Tooth Enamel EPR Dosimetry: Optimization of EPR Spectra Recording Parameters and Effect of Sample Mass on Spectral Sensitivity

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In order to improve the accuracy of the tooth enamel EPR dosimetry method, EPR spectra recording conditions were optimized. The uncertainty of dose determination was obtained as the mean square deviation of doses, determined with the use of a spectra deconvolution program, from the nominal doses for ten enamel samples irradiated in the range from 0 to 500 mGy. The spectra were recorded at different microwave powers and accumulation times. It was shown that minimal uncertainty is achieved at the microwave power of about 2 mW for a used spectrometer JEOL JES-FA100. It was found that a limit of the accumulation time exists beyond which uncertainty reduction is ineffective. At an established total time of measurement, reduced uncertainty is obtained by averaging the experimental doses determined from recorded spectra following intermittent sample shaking and sample tube rotation, rather than from one spectrum recorded at longer accumulation time. The effect of sample mass on the spectrometer's sensitivity was investigated in order to find out how to make appropriate corrections.

INTRODUCTION

Experimental determination of irradiation dose by the electron paramagnetic resonance (EPR) spectroscopy of tooth enamel (EPR dosimetry) is a very important tool for dose reconstruction at radiation accidents.[1] It is known that the spectrum of irradiated tooth enamel is composed of two components: the native background signal (BGS) and the radiation-induced signal (RIS), which overlap each other. The intensity of RIS depends on dose absorbed in enamel and it is used for dose reconstruction.

In the low dose region (less than 0.5 Gy, which is of interest in the radiation epidemiology) the accuracy of dose determination using EPR dosimetry method is essentially dependent on the quality of EPR spectra, which is defined by sensitivity of spectrometer, sample mass and spectrum registration parameters, such as microwave power, modula-

tion amplitude, accumulation time and others. At the same time, the accuracy depends on the procedure of spectra processing, which is used for determination of the RIS intensity. Therefore, quality of spectra should be appreciated by a criterion that it must provide maximal accuracy of dose determination regarding that a certain spectra processing procedure is used.

In the previous work,[3] optimization of the registration parameters giving the best accuracy of dose determination was performed for a BRUKER type spectrometer with the use of a developed spectra processing program.[2] It was stated that requirements to optimal modulation amplitude are the same for all types of spectrometers. However, the microwave power should be optimized for a specific type of spectrometer, since microwave field strength in the resonator cavity at given power input depends on the type of cavity. Effect of the spectra accumulation time on the accuracy of dose determination still has not been investigated yet to give definite recommendations for its selection. It is usually assumed that at longer accumulation time better signal-to-noise ratio is obtained, which supposedly leads to improving the accuracy. However, at long accumulation time influence of instability of the spectrometer is increased. Selection of the accumulation time is usually based on intuitive considerations based mainly on some reasonable maximal time, which can be spent on measurements. In the course of the

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international intercomparison,[4] different research groups used a wide range of accumulation time (from 20 to 110 min) but no correlation of the accuracy of dose determination with this parameter was observed.

In order to make the EPR dosimetry method suitable for wide routine use, process of its realization should be unified. For this reason, an automatic spectra processing procedure was developed,[2] which is used now in several research groups.[4,5] The next step is to elaborate a general approach for finding out the optimal spectra recording parameters suitable for different types of spectrometers.

The objective of this work is optimization of the microwave power and the accumulation time giving the best accuracy of dose determination at spectra registration using a JEOL JES-FA100 spectrometer and the previously developed spectra processing procedure.[2] The scope of this work is also to find out limitations of the spectrometer, in which it can be used in the automatic mode of spectra accumulation. Influence of the sample mass on the sensitivity of the spectrometer is also investigated in order to find out in what range of sample mass this effect is significant and how correction of the sensitivity may be performed.

MATERIALS AND METHODS

Sample preparation and irradiation

Tooth enamel was prepared from ten molar teeth extracted for medical indications in the Dental Clinic of Hiroshima University. The enamel was separated from dentin manually using hard alloy dental drills and it was crushed by a pair of nippers to chips sized 0.5–1.5 mm. Such grain size has been proved to be optimal from point of view of minimization of effects of sample anisotropy and surface effects at enamel crushing leading to distortion of the EPR signal.[6] Enamel from different teeth was mixed together to obtain pooled enamel.

Ten sample aliquots of 100 mg mass were irradiated by gamma rays of a laboratory ^{60}Co source in Hiroshima University in doses of 0, 100, 200, 300 and 500 mGy, by pairs for each dose. These samples were used for calibration of the EPR signal dose response and for dose determination at optimization of the spectra recording parameters. One sample aliquot of 200 mg was irradiated in dose of 100 Gy; it was used for measurements of the dependence of the spectrometer's sensitivity on the sample mass. Doses were controlled by a tissue-equivalent dosimeter with standard accuracy not worse then 3%. At irradiation, the samples were placed between two 4-mm polymethylmetacrylate plates. Dose absorbed in enamel at such conditions of irradiation is very close to dose measured by the tissue-equivalent dosimeter.[7,8]

EPR spectra measurements were performed not less than ten days after irradiation and sample preparation, so all transient radiation-induced and mechanical-induced signals should have been eliminated or come to an equilibrium state during this period.[9]

EPR spectra measurement

EPR spectra measurements were performed at stabilized room temperature of 21°C with a JEOL JES-FA100 EPR spectrometer operating in the X-band and equipped with a high quality factor (Q-factor) cylindrical resonator cavity ES-UCX2 of TE_{011} mode. According to the reading of the spectrometer, the empty cavity has Q-factor of 7440, and it is varied over 6000–7436 at loading with the sample tube and the enamel sample. The following spectra recording conditions and parameters were used: modulation amplitude 0.3 mT, modulation frequency 100 kHz, receiver time constant 30 ms, sweep time 30 s, sweep width 10 mT. Unless stated otherwise, receiver amplitude was set 1000, microwave power supplied to the cavity was 2 mW, number of spectra scans was 40 corresponding to spectra accumulation time of 20 min. At optimization of the recording conditions, different settings of the microwave power and the accumulation time were used.

A signal of a reference sample containing Mn^{2+} mounted in the cavity was recorded together with the spectrum of enamel sample. This signal was used for correction of field position at spectra processing and for monitoring of the spectrometer sensitivity at different sample mass. No additional signal of Mn^{2+} due to forbidden transition was detected.

The same JEOL's quartz sample tube with internal diameter of 3 mm was used for all samples. The tube was positioned in the resonator cavity so that the center of the sample coincided with the center of the cavity. No subtraction of the spectrum of the empty sample tube was performed. At changing the sample, the tube was cleaned out by blowing using a flow of gas.

The sweep time and the receiver time constant were set according to available recommendations.[1,10] The principals of setting these parameters are the following. It is accepted,[10] that in order not to disturb the line shape, the time of line passage should be at least ten times higher in comparison with the receiver time constant and spin lattice relaxation time (T_1). This condition produces the following relation:

$(Line\ passage\ time) = (Line\ width\ /\ Sweep\ width) \times (Sweep\ time) >$
$$10 \times (Receiver\ time\ constant\ or\ T_1). \quad (1)$$

The enamel spectrum consists of lines having width over 0.3 mT; the line width of Mn^{2+} signals is about 0.1 mT (in fact, the line width is 0.075 mT, but it is broadened because of relative high modulation amplitude of 0.3 mT). Taking into account the line widths, at sweep width 10 mT, the following ratio should be fulfilled in order not to disturb the spectrum having minimal line width 0.1 mT:

$(Sweep\ time) \geq 1000\ (Receiver\ time\ constant\ or\ T_1). \quad (2)$

Typical values of T_1 in the crystalline system are below 10^{-4} s.[10] Therefore, according to Eq. (2) the condition imposed by T_1 on the sweep time is fulfilled at 0.1 s, and reasonable limitations to this parameter are given by the receiver time constant. The signal to noise ratio characterizing the quality of the spectrum may be improved by increasing of the receiver time constant and/or by increasing of the number of spectra accumulation, which both result in increasing of the spectra accumulation time. The sweep time and the receiver time constant can be selected quite arbitrary taking into account the condition imposed by Eq. (2). In the present work, values of 30 s are selected for the sweep time and 30 ms for the receiver time constant.

Spectra processing

An automatic spectra processing computer procedure described in a recent publication[2] was used for separating RIS and evaluation its intensity. This procedure is able to make deconvolution of the experimental spectrum of irradiated enamel using the non-linear least squares fit by a model spectrum describing RIS and BGS in analytical form. The spectra processing procedure was applied in the mode with description of BGS by asymmetric narrow component and wide component both composed of combination of the derivative Gaussian functions. A fitting window was set having left border of –1.0 mT and right border of +2.0 mT relatively to the maximum of BGS. The following parameters were varied in the fitting procedure: amplitude of RIS, amplitudes of narrow and wide components of BGS, width of narrow component of BGS, vertical and horizontal offsets of the spectra. Other parameters of the model (parameters describing shape of RIS, shift between RIS and BGS, width of the wide component of BGS) were set constant as they were obtained at optimization of the procedure.[2] Base line correction was performed by fitting of the first order polynomial to two areas in the spectrum between enamel's signal and two Mn^{2+} signals.

Calibration, determination of experimental doses and their uncertainty

For each set of recording parameters, spectra of ten samples irradiated in different nominal doses were measured and processed to obtain the RIS amplitudes. Calibration line was determined for each set of parameters using the linear regression method[11] from dependence of the RIS amplitude on the nominal doses. Experimental doses were determined from the RIS amplitudes using the obtained calibration line. This procedure of calibration and experimental doses determination was performed automatically by the spectra-processing program for each set of spectra for ten samples measured at given recording conditions. The experimental doses were obtained as result of single spectrum measurement and also as averaged over results obtained at four repeated spectrum measurements at each set of recording parameters after sample shaking and sample tube rotation to an arbitrary angle. Mean square deviation of the experimental doses from the nominal doses (standard deviation) was used as a parameter characterizing an uncertainty of dose determination. This parameter was determined for experimental doses obtained at single measurement (SDS) and at averaging over results obtained for four repeated measurements (SDR).

RESULTS AND DISCUSSION

Measurement of spectra at different accumulation time and microwave power

Spectra of ten samples irradiated in different doses were recorded at 10, 20, 40, 80 and 120 scans (corresponding to the accumulation time of 5, 10, 20, 40, 60 min) and at the microwave power of 1, 2, 5, and 10 mW. The measurements were repeated four times for each sample after sample shaking and sample tube rotation to an arbitrary angle at each set of recording parameters. Thus, 40 spectra were recorded for each pair of accumulation time and power values, and 800 spectra were recorded in total.

Examples of spectra of one of the samples irradiated in dose of 500 mGy and recorded at different accumulation times at microwave power of 2 mW together with results of spectra processing are shown in Fig. 1. Examples of spectra of this sample recorded at different microwave power at accumulation time of 20 min are shown in Fig. 2.

Analysis of dependencies for residual sum

Quality of spectra fitting is characterized by residual signal, obtained as difference between the model and the experimental spectra after fitting at spectrum processing. The residual signal is characterized by residual sum (RS), which is derived from its mean-square amplitude. Averaged RS is obtained by averaging the values resulted from mathematical processing of four times repeatedly measured spectra of ten samples irradiated in different doses. Dependencies of the averaged RS on the accumulation time measured at different microwave power are shown in Fig. 3a. It is seen that RS is decreased significantly when the accumulation time is increased up to 20 min. At higher accumulation time, RS is reduced gradually at 1 mW and even has a tendency to grow at power of 2 mW and over. Therefore, it may be accepted that increasing of the accumulation time over some limit is not effective for improving of the quality of spectra fitting. In given conditions of spectrum measurements and given type of spectrometer this limit looks like to be 20–40 min. The observed effect is probably caused by operational instability of the spectrometer (base line drift, signals caused by electromagnetic disturbances, *etc*.) and becomes more prominent at longer accumulation time. Indeed, as it is seen from Fig. 1, at increasing of the accumulation time, though the high frequency noise component in the residual signal (spontaneous signals characterized by width below 0.1 mT)
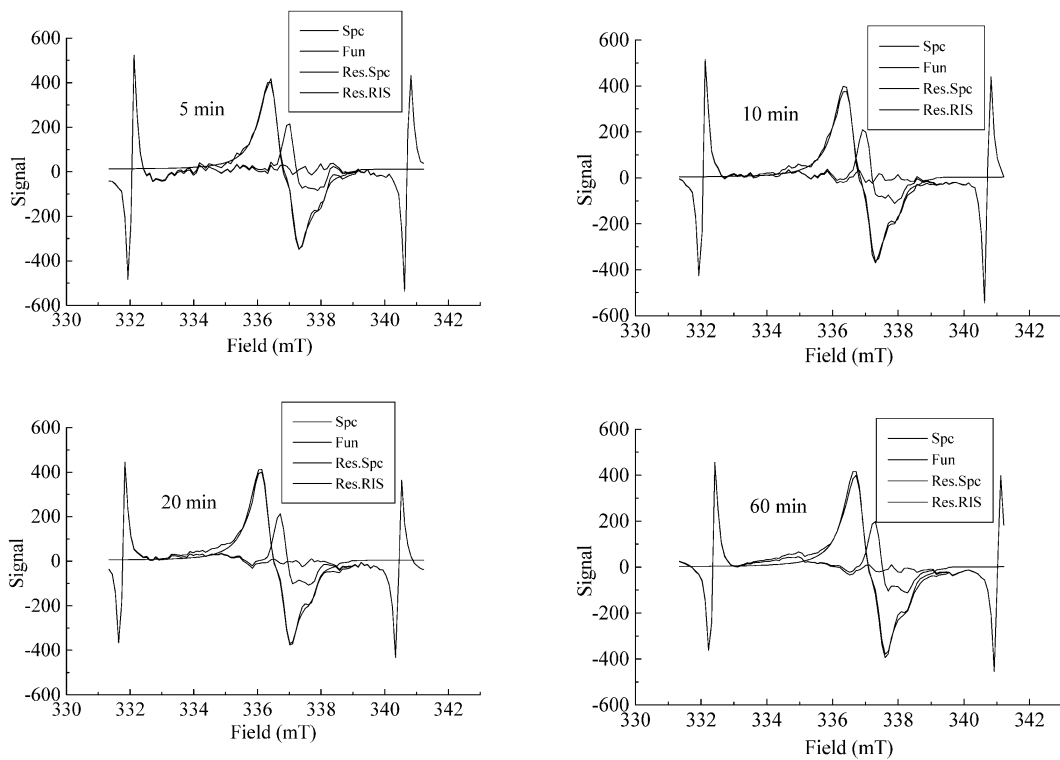
Fig. 1. Spectra of samples irradiated in dose of 500 mGy and measured at microwave power of 2 mW and different accumulation times. Curve notations: *Spc* – experimental spectrum; *Fun* – fitted model function describing the spectrum; *Res.Spc* – residual signal after subtraction of the fitted model spectrum; *Res.RIS* – residual signal corresponding to the RIS obtained after subtraction of the model background signal. The accumulation time values are indicated on the plot panels.
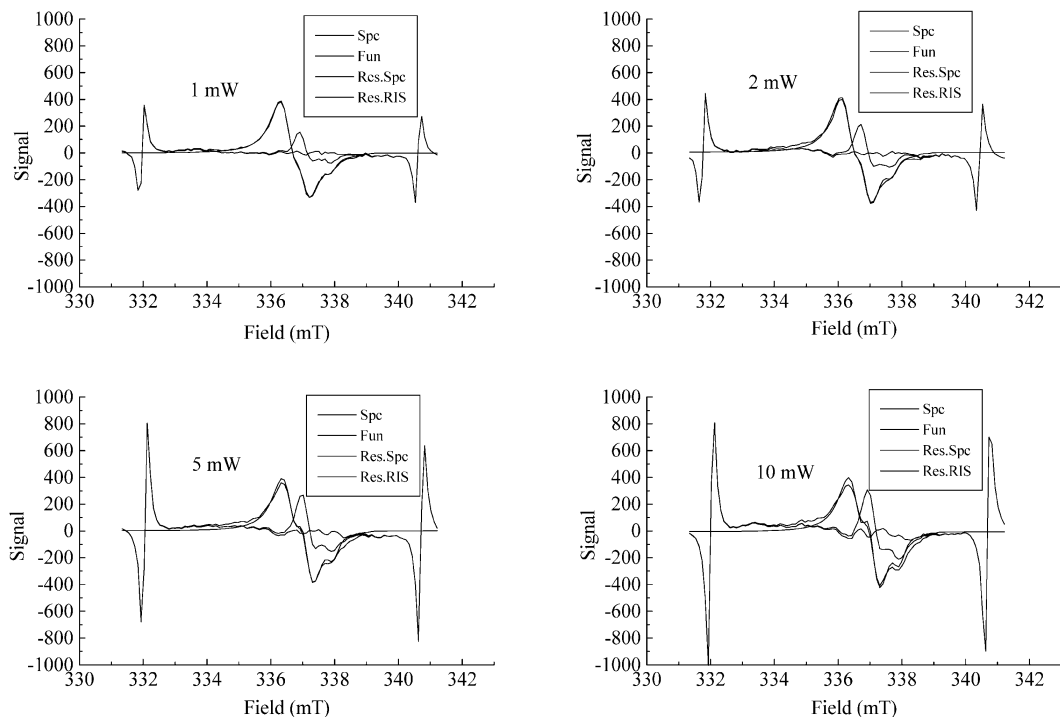
Fig. 2. Spectra of the sample irradiated in 500 mGy and measured at accumulation time of 20 min and different microwave power. Curve notations are the same as in Fig. 1. The microwave power values are indicated on the plot panels.
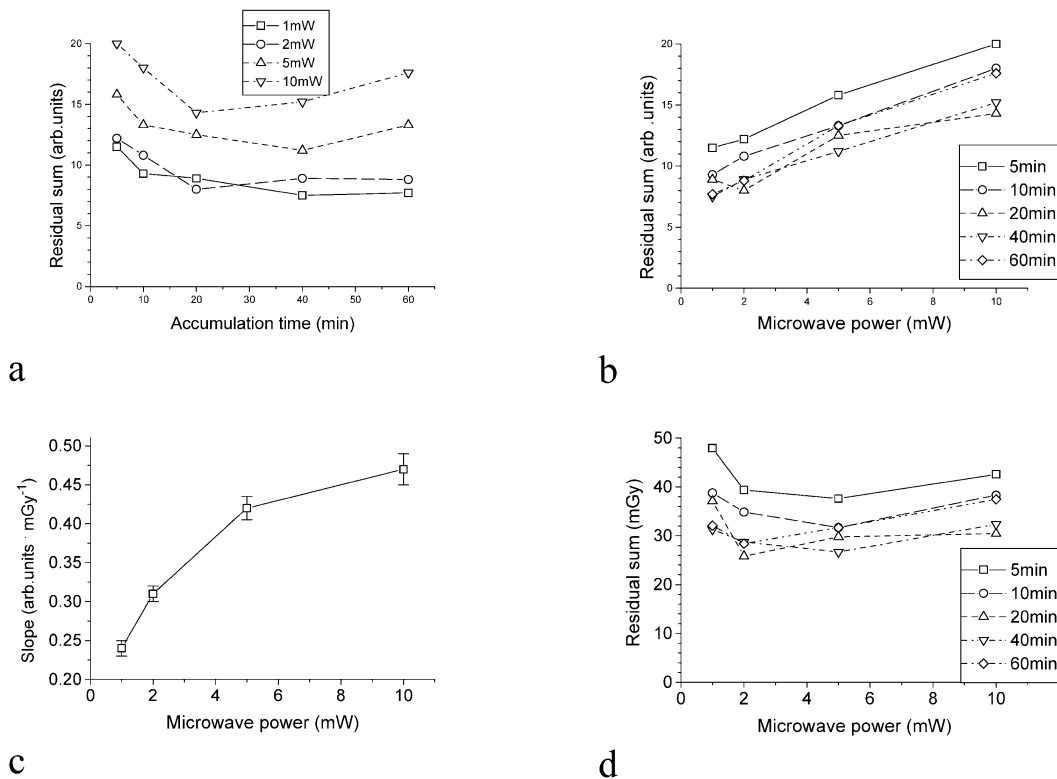
Fig. 3. Dependencies of the residual sum: (a) – on the spectra accumulation time determined at different microwave power; (b) – on the microwave power at different accumulation time. (c) – Dependence of the slope of calibration line of EPR signal dose responce on the microwave power. (d) – Dependence of the residual sum on the microwave power converted to units of dose absorbed in enamel according to the slope of calibration line. The microwave power and the accumulation time values are presented on the plot panels.

is decreased, but the low frequency noise component (characterized by width over 0.1 mT) has tendency to increase.

RS increases when microwave power is increased (Fig. 3b). It is due to increasing of both the low and high frequency noise components in the spectra, as it is seen in Fig. 2. If to convert the RS values to units of dose absorbed in enamel dividing them by the slope of calibration line (Fig. 3c), new details of the effects of the microwave power on RS become pronounced (Fig. 3d) showing a minimum in the region of about 2–4 mW. Presence of this minimum is probably caused by incomplete fitting of the model in wide range of the microwave power and by effects of spectral noises to which the signal of the empty sample tube may contribute along with other sources of noise.

Variation of the line shape of BGS at different microwave power is accounted for at spectrum processing by variation of the width of its narrow component and of the amplitudes of narrow and wide components. The shape of RIS is unchanged at fitting and is kept the same at different power. Improving of the model is necessary to take into account more completely modification of the spectrum at different power.

Signal of the empty sample tube was characterized by its mean square amplitude from a middle line determined in the

same range of magnetic field as the fitting window. Power dependence of this value converted to dose units according to the slope of calibration line is presented in Fig. 4. This signal is comparable with the RS and even exceeds it at some power values. Therefore, the signal of the empty tube gives essential contribution to RS. On the other hand, this
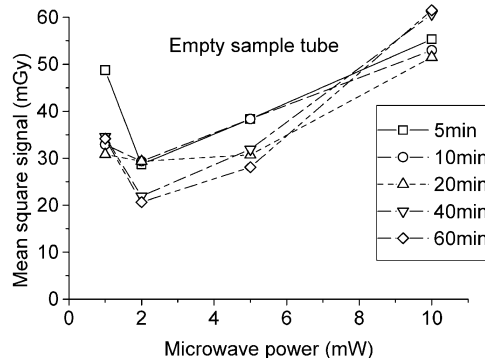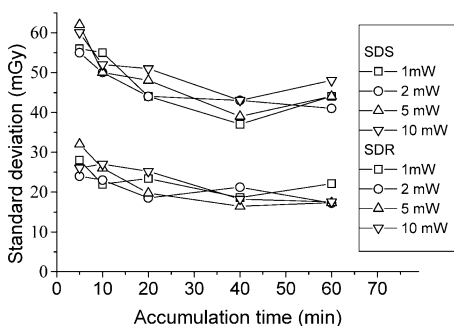

Fig. 4. Dependencies of the mean square signal of the empty sample tube converted to units of dose absorbed in enamel on the microwave power determined at different accumulation times. The accumulation time values are presented in the plot panel.
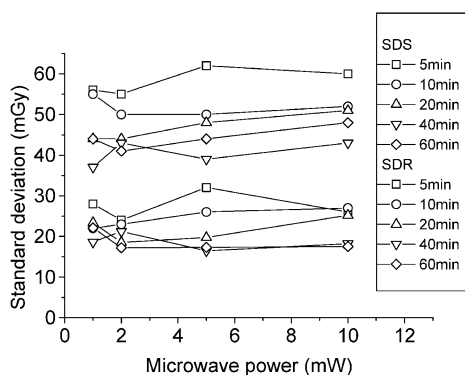
signal is not expected to influence significantly the results of dose determination, because it contributes into the bias level obtained at calibration. Nevertheless, effects of this signal on the accuracy should be investigated, to clarify whether it is necessary to make subtraction of this signal at spectra processing.

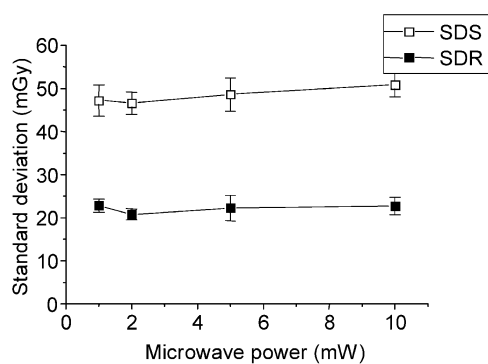### Analysis of dependencies for uncertainty of dose determination

In order to define the optimal spectra registration conditions, direct measurements of SDS and SDR characterizing the uncertainty of dose determination in dependence on the spectra accumulation time and the microwave power were performed. Dependencies of SDS and SDR on the accumulation time determined at different microwave power are presented in Fig. 5. It is seen that these parameters are decreased significantly at accumulation time increasing up to 20 min and then are decreased much weakly or even has
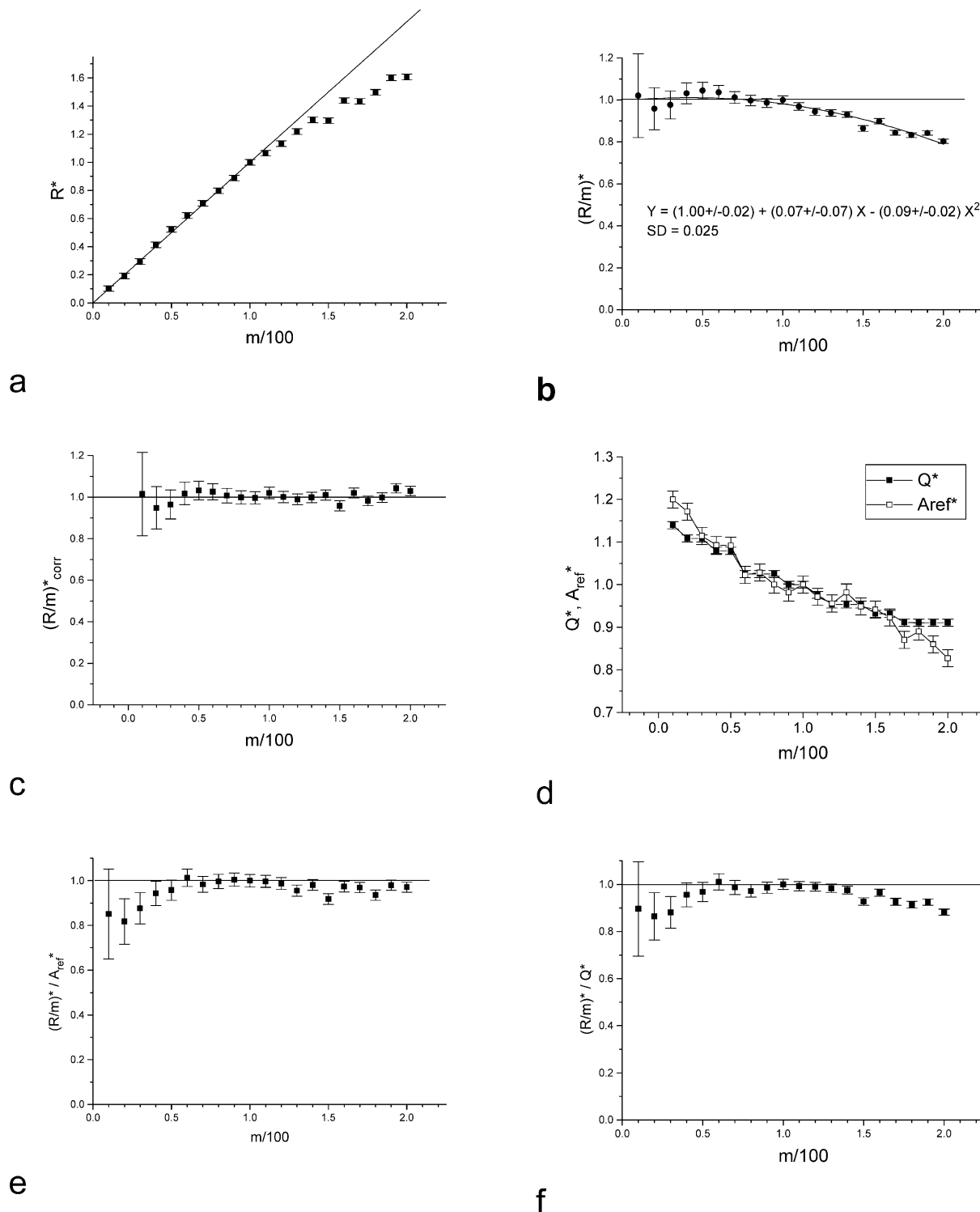
a tendency to increase for some power values. The reason of increasing of the uncertainty may be the same as for RS – effects of instability of the spectrometers becoming apparent at high accumulation time.

Averaging over four repeated measurements leads to reducing of the uncertainty of dose determination by a factor of about two. Increasing of the accumulation time by four times leads to reducing of the uncertainty by not more than 30%. Therefore, at given total time of measurement it is advantageously to take average of dose determination from multiple repeated spectra measurements following sample shaking and sample tube rotation than to use single spectra measurement with long accumulation time.

Dependencies of SDS and SDR on the microwave power determined at different accumulation times are presented in Fig. 6a. It is hardly to find out a distinct minimum in these dependencies. However, in dependencies averaged over different accumulation times (Fig. 6b), a vague minimum is observed at 2 mW. This minimum is not well pronounced; nevertheless, presence of this minimum together with observed minimum in the power dependencies of RS converted to dose units (Fig. 3c) gives priority to the value of 2 mW to be selected as the optimal power giving the least uncertainty of dose determination.

Effects of sample mass on sensitivity

For determining dependence of the spectrometer's sensitivity on the sample mass, spectra measurements of tooth enamel with different sample mass irradiated in 100 Gy dose were performed. The intensities of RIS and of the Mn^{2+} reference signal were determined as their peak-to-peak amplitudes. The uncertainties of RIS of the reference signal amplitudes are estimated from multiple repeated measurements and accepted to be 2% of these signal amplitudes at 100 mg sample mass. Q-factor was determined from the readings of

Fig. 5. Dependencies of the standard deviation of the experimental doses from the nominal doses for single measurement (SDS) and averaged over four repeated measurements (SDR) on the accumulation time determined at different microwave power. The power values are presented on the plot panel.

Fig. 6. Dependencies of the standard deviation of the experimental doses from the nominal doses on the microwave power determined at different accumulation times: (a) – for single measurement (SDS) and averaged over four repeated measurements (SDR). The accumulation time walues are presented on the plot panel. (b) – Dependensies of SDS and SDR on the microwave power averaged over different accumulation times. Error bars indicate uncertainties of the average values.

Fig. 7. Dependencies of parameters characterising sensitivity of the spectrometer on the sample mass normalized by theirs values at sample mass of 100 mg ($m/100$): (a) – the RIS amplitude (R^*); (b) – the RIS amplitude normalized by the sample mass ($(R/m)^*$, the relative sensitivity); (c) – the relative sensitivity normalized by the correction function ($(R/m)^*_{corr}$); (d) – the quality factor (curve denoted by Q^*) and the reference sample signal amplitude (A_{ref}); (e) – the relative sensitivity normalized by the reference signal amplitude ($(R/m)^* / A_{ref}^*$); (f) the relative sensitivity normalized by the quality factor – ($(R/m)^* / Q^*$)

the spectrometer.

Dependence of the signal intensity on the sample mass is presented in Fig 7a. In the range up to 100 mg it is linear within 1.5%. At higher sample mass, declination from linearity is observed up to 6% at sample mass of 130 mg and up to 25% at sample mass of 200 mg. The sensitivity defined as the signal amplitude divided by the sample mass and normalized by its value at 100 mg is presented in dependence on the sample mass in Fig. 7b. This dependence can be described by a function, which is presented in the panel of Fig. 7b. Result of correction of the sensitivity using this function is presented in Fig. 7c. Dependencies of the reference signal amplitude and of the *Q*-factor are presented in Fig. 7d. Results of correction of the sensitivity using normalizing by the reference signal amplitude and Q-factor are presented in Fig 7e and Fig. 7f respectively.

In the range of sample mass up to 100 mg, the effect of the sample mass on the sensitivity is negligible and it is within the uncertainty of sensitivity determination. At higher sample mass, normalizing of the sensitivity by the experimental correction function completely compensates the effect of sample mass on sensitivity within the uncertainty of its determination. Normalizing of the sensitivity by the reference signal amplitude provides satisfactory results with variation of the corrected sensitivity in the range of sample mass from 40 mg to 200 mg. Reduction of the corrected sensitivity at small sample mass is probably due to effects of sample geometry and local disturbance of microwave field in the cavity by material of the sample tube. Normalizing by the *Q*-factor provide satisfactory results in the smaller range from 40 mg to 140 mg. The probable reason of reducing of the range of correction is nonlinear dependence of the sensitivity on this parameter. However, the reference signal and Q-factor can be used for correction of the sensitivity in the full mass range if to derive appropriate correction functions.

CONCLUSIONS

Direct measurements of uncertainty of experimental dose determination in enamel from EPR spectra measured by the JEOL JES-FA100 spectrometer at different spectra accumulation time and microwave power are performed. Minimal uncertainty was found to be at microwave power of about 2–5 mW. Increasing the spectra accumulation time over some value of about 20–40 min does not leads to reducing the uncertainty probably because of time instability of the spectrometer. At a limited total time of measurement, it is advantageously to use average of dose determination results obtained from repeatedly measured spectra at reduced accumulation time.

Effect of the sample mass on the spectrometer's sensitivity is also investigated. At increasing sample mass, reduction of the sensitivity occurs, which does not exceed 1.5%, 6% and 25% in the sample mass range from 10 mg up to 100,

130 and 200 mg respectively. Correction of the sensitivity at high sample mass can be performed with the use of an experimental correction function or dividing by the reference sample signal amplitude.

ACKNOWLEDGEMENTS

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