exchange freely at the metal centre, as shown in Figure . Initial reports focused on the use of iridium complexes possessing phosphine ligands.[17, 18] Cationic iridium complexes with the formula $[Ir(COD)(PR_3)_2][BF_4]$ were synthesized where R = Ph, *p*-tolyl or *p*-C_6H_4-OMe.[17] Upon addition of *para*hydrogen and pyridine the main complex formed was $[Ir(PR_3)_2(py)_2(H)_2]^+$ along with small amounts of $[Ir(PR_3)(py)_3(H)_2]^+$. The effect of the phosphine substituent on the rate of exchange of hydride to hydrogen and equatorially bound pyridine to free pyridine was examined. At 335 K, the rate of equatorial pyridine dissociation from $[Ir(PR_3)_2(py)_2(H)_2]^+$ was fastest for when R = *p*-C_6H_4-OMe, which is the most electron rich carbene examined. A rate constant of 0.90 s^{-1} was determined for the loss of pyridine. However, the rate of equatorial pyridine dissociation from $[Ir(PR_3)(py)_3(H)_2]^+$ was found to be much quicker; the rate constants at 335 K for when R = Ph or *p*-tolyl are 1.16 and 2.00 s^{-1} respectively. The rate constant for when R = *p*-C_6H_4-OMe

could not be determined due to thermal decomposition. However, a rate constant could be determined at 308 K, and by using Eyring data to estimate the corresponding rate constants for R = Ph or *p*-tolyl, meant that a comparison could be made. The three constants determined were 0.066 s^{-1}, 0.068 s^{-1} and 0.064 s^{-1} for when R = *p*-C_6H_4-OMe, *p*-tolyl or Ph respectively. Thus the rate constants for the dissociation of equatorial pyridine from $[Ir(PR_3)(py)_3(H)_2]^+$ are comparable at 308 K but are considerably quicker than $[Ir(PR_3)_2(py)_2(H)_2]^+$ at 335 K. Furthermore, the values of $\Delta H^‡$ are larger for $[Ir(PR_3)_2(py)_2(H)_2]^+$ than $[Ir(PR_3)(py)_3(H)_2]^+$ which is suggestive that the Ir-N bonds are stronger due to the second phosphine ligand acting to strengthen it. As well as ascertaining what effect the phosphine identity has on the rate of dissociation, it was shown that ^{15}N-pyridine could be polarised using $[Ir(PR_3)_2(py)_2(H)_2]^+$, as the polarisation transfer catalyst, coupled with a *para*hydrogen INEPT+EXSY sequence. A 120-fold gain in signal strength was observed in the ^{15}N NMR spectrum.

Figure Chemical structures of the phosphines (PR_3) that were used to produce complexes of the type $[Ir(PR_3)(py)_3(H)_2][BF_4]$

The chemical identity of the phosphine of $[Ir(PR_3)(py)_3(H)_2][BF_4]$ was further extended in which the phosphine (PR_3) was PCy_3, PCy_2Ph, $PCyPh_2$, PEt_3, P^iPr_3, P^nBu_3, P^tBu_3 or P(1-naphtyl)$_3$.[18] Of this series, PCy_2Ph, led to the best enhancement for the three ^{1}H sites of pyridine following polarisation transfer at 0.5 x 10^{-4} T. In light of the previous study, this is not surprising as PCy_2Ph is the most electron rich ligand in the series. In addition, this report also investigated the effect of catalyst loading on the enhancement of the three ^{1}H sites of pyridine. It was found that enhancement was maximized at the 1 : 5 loading level when $[Ir(COD)(PCy_3)(py)][BF_4]$ was used as the catalyst. In particular, the *ortho* protons of pyridine were enhanced by 94-fold, following polarisation transfer at a magnetic field of 0.5 x 10^{-4} T, when this loading was used relative to those at thermal equilibrium.

Having established that the electron richness of the phosphine ligand is firmly linked to the enhancement of a substrate by SABRE, the utilisation of carbenes seemed to be a logical progression.[19-21] The first carbene complex to be used in conjunction with SABRE was published in 2011.[19] It focused on the use of IMes (1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene) to form the complex [Ir(IMes)(COD)Cl]Cl (COD = cyclooctadiene), which following activation by hydrogen in the presence of pyridine gave $[Ir(IMes)(H)_2(py)_3]Cl$. Thus, it was structurally analogous to the mono-

phosphine complexes reported previously. Comparison of [Ir(IMes)$(H)_2(py)_3$]Cl and [Ir(PCy_3)$(H)_2(py)_3$]Cl as SABRE catalysts, led to the observation that the former enhanced the *meta* peak of pyridine by 266-fold, whilst the latter only achieved 18-fold. Therefore, the inclusion of the more electron donating NHC ligand in the polarisation transfer catalyst led to a significant improvement in the observed enhancement.

Further investigations into the chemical identity of the carbene revealed that the nature of the groups appended to the imidazole through the nitrogen atoms resulted in differing enhancement values for pyridine. A range of carbenes were investigated by the two groups of Feiters[21] and Duckett.[20] Both groups synthesised complexes with the formula [Ir(NHC)(COD)Cl], where NHC is one of the carbenes shown in Figure , to give [Ir(NHC)$(py)_3(H)_2$]Cl upon the addition of pyridine under a hydrogen atmosphere. Feiters and co-workers noted that the variation in the electronic properties of the carbene, as expressed by their relative TEP (Tolman Electronic Parameter) values, bore no correlation with that of the hyperpolarisation of pyridine.[21] However, they did note that by increasing the buried volume ($\%V_{bur}$) of the NHC, the pyridine exchange rates also increased. This trend is in keeping with a dissociative mechanism that was shown experimentally and theoretically for [Ir(IMes)$(py)_3(H)_2]^+$.[19] When saturated NHCs were compared to their unsaturated analogues, the former were found to have significantly higher exchange rates. For example, the exchange rates for pyridine in [Ir(IMes)$(py)_3(H)_2]^+$ relative to [Ir(SIMes)$(py)_3(H)_2]^+$ were found to be ≈4-5 times smaller. The IMes carbene ligand was found to be optimal, which provided an enhancement of 680-fold for pyridine. Similarly, Duckett and co-workers found that the IMes ligand gave the best enhancement out of the series of carbene ligands studied, but by optimisation of the catalytic system, were able to obtain a 5500-fold enhancement for pyridine.[20] In addition, the hydride exchange rate was studied and led to the NHC ligands being ranked thus : SIPr > SIMes > IPr > IMes > $ImMe_2NPr^i_2$ > ICy > IMe. This contrasts with the order determined for the dissociation of pyridine from [Ir(IMes)$(py)_3(H)_2]^+$ which was found to be : SIPr > IPr > SIMes > IMes > $ImMe_2NPr^i_2$ > ICy > IMe. Interestingly, IMes, which gave the largest enhancement, sits in the middle of both trends, and is therefore suggestive of an optimal exchange rate.

Figure . Carbenes employed in the synthesis of SABRE polarisation catalysts.

Mixed NHC-phosphine systems have also been explored. A series of complexes which possess phosphine and NHC ligands has been described for use as SABRE catalysts.[22] It was found that for $[Ir(IMes)(PPh_3)(py)_2(H)_2]^+$ the signal enhancement of pyridine was ≈3-fold larger than that observed for $[Ir(PCy_3)(py)_3(H)_2]^+$. The enhancement is, however, lower than that observed when $[Ir(IMes)(py)_3(H)_2]^+$ is used (by about 50%). Pyridine dissociation rate constants measured using a 20-fold of excess of pyridine and a 5.5 mM concentration of catalyst were 0.46 s^{-1} for $[Ir(PCy_3)(py)_3(H)_2]^+$, 6.26 s^{-1} for $[Ir(IMes)(py)_3(H)_2]^+$, 1.94 s^{-1} for $[Ir(IMes)(PPh_3)(py)_2(H)_2]^+$ and 3.1 s^{-1} for $[Ir(IMes)(PCy_3)(py)_2(H)_2]^+$. Thus, the exchange rate of a substrate, such as pyridine, can be tuned by the synthesis of a complex with the appropriate ligands. The use of mixed phosphine-NHC systems effectively allow access to intermediate exchange rates, whereas NHC and phosphine systems enable access to the faster and slower exchange rates respectively.

Figure . Pincer complex used for SABRE catalysis by Duckett and co-workers.[23]

Thus far, complexes to catalyse SABRE have focused on the use of mono-dentate ligands. Duckett and co-workers explored the use of a PNP pincer ligand, with pyridine as the central linker.[23] When the complex was employed to do SABRE-based polarisation transfer, SABRE was observed in free and bound pyridine, and the pyridine moiety of the pincer ligand. Upon increasing the temperature from 298 K to 310 K, an increase in signal intensity was observed. The increase was attributed to a better rate of ligand loss at higher temperatures, although for polarisation transfer at 0.5 G, 300 K was noted to be better than 310 K. Deuteration studies were also completed which showed that efficiency of transfer was increased. This is covered in more detail in Section 3Controlling the number of acceptor nuclei in the molecule to be polarised.

As well as iridium complexes, there are emerging examples of rhodium catalysts being used for SABRE. In a study to promote polarisation transfer to ^{31}P, a rhodium complex was formed *in situ* from $[Rh(COD)(DPPB)]BF_4$ (DPPB = 1,4-bis(diphenylphosphino)butane) and demonstrated that polarisation transfer *via* SABRE was feasible.[24] It was noted that the signals were hard to assign due to the complex chemical equilibria that exists in solution coupled with the low concentration of polarised species. Nonetheless, this report does highlight that SABRE is not restricted to iridium complexes to act as polarisation transfer catalysts.

In this section, the structure of the SABRE catalyst has been focused upon and the role it plays in exchange rates of both the substrate and hydride ligands highlighted. The rate of dissociation of substrate and hydride ligands need to be commensurate, but not at the risk of increasing or decreasing the lifetime of the substrate to a point where the scalar coupling cannot be established, or that relaxation at the metal surface becomes a prominent characteristic of polarisation transfer (i.e. relaxation is greater than the rate of polarisation transfer). In the next section, the physical constraints that affect polarisation transfer, with respect to the SABRE methodology, are discussed and evaluated with respect to their optimisation.

2. **Optimising polarisation transfer**

The catalysis of spin transfer that is necessary in SABRE can be maximised in a similar fashion to normal catalysis. The main factors that influence the polarisation transfer are : a) magnetic field at which polarisation transfer is conducted; b) the temperature at which polarisation transfer occurs; c) the ratio of catalyst to substrate; d) the pressure of *para*hydrogen gas; e) the number of acceptor nuclei in the molecule to be polarised. All of these factors have been studied and will now be discussed in turn.

a) **Effect of magnetic field on polarisation transfer**

In Section 1, it was shown that the magnitude of the enhancement is linked to exchange rates of both the pyridine and hydride ligands. This is heavily influence by the structure of the polarisation transfer catalyst. SABRE is also field dependent as the polarisation transfer is relayed under the scalar coupling and chemical shift differences that exist between the *para*hydrogen derived hydride ligands and the spin-½ nuclei in the substrate molecule whilst ligated to the metal centre (see Figure).[25] Modulation of the polarisation transfer field (PTF) can be used to optimise the amplitudes of polarised states. It has been shown that for fused ring systems such as pyridine,[19] quinoline[26] and isoniazid,[27] the maximal amplitude for longitudinal magnetisation, I_z, for ^{1}H nuclei is in the magnetic field region of 60-80 G. The similarity of the PTF curves, for a range of N-heterocycles, has been studied by Dücker *et al.*, in which the molecular structure of the substrate was found to be a secondary concern relative to that of the strength of the coupling between the *para*hydrogen derived hydride ligands and the ^{1}H nuclei in the substrate.[28] Those substrates possessing a $^4J_{HH}$ coupling between one of their ^{1}H nuclei and a *para*hydrogen derived hydride ligand were found to be similar, whereas substrates with only a $^5J_{HH}$ coupling possessed a different PTF plot. Thus

were pyridine and pyrazole found to have similar PTF plots (both possess a $^{4}J_{HH}$ coupling) whereas as 3,5-dimethylpyrazole was found to be different ($^{5}J_{HH}$ coupling) (see Figure for *J*-couplings).

Figure J-couplings between either pyrazole or 3,5-dimethylpyrazole and a parahydrogen derived hydride ligand during the polarisation transfer process in SABRE

Through the investigation of acetonitrile as a model substrate, it has been shown that it is possible to theoretically predict both ^{1}H and ^{13}C NMR spectra over a magnetic field range of 0 – 140 G (field produced by a copper coil surrounding the sample) which accurately match those of the spectra produced by the experiment.[29] Figure provides a description of the methodology used to obtain the experimental magnetic field plots. In this work, the importance of minimizing the chemical shift difference between the hydride ligands and that of the ^{13}CN group of acetonitrile was paramount to transferring polarisation. Thus in this instance the coil was switched off to propagate polarisation transfer at earth's magnetic field, whereas transfer to the ^{1}H nuclei of acetonitrile was optimised by setting the coil to 80 G. Interestingly, when ^{13}C labelled acetonitrile was employed, an increase in signal intensity in the ^{13}C NMR spectrum results only if the cyano group is labelled. This is because the $^{2}J_{HC}$ coupling in acetonitrile is of the same magnitude as the hydride-hydride coupling whereas the $^{1}J_{HC}$ coupling is an order of magnitude larger. Consideration of the couplings within molecules to be polarised by SABRE, is therefore, highly important.

Figure Methodology for the collection of a magnetic field plot

b) **Effect of temperature on polarisation transfer**

SABRE relies on the exchange of molecules at a metal centre. It has already been documented in Section 1, the importance of dissociation rates on the efficiency of transfer of SABRE. The temperature at which polarisation transfer is conducted is, therefore, very important in the optimisation process. A number of reports, which examine the effect of temperature on polarisation transfer, have been published. Zhivonitko *et al.* noted that only upon heating their sample consisting of $(PPh_3)_3Ir(H)_2Cl$ and PPh_3 to 60 °C from room temperature did SABRE transfer occur, leading to the observation of a 120-fold signal gain in the ^{31}P NMR spectrum.[24]

The effect of temperature on the enhancement observed for all the proton spins of pyridine following polarisation transfer using $[Ir(NHC)(py)_3(H)_2]^+$, where NHC is SIMes, ICy, IPr, SiPr, $ImMe_2NPr^i_2$, IMes and IMe has been reported.[20] Temperature dependence, over the range of 215 – 335 K, was presented for all seven complexes. This data revealed the majority of complexes facilitated polarisation transfer most efficiently between 255 – 295 K. However, there were extremes. In particular, when the NHC was SiPr, optimal polarisation transfer resulted at 223 K. When this data is considered alongside the dissociation rates of pyridine and hydride ligand in this complex (261 and 358 s^{-1} respectively at 300K), then this is perhaps not unsurprising. This is because the exchange rates of both ligands were reduced considerably at the lower temperature, and this results in better polarisation transfer by prolonging the scalar coupling between the two sets of ligands at the metal centre (due to the residence time increasing). IMe coincidentally requires a much larger temperature, 323 K, because the exchange rates at 300 K are too slow (hydride and pyridine ligand dissociation rates are 0.07 and 0.6 s^{-1} respectively). Ultimately, at present, the utilisation of the NHC IMes provides the best enhancement because its pyridine and hydride ligand dissociation rates seem to be commensurate with propagating polarisation transfer efficiently at room temperature.

c) **Ratio of catalyst to substrate**

The SABRE catalyst's primary role is to act as a mediator during polarisation transfer. However, it also has a role to play in relaxation. Studies have shown that the presence of the polarisation transfer catalyst results in T_1 relaxation constants of ^1H nuclei in the substrate being shortened. In substrate molecules which already have short T_1's already, this can be entirely detrimental to the observation of a hyperpolarised signal. The effect on T_1 of the all of the *ortho*, *meta* and *para* ^1H nuclei of pyridine in the presence of $[Ir(NHC)(py)_3(H)_2]^+$, where NHC is SIMes, IPr, SiPr, $ImMe_2NPr^i_2$, IMes and IMe has been demonstrated.[20] The T_1 data for the *ortho* ^1H spins are shown in Table . The data showed that for the majority, there is a substantial reduction in T_1 values (50-70%). However, for the NHCs ICy, $ImMe_2NPr^i_2$ and IMe there was a small increase for the *ortho* ^1H resonance. Although, it should be stated that upon inspection of the *para* and *meta* ^1H resonance T_1 values, a decrease of between 5-7% is observed for these three NHCs. The presence of the polarisation transfer catalyst, therefore, has a substantial effect on the longevity of the polarisation. Removal of the catalyst is thus not only important for biological application (see Section Towards a biological application of SABRE) but also for prolonging the polarised states created.

Table 1 here

Table . T_1 values at 298 K for the ortho ^1H resonance of pyridine in the presence of [Ir(NHC)(COD)Cl] and $[Ir(NHC)(py)_3(H)_2]^+$ and 3 bar H_2. †Percentage denotes the increase or decrease in T_1

The effect on the T_1 values for nicotinamide in the presence of the SABRE catalyst has been shown.[30] In this study, the ratio of catalyst ($[Ir(NHC)(nicotinamide)_3(H)_2]^+$) to nicotinamide was tested. At a ratio of 160 : 1 catalyst : nicotinamide, the T_1 of the ^1H resonance alpha to both the ring nitrogen and amide group was 18.8 s. This shortened to 16.9 and 9.6 seconds when the ratio of catalyst : nicotinamide was 50 : 1 and 16 : 1 respectively. The same ^1H resonance in solutions without catalyst had a value of 28.8 s and 43.1 s when the solution concentration was 0.08 M and 0.025 M respectively. Thus the shortening of the T_1 values cannot be sufficiently offset by reducing the ratio of catalyst : nicotinamide. In this report, the use of lower concentrations of catalyst was highlighted, with the maximum amplitude of longitudinal magnetisation taking longer to achieve (with constant bubbling of *para*hydrogen). Thus, polarisation transfer needs to be carefully managed in terms of offsetting the relaxation caused by the presence of the catalyst and the rate at which polarisation builds up within the substrate of interest.

d) **Pressure of hydrogen gas**

Hydrogen has only limited solubility in the solvents typically employed in SABRE and this worsens when aqueous systems are used (hydrogen is more soluble in ethanol compared with water by a factor of ~14-fold).[31] In light of this, reports have highlighted that greater signal intensities are obtained by increasing the pressure of *para*hydrogen gas. When pyridine was polarised in the earth's magnetic field by [Ir(IMes)(COD)Cl], the *ortho* proton signal for free pyridine was increased from a factor of 39-fold at 1 atm. to 243-fold at 3 atm.[19] For nicotinamide, when polarised by $[Ir(IMes)(py)_3(H)_2]Cl$, an increase in the signal enhancement for the four ring protons of nicotinamide was observed; this relationship was linear at low pressures but showed evidence for plateauing at higher pressures.[30] The *para*hydrogen here was delivered *via* an automated system[19] and a plot of bubbling time against signal enhancement highlighted that a finite amount of time was required to achieve maximal enhancement. Similar experiments conducted using medium- and heavy-walled NMR tubes in which pyridine was the focus, displayed a similar relationship between signal intensity and pressure.[20] Thus, the use of higher pressures of *para*hydrogen are advisable when desiring the best signal enhancements.

e) **Controlling the number of acceptor nuclei in the molecule to be polarised**

The transfer of polarisation *via* SABRE occurs through the *J*-coupling network which is established between the *para*hydrogen derived nuclei and spin ½ nuclei in the substrate molecule. Effectively, due to spin-spin coupling that exists between nuclei in the substrate molecule, polarisation is shared over all the spins that can viably accept polarisation. The net result is that the polarisation becomes “diluted”. To circumvent this, it is possible to design the substrate molecule in such a way that the number of polarisation acceptors are reduced, which leads to increased signal intensities for the remaining polarisation acceptors. One way to achieve this is by using deuterium labelling; less efficient transfer results because ^{2}H nuclei compared with ^{1}H nuclei are very far apart in terms of frequency and thus restricts transfer to these nuclei. This approach was first exemplified when 3,4,5-d_3-pyridine was considered as a substrate.[19] Having noted that pyridine delivered a 6000-fold enhancement at 3 T, with the *ortho*, *meta* and *para* protons possessing longitudinal magnetisation polarisations of 7.2, 3.8 and 8.1% respectively, when 3,4,5-d_3-pyridine was tested in analogous way, the longitudinal magnetisation on the *ortho* protons increased by 60%. Subsequent images acquired using RARE (rapid acquisition with refocused echoes) demonstrated that 3,4,5-d_3-pyridine gave a brighter signal than pyridine at 9.4 T; 3,4,5-d_3-pyridine gave a contrast enhancement of 807 relative to a water reference sample whereas pyridine gave only 169.[32]

Another approach to using deuterated pyridine has been explored by Dücker *et al.*[28] Instead of labelling pyridine selectively, a mixture of pyridine and d_5-pyridine, in a ratio of 1 : 10 was employed, resulting in an increased signal intensity on the *ortho* protons of pyridine. Significantly, the magnetic field strengths for the maxima in the determined field dependency curves remain unchanged when this mixture is used instead of neat pyridine.

The use of fully deuterated ligands can also be advantageous when studying polarisation transfer to the catalyst itself. This approach was implemented in the study of polarisation transfer into the pincer backbone of the complex shown in Figure by employing d_5-pyridine to reduce the number of polarisation accepters.[23] As a result, it was observed that the signals for the two pyridyl resonances of the pincer motif were strengthened. Similarly, when the pincer ligand was deuterated, as shown in Figure , the *ortho* signals of the pyridine ligand were increased by 35% at 65 G, which is the result of diminished magnetisation wastage into the polarisation transfer catalyst.

Figure The two deuterated pincer complexes explored by Holmes et al. to probe magnetisation transfer[23]

Subsequent investigations by Fekete *et al.* have highlighted the significant importance of the labelling strategy in order to increase polarisation transfer.[22] When analysing the polarisation transfer to acetonitrile in the complex $[Ir(IMes)(PPh_3)(H)_2(py)(MeCN)]^+$ (Figure), polarisation was noted to be transferred to the pyridine and IMes ligands, and both the ^{31}P nucleus and aromatic protons of PPh_3 also. When polarisation transfer to acetonitrile was monitored at a magnetic field of 20 G, the enhancement increased from approximately 1-fold to ~115-fold when the IMes and pyridine ligands were both deuterated. Deuterating the PPh_3 ligand had only a marginal increase in the signal intensity whereas deuterating both the IMes and pyridine ligands led to a greater enhancement than the sum of the enhancements from experiments in which either the pyridine or IMes ligands were deuterated. Thus, the deuteration of ligands needs to be carefully considered, as deuterating some ligands will result in only a marginal increase in signal intensity for the analyte, whereas deuterating others can lead to significant signal intensity improvement.

Figure Structure of the SABRE catalyst used by Fekete et al.[22]

The transfer of polarisation to acetonitrile has been studied in more detail by considering the possible ^{13}C and ^{2}H isotopomers, when polarised by $[Ir(IMes)(H)_2(py)_2(MeCN)]^+$.[29] Firstly, the use of d_5-pyridine increased the enhancement from 20-fold to 60-fold following polarisation transfer at 80 G. Transfer of polarisation to ^{13}C was also investigated and it was shown that polarisation was readily evident to the quaternary of the nitrile group, whilst transfer to the methyl ^{13}C was negligible. Isotopically labelling either of the carbon centres as ^{13}C gave the same observations; the quaternary was significantly hyperpolarised as evidenced by the signal-to-noise of 105 following polarisation transfer at 0 G whereas the methyl carbon showed little evidence for polarisation transfer. Deuterating the methyl protons of acetonitrile resulted in no polarisation being transferred to either carbon nucleus. This is because of the complete lack of ^{1}H-^{1}H coupling between the substrate and *para*hydrogen derived hydride ligands. Therefore, when minimizing the number of polarisation acceptors, an initial route into the spin-system of the molecule must be available for polarisation to be initially transferred.

3. **Detection limits**

The large improvement in signal-to-noise that SABRE offers, means that there is the opportunity to detect analyte molecules at much lower concentrations than NMR would normally allow. For quinoline, it was possible to detect down to 0.2 μmol level, as exemplified by ^{1}H NMR spectroscopy in that the proton α to the ring nitrogen possessed a signal-to-noise ratio of 15 : 1 following polarisation transfer at 50 G.[26] It was also possible to obtain 2D ^{1}H-^{1}H spectra at a level of 2 μmol of quinoline. This was possible through the implementation of the OPSY (Only *Para*hydrogen SpectrsocopY)[33, 34] sequence, which was set-up to select the double quantum coherence only, thus maximising cross-peak encoding prior to the start of the COSY sequence. The resulting ^{1}H OPSY-COSY spectrum was acquired in 64 increments. This could be significantly reduced by using ultrafast methods; a 2D COSY measurement was acquired in just a single transient (1 s) using this method.

Further investigation in pushing SABRE towards the nanomolar regime has been performed by Tessari and co-workers.[35] It was shown that in the low micromolar regime, a linear dependence exists between SABRE derived signal intensity and substrate concentration. Furthermore, by using a co-substrate whose principal role is to help prevent the formation of $[Ir(SIMes)(MeOH)(py)_2(H)_2]^+$ by possessing a higher affinity for the metal centre than the solvent, whilst still having a lower affinity than the analyte under investigation (in this instance pyridine), it was shown that it was feasible to detect pyridine at a limit of <1 μM. In this study, 1-methyl-1,2,3-triazole (mtz) was used as the co-substrate. Interestingly, the carbene SIMes was preferred over IMes as a ligand in the polarisation transfer catalyst, because the exchange rates for *para*hydrogen and pyridine in the complex $[Ir(SIMes)(H)_2(mtz)_2(py)]Cl$ were more commensurate than the analogous IMes based system. As such, the SIMes system yielded a ~3-fold increase in signal intensity over the IMes system.

The ability of SABRE to detect substrates from a complex mixture has also been reported upon, highlighting that low concentrations of analyte are detectable against a strong background. Nicotinamide was detected following polarisation transfer from a solution containing fifteen co-substrates whose concentrations were >700-fold larger.[36] Using a standard addition procedure coupled with SABRE, it was possible to calculate the original concentration of nicotinamide to within 0.2 μM of that calculated by conventional means (obtaining a fully relaxed ^{1}H NMR spectrum and then comparing the integral of nicotinamide to that of the methylene group of ethanol which had been added as an internal reference). Comparable results were also obtained for pyrazine, isoxazole and quinazoline in similar mixtures.

In Section Low-field detection of SABRE hyperpolarised substrates, there are further examples of the limits of detection achieved by SABRE which were obtained at low-magnetic field.

4. Towards a biological application of SABRE

In this section, the focus of the discussion relates to the advancements in SABRE towards its use in producing a polarised biologically relevant contrast agent for MRI. To achieve such an aim, the removal of the catalyst needs to be considered, as well as working in biologically compatible media to aid the rapid injection of a hyperpolarised bolus.

a) Heterogeneous SABRE catalysis

The desire to use SABRE to polarise biologically relevant molecules, and use them as polarised contrast agents for MR imaging, has resulted in interest in supporting the catalyst on a heterogeneous support. Goodson and co-workers took [Ir(IMes)(COD)][PF_6] and tethered it to a commercially available polymer microbead functionalized with 4-dimethylaminopyridine.[37] The structure of this solid supported complex is shown in Figure . The use of this complex to polarise pyridine in the presence of *para*hydrogen, resulted in the observation of emissive signals for all three of the pyridine ^{1}H resonances. These signals were enhanced between 3 – 5-fold following polarisation transfer at 100 G, with the *ortho* resonance being the most strongly enhanced. This contrasts with an approximate 70-fold emission signal being observed when [Ir(IMes)(COD)Cl] was used. It was noted that extracted supernatant liquid from the heterogeneous sample resulted in the observation of no SABRE based polarisation transfer. Thus, it was concluded that the catalyst remains firmly bound to the support during polarisation transfer, which is important for translating the SABRE approach towards the delivery of a hyperpolarised bolus.

Figure . Structure of the heterogeneously solid supported SABRE catalyst synthesised by Goodson and co-workers.[37]

SABRE catalysts have also been prepared on the nanoscale. Two nanoscale catalysts were prepared by immobilising [Ir(IMes)(COD)][PF_6]; one had a TiO_2/PMAA (poly(methacrylic acid) core/shell, whereas the other used polyvinylpyridine (PVP) to form a polymer comb.[38] The PVP comb polymer based catalyst gave a 7-fold enhancement of the *ortho*-proton of pyridine following polarisation transfer at ~100 G. The *meta* and *para* protons were similarly enhanced by 3- and 6-fold respectively. The enhancements obtained when the TiO_2/PMAA core/shell catalysts were

used were significantly better; the *ortho*-proton of pyridine was enhanced by 18-fold when this catalyst was employed. Significantly, this was improved by increasing the *para*hydrogen bubbling time from 30 s to 300 s which was evidenced by the enhancement rising to 26-fold. Both sets of experiments employed *para*hydrogen bubbling at near ambient pressure, and thus the enhancements would be significantly improved upon by increasing the pressure over the heterogeneous solution during polarisation transfer (see Section 2Pressure of hydrogen gas). Extraction of the supernatant from solutions containing both nanoscale catalysts gave no evidence for SABRE which was supported by atomic absorption spectroscopy (AAS) measurements being unable to detect the presence of iridium. The feasibility of reusing the catalyst, even though a lower enhancement was obtained due to possible lower catalyst concentrations and catalyst deactivation due to the recovery process, was also demonstrated. Furthermore, the improvement in signal enhancement is five times larger compared to the work previously published using microbeads[37] as the support.

b) **Use of biologically compatible media**

The vast majority of studies reported in the literature make use of methanol as the solvent in which to perform the polarisation transfer necessary for SABRE. As methanol is not biologically compatible, alternative solvents to conduct the polarisation transfer within have been sought, with a view to polarising biologically relevant substrates for MRI studies.

Polarisation transfer of the proton nuclei of 3-amino-1,2,4-triazine (ATZ) *via* SABRE has been accomplished in 90% water 10% methanol mixtures.[39] Initially, a methanolic sample containing the catalyst, [Ir(IMes)(COD)Cl], was treated with hydrogen for 2 hours. The subsequent removal of methanol under vacuum whilst heating the sample to 40 °C for 10 minutes, yielded the desired mixture. After filtering, the sample was ready for use in polarisation measurements. After polarisation transfer for 30 s at 65 G and at a temperature of 54.4 °C, an enhancement of 170 ± 45-fold was achieved. This corresponded to 1% polarisation. However, it was noted that this method could not be used for pyridine, isoniazid and pyrazinamide despite these compounds having comparable enhancement values to AZT when measured in methanol. Hövenor and co-workers were able to use a 9:1 H_2O-ethanol mixture to polarise pyridine, but only achieved a 0.02% polarisation level at 5.4 mT.[32]

The use of ethanol / water mixtures have been utilised to effect the polarisation transfer to nicotinamide, the amide of vitamin B_3.

Nicotinamide polarised by [Ir(IMes)(COD)Cl] in a ratio of 16 : 1 yielded a 70-fold enhancement for the proton that is α to both the ring nitrogen and the amide functional group.[30] This was shown to increase to 105-fold when a 50 : 50 D_2O : d_6-ethanol solution was employed. Both enhancements were noted to be lower than those obtained in methanol and this was attributed to a change in the reaction kinetics of ligand exchange. Nicotinamide can be polarised in neat water; it requires, however, a change in the way the solution containing the SABRE catalyst and nicotinamide is prepared. The change focuses around activating the SABRE catalyst and nicotinamide in neat ethanol and then removing the solvent *in vacuo*, prior to reconstituting in D_2O. Enhancements of ~ 30-fold were observed for most proton signals of nicotinamide at 9.4 T, following polarisation transfer at 6 ± 4 mT, when this method was employed.[40]

In this section, examples for supporting the polarisation transfer catalyst were highlighted as well as presenting reports that demonstrate that SABRE can be used in biological solvent mixtures. Combining both of these elements will be necessary to realise the potential of SABRE in delivering a hyperpolarised bolus.

5. Low-field detection of SABRE hyperpolarised substrates

Low-field NMR is an exciting prospect because it doesn't require cryogenic liquids and it can be made portable. The level of signal enhancement that SABRE offers makes this an even more attractive prospect. Initial experiments into this area, in which SABRE was employed, focused on the detection of hyperpolarised pyridine at 3.9 mT.[41] Compared to the ^{1}H NMR spectrum of non-hyperpolarised pyridine (which was thermally pre-polarised at 2 T), SABRE enabled the detection of pyridine by a further three orders of magnitude. In fact, it was possible to detect 4.9 nL of pyridine using the experimental set-up the authors describe.

This approach was further extended to evaluate the use of low-field NMR coupled with SABRE to detect the drug molecules harmine, morphine and nicotine at 3.9 mT.[42] These drug molecules were detected down to the milligram level, which in the case of nicotine is representative of the amount of substance that would be found in a single cigarette. These results were obtained using only 50% *para*hydrogen, and so a greater level of detection could be envisaged, for example down to the microgram level, if 100% *para*hydrogen was employed.

Larger fields have also been utilised to study SABRE at low magnetic field. Barsiky *et al.* used a field of 47.5 mT to detect hyperpolarised pyridine produced by SABRE.[43] In this study, it was not possible to discern between the signal originating from the pyridyl protons and those of

*ortho*hydrogen, but the hydride ligands were sufficiently resolved to be observed as an independent signal. The spectra were acquired during constant bubbling with *para*hydrogen, which caused significant line broadening due to susceptibility-induced magnetic field gradients. For heterogeneously catalysed reactions, where a constant supply of *para*hydrogen is required, this is especially problematic. However, the spectra obtained are not visually affected by this process and hence highlights an advantage of low-field NMR coupled with SABRE.

6. **Performing SABRE at high-magnetic field**

Typically, the polarisation transfer of SABRE is performed at low magnetic field to create strong coupling conditions to facilitate the transfer of polarisation. The sample is then shuttled to high-magnetic field, in the majority of cases, for measurement to occur. Performing SABRE in the magnet by the use of spin-locking or continuous-wave (CW) pulses, would therefore, be advantageous as it removes the need for the rapid transportation of the sample from low- to high-magnetic field to facilitate the acquisition of NMR data.

The transfer of spin order, during SABRE, occurs optimally in the level anti-crossings (LACs) regions.[44] LACs occur when two energy levels intercept one another but due to spin-spin coupling, the degeneracy is removed and the crossing is avoided. Thus, LACs enable the exchange of populations between Eigen-states due to the Eigen-states combining. For SABRE, the mixing of the Eigen-states results in the depletion of singlet order of the hydride ligands whilst yielding a net polarisation. Typically, LACs occur at magnetic fields below 20-30 mT. To access them at high-magnetic field, spin-locking can be utilised. The physical principles behind this methodology have been described and the technique has been used to successfully polarise, *via* SABRE, a range of N-heterocycles in a magnetic field of 4.7 T.[45] Crabtree's catalyst, as well as Ir(IMes)(COD)Cl, were employed as the polarisation transfer catalysts. Typically, *para*hydrogen was bubbled through solutions containing the catalyst and substrate for 6 s; this was achieved whilst the sample was located inside the bore of the spectrometer. After a small wait time, spin-locking was turned on for 0.1 – 7 s, the duration of which being sufficient to enable multiple complex formation and dissociation steps to occur. The resulting phase of the lines observed in the resulting ^{1}H NMR spectrum were dependent on the frequency of the spin lock applied. For d_4-pyridine (single *ortho* position non-deuterated), an enhancement of 360-fold was obtained which is substantially more than that obtained by transferring polarisation spontaneously at high magnetic field, which was reported by Barskiy *et al*.[46]

Low-power CW pulses have been used to polarise ^{15}N-labelled pyridine via SABRE at high-magnetic field.[47] The use of CW pulses can cause the transfer of large amounts of coherent polarisation, provided that the *para*hydrogen derived hydride ligands in the polarisation transfer complex are not magnetically equivalent but are chemically equivalent. To facilitate this transfer of polarisation to ^{15}N, the power of the CW pulse must match the sum or difference of the hydride-hydride and the ^{15}N-^{15}N *J*-coupling. Following CW-irradiation, a $\pi/2$ pulse is applied selectively to the bound substrate in order to convert x-magnetisation to z-magnetisation, which is retained upon dissociation. The application of the CW-pulse followed by a subsequent $\pi/2$ pulse is repeated as appropriate so that polarisation is built up in the analyte molecules. A broad-band $\pi/2$ pulse finally enables the magnetisation to be read-out and a FID acquired. This approach can be applied to other nuclei other than ^{15}N and so the technique has general applicability. The pioneers of this work coined the phrase Low Intensity Generation of High Tesla (LIGHT) SABRE to describe their approach. The implementation of this technique for polarising ^{15}N-labelled pyridine resulted in significant signal gains. For a catalyst to substrate ratio of 1 : 10, a signal enhancement for the ^{15}N peak of 150-fold was obtained when the ^{15}N-labelled pyridine concentration was 63 mM. Dropping the concentration down to 2.5 mM yielded an even higher enhancement of 480-fold.

Figure LIGHT-SABRE pulse sequence. The CW pulse is applied selectively to ^{15}N-pyridine which is ligated to the iridium centre for a period τ_{CW}. This is repeated n-times prior to the application of a broad band $\pi/2$ pulse and subsequent acquisition.

The polarisation transfer of polarisation to ^{15}N-labelled substrates, namely pyridine and nicotinamide, with a µ-magnetic shield has been investigated.[48] Principally, this is a very simple technique to perform; *para*hydrogen is bubbled through a sample containing the catalyst and the ^{15}N-labelled substrate which is hosted within a µ-magnetic shield. The sample is then rapidly transferred to the spectrometer where a pulse-acquire experiment was employed. This method has been dubbed SABRE-SHEATH (SABRE in SHield Enables Alignment Transfer to Heteronuclei). When this technique was applied to a solution comprising 4 mM ^{15}N-pyridine and 0.24 mM [Ir(IMes)(COD)Cl], a 30,000-fold polarisation enhancement over the thermal level was achieved. This corresponds to 10% polarisation. Similar enhancements were obtained for ^{15}N-nicotinamide; in the hyperpolarised ^{15}N NMR spectrum, an enhancement of ~20,000-fold (~7% polarisation) was observed for the free material. In

both instances, the signals for the bound materials was also evident in the corresponding ^{15}N NMR spectra.

7. Continuous production of hyperpolarised molecules produced by SABRE

An advantage that SABRE has over DNP, is that the level of polarisation can be surveyed prior to using the hyperpolarised material for its desired purpose. This is because the re-hyperpolarisation of a sample by SABRE takes a few seconds, whereas the same process with respect to DNP takes a lot longer. Hövener *et al.* demonstrated that a hyperpolarised pyridine solution maintained the same level of polarisation at 5 mT over a time course of 700 s.[49] Furthermore, it was shown that if the polarisation was read-out using a series of $\pi/2$ pulses, the level of polarisation prior to read-out could be obtained again within a few seconds. The lifetime of the hyperpolarised state, therefore, is no longer of concern because the rate of depletion is matched by the rate of hyperpolarisation transfer. The equilibrium polarisation obtained by using continuous polarisation *via* SABRE was equivalent to that of a few hundred Tesla and thus no superconducting magnet is required. Low-field MRI was the modality used to exemplify the application of this method; a two-dimensional image was obtained in 4.16 minutes for hyperpolarised pyridine *via* continuous hyperpolarisation whereas 2.16 hours were required to obtain a water image that was 700 times more concentrated than the pyridine sample. The work by Chekmenev and co-workers,[43] which was highlighted in Section Low-field detection of SABRE hyperpolarised substrates, is another significant example of data being acquired when the sample is being continuously polarised. In this case, the spectrum is sufficiently resolved to enable the distinction between polarised pyridine and the hydride ligands of the polarisation complex at 47.5 mT, despite a difference in frequencies of only 64 Hz. A theoretical assessment of continuous hyperpolarisation has been detailed, and when compared to experimental data, replicates the major features very well.[50] Importantly, a distinct distribution of polarisation occurred, in that the loss of singlet order from *para*hydrogen did not correlate directly to the generation of longitudinal polarisation; non-observable spin order was also transferred, which has been highlighted previously.[25]

8. Imaging Studies

The range of molecules which SABRE can polarise has resulted in a number of groups working towards its use in MRI with respect to producing hyperpolarised contrast agents. This was first exemplified by examining pyridine, which was proposed as a possible target due to its

incorporation into drug molecules. ^{1}H True-FISP (fast imaging with steady-state precession) MR images were collected over a time period of 0.7 s of an 8 mm tube containing ten cylinders which were of 1 mm internal diameter.[16] The image produced when hyperpolarised pyridine was used (polarised by $[Ir(H)_2(PCy_3)(pyridine)_3][BF_4]$), enabled the cylinders to be clearly identified. The contrast increase is even more evident when compared to the same image collected under thermal polarisation; despite increasing the slice thickness sampled to 20 mm from 0.5 mm, no image was visible. The collection of low-field images of pyridine[49] was discussed in Section Continuous production of hyperpolarised molecules produced by SABRE, in which continuous hyperpolarisation was used. Chekmenev and co-workers extended upon this work by acquiring 2D MR images of pyridine at a single magnetic field for both polarisation transfer and detection (*in situ* detection).[43] This negated the need for field-cycling and also reduced the repetition time (TR) of measurements by a factor of 40, so that TR was 0.2 s. Furthermore, a spatial resolution of 0.75 x 0.75 mm^2 was achieved. *Ex situ* MR images were also acquired, whereby polarisation transfer occurs at 5.75 mT whilst detection is at 47.5 mT, and gave improved in-plane spatial resolution (0.125 x 0.125 mm^2) and ≤7.7 s temporal resolution. Both methods enabled the collection of MR images of a 10 mm NMR tube which contained 100 mM of pyridine and 7 mM of [Ir(IMes)(COD)Cl].

As well as the collection of ^{1}H MR images, ^{13}C images have also been obtained. ^{13}C-labeled nicotinamide was polarised under SABRE conditions and ^{13}C-RARE images were acquired at 7 T.[32] The image obtained was enhanced by a factor of 65-fold; although this is a low enhancement, an image acquired under thermal equilibrium conditions took 18 hours and 20 minutes (1100 averages) to obtain. This contrasts with just a single second for the hyperpolarised image. Given the natural low abundance of ^{13}C in the body, the low enhancement is not that detrimental to investigating further ^{13}C-labeled materials.

A further heteronucleus, ^{31}P, has been explored using SABRE to obtain MR images. Transverse cross-sections of a 15 mm sample tube filled with $[Ir(PPh_3)_3(H)_2Cl]$ (6 mM) in toluene gave a strong ^{31}P image.[24] Conversely, from the corresponding thermal image acquired after 128 accumulations (1.5 hours), it was not possible to discern the outline of the sample tube. The hyperpolarised image was produced following the bubbling of *para*hydrogen through the solution at 80°C for 3 seconds, whilst the sample was retained within a μ-metal shield.

In this section, it has been shown that SABRE is not restricted to ^{1}H MR imaging, in that both ^{13}C and ^{31}P are also nuclei which show promise. At

present, only phantoms have been imaged, but when this is viewed alongside the advances detailed in Section Towards a biological application of SABRE, an *in vivo* image will not be a too distant reality. Furthermore, the applicability of SABRE in low- and high-magnetic field imaging applications is suggestive that a great deal more can be accomplished within both of these areas.

9. **Conclusions**

In this mini-review, the literature reports relating to the hyperpolarisation technique SABRE were discussed, and their impact on NMR and MRI highlighted. The chemical composition of the SABRE catalyst was focused upon, with specific attention on how the structure related to the exchange rates of both the hydride and analyte ligands. In addition, the importance of the catalyst possessing an N-heterocyclic carbene ligand instead of a phosphine ligand was emphasised. Furthermore, the chemical structure of the catalyst was linked to polarisation transfer efficiency and hence the signal enhancement observed. The physical constraints, which dictate the polarisation transfer efficiency, were also highlighted. These constraints were placed into a context whereby they were treated in turn to demonstrate how they could be tuned to maximise the transfer efficiency. Examples of analyte molecules in which hyperpolarisation transfer to ^{1}H, ^{13}C, ^{19}F and ^{31}P were discussed, to illustrate the wide utility of this approach.

The use of SABRE, at both high and low magnetic field, was discussed with reference to the detection of, or imaging of, hyperpolarised analytes *via* the acquiring of ^{1}H, ^{13}C, ^{19}F and ^{31}P spectra or images. In these sections, the ease of detecting low concentrations of analytes in the absence of magnetic fields was scrutinised, and the possibility of portable SABRE evaluated. The notion of performing SABRE completely at high magnetic field is an important step forward in these areas, as it negates the need for the rapid transfer of the hyperpolarised sample from low- to high-magnetic field. Two approaches were highlighted; one which used field-cycling and the other which utilised low power CW pulses.

The latter sections of the review dealt with literature reports in which the emphasis was on translating the SABRE technique towards biomedical imaging. Performing the polarisation transfer in biocompatible solvent mixtures was highlighted as a major thrust in this area with the emphasis being placed on working towards biologically relevant solvent mixtures. Furthermore, advances in catalyst design whereby the catalyst is tethered to a heterogeneous support, to ease its removal prior to *in vivo* delivery of the hyperpolarised bolus, represents an exciting step forward in this area.

Abbreviations

AAS	Atomic Absorption Spectroscopy
ATZ	3-amino-1,2,4-triazine
COD	Cyclooctadiene
COPD	Chronic Obstructive Pulmonary Disorder
CW	Continuous wave
DNP	Dynamic Nuclear Polarisation
DPPM	1,4-Bis(diphenylphosphino)butane
FID	Free Induction Decay
IMes	1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene
LIGHT-SABRE	Low Intensity Generation of High Tesla SABRE
mtz	1-methyl-1,2,3-triazole
NHC	*N*-heterocyclic carbene
PHIP	*Para*Hydrogen Induced Polarisation
PMAA	poly(methacrylic acid
PTF	Polarisation Transfer Field
PVP	polyvinylpyridine
RARE	Rapid Acquisition with Refocusing Echoes
SABRE	Signal Amplification By Reversible Exchange
SABRE-SHEATH	SABRE in SHield Enables Alignment Transfer to Heteronuclei
SEOP	Spin Exchange Optical Pumping
TEP	Tolman Electronic Parameter
TR	Repetition Time

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NHC	*Ortho* ^{1}H T_1 in the presence of [Ir(NHC)(COD)Cl] /s	*Ortho* ^{1}H T_1 in the presence of [Ir(NHC)(py)$_3$(H)$_2$]$^+$ and 3 bar H_2 /s†
IMes	28.8	12.6 (−56%)
IPr	30.5	9.2 (−70%)
SiPr	31.1	14.4 (−54%)
SIMes	32.5	15.9 (−51%)
ICy	16.4	17.2 (+4%)
ImMe$_2$*N*Pr$^i_2$	18.7	20.1 (+7%)
IMe	22.3	22.9 (+3%)

Table 1. T_1 values at 298 K for the ortho ^{1}H resonance of pyridine in the presence of [Ir(NHC)(COD)Cl] and [Ir(NHC)(py)$_3$(H)$_2$]$^+$ and 3 bar H_2. †Percentage denotes the increase or decrease in T_1