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Advancements in accuracy of the alanine dosimetry system. Part 1. The effects of environmental humidity

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Abstract

A one-year study of the EPR signal of γ-irradiated (^{60}Co) L-α-alanine with simultaneous monitoring of the cavity *Q*-factor was undertaken. The widespread opinion that the EPR signal remains absolutely stable under normal laboratory storage conditions is inaccurate. At 0% humidity, the signal can be regarded as stable within ±1% of its initial value for 6 months for 1 and 10 kGy doses, but for only 3 months for 100 kGy. When stored at the same relative humidity values up to 60%, the fading rates for dosimeters irradiated to 1 and 10 kGy are similar, whereas signals of dosimeters irradiated to 100 kGy fade considerably faster for all humidities. The rates of fading increase with the relative humidity, especially above 60% R. H. Environmental humidity also deteriorates the accuracy of alanine dosimetry by changing the resonant cavity *Q*-factor. This is particularly important when irradiated alanine dosimeters are used as instrument calibration standards. Short-term changes in alanine EPR signal amplitudes were recorded upon removal of the irradiated dosimeters from their storage environments. The importance of an in situ standard to correct for measurement errors due to environmental effects is demonstrated. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Alanine; EPR dosimetry; Humidity; Fading

1. Introduction

Changes in the radiation-induced electron paramagnetic resonance (EPR) signal between the irradiation and subsequent EPR measurements are an important factor directly affecting the accuracy of alanine-EPR dosimetry. It is well known that radiation-induced radicals in alanine are far more stable than the free radical species produced by ionizing radiation in most other organic substances; this is the primary reason for alanine being the most commonly used material for high-dose EPR dosimetry. However, the stability of the alanine radicals is not absolute, and they are known to decay at elevated temperatures and higher humidity. Over the past two decades, many sources of inaccuracies in alanine dosimetry have been successfully eliminated such that errors even below even 1% are significant. Therefore, EPR dosimetrists now need very precise information on the stability of alanine radicals under normal environmental conditions of dosimetry.

Previous published studies addressing EPR signal stability of irradiated alanine are summarized in Tables 1 and 2. The prevailing opinion is that the degree of fading for the alanine signal, under normal laboratory conditions, remains below 1% for at least 1 year. Unfortunately, the studies claiming high stability generally provide neither enough original numeric

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Table 1
Reported results of the studies of alanine signal fading at room temperature: Experiments with controlled humidity conditions

Author/Year	Dosimeter characteristics	Dose (kGy)[a]	Relative humidity (%)	Period of observation (month)	**Fading (%)**	Number of measured sessions	Uncertainty (%)[b]	Reference sample used	Comments
Deffner and Regulla (1980)	?-Ala + paraffin (? + ?); D = 5 mm, h = 7.5 mm	1–100	40–60	1	**~0**[c]	NA[d]	NA	NA	
		1–5	70	1	**0**	NA	NA	NA	
		20	70	1	**2**	NA	NA	NA	
		100	70	1	**5**	NA	NA	NA	
		1–5	90–100	1	**3**	NA	NA	NA	
		20	90–100	1	**4**	NA	NA	NA	
		100	90–100	1	**7**	NA	NA	NA	
Regulla and Deffner (1982)	L-Ala + paraffin (90 + 10); D = 4.9 mm, h = 10 mm	1	40–60	1	**~0**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		5	40–60	1	**~0**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		25	40–60	1	**~0**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		40	40–60	1	**~0**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		100	40–60	1	**~0**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		1	70	1	**~0**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		5	70	1	**0.5**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		25	70	1	**2**	5	3–5	Alanine pellet, checks before and after each series of measurements	

Table 1 (*continued*)

Author/Year	Dosimeter characteristics	Dose (kGy)[a]	Relative humidity (%)	Period of observation (month)	**Fading (%)**	Number of measured sessions	Uncertainty (%)[b]	Reference sample used	Comments
		40	70	1	**3**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		100	70	1	**6**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		1	90	1	**2**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		5	90	1	**3**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		25	90	1	**5**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		40	90	1	**6**	5	3–5	Alanine pellet, checks before and after each series of measurements	
		100	90	1	**8**	5	3–5		
		NA	Below room humidity	24	**< 1**	NA	NA	Alanine pellet, checks before and after each series of measurements	Stored at 6°C
Regulla and Deffner (1985)	L-α-Ala + paraffin (90 + 10); D = 4.9 mm, h = 10 mm	NA	40–60	36	**< 1**	NA	NA	Alanine pellet, once a day	
Kojima and Tanaka (1989)	DL-Ala + polystyrene (70 + 30); D = 3 mm, h = 30 mm	NA	60	NA	**"very stable"**	NA	NA	NA	
Kojima et al. (1992)	DL-α-Ala + polystyrene (70 + 30); D = 3 mm, h = 30 mm	1.4	50	5.3	**~ 0**	NA	NA	Alanine pellet, before and after measurement series	
		14	50	3.3	**< 2**	NA	NA	Alanine pellet, before and after measurement series	

(continued on next page)

Table 1 (*continued*)

Author/Year	Dosimeter characteristics	Dose (kGy)[a]	Relative humidity (%)	Period of observation (month)	**Fading (%)**	Number of measured sessions	Uncertainty (%)[b]	Reference sample used	Comments
		100	50	0.2	**1**	NA	NA	Alanine pellet, before and after measurement series	
		100	50	5.3	**6**	NA	NA	Alanine pellet, before and after measurement series	
Arber and Sharpe (1993)	L-Ala + paraffin (90 + 10); $D = 5$ mm, $h = 2.5$ mm	1	0	3.3	**0**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		1	40	3.3	**2.3**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		1	60	3.3	**1.7**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		1	80	3.3	**3.6**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		10	0	3.3	**(0.4)**	10	0.3–0.5	NA	Preconditioned at the same humidity before irradiation for 1 week
		10	40	3.3	**1.7**	10	1.0–1.3	NA	Preconditioned at the same humidity before irradiation for 1 week
		10	60	3.3	**1.7**	10	0.3–0.6	NA	Preconditioned at the same humidity before irradiation for 1 week
		10	80	3.3	**2.2**	10	1.0–1.3	NA	Preconditioned at the same humidity before irradiation for 1 week

Table 1 (*continued*)

Author/Year	Dosimeter characteristics	Dose (kGy)[a]	Relative humidity (%)	Period of observation (month)	**Fading (%)**	Number of measured sessions	Uncertainty (%)[b]	Reference sample used	Comments
		40[e]	0	3.3	**(0.1)**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		40	40	3.3	**1.5**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		40	60	3.3	**1.6**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		40	80	3.3	**2.9**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 1 week
		1	0	3.3	**(0.1)**[f]	NA	NA	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		1	40	3.3	**0.8**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		1	60	3.3	**0.7**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		1	80	3.3	**2.3**	NA	NA	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		10	0	3.3	**0.3**	10	0.5–1	NA	Preconditioned at the same humidity before irradiation for 4 weeks

(continued on next page)

Table 1 (*continued*)

Author/Year	Dosimeter characteristics	Dose (kGy)[a]	Relative humidity (%)	Period of observation (month)	**Fading (%)**	Number of measured sessions	Uncertainty (%)[b]	Reference sample used	Comments
		10	40	3.3	**0.9**	10	0.5–1	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		10	60	3.3	**0.6**	10	0.5–0.7	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		10	80	3.3	**1.4**	10	0.5–1	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		40	40	3.3	**1.1**	10	0.5–1	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		40	60	3.3	**1.6**	10	0.5–1	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		40	80	3.3	**1.9**	10	0.5–1	NA	Preconditioned at the same humidity before irradiation for 4 weeks
		1	0	3.3	**0.1**	10		NA	Preconditioned at the same humidity before irradiation for 8 weeks
		1	40	3.3	**(0.4)**	10		NA	Preconditioned at the same humidity before irradiation for 8 weeks
		1	60	3.3	**(0.1)**	10		NA	Preconditioned at the same humidity before irradiation for 8 weeks

Table 1 (*continued*)

Author/Year	Dosimeter characteristics	Dose (kGy)[a]	Relative humidity (%)	Period of observation (month)	**Fading (%)**	Number of measured sessions	Uncertainty (%)[b]	Reference sample used	Comments
		1	80	3.3	**(1.1)**	10		NA	Preconditioned at the same humidity before irradiation for 8 weeks
		10	0	3.3	**(0.4)**	10	0.8–1	NA	Preconditioned at the same humidity before irradiation for 8 weeks
		10	40	3.3	**0.2**	10	0.2–0.3	NA	Preconditioned at the same humidity before irradiation for 8 weeks
		10	60	3.3	**0.6**	10	0.2–0.5	NA	Preconditioned at the same humidity before irradiation for 8 weeks
		10	80	3.3	**1.5**	10	0.5–1.0	NA	Preconditioned at the same humidity before irradiation for 8 weeks
		40	0	3.3	**(0.5)**	10	0.5–1.0	NA	Preconditioned at the same humidity before irradiation for 8 weeks
		40	40	3.3	**0.2**	10	0.5–1.0	NA	Preconditioned at the same humidity before irradiation for 8 weeks
		40	60	3.3	**(0.4)**	10	0.5–1.0	NA	Preconditioned at the same humidity before irradiation for 8 weeks
		40	80	3.3	**1.9**	10	0.5–1.0	NA	Preconditioned at the same humidity before irradiation for 8 weeks

(continued on next page)

Table 1 (*continued*)

Author/Year	Dosimeter characteristics	Dose (kGy)[a]	Relative humidity (%)	Period of observation (month)	**Fading (%)**	Number of measured sessions	Uncertainty (%)[b]	Reference sample used	Comments
Wieser et al. (1993)	L-α-Ala + paraffin (85 + 15); D = 4.9 mm, h = 10 mm	0.01	50	1.6	**~0**	7	1	NA	
		0.01	80	1.6	**13**	7	1	NA	30°C
Juncheng et al. (1996)	L-α-Ala + paraffin (? + ?), rods; D = 4.5 mm, L = 10 mm	0.1	12	6.6	**0**	NA	NA	Alanine rod	
			33	6.6	**0**	NA	NA	Alanine rod	
			58	6.6	**3**	NA	NA	Alanine rod	
			76	2.5	**84**	NA	NA	Alanine rod	
			98	0.3	**>98**	NA	NA	Alanine rod	
Kojima et al. (1997) (S♯2000)	DL-α-Ala + polystyrene (50 + 50); D = 3 mm, h = 20 mm	0.1–100	40	2	**< 1**	NA	NA	NA	
	DL-α-Ala + polystyrene (50 + 50); D = 3 mm, h = 20 mm	1	40	12	**< 1**	NA	NA	NA	

[a] $^{60}\gamma$-Co gamma rays are assumed unless specified otherwise in the "Comments" column.

[b] In many cases, values in this column were determined from plots in small figures and, thus, should be regarded as very rough estimates.

[c] Signal changes are within uncertainties of the experiment.

[d] NA = no explicit information on this point is available in the publication.

[e] Values of fading for 40 kGy in this study are approximate because of the nonlinearity of the calibration plot in this range used for their determination. The error due to non-linearity is likely to be very small as compared to other uncertainties.

[f] Values in parentheses represent increase in the signal with respect to the initial value.

data, nor sufficient experimental details to support the validity of this statement. Indeed, it is difficult to monitor reliably small changes in alanine signal amplitudes over extended periods of time. The necessary long-term standardization or control of the EPR spectrometer sensitivity with a high accuracy is a formidable problem in itself. Yet, as can be seen from the tables, most of the authors do not mention any reference samples. Many who monitor the sensitivity with a reference sample use an irradiated alanine dosimeter for this purpose; in principle the signal of such a sample is prone to the same changes as signals to be monitored. Even those rare EPR spectrometrists who employed a non-alanine reference sample almost invariably did not record the reference signal with the alanine dosimeter *simultaneously* present in the cavity, and, thus, did not take into account variations in the cavity Q-factor. This parameter is particularly important because of the varying moisture content of the dosimeters. The difficulties in evaluating the reliability of previous studies claiming extremely high signal stability are compounded by the relatively small number of measurements taken and the considerable scatter of data points in the published plots.

Our routine experience has suggested that the fading rate of alanine radicals under "normal laboratory conditions" is actually somewhat higher than has been commonly reported. This prompted us to undertake a new investigation of the problem with special attention to several important experimental details and the acquisition of a large number of measurements sufficient for statistically significant conclusions.

2. Experimental[1]

2.1. Controlled humidity environments

The humidity in an enclosed volume above a saturated aqueous solution of a particular salt in a closed space depends only on temperature (Rockland, 1960); this principle is used to create environments with standardized humidities. An important advantage of such three-phase systems over the two-phase ones, such as sulfuric acid or glycerol, is the independence of the relative humidity produced from moisture being absorbed or evolved by substances stored in the chamber. Of the numerous salts recommended for this purpose, we selected those exhibiting the weakest temperature dependence in the typical room temperature range (Table 3). Because the temperature in our laboratory remained within the 22–25°C range, these systems reliably provided constant relative humidities (R. H.) accurate to $\pm 1\%$. Polycarbonate desiccators (Nalgene) were used as controlled humidity chambers. A saturated solution with some amount of the undissolved salt was located on the vessel bottom, while alanine dosimeters in open glass vessels were stored on the perforated plate. As the volume of the desiccator was only about 1 L, while the saturated solution surface area was about 100 cm^2, there was no need for special means to eliminate humidity gradients. An additional desiccator with anhydrous P_2O_5 was used to provide a 0% relative humidity environment. Between measurement sessions, the desiccators were kept sealed with a silicon grease, and special attention was paid to keeping the solutions saturated and the phosphorus pentoxide powder dry at all times. To reduce the chance of any photochemical effect, the desiccators with alanine pellets were stored in the dark.

2.2. Dosimeters

In most of the measurements described in this paper, commercially available Bruker alanine dosimeters (Bruker Instruments: Batch No. 3) have been used. They contain 80% L-α-alanine and 20% polyethylene and are 4.9 mm in diameter and 5 mm in height. The dosimeter masses range from 84 to 87 mg. To simulate the situation most frequently occurring in practice at present, the dosimeters were used "as delivered," without any preconditioning additional to that performed by the manufacturer (Bruker Instruments: 55% R. H.; 24°C; 3 months). The dosimeters were irradiated with ^{60}Coγ-rays to absorbed doses of 1, 10, and 100 kGy in a Gammacell (Nordion) with a dose rate of 9.5 kGy/h under electron equilibrium conditions and controlled temperature (22–24°C). The first signal measurements were performed 2–5 h after the irradiation. The dosimeters were then placed into the controlled humidity chambers. Three replicate dosimeters were monitored simultaneously for each humidity–dose combination. Before each measurement, the pellets were removed from the chamber and stored in the open air in a well-ventilated laboratory for about 1 h. This period was sufficient for obtaining a stable, highly reproducible signal for all of the humidities studied (this effect is explained in Section 4.1).

Some preliminary experiments mentioned below were performed with L-α-alanine (Aldrich, 99 + %) pellets manufactured at NIST. They contain 10% polyethylene (Polysciences, MW = 700, 60 μm) as a binder and are 4.9 mm in diameter and 2.7 mm in height. They were irradiated as described above and kept

[1] The mention of commercial products throughout this paper does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that products identified are necessarily the best available for this purpose.

Table 2
Reported results of the studies of alanine signal fading at room temperature: experiments with uncontrolled humidity conditions (See footnotes to Table 1)

Author/Year	Dosimeter characteristics	Dose (kGy)	Observation period (months)	**Fading (%)**	Number of measurement sessions	Uncertainty (%)	Reference sample	Comments
Randolph and Parrish (1959)	?-α-Ala powder	170	6	**< 20**	NA	NA	NA	
Bradshaw et al. (1962)	?-α-Ala powder	0.01–1	0.5	**~0**	NA	5	Coal sample, before and after each ala sample	
		1	3	**5**	NA	5	Coal sample, before and after each ala sample	
Bermann et al. (1971)	L-α-Ala powder	0.762	24	**~0**	9	2–3	NA	
Hansen and Olsen (1985)	L-α-Ala + cellulose (95+5); D = 4.5 mm, h = 2 mm	< 10	18	**~0**	NA	NA	NA	
		500	NA	**Significant**		NA	NA	
Kojima et al. (1986)	DL-α-Ala + EPR (77+33), D = 3 mm; h = 30 mm; 2 × 3 × 30 mm	1	48	**2**	Calculated	NA	NA	Calculated from the rate of signal fading at 80–120°C; no mention of controlled humidity conditions
Hansen et al. (1987)	L-α-Ala + polyvidone (95+5); D = 4.5 mm; h = 2 mm	< 10	5.5	**< 0.5**	NA	NA	NA	
		700	5.5	**12**	NA	NA	NA	10 MeV electrons
		500	5.5	**16**	NA	NA	NA	16 MeV protons
		1000	5.5	**22**	NA	NA	NA	Stopping 21 MeV ^{7}Li ions
Olsen et al. (1989)	?-α-Ala + polyvidone (95+5); D = 4.5 mm; h = 2 mm	0.01–0.1	12	**~0**	NA	1	DPPH, alanine, daily checks	
		0.1	48	**1.8**	NA	NA	DPPH, alanine, daily checks	
Panta et al. (1989)	L-α-Ala powder?	$0.01–10^{3}$	36	**~0**	NA	5	Mn(2+) in MgO, adj.	
Kojima and Tanaka (1989)	DL-α-Ala + polysterene (70+30); D = 3 mm, h = 30 mm	NA	NA	**"very stable"**	NA	NA	NA	

Table 2 (*continued*)

Author/Year	Dosimeter characteristics	Dose (kGy)	Observation period (months)	**Fading (%)**	Number of measurement sessions	Uncertainty (%)	Reference sample	Comments
Hansen and Olsen (1989)	L-α-Ala + polyvidone (95 + 5); $D = 4.5$ mm, $h = 2$ mm	"low dose"	1.4	**< 1**	2	2.6	Alanine pellet, once a session	
Len (1994)	?-α-Ala + polyvidone (95 + 5)	0.1, 10 kGy	2	**~ 0**	NA	NA	NA	
Mehta (1996)	DL-α-Ala + polystyrene, (70 + 30), $D = 3$ mm, $h = 30$ mm	15	8	**1–1.4**	20	0.2–0.3	NA	Preconditioned at 50% RH, 3 irradiation temperatures (15, 27.5, 40°C)
	DL-α-Ala + polystyrene, (70 + 30), $D = 3$ mm, $h = 30$ mm	45	8	**1–1.4**	20		NA	

under ordinary laboratory conditions (20–60% R. H., 22–25°C), unless specified otherwise.

2.3. EPR spectrometry

All measurements with Bruker pellets have been performed on an ESC106 spectrometer (Bruker Instruments) with a 4103TM cavity at room temperature. The modulation frequency was 50 kHz. The microwave power was 0.25 mW, the highest within the linear range of the signal amplitude dependence on the square root of the microwave power, and the sweep rate was low enough to make the passage between the derivative extreme longer than 10 time constants. A 1.8-mT central portion of the alanine spectrum was measured.

The EPR spectrum of each dosimeter was measured at two dosimeter orientations differing by approximately 90° with respect to Zeeman field. A high-precision tube, with an inner diameter (5.0 mm) that closely matched the diameter of the dosimeters (4.9 mm and an inner fused-quartz pedestal) was permanently mounted in the cavity. This sample tube provided reproducible positioning of the dosimeters in the cavity; the dosimeters were inserted and removed with a pneumatic manipulator. The sample tube remained in the cavity for the whole duration of the study.

To account for variations in the cavity Q-factor, which are inevitable in this kind of experiment, an adjacent ruby reference sample was permanently present in the cavity. It was inserted through the slot for the illumination grid in the front wall of the cavity. This design (courtesy of Bruker Instruments) made it possible to independently adjust the depth of the ruby crystal insertion into the cavity and the ruby crystal orientation with respect to Zeeman field, which allowed both the positions of Cr(3+) spectral lines and their intensity to be easily changed. The position and orientation of the ruby crystal selected in the beginning of the study remained unchanged during the whole series of measurements. The selected ruby line was automatically recorded immediately after recording the central line of the alanine spectrum of each dosimeter at each of its two orientations (settings for the critical operational parameters of the spectrometer remained the same). All alanine signal amplitudes measured in this study have been normalized to the amplitudes of the ruby line recorded with the alanine dosimeters at the selected orientations. The ruby-normalized signals have been further normalized to the initial individual masses of the pellets, and the reported values are an average of three pellets for each dose–humidity combination (in preparing the plots, the signal intensities have been further normalized to make all their initial values equal to 100%). The alanine signal of each irradiated dosimeter was measured once or

Table 3
Relative humidity variations with temperature of saturated salt solutions used in the study (Rockland, 1960)

Salt	Relative humidity (%C)			
	15°C	20°C	25°C	30°C
Magnesium chloride	33	33	33	32
Potassium carbonate	45	44	43	42
Sodium bromide	58	57	57	57
Sodium chloride	75	75	75	75
Potassium nitrate	95	94	93	92

twice every week during the period of observation. The measurement of uncertainty is 0.8%.

3. Results

Fig. 1(A–F) shows long-term changes of the EPR signal of alanine dosimeters irradiated to 1, 10, and 100 kGy and stored in the dark at various constant humidities. For pellets stored at low and moderate humidities (Fig. 1A–D), the signal amplitude slightly increases during the first days after irradiation, and then begins to decrease steadily. The initial increase in amplitude of approximately 0.5% has been previously characterized by Nagy and Desrosiers (1996). The weak initial rise in signal, however, is not observed in Figs. 1E and F due to the strong fading (up to 8% at 96 R. H.) induced at high relative humidities.

The fading is relatively slow at low and moderate humidities. After a 3-month storage period under "normal" humidities 33–44%, the signal amplitudes deviate from their *maximal* observed values by 1.3, 1.7, and ~2.5% for 1, 10, and 100 kGy, respectively. If one compares the observed normalized amplitudes with the amplitudes of not the *maximal*, but the *initial* signals, the decrease in 3 months is below 1% for 1 and 10 kGy, and about 2–2.5% for 100 kGy. At the end of a 1-year storage, the amplitudes for these doses decline by 4.6, 5.2, and 6.2% of their maximal values, respectively.

Fading rate increases with relative humidity especially at R. H. greater than 60%. At 94% R. H., three months after 1 and 10 kGy irradiation, the EPR signal amplitudes decrease by 13–14% and 18% for 100 kGy. At the end of one year, the decrease is about 20% for 1 and 10 kGy and as large as 25% for 100 kGy.

Even for pellets stored under anhydrous phosphorus pentoxide, which is one of the strongest dehydrating reagents known (essentially 0% humidity), the signal decrease with respect to the *maximal* value can be regarded as statistically insignificant for 3 months at 1 and 10 kGy, and only 1–2 months for pellets irradiated to 100 kGy. After one year, the decrease in signal is about 2% of the *initial* values for doses of 1 and 10 kGy, and 3.5% for 100 kGy.

Fig. 2 presents the same data in a way convenient for observing the effect of the dose at a constant humidity. Also, the expanded scale provides an opportunity to evaluate signal values at various stages of the storage with a higher precision. Generally, the difference between the signals of dosimeters irradiated to 1 and 10 kGy is small (in most cases less than 1%) and is probably statistically insignificant.

4. Discussion

Our study of the effect of humidity on the alanine signal differ from other researchers not only in the number of measurements, but, more importantly, in the use of a ruby crystal as a reference sample permanently present in the cavity. The "external" reference samples used by others, such as an irradiated alanine dosimeter or "strong pitch" measured before and after each measurement series, attempt to monitor and correct for uncontrolled spectrometer sensitivity variations. Even in this capacity they are far from being perfect: significant changes in the spectrometer sensitivity during a measurement session, which are comparable with the small signal changes due to the radical decay, are commonly observed. Most important, this type of reference technique provides absolutely no information about the Q-factors of the cavities loaded with dosimeters, which depend on the amount of polar substances, such as water, present in the cavity. The cavity Q-factor directly affects the amplitudes of recorded spectra; the same number of paramagnetic centers will produce different signal amplitudes at different Q-values, so much so that methods to determine moisture content of various materials have been proposed that are based on this effect (Kozlov et al., 1978). Clearly, this effect has to be properly taken into account in a study where measured samples are stored at different humidities, and water content of each pellet may change with time. The changes of Q-factor due to variations in water concentration in dosimeters are significant and must be characterized independently to measure changes in the alanine signal amplitude accurately due to radical decay. Fig. 3 shows the relative values of the ruby crystal signal from the fixed reference device and measured in the presence of dosimeters that have been stored at various humidities. For storage relative humidity ranging from 20 to 80%, variations in the cavity Q-factor due to the differences in water concentrations in dosimeters may reach 7%. Obviously, these dependencies cannot be ignored.

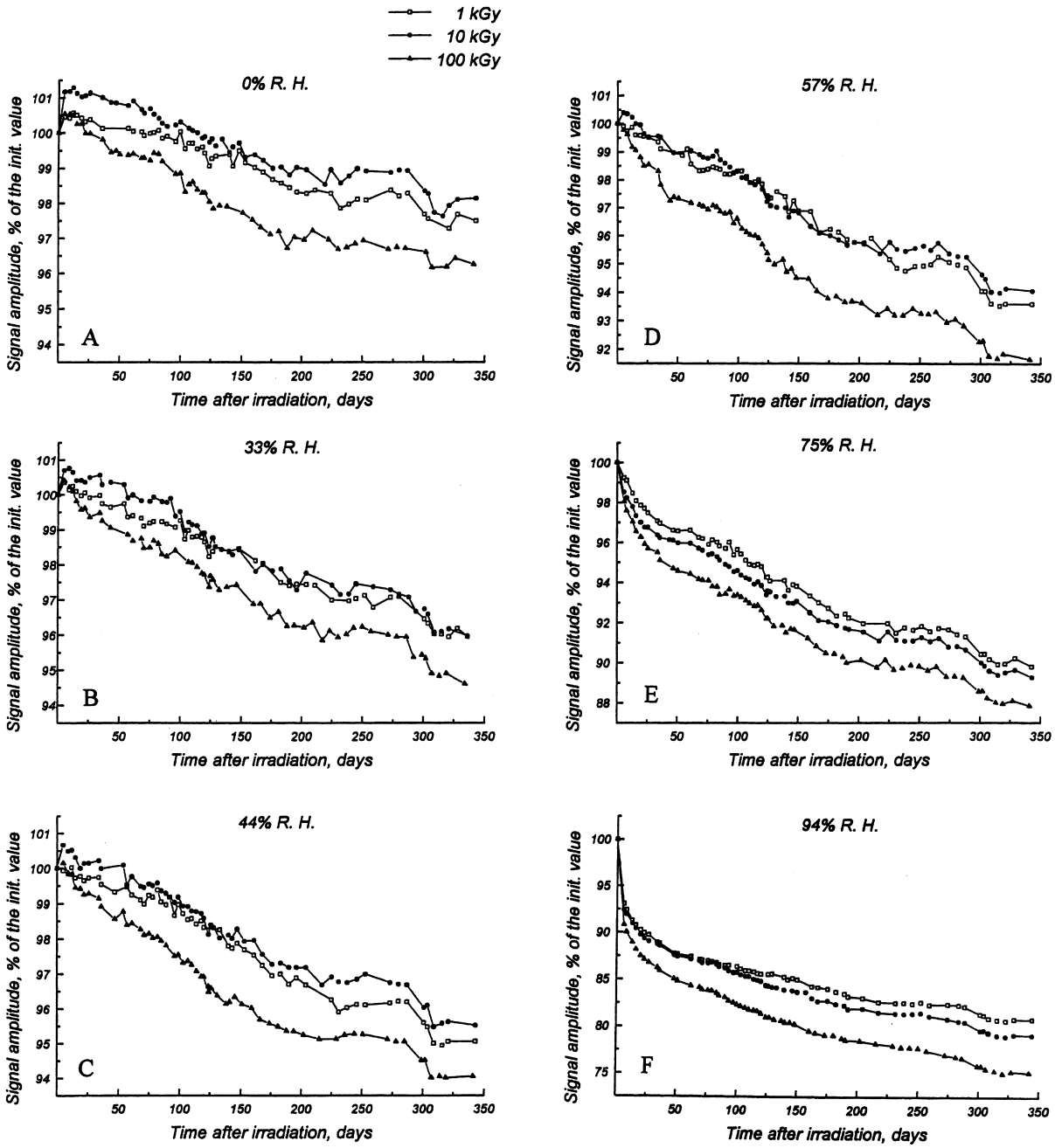

Fig. 1. Long-term time dependence of the EPR signal amplitudes of Bruker alanine dosimeters stored at different humidities.

A ruby crystal permanently present in the cavity alleviates this problem. Its signal is recorded with the alanine dosimeter in question present in the same cavity, and the dosimeter moisture content affects the signals of the radicals and Cr(3+) in ruby approximately to the same extent. Therefore, the alanine signal normalized to the ruby signal becomes independent of water concentration in the dosimeter.[2] Thus, the ruby

[2] This is true to the first, but yet, very good approximation. There are also effects of the microwave field perturbation, which make signal intensity not exactly proportional to Q-value (Nagy, 1994), but, in most cases, the deviations from proportionality are about 2 orders of magnitude smaller than the changes of signals due to Q-factor variations.

reference sample serves to reliably compensate for both the variations in the spectrometer sensitivity and the Q-factor variations that occur due to unpredictable varying concentration of water in the dosimeters. In contrast to irradiated-alanine reference samples, the Cr(3+) signal from ruby is known to be absolutely stable under normal laboratory conditions. Moreover, because the crystal is rigidly fixed, the signal is also free of the undesirable variations that are observed with alanine reference pellets due to the slight anisotropy of the alanine signals. Normalizing alanine signals under study to the Cr(3+) signal of a ruby crystal effectively converts them to a universal scale, making them free of most temporary influences and valid for intercomparison during very long periods of time. The specifics of our experiment, as well as the much larger body of measurements taken in this study on the effects of relative humidity, add considerable credence to the reliability of our data over those previously reported.

Close analysis of the literature shows that some of the earlier observations are in reasonable agreement with our data. In the cases of low and moderate humidities, the increase in the signal amplitude during the first days after irradiation is consistent with the results of our previous studies of the short-term behavior of the alanine signal (Nagy and Desrosiers, 1996); this effect was also observed in a study by Mehta (1996). The former study showed that, after a sharp decrease within the first 2 h after irradiation, the amplitude of the alanine signal begins to slowly increase, reaching a maximum within a few days. Although there are no explicit details on the timing of measurements in each publication, it is reasonable to suppose from the reported frequencies of measurements that, in many cases, the first measurement was taken on the first day after irradiation, while the next one occurred a considerable time later. This approach overlooks the period of maximum amplitude and the actual range of the signal variations turns out to be underestimated. If we relate the amplitudes of our signals observed over the time not to the *maximal*, but to the *initial* values that occurred on the days of irradiation, the decrease in 100 days for 1 and 10 kGy at 33 and 44% R. H. will be within 1%, which agrees with the results of Kojima et al. (1997), Len (1994), Hansen and Olsen (1989), Van Laere et al. (1989), Regulla and Deffner (1982), and Bradshaw et al. (1962).

The study performed by Arber and Sharpe (1993) differs from ours in that a good portion of its focus is on preconditioning and the effect of *changes* in storage humidities. Where comparable, our study is often in good agreement with the results of that work. For example, for doses of 1 and 10 kGy, Arber and Sharpe report similar or even bigger fading degrees for ambi-

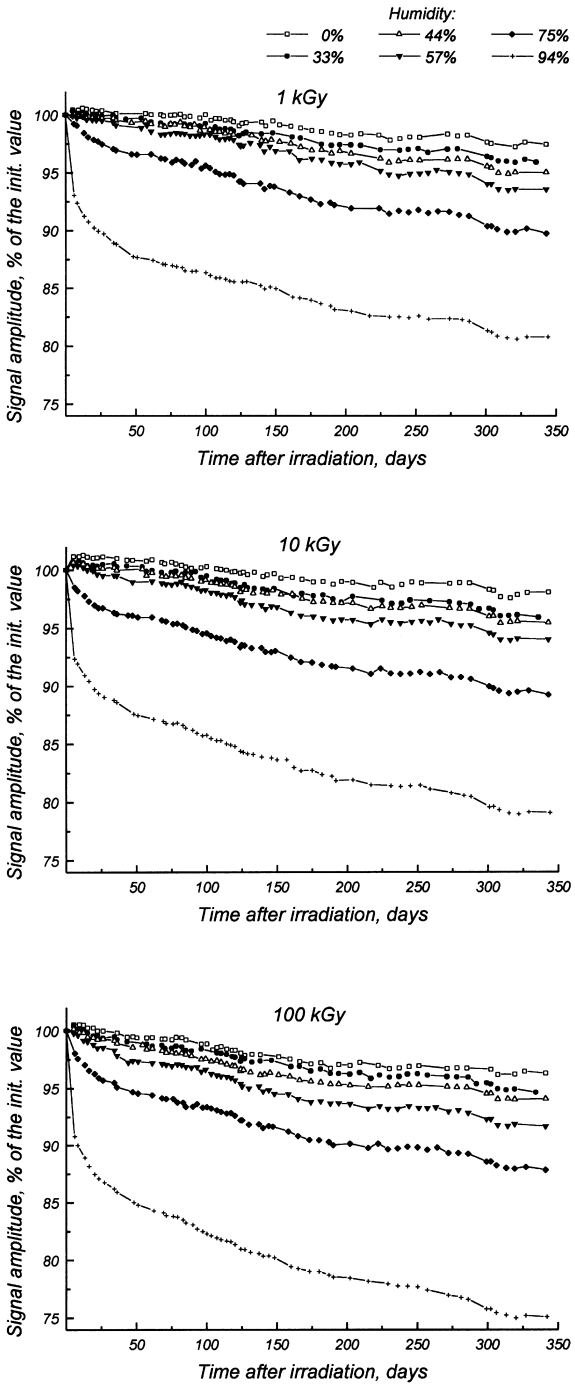

Fig. 2. Effect of the irradiation dose on the alanine signal fading at different humidities.

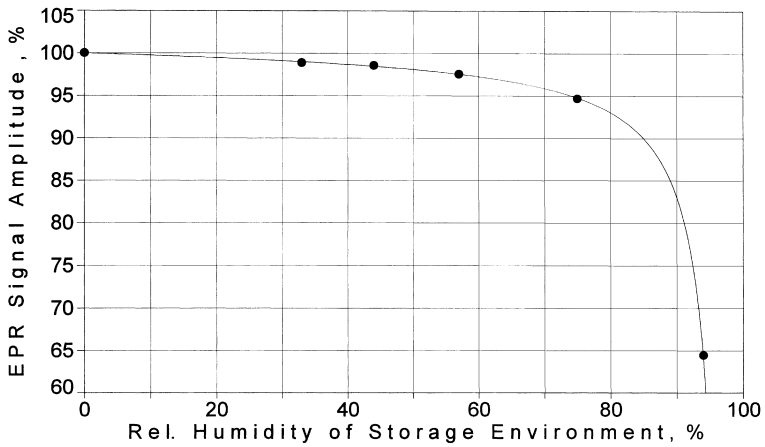

Fig. 3. Amplitudes of the EPR signal of a ruby reference sample permanently present in the cavity in the presence of alanine dosimeters, which have been stored at various constant humidities for about 1 year.

ent humidities (40 and 60%) in the case of 1-week (shortest used) preconditioning. They also point out that their results demonstrate a stronger fading rate than was previously believed. However, they measured smaller fading rates for high humidities than we observed. Unfortunately, a closer comparison is not possible because no adjacent reference sample was used in the published study to monitor cavity Q-factors. Also, data presentation in the form of apparent doses without a calibration curve hampers exact analysis.

Regulla and Deffner (1982) observed 5% fading over four weeks of storage at 70% R. H. for dosimeters irradiated to 100 kGy. Our data for the same dose and period of storage at 75% R. H. gives 4.6% fading. However, to our surprise, only slightly higher (6.5%) fading was reported by Regulla and Deffner at 90% R. H., whereas, according to our observations, the fading under the same conditions is greater by a factor of two. Kojima et al. (1992) reported 6% fading for DL-alanine dosimeters irradiated to 100 kGy and stored at 50% R. H. for 160 days; this is in very good agreement with our data for 57% R. H. (6%). Juncheng and Zaiyong (1996) reported 3% fading in 6.6 months for 0.1 kGy at 58% R. H., our data for 1 kGy and the same humidity and storage period show 4% fading. However, these authors reported fading much faster than ours for higher humidities and much slower than ours for the lower humidities.

It is often difficult to strictly compare our results, or results of others, with the previously published data due to the lack of experimental details in the publications, the small number of observations taken under conditions of considerable uncertainties, and differences in dosimeter composition. Some authors claim stability considerably higher than the uncertainty of their measurements.

Careless citations of earlier original papers also contribute to the common but erroneous belief in "absolute stability" of the alanine signal. For example, many authors cite one of the very first papers in alanine dosimetry (Regulla and Deffner, 1982) as a proof that the signal of alanine dosimeters remains constant within 1% over a two-year storage. However, the pellets in the study by Regulla and Deffner were stored at 6°C, which does not correspond to the "normal laboratory conditions." According to Van't Hoff's rule, a decrease in temperature by 20°C usually results in a 4- to 16-fold decrease in reaction rates. Also, the humidity in the refrigerator was presumably lower than typical laboratory conditions.

We also have some observations comparing NIST and Bruker alanine pellets stored under uncontrolled room humidity. These results are less accurate by definition, because no adjacent reference sample could be used in EMS104 EPR spectrometer to monitor the Q-factor and the storage humidity was not controlled. Nevertheless, the data are of interest since it is the only direct comparison of humidity effects on pellets of different brands and the measurement conditions are more similar to those encountered at industrial radiation facilities. These comparisons reveal that NIST dosimeters demonstrate a significantly (approximately two times) faster signal fading than Bruker dosimeters. Because NIST and Bruker pellets differ only slightly in composition (10% polyethylene in the NIST's pellets vs 20% in the Bruker's), this discrepancy in behavior is likely due to the difference in the pellet shape. Calculations show that NIST pellets have about 30% larger surface area per unit mass and, thus, a significantly more favorable condition for interface interactions than Bruker pellets. If this hypothesis is correct, dosimeter shapes that approximate a sphere (i.e., the shape with the smallest surface/volume ratio)

should have advantages in this respect over more extended shapes, especially long narrow rods and films, unless the alanine/binder proportions, binder type, or manufacturing processes are adjusted to compensate for shape differences. Another interesting observation from this preliminary study is that when pellets are removed from a humid environment and placed in a desiccator their signal fading slows down considerably (approximately ten times slower).

Though semiquantitative, these results demonstrate that the extensive data provided for Bruker dosimeters do not necessarily translate to dosimeters of other formulations.

All experiments performed in this study used L-α-alanine. It is known that, of the three optical isomers of α-alanine, this one has the highest activation energy of the free radical decay (Horan, 1968). Therefore, D-α-alanine and DL-α-alanine may actually exhibit an even faster decay than we observed.

4.1. Practical implications of the dependence of alanine signal intensity on the cavity Q-factor

The detrimental effect of cavity Q-factor variations on the alanine signal intensity is an important issue not only in special studies of long-term fading rates like the one described above, but also in routine practical dosimetry. Variations in Q-factor on the order of several percent are not easily noticeable during microwave bridge tuning, but they do produce errors of the same relative value in measured alanine signals. In most cases, Q variations result from the differing moisture content of alanine pellets, which, in turn, depends on the relative humidity of the storage environment and of the room where the EPR measurements are performed. To determine the doses correctly, it is important to make sure that either the cavity Q-factor actually remains constant during the whole series of measurements involving both calibration and test pellets, or proper corrections for its variations are applied.

This requirement is widely ignored in practice. Quite often in measurements, test pellets preconditioned at room humidity are used in combination with calibration pellets just removed from a desiccator, or doses to freshly irradiated test pellets are determined with a calibration curve constructed on a different day with a different relative humidity. If no reference sample is permanently present in the cavity, errors as large as 5% can occur.

Fig. 4 shows short-term changes in the amplitudes of EPR signals of alanine pellets measured immediately after removal from a controlled humidity environment after storage for months. The curves for absolute alanine amplitudes (square point symbols) reflect what most EPR alanine dosimetrists deal with in practice. In the case of pellets just removed from the 0% humidity desiccator, the alanine signal amplitude noticeably decreases for at least 5 h, and the decrease during the first two hours is particularly fast. The amplitude of the ruby signal decreases accordingly; these changes reflect the Q-factor decrease with time due to the process of absorbing humidity from the laboratory (about 30–40% R. H.). It is worth noting that even in the case of this moderate relative humidity in the laboratory the signal decreases approximately 1% in 2 h.

If storage humidity is relatively high (44%, 57%, 75%, and 94% R. H.), the absolute amplitude of the alanine signal increases during the first hours after entering the EPR laboratory environment; so does the amplitude of the ruby signal. This reflects the process of pellet drying in the cavity. The greater the initial moisture content of the pellet, the stronger is the signal increase in time. The rate of drying also increases with modulation amplitude in the 0.28–1.4 mT range, because higher modulation amplitudes provide higher temperatures of the pellets during the period of spectrum monitoring.

If the storage humidity (33%) is close to the laboratory humidity, the changes in alanine signal amplitude are very small.

It should be noted that normalization to the ruby amplitude keeps the alanine signal constant within 0.2% of its initial value regardless of these drying/wetting processes in all cases except at 94% R. H. In the latter case, the initial moisture content is so high that microwave field perturbation effects mentioned above are very pronounced. This results in a more noticeable deviation from strict proportionality between the alanine and ruby signal amplitudes, as mentioned earlier. In this extreme case, ruby normalization does not provide an absolutely perfect signal correction, but, nevertheless, it diminishes the error resulting from Q changes by as much as a factor of 20. However, the ruby correction works very well even at relatively high humidities (75% R. H.).

The magnitudes of the alanine signal changes shown in Fig. 4 and Table 4 agree well with the data of Fig.

Table 4
Percentage change in the alanine signal amplitude relative to the initial measurement

Time after removal (h)	Relative humidity (%)					
	0	33	44	57	75	94
0.5	−0.3	0.15	0.35	0.65	2.1	5.1
1	−0.7	0.2	0.4	0.8	3.3	10.1
2	−1.1	0.25	0.4	0.9	4.1	19.6

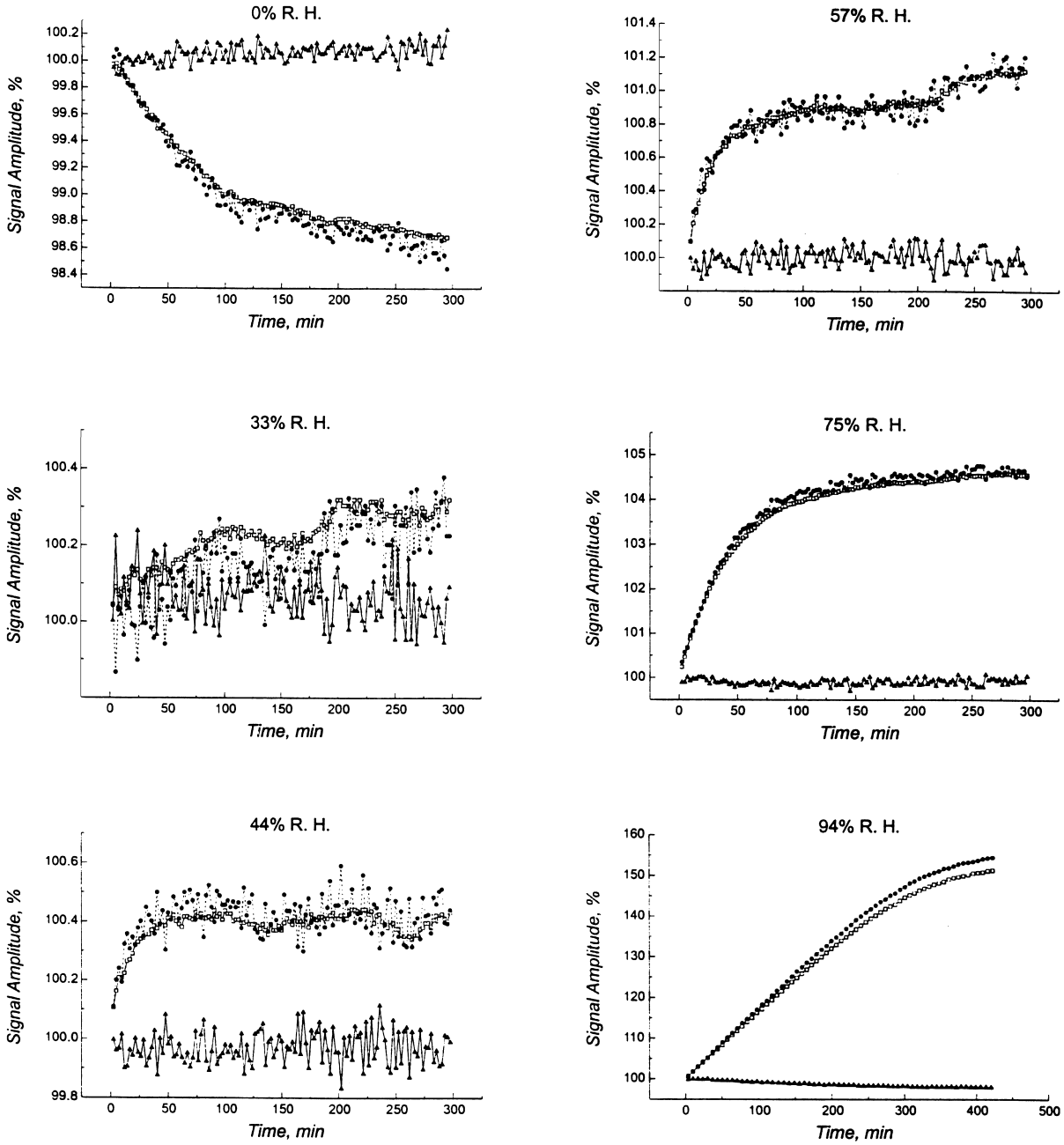

Fig. 4. Short-term time changes in EPR signal amplitudes of alanine (□), ruby (●), and the alanine-ruby signal ratio (▲) recorded after the removal from the storage environment.

3. In comparing them, the reader should bear in mind that signals in Fig. 3 are normalized to the signal under "absolutely dry" conditions, whereas signals in Fig. 4 are normalized to the signal at a specific storage humidity.

The data presented above clearly demonstrate that EPR dosimetrists should take the Q-factor value into account at all times during their measurements. If no adjacent reference sample, such as a ruby crystal, is used, one should ensure that the moisture content of all the pellets whose signals are to be compared is the same and stable. The following obvious guidelines should be followed.

- Test pellets that were preconditioned at an ambient

humidity before measurements should not be directly used in combination with calibration pellets just removed from a desiccator containing a strong dehydrating agent.
- Both the test and the calibration vials should contain pellets that were stored together or under identical conditions.
- Test pellets should have moisture content as close as possible to the calibration pellets on the days of measurements.
- Irradiated pellets should be preconditioned overnight for moisture content stabilization at the ambient relative humidity of the EPR measurement laboratory.
- Modulation amplitude should be kept small enough as not to cause heating sufficient to noticeably change the moisture content of pellets during measurement.
- One should not overly rely on numerical corrections for Q-factor changes derived from the experimental plots like those shown in Fig. 4. (Although these dependencies characterize the typical situations, we observed noticeable deviations from these patterns for some pellets. Ensuring identical experimental conditions is a more reliable approach than the introduction of correction factors.)

All these precautions are unnecessary if an adjacent reference sample is used. A convenient reference sample reliably fixed in the cavity, whose non-overlapping signal is recorded immediately before or after the alanine spectrum with the alanine dosimeter still hosted in the cavity and minimum number of parameters changed, solves this problem. A high-quality ruby crystal is particularly good for this purpose, because a convenient line position can easily be provided just by crystal reorientation, its signal shows no tendency to saturate at power levels used in alanine dosimetry, and the signal is very stable. Use of a dual cavity is less desirable than fixing a reference sample in a peripheral area of the conventional cavity, because the on and off switching of the modulation coils in two chambers keeps a dual cavity in permanent thermal instability. In conducting very precise measurements of alanine signal evolution with immobile samples (Nagy and Desrosiers, 1996), good reproducibility was not achievable with a dual cavity.

Arber and Sharpe (1993) concluded that the rate of the signal fading depends not on the storage humidity itself, but on its *changes*. They report that dosimeters preconditioned at certain humidity for months before irradiation exhibit much higher stability of the signal when stored at the same humidity after irradiation. This might be a very convenient way for handling the long-term fading problem, but it needs additional verification, as the measurement procedure used by those authors apparently did not involve monitoring the cavity Q-factor. Our data suggest that, in the absence of an adjacent reference sample, dosimeter preconditioning before EPR measurements is at least as important as dosimeter preconditioning before irradiation.

5. Conclusions

Our results show that the widespread opinion that the radiation-induced alanine signal is stable under normal laboratory conditions for years is incorrect. The changes in alanine signal are not large enough to compromise alanine dosimetry as a method, but they do require attention of dosimetrists. Even at 0% humidity the signal can be regarded as stable to within $\pm 1\%$ of its initial value for not longer than 0.5 year for doses of 1 and 10 kGy, and only for 3 months for 100 kGy. The rate of fading increases with the relative humidity, especially above 60%. Fading rates for 1 and 10 kGy at the same humidity are comparable, while signals of dosimeters irradiated to 100 kGy fade considerably faster. This pronounced fading should be taken into account in practice.

Fading characteristics of alanine dosimeters vary with the shape and possibly the composition of the dosimeters. Therefore, verification of these effects is recommended for users when dosimeters are from different sources.

Environmental humidity unfavorably affects the accuracy of EPR dosimetry not only by facilitating the radical decay, but also by uncontrollably affecting the cavity Q-factor. Variations in the cavity Q-factor are easily compensated when an appropriate reference sample is permanently mounted in the cavity. The absence of such a reference sample requires that the EPR dosimetrist implement special precautions and guidelines.

Noticeable fading of the EPR signal of irradiated alanine over extended periods of time along with the complications from the cavity Q-factor instability due to varying moisture content of the pellets make it undesirable to use "standard" irradiated alanine pellets for monitoring variations in sensitivity of EPR spectrometers.

It is the recommendation of these authors that the measurement practices described here should be used in place of those described in the current ASTM standard on alanine dosimetry (American Society for Testing and Materials, 1998).

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