Improved FBX chemical dosimeter system with enhanced radiochemical yield for reference dosimetry in radiobiology and radiotherapy research

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A B S T R A C T

Radiation dosimetry plays important role in the reproducibility of radiobiology experiments, in the replicability of results, as well as in the successful and safe use of radiotherapy procedures. The consistency and accuracy of the applied dosimetry methods pre-define the outcomes of these applications. This paper presents a version of the well-known ferrous sulphate – benzoic acid – xylenol orange (FBX) chemical dosimeter with improved sensitivity, accuracy and precision. Sensitivity is increased due to a slight modification in composition and the preparation procedures. We use stock solutions for the preparation of the dosimeter solution, which consists of 1 mM ferrous sulphate and 16 mM benzoic acid with 0.25 mM xylenol orange added post-irradiation. The nonlinear response to the absorbed dose of this system is eliminated by the increased ferrous sulphate concentration, permitting the calculation of the absorbed dose by a linear relationship between the absorbed dose and the optical absorbance of the solution. The measured chemical yield of our dosimeter is $9.08 \cdot 10^{-6}\ mol/J$ for 6 MV photon beams and $6.42 \cdot 10^{-6}\ mol/J$ for 250 kVp x-rays. This is a 24% enhancement over the original FBX solution, which permits a finer dose resolution. The accuracy and precision of our method is assured by a well-designed and consistently used practice. A custom designed multipurpose PMMA slab phantom was used for irradiation in reference conditions. This phantom can be used for irradiation in reference conditions of dosimetric solutions, dosimetric films and chemical or biological samples. The combined standard uncertainty of this system is 1.12%, which can be improved by using an appropriate temperature correction factor. Furthermore, a working protocol has been established which allows dosimetry measurements using less than 1 mL dosimetric solutions.

1. Introduction

Ferrous ammonium sulphate based chemical dosimeters are well known in dosimetry. Their most common form, known as the Fricke dosimeter system, is made from 1 mM ferrous ammonium sulphate, 1 mM sodium chloride and 0.4 M sulphuric acid, which can be used in the absorbed dose range from 20 Gy to 400 Gy. The ISO/ASTM51026-15 (2015) international standards recommend that the Fricke dosimetry system can be used as a reference standard dosimeter. However, due to time and technical constraints, it is not used in routine radiotherapy practice or radiobiological experiment dosimetry. These applications require a lower detection window with an upper dose limit of 20 Gy.

Ferrous ammonium sulphate – benzoic acid – xylenol orange (FBX) dosimeters belong to the group of ferrous sulphate based chemical dosimeters which can be used for low dose applications, up to 20 Gy. The original FBX chemical dosimeter was developed by Gupta, B.L. (1970) and is made from 0.2 mM ferrous ammonium sulphate, 5.0 mM benzoic acid and 0.2 mM xylenol orange in 25 mM sulphuric acid. In this system benzoic acid increases the radiolytical oxidation of Fe^{2+} ions, which subsequently bind to the xylenol orange dye molecules and form a complex with an absorption line around 540 nm. This line can be subsequently detected and measured.

The radiation chemistry of this system has been studied in detail by Geisselsoder et al. (1963); Gupta et al. (1978); Gupta and Nilekani (1998); Gupta et al. (1997); Gupta (1989); Gupta et al. (1983); Jia-Shan et al. (1982). Its chemical yield has been determined for different beam qualities. For example, Gupta et al. (1976) measured the chemical yield for ^{10}B(n, α)^{7}Li neutron beam, while Bhat et al. (2003) and Semwal et al. (2002) measured the response to ^{12}C and ^{7}Li ions. Low dose rate and dose fractionation FBX dosimeter responses were also studied by Gupta and Madhvanath (1985); Gupta et al. (1981).

Thanks to its high sensitivity, the FBX dosimeter can be used for a

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variety of applications. Gupta et al. (1992) studied the possibilities of using the FBX dosimeter for ^{60}Co therapy unit calibration and quality assurance Gupta et al. (1992, 1982). Moussous et al. (2011) used the FBX dosimeter for measuring dosimetric parameters, and Gupta et al. (2000) studied the usability of chemical dosimetry techniques, including the FBX solution, for various applications under different geometries.

To date, several attempts have been made to further enhance the FBX dosimeter. For example, Brindha et al. (2002) introduced the stock solution techniques in the preparation process to increase the shelf-life of prepared solutions, while Upadhyay et al. (1982) modified the FBX composition to obtain a more tissue-equivalent composition, resulting in its use for neutron dosimetry.

Moreover, xylenol orange (XO) is used as an analytical tool in chemistry for the determination of iron ion concentration. The protocol established by Gay et al. (1999) to measure Fe^{3+} concentration differs slightly from the FBX method. The measured wavelength is stated as 560 nm, and the amount of the xylenol orange is increased to ensure that the absorbance of the XO-Fe complex is independent of the concentration of XO. This occurs when the XO: Fe^{3+} ratio is above 3. Xylenol orange dye is added to the Fe^{3+} ion solution before the spectrophotometric measurements using pre-prepared stock solutions. The method described by Gay et al. (1999) is recommended to measure the Fe^{3+} ions in concentrations between $5\,\mu M$ and $50\,\mu M$. We started out from this study to improve the sensitivity of our FBX dosimeter.

Ferrous ammonium sulphate based dosimetry can be considered as a precise method to measure Fe^{3+} concentration produced by ionizing radiation. This can be performed measuring the absorbance at a certain wavelength, and the dose can be calculated from this value with the following equation:

$$D = \frac{A - A_0}{\varepsilon \rho d G(Fe^{3+})}, \tag{1}$$

where A and A_0 are the absorbance of irradiated and non-irradiated reference solutions, respectively, ε is the molar absorption coefficient, ρ is the density of the solution, d is the optical path length used in spectrophotometric determination and $G(Fe^{3+})$ is the radiochemical yield of the Fe^{3+} ions.

All quantities in Equation (1) are subject to some uncertainties. Density can be measured with relatively high precision, and its variation in the prepared solutions is negligible. The cuvette optical path length is well defined by the manufacturer, and in most cases it has a 0.5 % error. Both the molar absorption coefficient and the radiochemical yield depend on the accuracy of the measurements of the optical absorbance of solutions with different Fe^{3+} concentrations.

The relation between the relative uncertainty of the measured absorbance can be derived using the Lambert-Beer law of optical absorption. It has a minimum at $A = 0.43$, where the theoretical relative uncertainty is 0.2% Sommer (1989). Below $A = 0.2$, relative uncertainty increases considerably, and for very low absorbance, this tends to infinity. The concentration of Fe^{3+} ions in a freshly prepared FBX solution is around $1.36\,\mu M$, depending on the purity, storage time and conditions of the Fe^{2+} salt. This concentration corresponds to an absorption of $A = 0.02$ and a theoretical relative uncertainty of 1.6 % in spectrophotometric measurements.

It is well known that a more than 5% difference in absorbed dose can lead to considerable differences in the outcomes of radiobiological experiments or radiotherapy procedures. Therefore, the reproducibility of radiobiological experiments or the replicability of treatment results depend on the precision and accuracy of dosimetric measurements. The main objective of this work was to develop an FBX formula based, enhanced chemical dosimetry system to be used in radiobiological research as a routine reference dosimetry method in various irradiation arrangements where common dosimetry tools are not suitable or are difficult to use. To achieve this, a standard operational procedure has been established and validated to guarantee the necessary accuracy and

precision. Related to this, we have developed a custom designed multipurpose PMMA slab phantom to ensure reference irradiation conditions.

2. Methods

Dosimetric solutions were prepared from analytical grade reagents and HPLC grade water using suitably cleaned glassware. A high level of laboratory cleanliness was established and maintained through an in-house practice protocol. Reagents were weighed with the help of a calibrated analytical balance, precision pipettes, class A cylinders and volumetric flasks. Apart from the ferrous ammonium sulphate stock solution all prepared solutions were stored in dark media bottles with screw caps at room temperature.

2.1. Dosimeter preparation

The dosimetric solutions were prepared from pre-prepared stock solutions, based on the following preparation procedure:

- Sulphuric acid stock solution – All stock solutions were made with 25 mM H_2SO_4, which was prepared before use from 0.5 M analytical grade sulphuric acid solution (5 mL 0.5 MH_2SO_4 dissolved in 100 mL HPLC water). The latter was obtained from concentrated H_2SO_4 solution (an ampoule contains 49.04 g H_2SO_4, Firma Chempure) and stored in dark screw-cap bottles at room temperature.
- The ferrous ammonium sulphate stock solution (FS) – ammonium Fe^{2+} sulphate hexahydrate $((NH_4)_2Fe(SO_4)_2 \times 6H_2O$, 3.921 g, Sigma-Aldrich) was dissolved in sulphuric acid (H_2SO_4, 25 mM, 50 mL). This was further diluted with 25 mM H_2SO_4 (100 mL). The resulting solution of ferrous ammonium sulphate (100 mM) was stored in a refrigerator to prevent the thermal oxidation of iron ions.
- The benzoic acid stock solution (BA) – benzoic acid ($C_7H_6O_2$, 1.974 g, Sigma-Aldrich) was dissolved in sulphuric acid (H_2SO_4, 25 mM, 900 mL). This was further diluted with 25 mM H_2SO_4 (100 mL). The final solution was 16.16 mM benzoic acid. (Dissolution can be accelerated by heating.)
- The xylenol orange stock solution (XO) – xylenol orange disodium salt (, 179.16 mg, Sigma-Aldrich) was dissolved in sulphuric acid (H_2SO_4, 25 mM, 50 mL) and was then further diluted with 25 mM H_2SO_4 (100 mL).

The dosimetric solution was prepared in a 100 mL volumetric flask using 1 mL FS stock solution diluted with the BA stock solution, which resulted in a 1 mM ferrous ammonium sulphate, 16 mM benzoic acid solution in 25 mM sulphuric acid. This solution was stored, until use, in dark screw-cap bottles or in sterile Eppendorf tubes in a refrigerator.

2.2. Reference irradiation

All irradiation was performed in reference conditions using an in-house made PMMA slab phantom and different beam qualities. A conventional LINAC ($SSD = 100$ cm, field size 15 cm $\times$ 15 cm) was used for 6 MV and 15 MV photon beams and for 6 MeV, 9 MeV and 12 MeV electron beams, respectively. A cell and small animal irradiator facility was used (Xstrahl, RS320 type self-contained X-Ray irradiator) for 250 kVp X-ray beams ($HVL = 1.53$ mm Cu equivalent).

Fig. 1 shows the PMMA slab phantom which measures 30 cm $\times$ 30 cm and has variable heights which can be changed using slabs with different thicknesses. The 2.5 cm thick sheet has a 15 cm $\times$ 15 cm square hollow in the middle with a depth of 1.5 cm, in which different inserts with the same dimensions can be placed. These inserts are prepared to hold different sample vials: 0.5, 1.5, 2 mL Eppendorf tubes, well plates and cell culture dishes. This slab phantom can be used for the reference irradiation of dosimetric solutions, dosimetric films or both at the same time, and for the irradiation of different
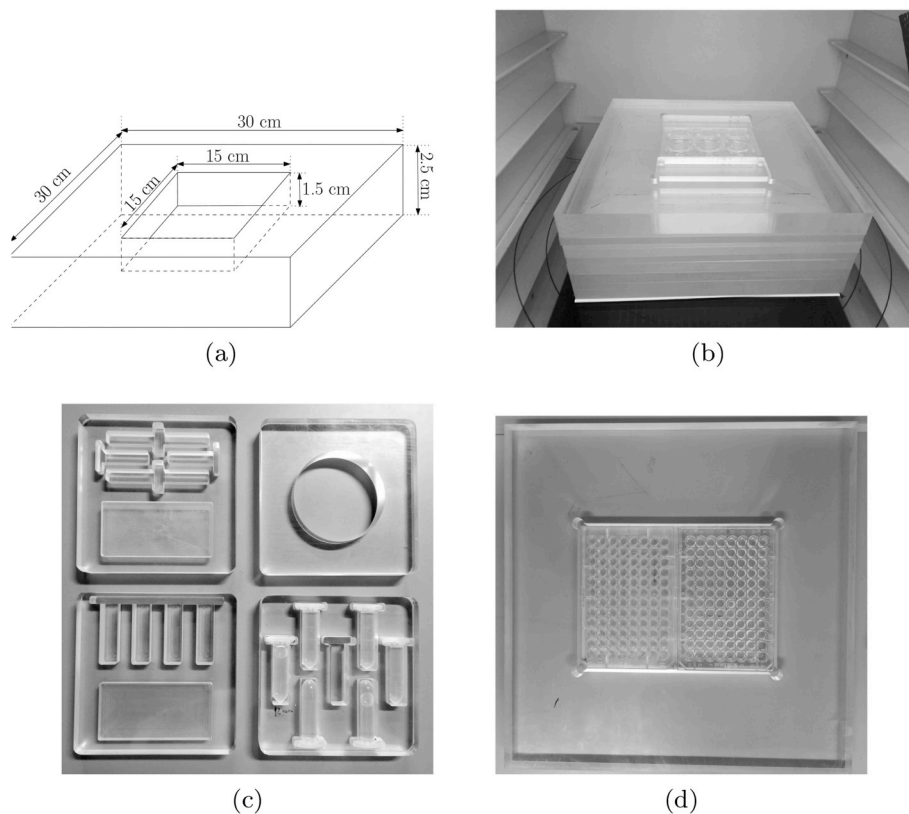
Fig. 1. Acrylic slab phantom for reference irradiation. The phantom has two functional parts: a 30 cm × 30 cm PMMA sheet with different thicknesses for variable PMMA phantom heights (1b) and a PMMA sheet with thickness of 2.5 cm with a 15 cm × 15 cm and 1.5 cm deep hollow in the middle of it which can accept inserts with the same dimensions (1a). These inserts are designed to accept different types of sample holders (1c). Another piece of 2.5 cm thick PMMA sheet is designed so it can be inserted in it one or two multi-wells plates with dosimetric solutions or biological samples (1d).

radiobiological samples (cell cultures, Zebrafish embryos etc.).

Samples for conventional LINAC irradiation were placed at an equivalent depth chosen so that 1 MU would yield the same dose as in water. This was determined by a series of measurements made with a PTW Unidose universal dosimeter and a Farmer type ionization chamber calibrated with a secondary standard in terms of absorbed dose to water.

In the cell and small animal irradiator, a beam hardening filter (consisting of a 1.06 mm aluminium foil and a 0.51 mm copper foil) was used to obtain an X-ray beam quality equivalent to $HVL = 1.53$ mm Cu. The correction factor of $k_{QQ_0} = 0.9978$ was determined through the interpolation of the beam quality factors provided by the calibration laboratory. The reference dose measurements were carried out in a PTW RW3 type solid slab phantom with a PTW Farmer Chamber type 30013, and a PTW Unidos$^{\text{webline}}$ dosimeter calibrated relative to water.

The absorbed dose was measured in a RW3 solid slab phantom used for daily calibrations. The measured dose was converted into absorbed dose to water using the manufacturer's conversion factors. Data were available neither for the 250 kVp X-ray RW3 solid slab phantom, nor for the plexi slab phantom. Consequently, a phantom dose conversion factor was determined using the procedure published by Seuntjens et al. (2005). The depth dose was measured in water at different depths (z_{ref}) and in the RW3 and PMMA slab phantoms at an equivalent depth (z_{eq}). The phantom dose conversion factor was calculated with the following equation:

$$k_{s,w}^Q = \frac{M_w^Q}{M_s^Q},$$

(2)

where $k_{s,w}^Q$ is the phantom dose conversion factor, M_w^Q is the ionization chamber readings in water at reference depth, z_{ref} and M_s^Q are the ionization chamber readings in the slab phantom at equivalent depth z_{eq}

with the same Q beam quality and corrected for influence quantities.

The equivalent depth is the inverse ratio of the relative electron densities (ρ_e) in the two phantom materials using the following equation Seuntjens et al. (2005):

$$\frac{z_{eq}}{z_{ref}} = \frac{\rho_e^w}{\rho_e^s}.$$

(3)

This Equation (3) should be used in conjunction with a similar equation for the ratio of the reference and equivalent field size. However, for the lack of an appropriate collimator, these conditions were not reproducible and because it does not significantly affect the final results, we neglected this condition.

Table 1 shows the equivalent depth and the phantom dose conversion factor for RW3 and PMMA, and for the 250 kVp X-ray ($HVL = 1.53\ mm$).

Table 1
Phantom dose conversion factor for RW3 and PMMA materials.

Water	RW3		PMMA	
z_{ref} (cm)	z_{eq} (cm)	$k_{s,w}^Q$	z_{eq} (cm)	$k_{s,w}^Q$
0.5	0.5	0.9539	0.4	0.9329
1.0	1.0	0.9492	0.9	0.9262
1.5	1.5	0.9421	1.3	0.9128
2.0	2.0	0.9241	1.7	0.8874
2.5	2.5	0.9158	2.2	0.8762
3.0	3.0	0.9122	2.6	0.8633
3.5	3.5	0.9083	3.0	0.8490
4.0	4.0	0.8893	3.5	0.8270
4.5	4.4	0.8837	3.9	0.8183
5.0	4.9	0.8785	4.3	0.8011

2.3. Spectrophotometric measurements

After irradiation, 0.1 mL xylenol orange stock solution (XO) was added to each 0.9 mL of irradiated dosimetric solution. The final solution contained 0.25 mM xylenol orange disodium salt. After waiting at least 10 min for the xylenol orange to form complexes with the Fe^{3+} ions Gay et al. (1999), we measured the solution's absorbance at 560 nm against the xylenol orange blank solution. The blank sample was prepared in a sample vial from the XO stock solution (0.1 mL) and sulphuric acid (0.9 mL 25 mM), in the same manner as the dosimetric samples.

Absorption measurements were performed using an UV-VIS spectrophotometer (Lambda 35, PerkinElmer, double light path) and a quartz cuvette with a 1 cm path length. The recommendation of the ISO/ASTM 51026:2014 standard was followed to ensure the reproducibility and accuracy of the measurements.

2.4. Density measurements

The density of the eFBX solution was measured with an Anton Paar DMA 35 portable density meter (accuracy, 0.001 g/cm^3) and the classical gravimetric method. The results of the two methods were compared and a mean value was generated.

2.5. Determination of the molar mass absorption coefficient

A simplified version of the recommendations of ISO/ASTM 5106:2014 were followed to determine the molar absorption coefficient. An Fe^{3+} stock solution was prepared by dissolving ammonium iron(III) sulphate dodecahydrate (Sigma Aldrich, 4.82 mg) in sulphuric acid (25 mM, 50 mL), and was then diluted down to 100 mL. From this stock solution and the BA stock solution a series of samples were prepared with Fe^{3+} ion concentrations between 10 μM and 60 μM, and 0.1 mL XO stock solution was added to each 0.9 mL solution.

The concentration of Fe^{3+} ions for each sample was determined by measuring absorption at 560 nm. The molar linear absorption coefficient was obtained from the gradient of the plot of the absorbance/path length versus concentration.

2.6. Determining the radiochemical yield of ferric ions

The radiochemical yield of ferric ions $G(Fe^{3+})$ was determined using a freshly prepared eFBX solution that was irradiated with different doses in 2 mL Eppendorf tubes using the PMMA slab phantom and the reference irradiation geometry. Optical absorption was measured for irradiated and nonirradiated samples and $\Delta A_i = A_i - A_0$ were calculated, where A_i is the absorption of the irradiated sample and A_0 is the absorption of the nonirradiated sample.

Plotting $\Delta A / \varepsilon \rho$ values as a function of dose results in a linear correlation where the gradient corresponds to the radiochemical yield of ferric ions ($G(Fe^{3+})$).

2.7. Dose determination

Whenever possible, we irradiated at least three FBX samples, together or separately, with the same dose. After irradiation we performed spectrophotometric measurements, and using Equation (1) we calculated the absorbed dose from the obtained absorption value.

The absorbed dose to water, D_w, can be determined from the mean absorbed dose using the relation:

$$D_{\text{water}}^{zref} = k_{\text{w,PMMA}} \cdot D_{\text{PMMA}}^{zeq}, \tag{4}$$

where $k_{\text{w, PMMA}}$ is the PMMA to water conversion factor, calculated as the ratio of the measured dose in water at reference depth (D_{water}^{zref}) and the dose in PMMA at equivalent depth (D_{PMMA}^{zeq}).

2.8. Diluted eFBX solution measurements

For the measurement of doses with volumes less than 1 mL, the irradiated eFBX solution was diluted with a well defined amount of nonirradiated eFBX solution. XO stock solution was added to these diluted eFBX solutions. Based on the degree of dilution, the change in absorbance induced by the absorbed dose can be calculated with the following formula:

$$\Delta A = \frac{A_m - \left(1 - \frac{1}{9}\beta\right)A_0}{0.9 - \beta}, \tag{5}$$

where ΔA is the change in absorbance, A_m is the measured absorbance of samples without correction, A_0 is the absorbance of the nonirradiated solution and β is equal to the ratio of the solvent volume and the total volume of samples $\beta = V_0/V_T$.

3. Results and discussions

To improve the sensitivity of the FBX dosimeter, attempts have been made to increase the chemical yield of ferric ions and to enhance the photometric measurement techniques by applying Gay et al. (1999) recommendation. We performed a series of experiments varying the chemical composition to increase the sensitivity of the FBX dosimetric solution.

It is well known that the radiochemical yield of Fe^{3+} ions is higher in the ferrous sulphate – benzoic acid system (FB) than in the ferrous sulphate – benzoic acid – xylenol orange system (FBX)Gupta et al. (1978).

The primary species formed by radiolysis in aqueous solutions are the H, OH, H_2O_2 and H_2 radicals. In the FB system each hydrogen atom oxidizes two ferrous ions, and each hydrogen-peroxide molecule oxidizes one ferrous ion, while one hydroxyl radical is produced. This secondary hydroxy radical reacts with benzoic acid and forms hydroxycyclohex-adiemyl radicals, which oxidize the ferrous ions in a chain reaction Jia-Shan et al. (1982):

$$(OH)\dot{C}_6H_5COOH + nFe^{2+}H^3, O_2$$
$$\rightarrow nFe^{3+} + C_6H_4(OH)COOH. \tag{6}$$

Concurrently, benzoic acid can effectively compete with oxygen for H atom releasing HO_2 radicals Gupta et al. (1978). Gupta et al. (1978) showed that the radiochemical yield for the FB system can be written as:

$$G(Fe3+) = 12 \cdot G_{H_2O_2} + 11 \cdot G_{OH} + 13 \cdot G_H, \tag{7}$$

where $G_{H_2O_2} = 0.79 \cdot 10^{-7}$ mol/J, $G_{OH} = 2.9 \cdot 10^{-7}$ mol/J and $G_H = 3.5 \cdot 10^{-7}$ mol/J are the radiochemical yield of hydrogen peroxide, hydroxyl radical and atomic hydrogen, respectively.

However, in the FBX system, H_2O_2 oxidizes 8 ferrous ions, instead of 12, and the xylenol orange competes for OH radicals with benzoic acid, producing $XO \cdot OH$ radical, which can oxidize one ferrous ion. According to Jia-Shan et al. (1982) and Gupta et al. (1978) the reaction chain length of the oxidation of Fe^{2+} decreases and the Equation (7) can be written as:

$$G(Fe3+) = 8 \cdot G_{H_2O_2} + 7 \cdot G_{OH} + 11 \cdot G_H. \tag{8}$$

The main drawback of the FB system is that there is a nonlinear response to dose. This behaviour was reported by Gupta et al. (1978) and shown in Fig. 2, where the dose response of the Fricke and FBX dosimeters is nonlinear above 5 Gy in the FB system. This behaviour can be qualitatively explained by the spur theory of water radiolysis, which states that the initial radiolysis species of water exist in a spur. These species then diffuse in the medium and react forming intermediates and the final product, Fe^{3+} ions. The probability of interaction with Fe^{2+} ions is determined on the basis of the concentration of Fe^{2+} ions and the initial radiolysis species, as well as the spatial distribution
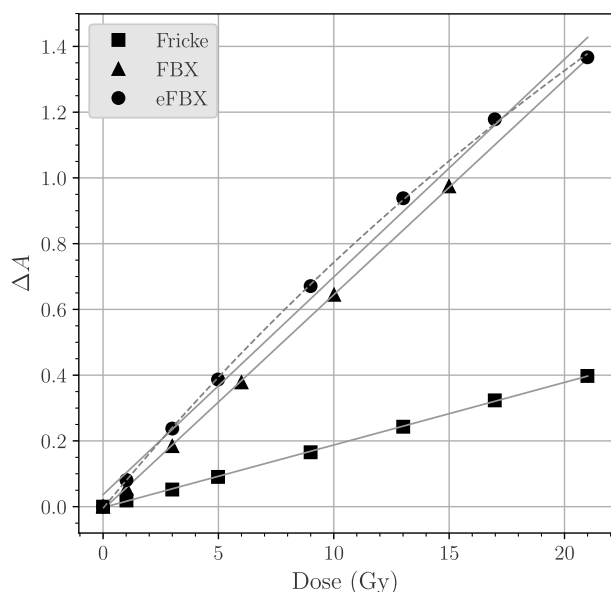
Fig. 2. Dose response curve of different dosimetric solutions for a standard Fricke solution, measured with xylenol orange techniques; an FBX solution and an FB solution.
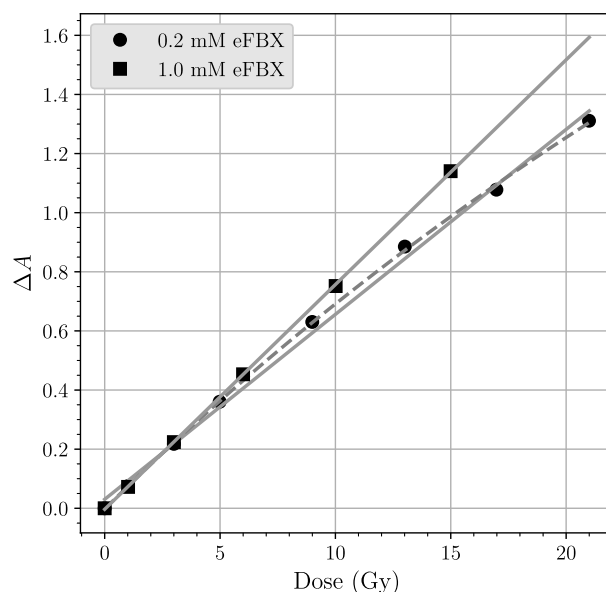

Fig. 3. The effects of increased ferrous sulphate concentrations. The lower line is the graph of linear fitting of the measurement points (marked with circles) in case of eFBX solutions with 0.2 mM ferrous sulphate concentration. The dashed line is the graph of fitting with a rational function of the same measurements points. The upper line represents the graph of linear fitting of the measurements points (marked with squares) obtained with 1 mM ferrous sulphate concentration.
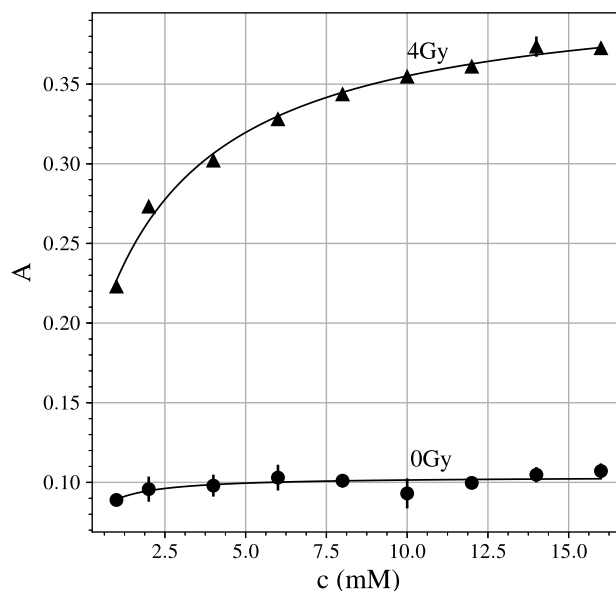
of these spurs. The spatial distribution of spurs is strongly related to the linear energy transfer (LET) value of radiation. At high LET, spurs are superposed, whilst at low LET the spatial distribution of spurs is scattered. This means that:

- the radiochemical yield of ferric ions is lower at high than at low LET radiations, because the probability of a reaction with a Fe^{2+} ion is higher when the spatial distribution of spurs is scattered, and
- the probability of a reaction with a Fe^{2+} ion at the same LET radiation level is higher in case of higher Fe^{2+} ion concentrations.

Consequently, the nonlinearity of the FB solution suggests that the solution becomes saturated at high doses, which means that there is a reduced probability of a reaction with a Fe^{2+}. This in turn leads to a decreasing chemical yield, which prompted us to remove the nonlinearity of the system by increasing the Fe^{2+} concentration. Fig. 3 shows the effects of increasing the ferrous ammonium sulphate concentration from 0.2 mM to 1 mM, which corresponds to the ferrous ammonium sulphate concentration of the classical Fricke dosimeter.

Studying the relation between the response of the FB system and the concentration of benzoic acid has revealed that the chemical yield of Fe^{3+} ions can improve if the amount of benzoic acid is increased in the system. The variation of absorbance as a function of BA concentration is shown in Fig. 4. When the FB samples with increased benzoic acid concentration were irradiated with 4 Gy, the response showed saturation effects, which became significant above 7.5 mM of benzoic acid. However, the response curve was not completely saturated in the measured concentration range. The extrapolation of the data revealed that complete saturation occurs above 16 mM.

On the other hand, variations in benzoic acid concentrations, due to poor measurement or improper dilution do not significantly influence the dosimetric response of the solution. Fig. 4 shows that a change in the BA concentration from the standard 5 mM–16 mM results in an 8% increase in absorbance, which corresponds to an 8% growth in radiochemical yield. At the same time, there is a 0.3% difference in absorbance at 14 mM and 16 mM. Therefore, at higher benzoic acid concentrations the error which may occur in the solution preparation process is negligible.

A further increase in benzoic acid concentration is hindered by its poor solubility in water (21.9 mM at $18\ ^oC$). No further improvements in

Fig. 4. Optical absorbance as a function of benzoic acid concentration for irradiated solution and for nonirradiated solution.

absorbance are expected above 16 mM, so there is no point in experimenting with even higher concentrations.

The radiochemical yield of Fe^{3+} ions in an FB solution with 5 mM benzoic acid concentration can be calculated using Equation (7) and is found to be $8.75 \cdot 10^{-6}$ mol/J. These measurements show that the radiochemical yield of dosimetric solutions with 16 mM is $9.08 \cdot 10^{-6}$ mol/J for a beam quality of 6 MV. This equals a 3.9% increase in yield, and is due to the higher benzoic acid concentration. Using the logic proposed by Jia-Shan et al. (1982) we can conclude that in the presence of 16 mM benzoic acid, the OH radical oxidizes 12 rather than 11 Fe^{3+} ions, and Equation (7) becomes:
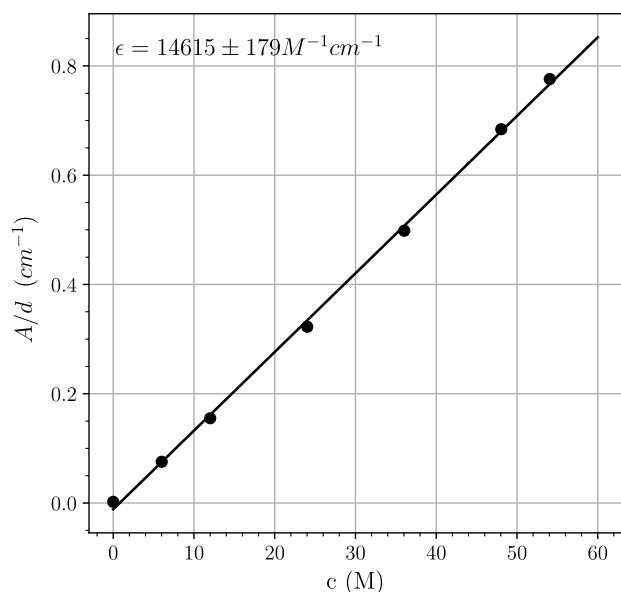
Fig. 5. Graphical representation of absorbance variation as a function of Fe^{3+} ion concentrations. The gradient is the molar attenuation coefficient, $\varepsilon = 14615 \pm 18\ M^{-1}cm^{-1}$.

$$G(Fe3+) = 12 \cdot G_{H_2O_2} + 12 \cdot G_{OH} + 13 \cdot G_H. \tag{9}$$

By using a concentration of 16 mM benzoic acid, and by irradiating an FB solution instead of an FBX solution, the radiochemical yield can be increased from a mean literature value of $6.89 \cdot 10^{-6}$ mol/J, to $9.08 \cdot 10^{-6}$ mol/J, which equals to a 24% enhancement. We named this system enhanced FBX (eFBX).

Our eFBX dosimetric solution has a density of 0.9978 g/cm^3, and the measured molar attenuation coefficient is $14615 \pm 18\ M^{-1}cm^{-1}$, which was obtained by fitting the measured absorbance at different Fe^{3+} concentrations (Fig. 5). This value is in agreement with literature values, and any difference may be attributed to the degree of purity of xylenol orange dyes, which varies from manufacturer to manufacturer. This is a common problem, and it is always recommended to measure the molar attenuation coefficient when a new lot of xylenol orange is used. A summary of different molar absorption coefficient values was published by Moussous et al. (2008).

The radiochemical yields of ferric ions resulting from different beam qualities are presented in Table 2. All values are in terms of dose to water, and were determined by applying the appropriate conversion factors.

The effects of the dilution of the irradiated solution were investigated to perform measurements with dosimetric solutions of less than 1 mL, for example solutions from single wells of a 96-well plate, 250 μl. Table 3 shows $G(Fe^{3+})$ values for various diluted eFBX solutions, and it can be seen that to some extent the radiochemical yield values

Table 2
The radiochemical yield of the eFBX dosimetric solution for different beam qualities.

Beam	$G(Fe^{3+})$
	$mol \cdot J^{-1}$
6 MV photon	$9.08 \pm 0.17 \cdot 10^{-6}$
15 MV photon	$9.10 \pm 0.17 \cdot 10^{-6}$
6 MeV e⁻	$8.98 \pm 0.15 \cdot 10^{-6}$
9 MeV e⁻	$9.03 \pm 0.08 \cdot 10^{-6}$
12 MeV e⁻	$8.97 \pm 0.26 \cdot 10^{-6}$
250 kVp X-ray	$6.46 \pm 0.08 \cdot 10^{-6}$

Table 3
The radiochemical yield of diluted eFBX dosimetric solution for different dilutions grades. All values refer to 250 kVp X-ray beam quality.

Dilution	Sample	Solvent	$G(Fe^{3+})$
	ml	ml	$mol \cdot J^{-1}$
4/0.9	0.9	2.6	$6.07 \pm 0.18 \cdot 10^{-6}$
4/0.5	0.5	3.1	$5.86 \pm 0.23 \cdot 10^{-6}$
4/0.25	0.25	3.35	$5.88 \pm 0.23 \cdot 10^{-6}$

differ for diluted and nondiluted solutions. This can be attributed to some matrix effects the origins of which have not yet been identified.

We established a standardized measurement protocol to ensure that eFBX is an easy-to-handle chemical dosimeter. For this purpose, we started out from the ISO standard on the practice for using the Fricke reference standard dosimetry system. Our protocol describes the preparation, handling and storage of the solution; irradiation in reference conditions; the photometric measurements and subsequent data processing.

Stock solutions were introduced similarly to Brindha et al. (2002) with some modifications for the preparation of dosimetric solutions. All but one of the stock solutions were stored at room temperature. The 1 mM FS stock solution was stored in a refrigerator due to its short, two-week shelf-life (caused by the relatively high thermal oxidation rate of ferrous ammonium sulphate, which is a function of Fe^{2+} ion concentration and temperature). The BA and XO stock solutions can be stored at room temperature for a long time without any observable degradation.

The initial ferric ion concentration depends on the thermal oxidation of the dosimetric solution, which reduces the measurable dose range. The freshly prepared eFBX solution has an optical absorption of 0.1, which can increase to 0.3 with a change in ferric ion concentration over time. For this reason it is always recommended to use freshly prepared FS stock solutions to avoid any uncontrolled change in dosimetric solution consistency, and to ensure complete control over the quality of dosimetric solutions.

Fig. 6 shows that the blank solution has considerable absorption at the maximum absorption wavelength (525 nm) of the eFBX system. This means that the Beer-Lambert law cannot be used here. A
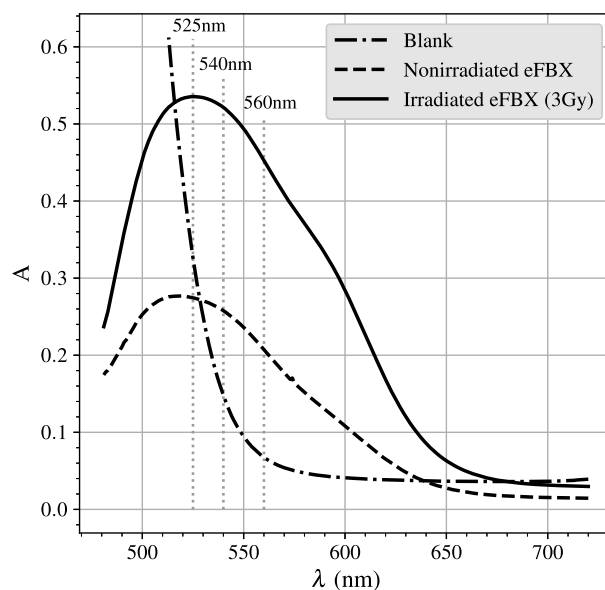

Fig. 6. The absorption spectra of the blank solution and of the irradiated eFBX solution. The dotted vertical lines represent the wavelengths for maximum absorptions and for the actual measurement wavelength.

Table 4

Uncertainty budget of the eFBX solution. Temperature correction was calculated based on the relations used for the Fricke dosimeter ISO/ASTM51026-15 (2015) as the temperature dependence measurement has not yet been performed. The combined standard uncertainty without this term becomes 1.0%.

Source of uncertainty	Relative standard uncertainty (%)	
	Type A	Type B
Reference dose rate		
N_k secondary standard	–	0.20
Positioning	–	0.02
Temperature and pressure correction	0.03	0.10
Measurement of current	0.05	0.10
Calibration of OFBX solution		
Positioning of dosimeter	–	0.02
N_{pw} plexi-water conversion	0.20	–
Photometric and volumetric correction	0.35	0.61
G value determination	0.6	–
ε determination	0.2	–
Temperature correction	–	0.50
Quadratic summation	0.75	0.83
Combined standard uncertainty	1.12	
Extended uncertainty (k = 2)	2.24	

Fig. 7. The relative uncertainties for the eFBX dosimeter due to the photometric measurements (solid curve) and due to the combination of photometric and volume measurements (dashed line).

convenient method is to measure absorption at a different wavelength where there are no considerable xylenol orange absorptions Gay et al. (1999). Hence, all photometric measurements were conducted at 560 nm, in accordance with the observations of Gay et al. (1999).

It is also interesting to note that the xylenol orange technique can be successfully applied to standard Fricke solutions in the low dose domain. The initial concentration of ferric ions is the same as in the case of the eFBX solution, and the chemical yield of the Fricke solution is 1.6 mol/J ISO/ASTM51026-15 (2015). The concentration change induced by irradiation can be measured with the xylenol orange technique. If we apply this technique to the Fricke solution, the chemical yield is $1.62 \cdot 10^{-6}$ mol/J for a 6 MV photon beam and $1.44 \cdot 10^{-6}$ mol/J for a 250 kVp X-ray beam, respectively.

3.1. Uncertainty budget

Table 4 summarizes the uncertainty budget for eFBX dosimeter calibration, made using the recommendations of IAEA International Atomic Energy Agency (2009). In this phase we encountered two major problems: the uncertainty of spectrophotometric measurements and that introduced by pipetting.

Theoretically, the most precise value for absorbance is $A = 0.434$, which is the minimum of function:

$$\frac{dA}{A} = -\frac{0.434}{10^{-A}A} dT, \qquad (10)$$

where A is the absorbance and dT is the standard deviation of transmittance. Equation (10) can be obtained using the Lambert-Beer law Sommer (1989) (Fig. 7). The graph shows that the uncertainty of measurements at low absorbance is relatively high. In a freshly prepared eFBX dosimeter solution, absorbance is usually between 0.01 and 0.02, and increases with storage time. The relative uncertainty of absorption is between 3.12% and 1.6%. However, a rise in the concentration of ferrous sulphate to 1 mM in the eFBX dosimetric solution increases the absorbance of the freshly prepared solution to 0.1 with a theoretical relative uncertainty of 0.38%.

The final ferric ion concentration of the eFBX solution is determined both by the absorbed dose and by the pipetting of the solution after irradiation. Section 2 states that for sample preparation 0.9 mL eFBX
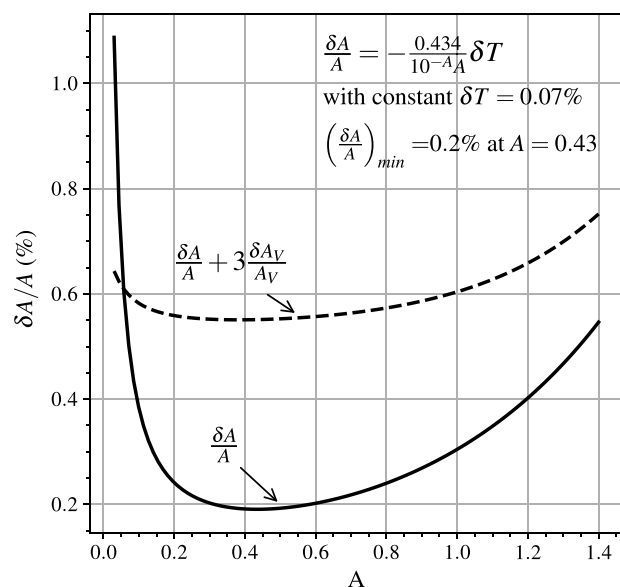
solution was extracted from the irradiated sample and 0.1 mL XO stock solution was added. Therefore, the final concentration of the solution was 0.9 ml·c_0/(0.9 + 0.1) ml, where c_0 is the initial ferric ion concentration. If we apply the rule of propagation of uncertainty we obtain:

$$\left(\frac{\delta A_B}{A_B}\right)^2 = n \cdot \left(\frac{\delta V}{V}\right)^2 + \left(\frac{\delta A_m}{A_m}\right)^2 + \left(\frac{\delta A_0}{A_0}\right)^2, \qquad (11)$$

where $\delta A_B/A_B$ is the total uncertainty of absorbance of the irradiated solution; $\delta A_m/A_m$ is the uncertainty of absorbance of the irradiated solution; $\delta A_0/A_0$ is the uncertainty of absorbance of the nonirradiated solution, which is discussed here separately, because low absorbance results in higher uncertainty, and $\delta V/V$ is the uncertainty associated with pipetting. The parameter n represents the volumes which were measured with pipette. For example, two samples of 0.9 mL can be extracted with a 1 mL pipette from a single 2 mL Eppendorf tube. With the addition of the XO stock solution the parameter n increases to 3. In an ideal situation, the uncertainty attributed to volume determination with a precise pipette is 0.2%. Equations (10) and (11) provide the combined uncertainties for absorbance measurements represented in Fig. 7 (dotted line).

Fig. 7 shows that the uncertainty introduced by pipetting resulted in a greater but flatter relative uncertainty in absorbance measurements. In the 0.1 to 1.2 absorbance interval, the relative uncertainty is between 0.64% and 0.65% with a minimum of 0.55%. This absorption interval corresponds to an absorbed dose range up to 9 Gy for 6 MV photon beams and up to 12 Gy for 250 kVp X-ray (with a $HVL = 1.53$ mm Cu equivalent).

Table 4 summarizes these calculations and considers other sources of errors. The combined standard uncertainty can be considerably improved by eliminating uncertainty type B assigned to temperature correction. Temperature correction was not considered in this study, but on the basis of the spur theory and the increased amount of ferrous sulphate in the final composition, a similar relation can be assumed between temperature and radiochemical yield as in case of the Fricke dosimeter ISO/ASTM51026-15 (2015). The same connection is true for the temperature correction of absorbance measurements, which is related to the associated volume change with temperature. Correction for this variation means that the combined standard uncertainty becomes 1.0%.

4. Conclusions

In this paper we studied some of the properties of the well-known FBX dosimetric solution in terms of sensitivity and dose range. The photometric determination of ferric ions is the major obstacle to obtaining good results with low uncertainties. This problem can be eliminated by improving the sensitivity of the solution, which we achieved by increasing the benzoic acid concentration and by adding xylenol orange dye after irradiation. Nonlinearity, a hallmark of an FB system, was eliminated by increasing the ferrous sulphate concentration from the original 0.2 mM value to 1 mM.

For reference irradiation, we constructed a special, multipurpose PMMA slab phantom to hold Eppendorf tubes filled with dosimetric solutions. This phantom can also be used for irradiation under the same conditions as those used for biological and/or chemical samples kept in cell culture dishes or multi-well plates. Moreover, one can perform the cross-calibration of the eFBX dosimeter with other dosimeters, such as films, ionization chambers etc.

Further improvements to the system can be made by studying the influence of temperature on chemical yield and absorbance measurements. If there is indeed a relation between chemical yield and temperature, and between absorbance and temperature, the obtained 1.12% combined standard uncertainty can be further reduced to 1.0%.

We are planning to conduct further experiments to make our reference dosimetry system more widely accepted. Our aim is to further optimize and refine the dosimetric characteristics and measurement practices and to ensure the reliability of this system as a reference dosimeter. It is also crucial to prove its suitability in various practical situations, especially in the dosimetry of radiobiological experiments, as well as in radiotherapy measurements.

CRediT authorship contribution statement

R. Polanek: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing - original draft. **Z. Varga:** Methodology, Investigation, Validation, Supervision. **E. Fodor:** Validation, Visualization. **Sz. Brunner:** Investigation, Resources. **E.R. Szabó:** Investigation, Resources. **T. Tőkés:** Investigation, Writing - review & editing. **K. Hideghéty:** Conceptualization, Supervision, Writing - review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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