EPR dosimetry in human fingernails: Investigation of the origin of the endogenous signal and implications for estimating dose from nail signals

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EPR dosimetry in human fingernail. Origin of the endogenous signal and implications for estimating dose from nail signals

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Human fingernails have been studied for many years for potential use for dosimetry, based on the EPR signals induced by ionizing radiation, but a fully validated protocol to measure doses retrospectively has not yet been developed. The major problem is that the EPR spectrum of irradiated fingernails is complex and its radiation-induced signals (*RIS*) overlap with an endogenous signal called the background signal (*BKS*). *RIS* and *BKS* have similar spectral parameters. Therefore, detailed characterization of the *BKS* is required to develop a method for measuring the amount of RIS by removing the signal due to *BKS* from the total spectrum of irradiated fingernails. Effects of reducing and oxidizing treatments of fingernail samples on the *BKS* were studied. Numerical simulations of the observed *BKSs* were performed. Common features of the EPR spectra in fingernails are discussed. We also found that *BKS* can be generated in the fingernail clippings by oxidation in ambient air with dioxygen. Results support the hypothesis that *BKS* is an o-semiquinone radical anion. Comparison of the chemical and spectral properties of the BKS and with the RIS 5 (the stable signal suitable for dose assessment) suggest that both sets of radicals underlying these signals are o-semiquinone radicals. Given the common chemical properties of the *BKS* and *RIS 5* it is unlikely that chemical treatment methods will provide a means to differentiate these two signals in irradiated nail spectra. Instead, other methods (i.e. dose additive methods, population-derived *BKS* means) may be necessary to selectively estimate the content of *BKS* and *RIS 5* in irradiated nail spectra.

Keywords: radiation dosimetry, fingernails, electron paramagnetic resonance spectrometry, semiquinone radicals

1. Introduction

Significant progress in retrospective dosimetry has been achieved during last two decades[1,2]. Electron Paramagnetic Resonance (EPR) measurements of isolated tooth enamel and bone fragments collected from radiation accident casualties is one of the most used dosimetry techniques. It has been successfully proven its capability to produce accurate dose estimations [3–15]. However, this dosimetric method has a significant limitation due to the invasive nature of the collection of tooth enamel and bone samples for dose measurements. However, advances in EPR instrumentation, resonator technology, and ergonomic designs have led to the development of non-invasive in vivo approaches to making measurements of radiation induced tooth signals [16–19]. With these advances, and the portability of the instrumentation, in vivo tooth dosimetry can now be applied to retrospective dosimetry in accidental and, more importantly, mass radiation exposure events. Beside in vivo EPR developments, efforts have been made to minimize the invasiveness of sampling of calcified tissue, especially using Q-band EPR spectrometer. Because Q-band has a higher sensitivity than X-band it only requires small sample masses of only a few mg to perform an EPR analysis [20–23]. It has been successfully applied in the last decade in support of the medical management of actual overexposed individuals with tooth enamel, bones and also fingernails [7,11,24] (. It is worth noting that for localized irradiation to hands, for example, it is difficult to estimate dose and dose distribution on hands with conventional approaches to estimating accident dosimetry, even with the use of numerical modeling methods. First clinical signs are not always a good indicator of the severity of the exposure [7] and bone EPR is only possible after surgical intervention (biopsy or after amputation) [7,13]. In the absence of other means to estimate dose distribution on irradiated hands/feet, finger/toenails are up to now the best and sole tools to estimating the severity of the exposure in support of medical decision making. It offers the unique advantage of estimating doses on tips of each finger or toe. Even if this is not sufficient to estimate dose distribution on the whole hands or feet, it has provided improvements in dose reconstruction for accident dosimetry.

An attractive feature of nails in regard to dosimetry is they are easy to collect and can be used for a dose mapping when measuring nail samples from each finger and foot separately, providing highly needed and essentially unique dose information. Obtaining nail clippings could readily be done in the field and the samples could be processed on site or at a distant lab. It is also envisioned that measurements of the nails can be conducted *in vivo*, including in the field.

Dosimetry based on radiation-induced signals in nails would be especially useful. Nails have been extensively studied [21,25–34], especially in the light of a potential act of radiological terrorism [35]. Recently, promising results were obtained when estimating high doses received by several workers who handled ^{192}Ir radioactive sources used in radiography cameras in Tunisia, Gabon, and Peru [7,11,24]. The proposed approach is limited to high dose exposures (> 10 Gy). A practical protocol for low doses (lower than 10 Gy) is highly desirable but is not available yet. The key to this may be the ability to differentiate between the principal radiation induced signal (RIS) and the background signal.

EPR spectra of irradiated fingernails samples contain a number of separate EPR spectra from differing free radicals in the nail. The signals are [34] :

- Four mechanically-induced signals (*MIS 1* to *MIS 4*) have been identified [34]. These are generated by mechanical stress while sample collection[31,32,34,36–38] (. These signals do not present a significant problem for dosimetry purposes because most of the *MISs* can be eliminated by soaking samples in water.

- Five radiation-induced signals (*RIS 1* to *RIS 5*) have been identified. The *RIS 2* and *RIS 5* are generated at relatively low doses (< 1 Gy) whereas the other *RIS* components are only measurable at doses higher than several hundreds of Gy [34]. Similar to the mechanically induced signals (*MIS*), most of the *RISs* can be eliminated by soaking the nail clippings in water. Some authors, however, have reported a residual *RIS* that can be observed even after long-time water soaking [34,39,40] (. This RIS component, labelled as *RIS 5*, shows a non-linear dose response with a saturation above 35-65 Gy. As it was discussed in Trompier et al. (2014b) in situations of real accidents, only the *RIS 5* can be used for dose assessment [34]. This is because all other components are reduced or completely eliminated by the natural moisture content of fingernails irradiated in vivo or through water contact (sweat, hand washing, etc.) between the time of the accidental exposure and EPR analysis of the nail. In fact, the RIS 5 nail signal was used in deriving estimated dose in several accidental exposures that will be discussed later [7,11,24] .

- The endogenous signal known as a background signal (*BKS*). This *BKS* is resistant to various chemical treatments and has spectral characteristics that are very similar to the unstable RIS component (*RIS 2)* and the stable RIS component (*RIS 5)*. It is impossible to resolve the *BKS* from the *RIS 5*, even at W-band EPR, which has provided the highest EPR spectral resolution achieved in the study of nail signals to date [41]. Therefore, only a superposition of the *RIS* 5 and *BKS* can be measured in reality. Hence, one of the major problems of EPR dosimetry in nails lies in how to extract the *RIS* from the experimentally measured signal. Several methods have been proposed to overcome this problem [11,24,34,41–44]. However, for doses <10 Gy, all proposed methods to date have limitations due to the fact that in this dose range *BKS* is typically more intense than the stable *RIS 5* and that *BKS* is known to be variable. The inter- and intra-person variability of the *BKS* has not been studied in detail. However, it is known that the *BKS* intensity depends on factors such as humidity during sample harvesting, storage, origin, age, and gender of the donors [34,45–48]. Therefore, in order to develop a low dose protocol, it is very important to identify the radical(s) associated with the *BKS* and identify and characterize the different factors that can influence the *BKS* intensity.

Most researches on EPR fingernail dosimetry were performed at X-band because such spectrometers are broadly available. Higher frequency Q-band spectrometers provide higher resolution of the spectrum and require less sample material for the measurements and therefore may be very valuable for the identification of the origin of the BKS, as well as for dose estimation. This paper is the first part of a systemic investigation of the different components of the EPR spectrum of irradiated fingernails with the aim here to identify the origin of the *BKS* and factors affecting its intensity using X- and Q-band EPR spectroscopy methods. Understanding the nature of the BKS is a key first step towards the development of new protocol in nails dosimetry, especially at dose below 10 Gy. In early works on nails dosimetry, BKS was thought to be a stable residual signal induced by mechanical stress [31,32] (. This hypothesis has driven development of sample preparation protocols trying to minimize as much as possible the MIS and also the development of in vivo approach, because in vivo measurement was thought to be at that time free of the BKS. In Trompier et al (2014b), it could be demonstrated that BKS was not induced by the mechanical stress, but no alternative hypothesis on its nature was provided [34]. In this paper, we will provide evidence that BKS is likely to be a semiquinone anion radical that can be produced by oxidation from reaction with oxygen in air and reduced by interaction with water, explaining the odd reported effects of drying and humidifying process on BKS intensity as

reported in Reyes et al. (2008) or Trompier et al. (2014b) [34,47]. Subsequent papers will be devoted to the origin of other spectral components with the ultimate goal to develop a comprehensive protocol of the radiation dose measurements in fingernails.

2. Materials and methods

Fingernail samples that had no apparent abnormalities were collected from thirty Caucasian male and female employees of IRSN. After harvesting, each sample was cut into 1 mm wide and 2 mm long pieces to ensure that they fit into both X- and Q-band sample tubes.

The nail samples were carefully washed and soaked in distilled water before measurements to eliminate the possibility of surface contaminates from contributing to the measured EPR nail signal. This precludes a possible origination of an additional signal from an impurity(ies) on the fingernails' surface. In addition, it was established that the fingernail donors did not use any special cosmetic products like hardeners and nail polish, which can be responsible for a generation of some additional EPR signals [49] .

The Q-band (34 GHz) measurements were carried out using a Bruker EMX+ spectrometer equipped with an ER5106QT/W resonator. The X-band (9 GHz) measurements were performed using a Bruker EMX equipped with a high-Q cavity (SHQ). The parameters used for spectra acquisitions are provided in Tables 1 and 2. The mass of fingernails used for EPR spectra acquisition was 1 to 3 mg for Q band and 10 to 30 mg for X band.

In order to measure only *BKS*, the mechanical induced radicals were eliminated by soaking fingernails in distilled water (18.2 $M\Omega.cm^{-1}$) for 20 minutes. After soaking, all samples were dried for 16 hours overnight in a vacuum dryer with silica gel, as described by Trompier et al. (2007b) [32]. Oxidation-reduction and acid-base sample treatments were used to assist in the identification of the radical(s) responsible for the *BKS* by soaking the samples for 20 minutes with one of the following chemical treatments:

- HCl (pH 1) aqueous solution;
- KOH (pH 13) aqueous solution;

- $K_3Fe[CN]_6$ (0.1 M) in base media of 0.1 N of potassium hydroxide (KOH) as suggested by Tipikin et al. (2016) [41];
- Dithiothreitol (DTT) (0.1 M), which was known to reduce disulfide bonds as suggested by Romanyukha et al. (2007b).

The use of 0.1 M DTT in that paper reduced the offset of the combined effect of the *MIS* and *BKS* from 10 Gy to approximately 0.3 Gy RIS equivalent signal intensity [31] .

Simulations of the EPR spectra of semiquinone radical were made using the Matlab Toolbox EasySpin [50] .

Previously, it has been shown that *BKS* intensity measured after water soaking increases gradually when it is left in ambient air, eventually reaching a maximum signal intensity [47] . In order to study the impact of sample storage on BKS signal intensity, one fingernail sample was split into two parts after water soaking and dried. One part was left for 72 hours under ambient air in darkness. The other part was kept in the dark but in a vacuum dryer with silica gel for the same time duration.

3. Results

3.1 Spectral features of *BKS*

The g-factors were determined utilizing the spectra of Mn^{2+} at Q-Band with the 3rd and 4th EPR lines of Mn^{2+} sextet located on both wings of the major EPR nail signals (endogenous, mechanically and/or radiation-induced signal). It was found that the amount of Mn^{2+} varies significantly among donors as was also shown by Ayodele and Bayero, (2010) in their study of Kano inhabitants [51].

Fig. 1 shows typical spectra of the *BKS* in fingernails samples recorded at X and Q bands. Three components at $g_1=2.0060(2)$, $g_2=2.0054(2)$ and $g_3=2.0024(2)$ were identified in Q-band; whereas only one singlet at $g=2.0044(2)$ was found in X-band, illustrating the greater spectral resolution obtained with Q-Band in the nail samples.

3.2 Variability of the *BKS* spectrum

We observed a certain variability in the appearance of *BKS* signal for different donors. Fig. 2 shows *BKS* signals observed on fingernails collected from three different donors. The typical shape of the endogenous *BKS* signal has 3 apparent g-factors: $g_1=2.0060(2)$, $g_2=2.0054(2)$ and $g_3=2.0024(2)$. For a small number of donors (~10% of the study

cohort), a very intense singlet at *g=2.0024(2)* was also observed. This signal has not been previously described in the literature. Similar to the typical endogenous signal *BKS*, the additional singlet signal at *g=2.0024* seems to be intrinsic; i.e. it did not appear to be related to external factors such as surface contaminates or nail pathology. The appearance of this signal did not vary with sample orientation relative to the magnetic field. Microwave power saturation studies show the intensity of the *BKS* endogenous signal saturated at about 1 mW and then decreased with additional microwave power, whilst the singlet did not show any power saturation up to 10 mW (Fig. 3). These differing power saturation behaviors can be used as part of the spectral decomposition procedure to preferentially detect and extract the singlet from the nail signal when determining the *RIS 5* and *BKS* in the signal. Given the ease of removing the singlet and its rare occurrence (only in 10% of the studied donors), we focused our study on the endogenous *BKS* signal.

3.3 Possible origin of the endogenous *BKS* signal in nails

The radical species responsible for the endogenous *BKS* signal has not yet been definitively identified. According to Strzelczak et al. (2013), a semiquinone radical appears to be one of the possible candidates [52]. They assumed that this radical originates from the ionization of melanin by UV or gamma-ray radiation. In biological tissues, melanin is produced by melanocytes. However, with the exception of a rare case of the nail pathology called melanonychia, melanocytes are quiescent in healthy nails and thus no melanin is expected in nails [53–55] .

A more plausible origin of the semiquinone radical in nails is oxidation of L-3,4-dihydroxyphenylalanine (DOPA) by γ, X- or UV irradiation. DOPA itself is derived from the partial oxidation of the amino acid tyrosine, which is naturally present in human nails [41] . It is important to emphasize that the semiquinone radical is most stable in its anionic form [56–58]. In order to test this hypothesis of the *BKS* origin, the spectrum of a o-semiquinone anion radical was simulated in both X and Q bands. As seen from Fig. 4, the simulated spectra are very similar to the experimental ones. A root mean square deviation (*RMSD*) between the simulated spectrum and the experimental one is 2.7% and 4.2% at X and Q band, respectively. This good agreement is consistent with the hypothesis of a o-semiquinone radical anion as the basis of the *BKS*, but because other radicals could have similar g-factors, the spectroscopic analyses are not completely definitive.

3.4 Effects of acid-base treatments on *BKS*

In order to test the hypothesis of an anionic o-semiquinone radical as the origin of the *BKS*, we studied the effects that fingernail treatments in a solution of hydrochloric acid pH=1 or sodium hydroxide at pH=13 have on the BKS signal.

On one hand, if the radical responsible for the endogenous signal *BKS* is in anionic form, then treatment of the nail sample in acidic medium should eliminate, or at least partially reduce, the EPR intensity. On the other hand, a basic solution treatment is expected not have any influence on the spectral intensity of the endogenous *BKS* signal. The results of this experiments are shown in Fig. 5.

The intensity of the endogenous signal *BKS* remains unchanged after treatment with the sodium hydroxide solution (pH=13), while its intensity is decreased by a factor of 3.7 after treatment with the hydrochloric acid solution (pH=1). This result is similar to results reported in the literature for the *RIS 2* that is also hypothesized to have originated from a semiquinone radical [41]. This result is in line with the greater stability of the anionic o-semiquinone radical compared to a cationic or a neutral semiquinone radical. Although the *BKS* is not fully reduced in acid conditions, these results remain compatible with the expected acid-base behavior. The radical entity responsible for the endogenous signal seems to be in anionic form, which is also consistent with the semiquinone radical literature [56–58]. The incomplete elimination of the *BKS* in acid conditions may be due to limited accessibility of some radicals in the keratin fibers. It is likely that the chemical solutions were unable to fully penetrate into the nail tissue during treatment, especially in the intermediate plate of the nails or deeply trapped in the nail matrix at specific sites within the dense keratin fibers, explaining the only partial removal of endogenous signal.

3.5 Oxidation-reduction properties of *BKS*

In a further test of the origin of the endogenous signal *BKS*, nail samples were treated either with an oxidant using 0.1M potassium ferricyanide $K_3Fe[CN]_6$ or a reducing agent using 0.1 M dithiothreitol (DTT), under basic conditions in KOH 0.1 N (pH = 13). Indeed, if the diamagnetic molecule at the origin of the anionic o-semiquinone radical is a dihydroquinone type molecule, then it should be possible to generate radicals by oxidative treatment [41]. Potassium ferricyanide is known to oxidize organic systems and it is expected to generate anionic o-semiquinone radicals from DOPA molecules. DTT is a reducing agent and it should therefore reduce anionic o-semiquinone radicals to DOPA. The results are shown in Fig. 6.

After oxidizing the nail sample, it was found that the amplitude of the *BKS* increased by a factor of 2.2, which suggests that the additional radicals were formed as a consequence of the oxidation of dihydroquinone type molecules in the nail sample. Conversely, the reverse behavior was observed after treating nails with the reducing agent. The *BKS* was found to decrease by a factor of 3.2 but was not entirely removed. Similar results were seen by Gonzales et al. (2020) in non-irradiated fingernails treated with 0.1 M DTT for 30 min [59]. These results suggest that those o-semiquinone

radicals accessible to the reducing treatment were likely reduced to the diamagnetic dihydroquinone. These results support the hypothesis that the endogenous *BKS* signal is linked to an anionic semiquinone radical resulting from the oxidation of DOPA molecules.

3.6 Impact of storage

It is known that storage of water-treated fingernails in air causes a significant increase in the intensity of the endogenous *BKS* signal until it saturates [34,47]. One possible explanation for this behavior may be a slow oxidation of nail proteins due to the contact of the nails with oxygen of the ambient atmosphere, giving rise to protein radicals detectable by EPR. To test this hypothesis, we prepared two identical samples from fingernails collected from the same donor by soaking in distilled water for 20 minutes. This allows preparing samples with the lowest possible intensity of the endogenous *BKS* signal [31,34]. One sample was dried in the dark at ambient air for 72 hours, and the other sample was placed into a vacuum desiccator in the dark with silica gel for 72 hours. Both samples were dried in the dark in order to avoid a potential effect of exposure to light. The advantage of vacuum drying is to dehydrate the nail samples without the presence of oxygen in order to investigate a potential effect of oxygen on the formation of *BKS*. From the results presented in Fig.-7, we find that the amplitude of the endogenous signal *BKS* was significantly greater by a factor ≈ 3 after drying in ambient air than after drying under vacuum. Over a period of 72 hours, the masses of the samples were equivalent (relative difference of 0.5%), indicating that the dehydration state of the two samples was approximately the same. Therefore, it is likely that some component of the nail proteins is undergoing oxidation in the presence of air. One possible mechanism for the *BKS* growth in open air is an oxidation of DOPA in the nail proteins to semiquinones radicals [60]. Indeed, the more the DOPA molecules are oxidized in open air, the more anionic o-semiquinone radicals are generated and the more intense the *BKS* becomes, which is in line with our observation.

4. Discussion

A comprehensive study of the endogenous signal is an important to nail based EPR dosimetry because the *BKS* is superimposed with the radiation-induced signals, *RIS 2* and *RIS 5*. With the characterization of the *BKS* it is hoped that strategies can be devised to discriminate the *BKS* from the *RIS 5* in irradiated nail samples. The results presented herein support the hypothesis that the endogenous *BKS* signal originates from stabilized anionic semiquinone radicals located in the bulk of the nail. Partial elimination of the anionic semiquinone radicals by soaking in distilled water or in a reducing solution can be explained by their neutralization to the original DOPA molecules. The semiquinone

radicals are generally unstable because they recombine rapidly to become quinones in air [56–58]5). That makes it difficult to explain the existence of a stable *BKS*. However, two explanations can be proposed:

(i) It is known that nails also contain a certain amount of positively charged metal ions such as Fe^{3+}, Cu^{2+}, Al^{3+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Na^{+}, K^{+} and ammonium ions $R\text{-}NH_3^{+}$ [61–63]. One possible explanation for the stability of some anionic o-semiquinone radicals in nails is the charge compensation of neighboring cations by coulombic interactions. Stability of the pairs of negatively and positively charged ions has been described in the literature [64,65]. These radicals can be very stable in the volume of the nail and therefore, observable by EPR spectroscopy over a long period. Determination of the concentrations and the nature of these metallic impurities is fundamental and therefore constitutes a perspective to be considered in support of future work related to the *BKS*.

(ii) Another possible explanation is based on the existence of some regions in the nails where there is insufficient water penetration because of the presence some hydrophobic molecules (lipids for example) or steric hindrances by large amino-acids side groups such as phenylalanine, proline and methionine [66]. Therefore, the limited accessibility of some radicals to water treatments would make it difficult to completely eliminate anionic o-semiquinone radicals from the nail samples.

The proposed chemical nature of the *BKS* as a semiquinone anionic radical presents a challenge in regard to devising a method to separate the *BKS* signal from the *RIS 5* signal in irradiated nails. From the work of Tipikin et al (2016) it has been proposed that the *RIS 5* may also be a semiquinone radical [41]. If so, then the *BKS* and *RIS 5* may be chemically identical [41]. This would imply then that chemical approaches to remove one or the other *BKS* or *RIS 5* signals from the nail spectrum may not be possible. The chemical processes responsible for the formation of the semiquinone radicals underlying the *BKS* or *RIS 5*, although mechanistically different, likely occur randomly within the nail proteins. The radicals associated with the *BKS* and *RIS 5* may not lend themselves to differences in distribution or localization of the two groups of radicals within the nail, although this remains to be investigated further. It has been suggested that *BKS* could be reduced through the use of an antioxidant during hand washing prior to starting work within an environment where exposure to radiation may occur as a part of radiological emergency preparedness in occupations such as first responders or medical workers [59]. However, this approach may not be amenable in situations where the radiation exposure has already occurred.

There are methods that can be used to estimate the content of the *BKS* or *RIS 5* in irradiated nail samples. For example, in ex vivo EPR nail dosimetry, dose additive methods could be used to estimate *RIS 5* in irradiated nail samples. This approach has been demonstrated in single incident accidental radiation exposures [11,24]. However, this approach is not suitable for in vivo nail EPR dosimetry. Also, this approach is less amenable for use with ex vivo nails in the field when addressing high throughput dosimetry needs in mass radiological exposure scenarios but could function well for follow-on off-site radiation dose distribution and verification needs. For in vivo EPR nail dosimetry applications, dose additive methods are not applicable. Instead, the means of the *BKS* signal amplitudes could be obtained from population studies of the BKS signal in nail plates measured in vivo. These population derived *BKS* means can then be used to subtract the BKS signal from the irradiated nail signals and thereby provide a means to estimate the *RIS 5* in the nail plate. The variability in this approach to estimating *RIS 5* in in vivo EPR nail measurements is dependent on the intra-individual variation in the *BKS* signal amplitudes. The extent of the intra-individual variability in the in vivo EPR measurements of the *BKS* has yet to be assessed but is expected to move forward now that instrumental methods for X-band in vivo EPR are becoming available [67–69].

5. Conclusion

This study aims at investigating the possible nature of the endogenous signal *BKS* in fingernails. The better sensitivity and spectral resolution of EPR at Q band revealed an anisotropic endogenous signal characterized by $g_1=2.0060(2)$, $g_2=2.0054(2)$ and $g_3=2.0024(2)$, giving only a unique line at $g=2.0044(2)$ at X band. The behavior of the *BKS* with both reducing and oxidizing agents can be explained by a semiquinone anion radical responsible for the *BKS*. Acid and base treatments also confirmed that the radical specie is in an anionic form. Results presented here show that *BKS* is generated through oxidation with ambient dioxygen. Finally, numerical simulations of o-semiquinone anion radical reproduce accurately the experiment spectra both in X and Q bands and support the proposed hypothesis. These results imply that the radicals underlying the *BKS* and *RIS 5* are virtually identical and that it is not possible to use chemical methods to eliminate selectively one of the two signals in irradiated nail spectra. Instead, other methods are needed (i.e. dose additive, population averaging of *BKS*) to estimate the *BKS* or *RIS 5* signal in irradiated nails.

Note

The views expressed in this paper are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense, nor the U. S. Government.

Author Contributions

The first draft of the manuscript was written by Nicolas Tkatchenko, who also performed the experiments under the supervision of F. Trompier and D. Gourier. All authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure Legends

Fig. 1. EPR spectra at X and Q band of a healthy nail previously treated in distilled water.

Fig. 2. EPR spectra at Q band of three healthy donors. The principal component of the *BKS* is defined by g_1=*2.0060(2)*, g_2=*2.0054(2)* and g_3=*2.0024(2)*. For a small number of donors (10% of the cohort), a very intense isotropic signal was observed *2.0024(2)* (light grey spectrum). Finally, the concentration of Mn^{2+} radicals also varies from one individual to another.

Fig. 3. Variation of the peak-to-peak amplitudes of the *BKS* (circles) and the particular singlet (squares) normalized as a function of the square root of the microwave power. It can be seen that the *BKS* saturates at approximately 1 mW, unlike the isotropic signal which exhibits no saturation behavior.

Fig. 4. EPR spectra at X-band (top) and Q-band (bottom) of the endogenous signal BKS of a healthy nail and the spectral simulation by an anionic o-semiquinone radical. The simulated spectrum at X-band used a g-value of 2.004; the simulated spectrum at Q-band used g-values of gx = 2.0059, gy = 2.0054, and gz = 2.0022 . Both simulations used a gaussian line function.

Fig. 5. Q band EPR spectra of the endogenous BKS signal of a fingernail without chemical treatment (black), after treatment in a KOH solution at pH = 13 (dark grey) and after treatment in HCl solution at pH = 1 (light grey).

Fig. 6. Q-band EPR spectra of the endogenous signal of a nail without chemical treatment (black), after treatment in an oxidizing solution at pH = 13 of 0.1M $K_3Fe[CN]_6$ (dark grey) and after treatment in a reducing solution of 0.1M of DTT (light grey).

Fig. 7. Q-band EPR spectra of the endogenous signal of a healthy nail from the same finger of the same donor. The two samples were soaked in distilled water for 1 hour and then dried for 72 hours in the dark in ambient air (grey) and in a vacuum desiccator (black), respectively.

Figure

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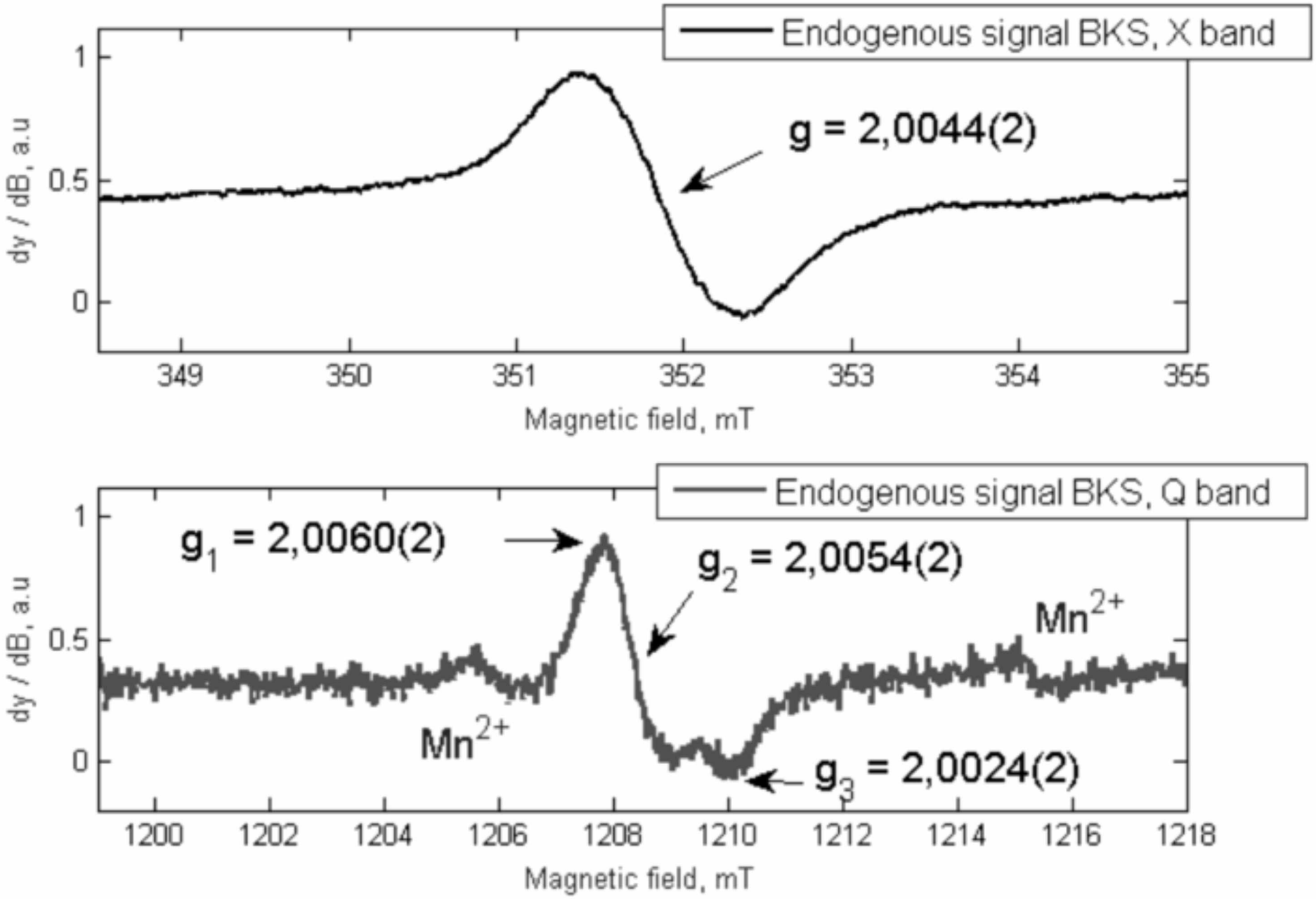

Figure

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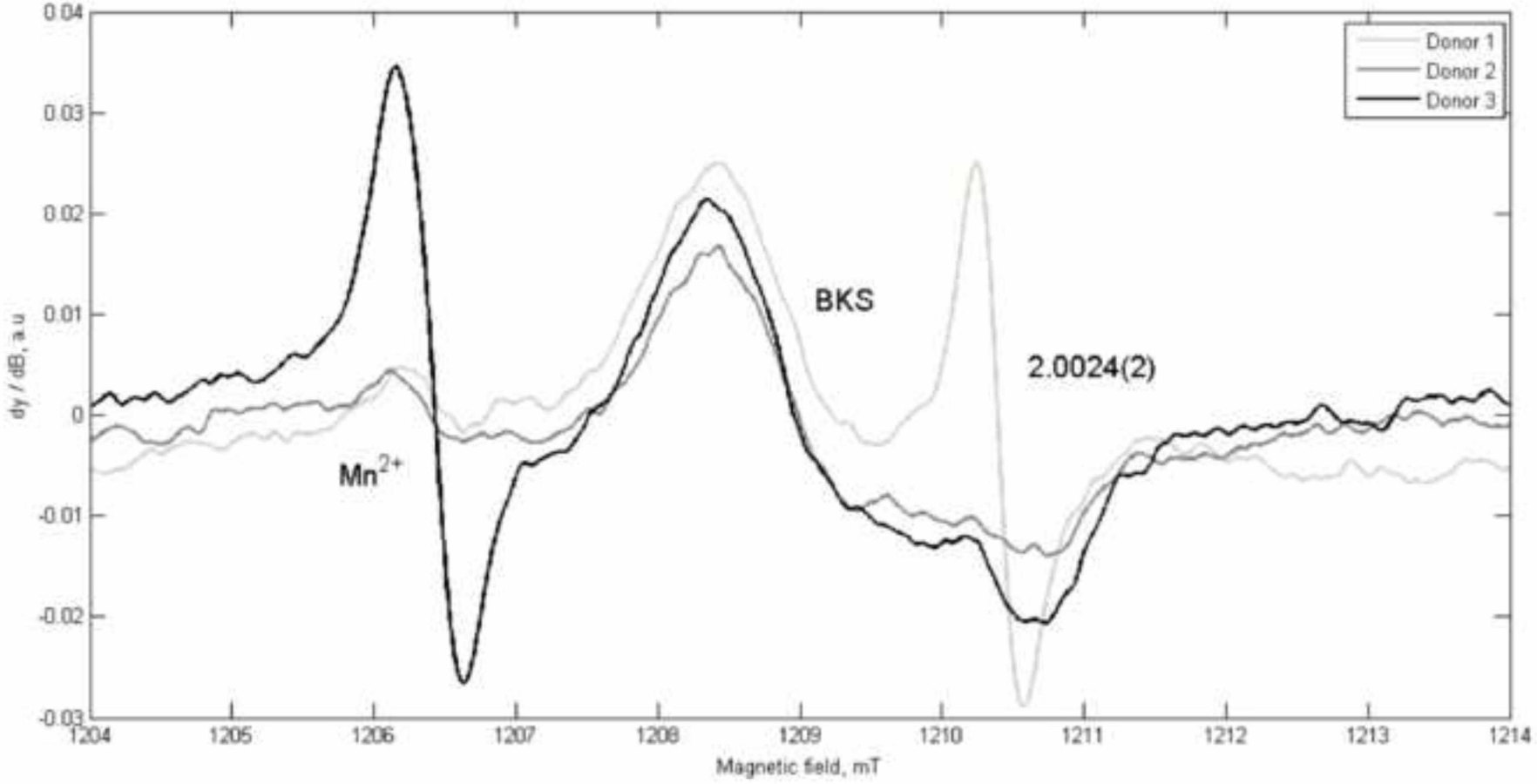

Figure

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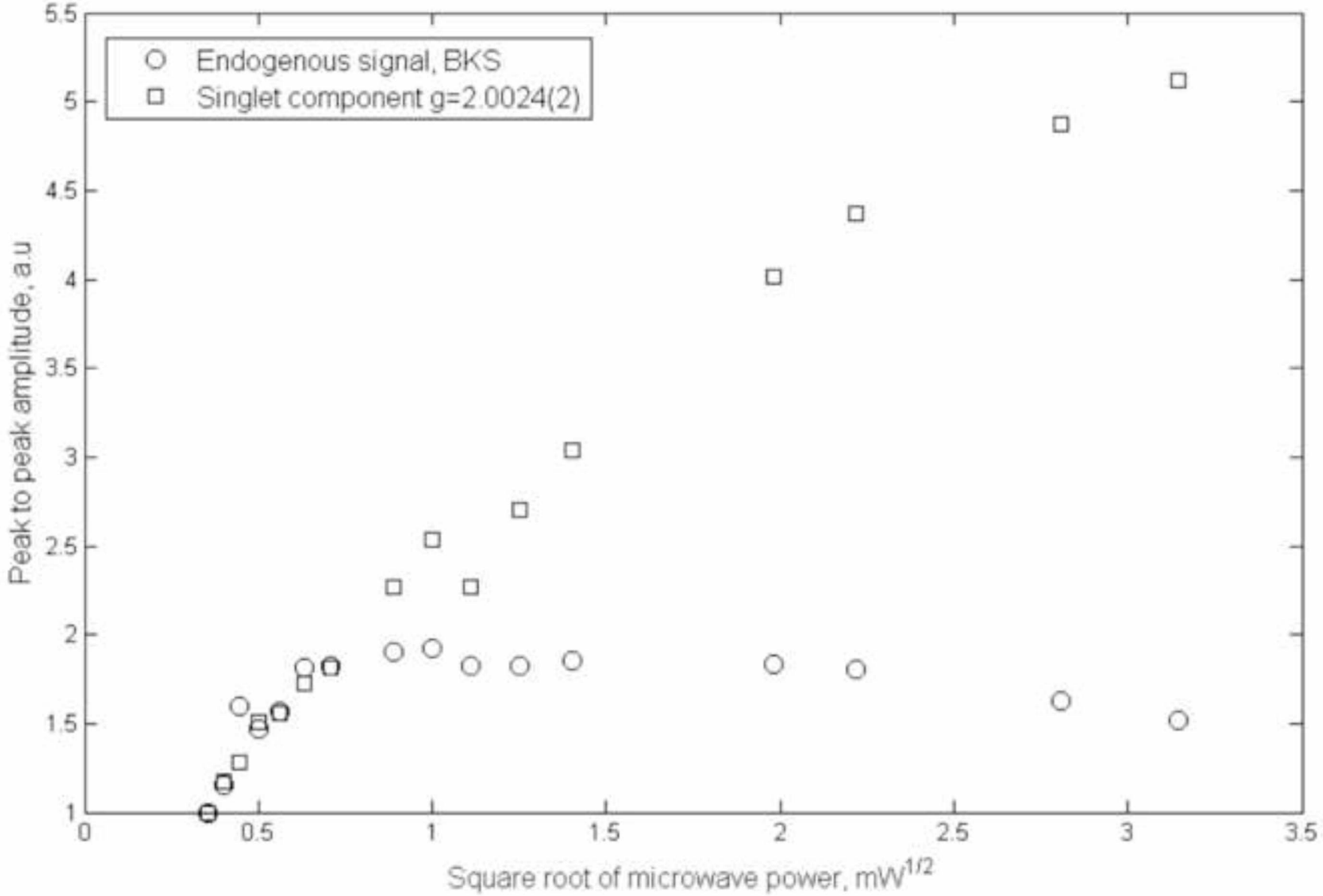

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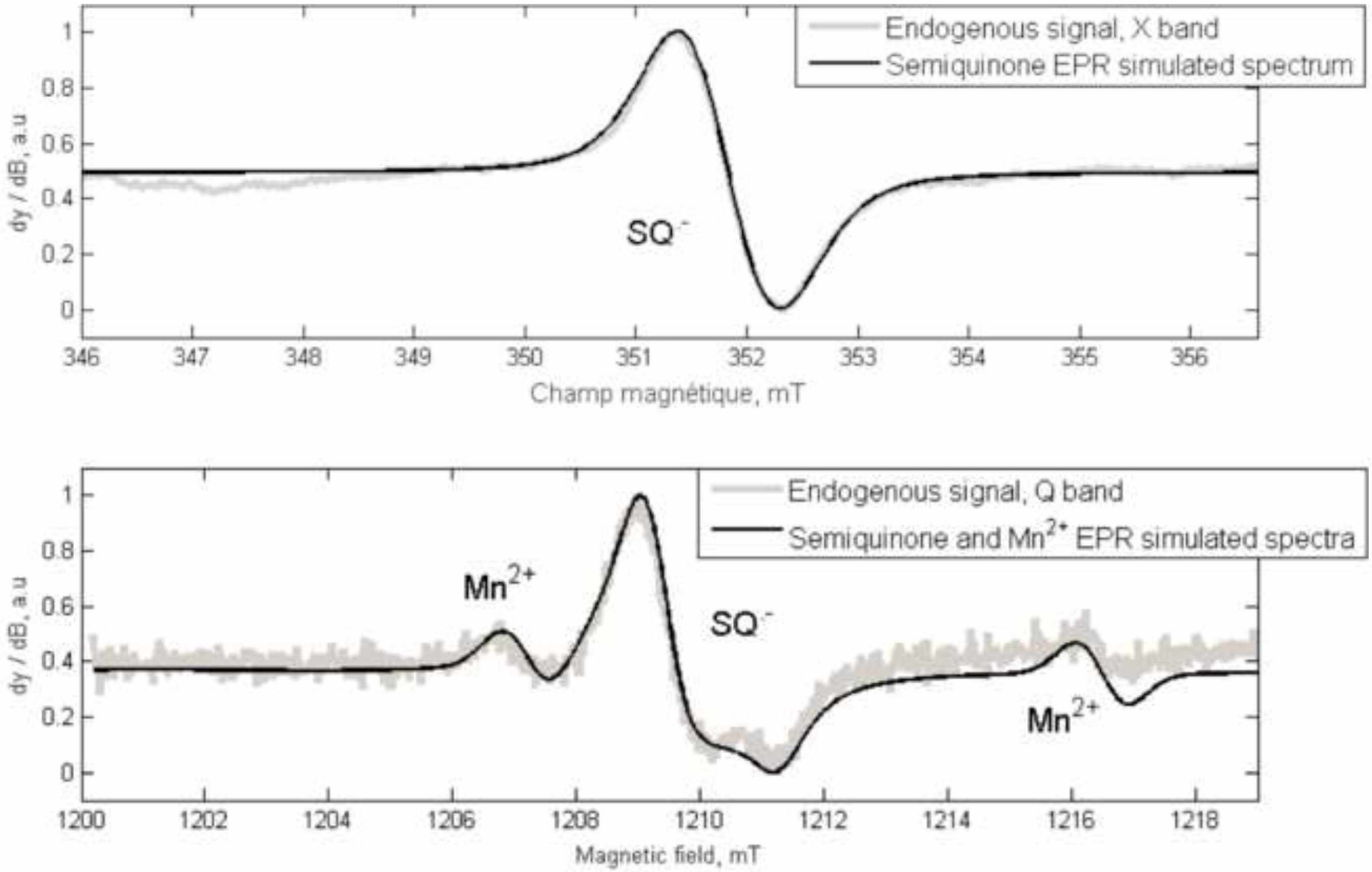

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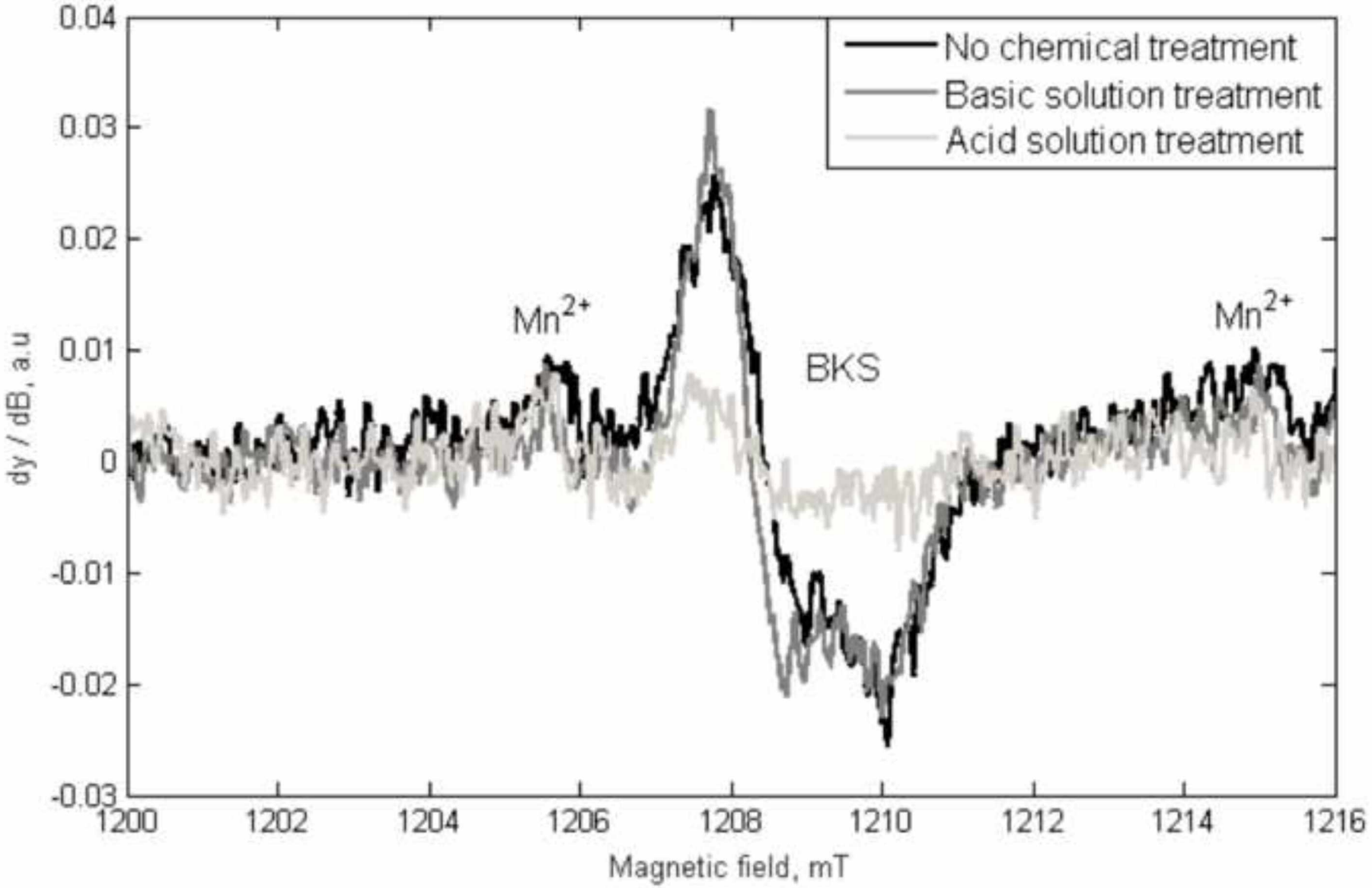

Figure

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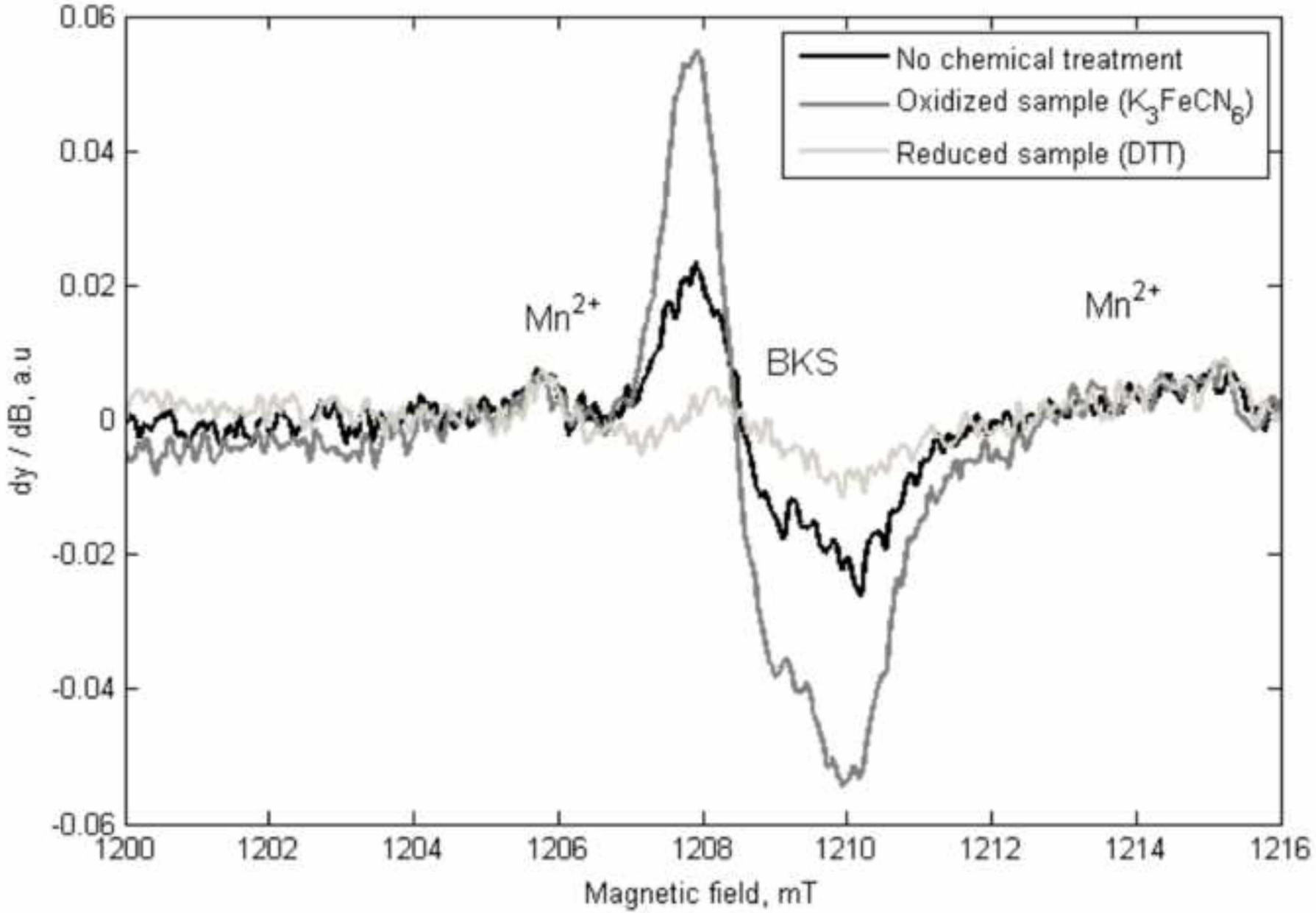

Figure

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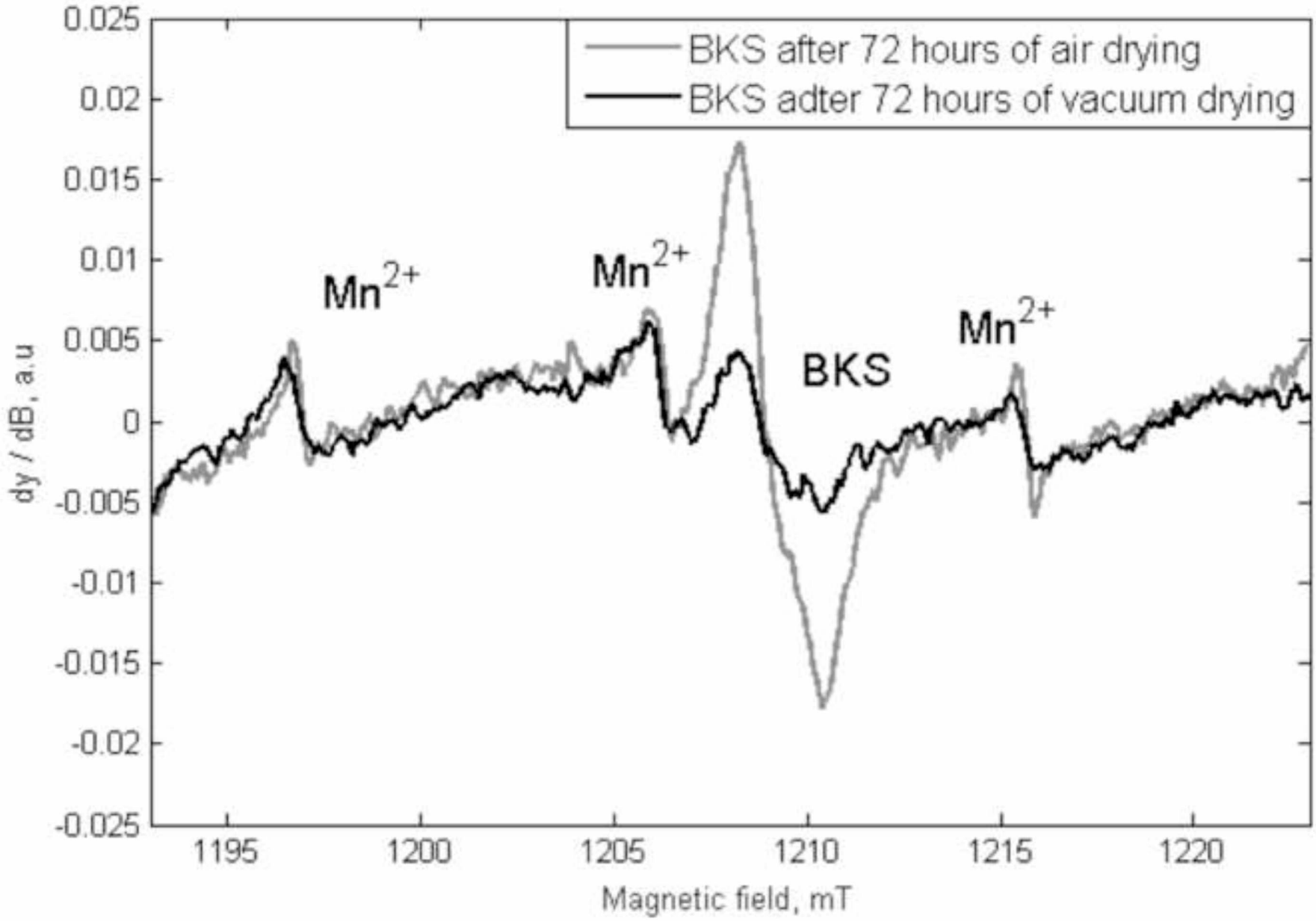

Table 1. Setting parameters for Q-band experiment.

Parameters	
Micro-wave frequency	34.00 GHz
Resonator model	ER5106QT
Fingernails mass	1.00 – 3.00 mg
Fingernails geometry	1.00 mm wide, 2.00 mm long
Inner diameter of the sample holder	2.00 mm
Incident micro-wave power	[0.1 ; 10] mW
Sweep width	[1200 ; 1220] mT
Modulation frequency	100.00 kHz
Modulation amplitude	0.3 – 0.5 mT
Receiver gain	23.00 dB
Time constant	10.24 ms
Number of points	1024
Sweep time	40 s
Number of scans	8 - 12

Table 1. Setting parameters for X-band experiment.

Parameters	
Micro-wave frequency	9.40 GHz
Resonator model	SHQ
Fingernails mass	15 - 30 mg
Fingernails geometry	1.00 mm wide, 2.00 mm long
Inner diameter of the sample holder	4.00 mm
Incident micro-wave power	[4 ; 50] mW
Sweep width	[346 ; 355] mT
Modulation frequency	100.00 kHz
Modulation amplitude	0.3 – 0.5 mT
Sweep time	30 s
Number of scans	8 - 12