An automated sample changer for Bruker ESR spectrometers

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***Abstract** : We have developed an automated sample changer which allows the measurement of up to 40 samples without operator attendance. It had not been possible to adapt commercially available robotic arms or auto-loaders. A performance test on enamel samples from a single tooth showed that (i) the changer worked reliably and was not an additional source of error and (ii) when using Fourier transformation to eliminate high frequency noise of the spectra, the uncertainty in the measurement of the ESR intensity was in the range of 1%. Furthermore, there was no difference in the uncertainty of an ESR measurement between aliquots that were weighed as precisely as possible to 40 mg before measurement and subsamples (ranging between about 33 and 50 mg) whose weight was used after measurement for spectrum weight normalisation.*

Equipment

The equipment consists of three major components: (i) a sample holder, (ii) a transfer arm and (iii) a microprocessor control unit. The components were constructed to allow maximum flexibility.

The sample holder is a barrel chain designed to hold the common 5 mm Wilmad quartz tubes. These have been shortened by the manufacturer to 100 mm (which makes them about 30% cheaper than the standard 178 mm tubes). In principle, the chain can be extended to accommodate any number of samples. The transfer arm was constructed from reinforced composite graphite which is non-magnetic, light weight and rigid. The 15 kG magnet of the ANU spectrometer requires a vertical transfer of about 500 mm. The arm can be optimised for any insertion depth. The sample tube is picked up by the use of a vacuum supplied by a small pump. The sample positioning in the cavity can be adjusted with an insertion vial (Figure 1). The system is controlled by a programmable micro-processor unit (Little Star, ZWorld Engineering) which is interfaced with the Bruker spectrometer.

After loading the sample tubes into the holder and switching the unit on, the first sample position is homed-in under the transfer arm. When the system is activated, the arm picks up the first tube and lifts it out of its barrel. The carriage holding the chain swings forward allowing the transfer arm to descend towards the cavity. The arm stops when mechanical resistance occurs. The optimal positioning of the samples in the cavity is ensured by the insertion vial (Figure 1). A scale at the top can be used to adjust the correct height for samples with different dimensions. The sample remains in the cavity until the unit receives a pulse from the spectrometer upon which the sample is lifted up and re-inserted into the holder. Provided another tube is in the next barrel, this tube will be transferred to the cavity. If the next position is empty, the micro-processor will recognise that no vacuum could be successfully applied and the unit will switch to stand-by mode.

Measurement

The timing of a sample change is controlled by the spectrometer. The recording of an ESR spectrum requires two files, the first is saved with the spectrum of the sample, whilst the second one is only used to send a pulse to the sample changer after the measurement has finished (called PULSE in step 2 of the automation routine). Before starting an automation routine, a representative sample is used to tune the spectrometer and work out the best measurement conditions. These will be applied to all following spectra. It seems advisable that the samples of one run have approximately the same volume and spectrum characteristics. The following automation routine will measure 40 samples, save spectra and show the measured spectra on 40 consecutive pages. The first page has been used to determine the measurement parameters.

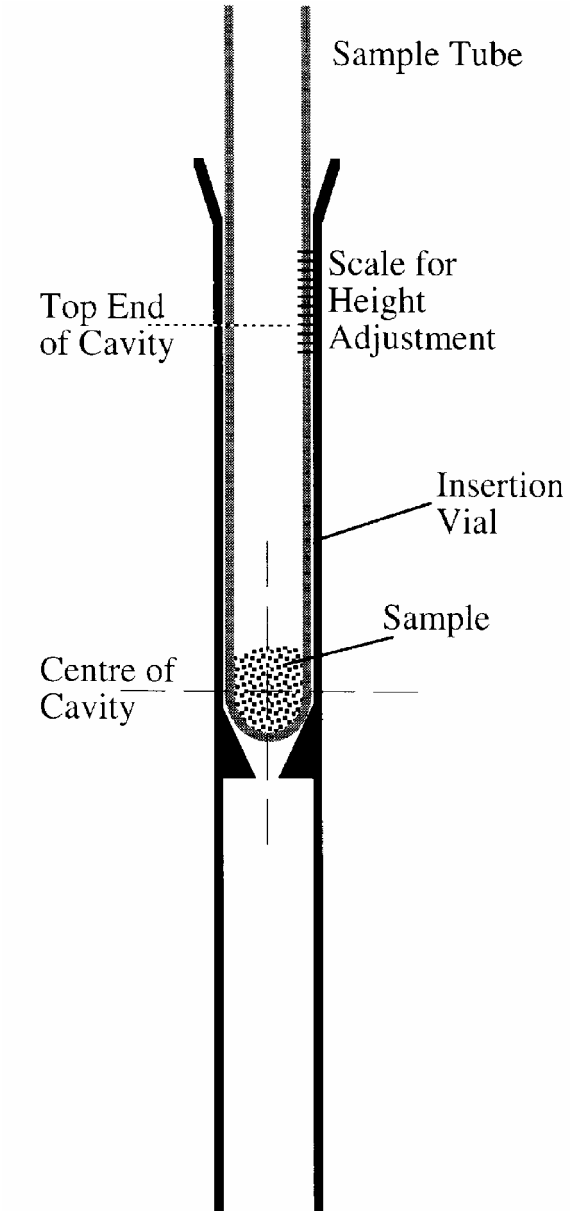

***Figure 1**: Schematic drawing of the insertion vial (not to scale).*

Example of an automation routine:

1> PG INC 1 'increase the page number by 1
2> READP PULSE 'read file that contains the pulse sequence (make sure it is in the selected directory)
3> SETP 'set parameters to page
4> TP INC 1 'copy parameters to next page (for next run)
5> DEL 'delete any previously stored spectra
6> RACQ 'measure (for sending pulse, the recorded spectrum will be over-written in the next run)
7> PG DEC 1 'decrease page number by one
8> SETP 'set parameters to page
9> WAIT 90 s 'time required to remove old sample and insert a new one
10> MTU 'fine tune
11> DEL 'delete any previously recorded spectra
12> RACQ 'measure sample
13> WRITE RUN1 'save recorded spectrum with the nam

When the sample is removed from the cavity, the spectrometer automatically resets the microwave power to 0.44 mW. During an automation routine it is not possible to switch the spectrometer to stand-by mode or to activate a complete auto-tuning cycle for each sample. The fine-tuning (step 10) optimises diode current and frequency. As long as the samples have similar ESR characteristics, the fine-tuning is sufficient to optimise the spectrometer. The sample changer is started during the first wait period. If a position is empty, no further sample will be re-inserted into the cavity. This has the disadvantage that in the subsequent fine tuning step, the spectrometer will be completely mis-tuned. It seems therefore advisable that the loop number (in step 16) corresponds to the number of samples to be measured, in which case the last sample stays in the cavity and the spectrometer remains tuned.

Performance

In order to test the performance of the sample changer, two sample sets were measured. About 3 g of tooth enamel was extracted from a hippopotamus tooth from the archaeological site of Florisbad. Two size fractions were prepared, 250-150 µm and < 150 µm. The first fraction was etched with acetic acid to remove any dust attached to the surfaces of the grains. It was thought that grains would be ideally suited for single aliquot dating (Grün, 1995) because the weight-loss should be minimal during the transfer of the sample from the measuring tube to the vial in which it is irradiated (and vice versa). The second fraction is used routinely for dating in this laboratory. Both sets were divided into 40 subsamples. Aliquots of the grains were weighed as closely as possible to 40 mg with a weighing error in the range of 0.2 mg. The powder samples were in the range of about 33 to 50 mg and were precisely weighed after they were filled into the sample tubes.

The weighing error of these samples is in the range

of < 0.05 mg. The measured powder ESR spectra were subsequently normalised on the weight. The measurement conditions were the ones that are routinely used for enamel samples with relatively large signals: accumulation of 20 scans with 1.015 Gpp modulation amplitude, 10.24 ms conversion factor, 20.48 ms time constant, 2048 bit spectrum resolution (resulting in a total sweep time of 20.972 s), 120 G sweep width and 2 mW microwave power. Each set was measured ten times in the course of about two weeks. The spectrometer had to be turned off several times between the runs. No standard was measured. As shown in Figure 2A, the spectra were quite noisy. In order to address this problem, the spectra were evaluated in two ways:

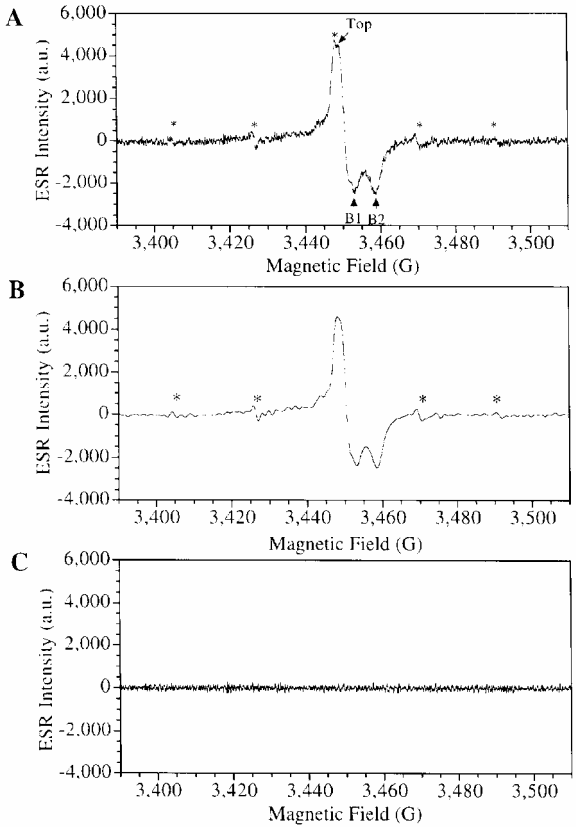

Figure 2
A: Raw spectrum of a grain sample. The asterisks indicate a quintet that has been ascribed to dimethyl (Bouchez et al. 1988).
B:Fourier-transform manipulated spectrum. Note that the split at the top of the wide signal has disappeared whilst the other four signals are still clearly resolved.
C: High frequencies that were stripped off the raw spectrum by the Fourier transformation procedure.

1) the raw spectra were manually evaluated (by setting markers and retrieving the corresponding intensities from the software of the spectrometer) from the top of the wide peak (as indicated in Figure 2A) to the base of the first trough (Top-B1 in Figure 3) as well as to the base of the second trough (Top-B2);
2) the spectra were Fourier transformed, the higher frequencies zeroed and the lower frequencies back-transformed. Figure 2B shows the manipulated spectrum. The peak intensities were then automatically evaluated by the software of the spectrometer.

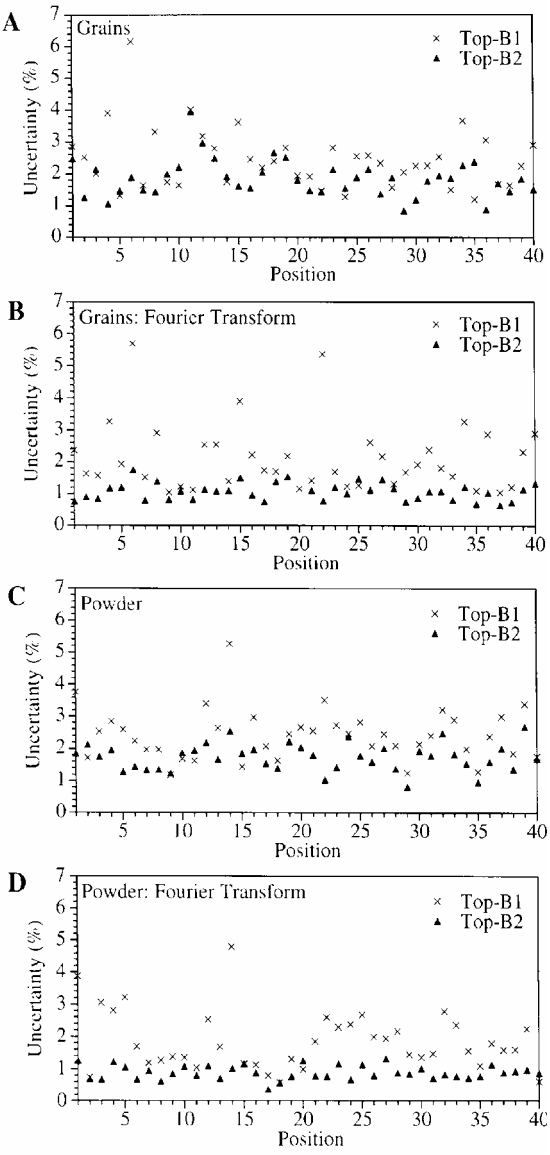

Figure 3*: Uncertainties involved in the repeated measurement of a specific sample. The two signal heights (Top-B1 and Top-B2) are indicated in Figure 2A.*

Figure 2C shows the higher frequencies that were stripped off the raw spectrum. This spectrum does not seem to contain any distinctive peaks. One particular advantage of the Fourier transform, henceforth FT, manipulation was that the dip at the top of the wide peak disappeared giving a more straight forward definition of the top-intensity. The other lines which are thought to be part of the same quintet (marked with asterisks) were smoothed but still resolved (compare Figure 2A and 2B). The difference between the average measured peak intensities of the raw and the FT manipulated spectra was less than 1%. The uncertainty values discussed below are the standard deviations expressed as percentage of the mean.

The performance test was used to address three questions:

1) Determination of the uncertainty of an ESR measurement of a particular sample. This has bearing on the estimation of the number of data points and distribution of dose steps required for the establishment of satisfactory dose response curves (Grün and Rhodes 1991, 1992) as well as the assessment of errors in dose determination (Grün and Brumby, 1994).
2) Determination of the over-all uncertainty of a run of the sample changer. This can be used to decide whether subsamples used for a dose response curve can be measured in the same run or are better measured in the same sample tube in subsequent runs.

3) Is there is a difference between weighing out aliquots before measurement and post-measurement spectrum normalisation? This can be used for minimising the efforts in sample preparation.

Figure 3 shows the uncertainties of the ten repeated measurements of each sample. These uncertainties arose from random measurement errors, equipment stability and positioning of the sample tube in the cavity. It is noteworthy that the signal intensity Top-B1 (crosses in Figure 3) showed nearly always larger uncertainties than the signal intensity Top-B2. The raw spectra of the grains and powders (Figure 3A and C) had uncertainties in the range 2 to 4%, whilst the FT manipulated spectra of the two sets showed uncertainties of around 2% for Top-B1 and about 1% for Top-B2.

Ten repeated measurements of a single sample without removing it from the cavity resulted in uncertainties of around 2% for the raw spectra. The uncertainty of the FT manipulated spectra Top-B2 was 0.89% which falls well into the respective ranges of the grains (1.07±0.27%) and powders (0.88±0.21%). Apart from the absence of mechanically removing the sample, the measurements of this sample were carried out within about two hours, minimising potential long term stability problems. One can conclude from these data that sample changing over longer periods of time did not introduce any significant errors.

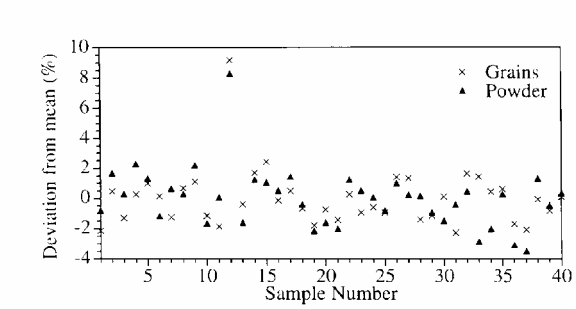

***Figure 4**: Deviation of the mean intensity at a particular position from the mean of all measurements.*

Additional sources for the uncertainty of the 40 measurements of a run were weighing errors, sample inhomogeneity and differences in glassware. Figure 4 shows the deviation of the mean intensity at each of the 40 positions from the mean of all measurements. Surprisingly, position 12 showed a very large increase of the intensity for both grains and powder. This was most probably due to the characteristics of the sample tube. The other positions showed random scattering in the range of ± 2%. Sample position 12 was excluded from the following evaluation. The uncertainties for a complete run were for the grains: 2.84±0.43% (Top-B1) and 2.15±0.32% (Top-B2) for the raw spectrum evaluation and 2.76±0.54% (Top-B1) and 1.55±0.15% (Top-B2) for the FT manipulated spectra. The values for the powder set were for raw spectrum evaluation: 3.37±.49 (Top-B1) and 2.24±0.28% (Top-B2); FT manipulated spectra: 3.05±0.39% (Top-B1) and 1.70±0.27% (Top-B2).

The uncertainties of the intensity measurement of complete runs (FT manipulated spectra, Top-B2) are nearly twice as high as the uncertainties of the repeated measurement of specific samples. This implies that subsamples used for the construction of a dose response curve ought to be measured in the same sample tube. Furthermore, single aliquot measurements minimise the problem of sample

inhomogeneity. However, so far it has not been demonstrated that the single aliquot technique is applicable to materials other than enamel.

The basic difference between the two sample sets is that the ESR spectra of the grains did not have to be weight-normalised. The uncertainties for the complete runs showed no quantifiable differences between the grain aliquots and the powders. Because precise weighing of aliquots requires more time and concentration than the precise weighing of subsamples whose absolute weight may scatter by up to about 15%, it seems preferable to use post-measurement weight normalisation.

It was thought that grains were particularly suitable for single aliquot measurements (Grün 1995) as weight-loss between measurements ought to be negligible. However, electrostatic problems made complete recovery impossible and up to 10% of the samples were lost. The weight-loss of powders can be kept below 1%.

Conclusions

The sample changer allows the measurement of up to 40 samples without operator attendance. Performance tests showed that the changing process introduced negligible uncertainties that were not quantifiable. The performance tests also showed that:

- the uncertainties in the ESR intensity estimation of relatively noisy spectra of tooth enamel were in the range of about 2%;
- FT manipulation could be successfully applied for the reduction spectrum noise and optimisation of the reproducibility, the uncertainties improving to about 1%;
- inhomogeneity of the samples and differences in the properties of the sample tubes introduced additional errors of about 1.3% which means that a single aliquot technique and measurement in the same sample tube ought to give best results for the construction of dose response curves;
- weight normalisation of spectra did not introduce additional errors for samples with weights between about 33 to 50 mg compared to pre-weighed 40 mg aliquots;
- it is more difficult to recover grains than powders from the sample tubes due to electrostatic problems.

Acknowledgment

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Reviewer
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