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Research Article

Radiosterilization of Fluoroquinolones and Cephalosporins: Assessment of Radiation Damage on Antibiotics by Changes in Optical Property and Colorimetric Parameters

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Abstract. A most common problem encountered in radiosterilization of solid drugs is discoloration or yellowing. By pharmacopoeia method, discoloration can be assessed by measuring absorbance of solutions of irradiated solid samples at 450 nm. We propose to evaluate discoloration of solid samples directly by recording their diffuse reflectance spectra. Further, the reflectance spectrum is used to compute various color parameters: CIE XYZ tristimulus value, CIE Lab, ΔE^*_{ab} (color difference), yellowness index (YI), dominant wavelength, and excitation purity by CIE method. The investigation of difference reflectance spectra and color parameters revealed that for fluoroquinolones, e-beam was more damaging than gamma radiation, whereas for cephalosporins, trend was reversed. The quantum of discoloration with gamma radiation and e-beam is found to be nearly equal when assessed by pharmacopeia method, and it is therefore inadequate to assess small color differences. The color parameters ΔE^*_{ab} and ΔYI are found to be reliable indicators of discoloration. The tolerance limits proposed in terms of ΔE^*_{ab} and ΔYI are ±2 and ±10 U, respectively. The dominant wavelength for all compounds has shifted to higher values indicating change in hue but defining color tolerance limit with this parameter requires adjunct excitation purity value.

KEY WORDS: cephalosporin; color difference; fluoroquinolone; radiosterilization; yellowness index.

INTRODUCTION

The sterilization of antibiotics is essential because any bio-burden may decrease potency of drug and reduce its shelf life. Most antibiotics are heat sensitive, and therefore, according to *European Agency for the Evaluation of Medicinal Products* (EMEA), decision tree can be sterilized by isothermal techniques, namely, radiation sterilization or membrane filtration (1). In EMEA decision tree, sterilization with high-energy ionizing radiation (gamma and e-beam) is preferred over membrane filtration because the former is a terminal sterilization technique, and unlike the latter, does not require aseptic handling after sterilization. Besides this, products shape and physical dimensions require less consideration due to high penetration ability of ionizing radiation. On the other hand, radiation induces free radical formation and causes radiolysis of active ingredient that may result in formation of toxic compounds in small amounts (2). Also, a most common problem encountered and reported in case of radiation sterilization is the yellowing or discoloration of the drug preparation due to radiolytic products formed (3–7). The yellowing sometimes may be perceptible, and it may pose limitation due to aesthetic unacceptability. In literature, the discoloration was reported to be assessed by measuring absorbance of drug solution at 450 nm (3) or studying UV-visible spectrum (4–6) or diffuse reflectance spectrum (7). In British Pharmacopoeia, two methods are described for determination of color of drug sample (a) by visual comparison of drug solution with color standards that are combinations of $FeCl_3$, $CoCl_2$, and $CuSO_4$ (brown–yellow–red color spectrum) or (b) by measuring absorbance of drug solution of known concentration at 450 nm (8).

Our objective is to study the effect of high-energy ionizing radiation (gamma and e-beam) on antibiotics by investigating optical properties of solid antibiotics, evaluate discoloration using device-independent color parameters, and propose color tolerance limit for irradiated solid drugs based on color parameters. The color analysis of solid drugs was

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ABBREVIATIONS: EMEA, European Agency for the Evaluation of Medicinal Products; CIE, Commission Internationale de l'Eclairage; CD, Cefdinir; CX, Cefixime; CU, Cefuroxime axetil; CP, Cefpodoxime proxetil; NFX, Norfloxacin; GFX, Gatifloxacin; SPX, Sparfloxacin; DIN, Official German Standard Color System.

done by standard procedures of "Commission Internationale de l'Eclairage" (CIE). The colorimetry has been extensively reported to be used for color analysis of pigments (9), image processing (10), and polymers (11), but its utility for color analysis of drugs has not been reported.

This study was performed on solid drugs belonging to cephalosporin and fluoroquinolone antibiotic classes. These are widely prescribed broad-spectrum antibiotics. The compounds selected from cephalosporin class are cefdinir (CD), cefuroxime axetil (CU), cefpodoxime proxetil (CP), and cefixime (CX); and from fluoroquinolone class are norfloxacin (NFX), gatifloxacin (GFX), sparfloxacin (SPX).

MATERIALS AND METHODS

Samples

The working standards of NFX, GFX, SPX, and CD were provided by Lupin Pharmaceuticals, India. CU, CP, and CX were from Orchid Healthcare, India. The samples were used as received from the manufacturer.

Materials

$BaSO_4$ and NaOH used were of AR grade (Merck, India). All solutions were prepared in doubly distilled water.

Irradiation

For irradiation, the solid samples were packed (1–2 mm thickness) in polythene bags. The samples were irradiated to an absorbed dose of 25 kGy: dose required to attain sterility assurance level of 10^{-6} (7) and 100 kGy: four times higher dose is applied to estimate increase in discoloration with dose. In order to study the effect of dose rate, samples were irradiated with gamma radiation and e-beam (electron beam) having dose rates 0.85 kGy/h and 5 kGy/min, respectively. The gamma irradiation was performed using GC-900 ^{60}Co source (Manufacturer: BRIT, BARC, India) at room temperature (27°C). The e-beam irradiation was performed on e-beam accelerator (ILU 6, Russian make, 2 MeV, 20 kW) at room temperature (30°C). The dose rate was calibrated with alanine dosimeter.

Transmittance Spectra of Solutions

The solutions of unirradiated and irradiated samples of all compounds were prepared in 0.1 N NaOH, as they were all readily soluble in alkaline medium. The transmittance spectra of cephalosporins were recorded within 30 min because the lactam ring undergoes hydrolysis under alkaline medium. The solution of fluoroquinolones in 0.1 N NaOH was found to be stable up to 1 h and, if kept in the dark, up to 2 h. The concentrations of sample solutions (unirradiated and irradiated) were 5% for NFX, GFX; 2% for SPX and 1% for CD, CU, CP, and CX. The visible spectra were recorded on *Cintra 20e* (Manufacturer: GBC, Australia) spectrophotometer using 0.1 N NaOH as reference.

Diffuse Reflectance Spectra of Solids

The reflectance spectra were recorded using integrating sphere assembly (Sphere diameter: 63 mm; Port/sphere area ratio: 8%; Sample incident angle: 8°) of *Cintra 20e* (Manufacturer: GBC, Australia) spectrophotometer. $BaSO_4$ was used as diluent and reference. The sample to $BaSO_4$ ratio was 1:1 for NFX, CD, and CX; 2:1 for GFX, CU, and CP; and 1:3 for SPX. Mixing was done for several minutes with agate mortar–pestle. The spectra were recorded in the wavelength range 380–780 nm (the visible region of electromagnetic radiation) at data interval of 1 nm. For each sample, three spectra were recorded, and averaged data points were converted to spectrum and used for color analysis.

Color Analysis

The color of drug sample before and after irradiation can be simply obtained by comparison of solution of sample with color standards of $FeCl_3$–$CoCl_2$–$CuSO_4$ (8). But due to mind-dependency of color, visual comparison is always subjected to error. Besides this in the method, the sample has to be solubilized. To determine color of solid drug samples, their diffuse reflectance spectra were recorded because it can be used as a mind-independent characteristic and is unique for every object. But with diffuse reflectance spectrum, the problem of metamerism (where physically distinct spectra will be percepted as same color) cannot be solved. Therefore, for correct evaluation of the color of an object, we require a method to convert reflectance spectrum data to color parameters which truly represents human eye capabilities. As we know, that color emerges from interaction of three components: light source, object's reflectance characteristic, and human vision system (Fig. 1). Thus, CIE XYZ color space (Fig. 2) is appropriate for this purpose because, in this color space, CIE XYZ tristimulus values are calculated from CIE standard observer function (2° observing field) considering illumination and reflectance spectrum of object's surface. The standard illuminant source being used is D65, which is based on actual spectral measurement of daylight with correlated color temperature of 6,504 K and is most commonly used illuminant source for color analysis. Under daylight condition, the vision process is mainly dependent on the fovea, a part of the retina occupying the area where the visual angle of observation is equal to 2° in the center of field of vision (12). Therefore, the hypothetical observer used is 2° CIE standard observer. The calculation for XYZ was performed using the following equations (12):

$$X = K\sum_{380}^{780}\rho_\lambda H_\lambda \bar{x}_\lambda \Delta\lambda$$

$$Y = K\sum_{380}^{780}\rho_\lambda H_\lambda \bar{y}_\lambda \Delta\lambda$$

$$Z = K\sum_{380}^{780}\rho_\lambda H_\lambda \bar{z}_\lambda \Delta\lambda$$

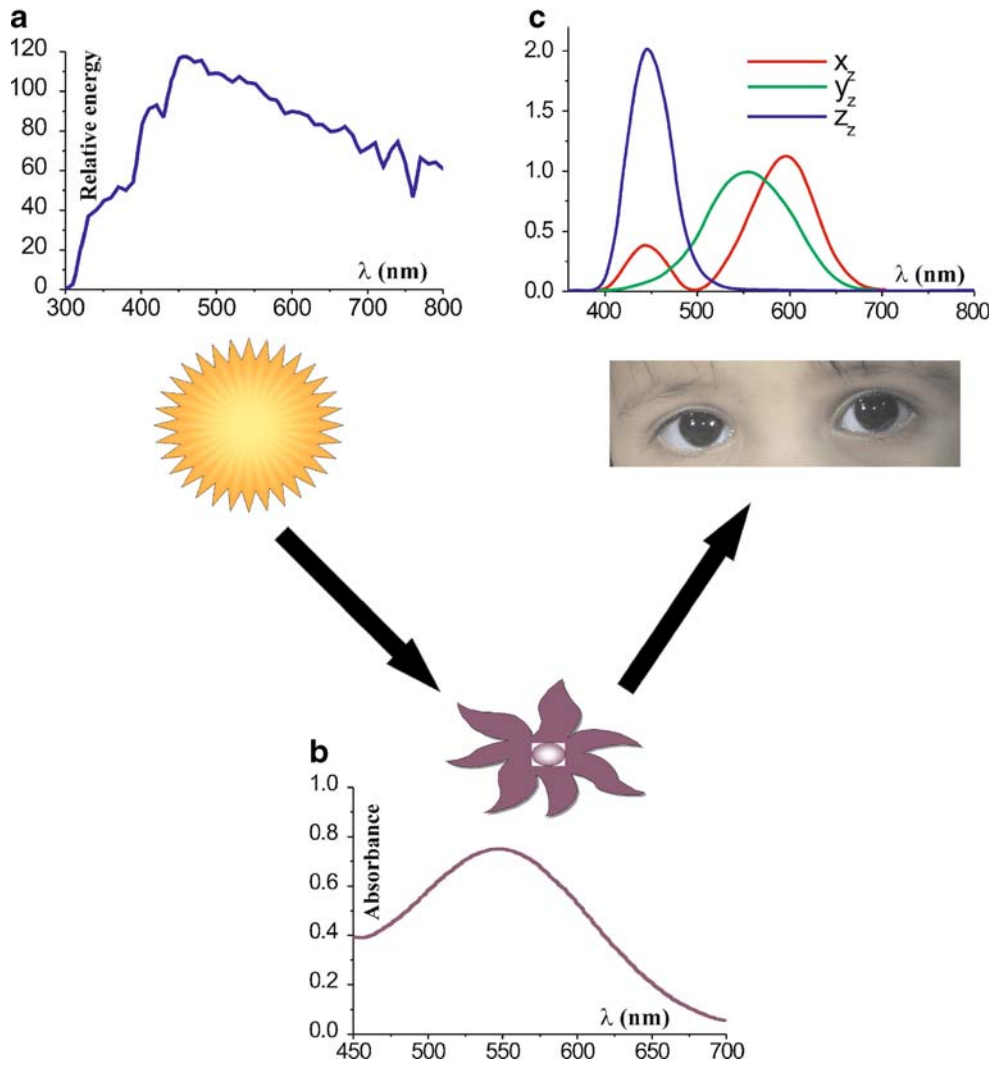

Fig. 1. The color triangle: interaction of illuminant source (**a**), object's reflectance characteristic (**b**), and human vision system (**c**); **a** Relative energy distribution of D65 illuminant; **b** CIE color matching function for 2° observer

Where,

ρ_λ	Spectral reflectance of the object at λ
H_λ	Illuminant function (D65) at λ
$\overline{x}_\lambda, \overline{y}_\lambda, \overline{z}_\lambda$	Color-matching functions of standard observer at λ
$\Delta\lambda$	Spectral resolution or data interval (1 nm)

The illuminant function and color-matching functions values are available in CIE 1931 document in wavelength range 380–780 nm at data interval of 1 nm (12), and the graphical representation of these functions are shown in Fig. 1a and b, respectively)

The normalizing factor "K" can be assigned any arbitrary value. In most cases, only relative values of $\rho_\lambda H_\lambda \Delta\lambda$ are provided because only relative values of X, Y, and Z are required. In such cases, the factor K is so chosen that Y has value 100.

$$K = \frac{100}{\sum_{380}^{780} H_\lambda \overline{y}_\lambda \Delta\lambda}$$

Thus, the tristimulus values X, Y, and Z calculated above are coordinates of a three-dimensional vector space and used to mathematically describe a color with color stimulus F given below,

$$\mathbf{F} = X.\mathbf{X} + Y.\mathbf{Y} + Z.\mathbf{Z}$$

A major disadvantage with CIE XYZ color system is that it does not constitute a physiologically equivalent color space, i.e., the same distance in different parts of the color space does not agree with the perceived color difference. Therefore, it cannot mimic the nonlinear response of human eye. However, the XYZ tristimulus values obtained are used as starting point for the calculation of other color parameters that are closer to human response.

CIE Lab and ΔE^*_{ab}

As discussed earlier, the human response to a stimulus is nonlinear in nature. Additionally, although the eye sensed color on RGB basis, at higher level of image processing in the brain, the human perception of chromaticity appeared to follow a space in which the two axes were redness *vs.*

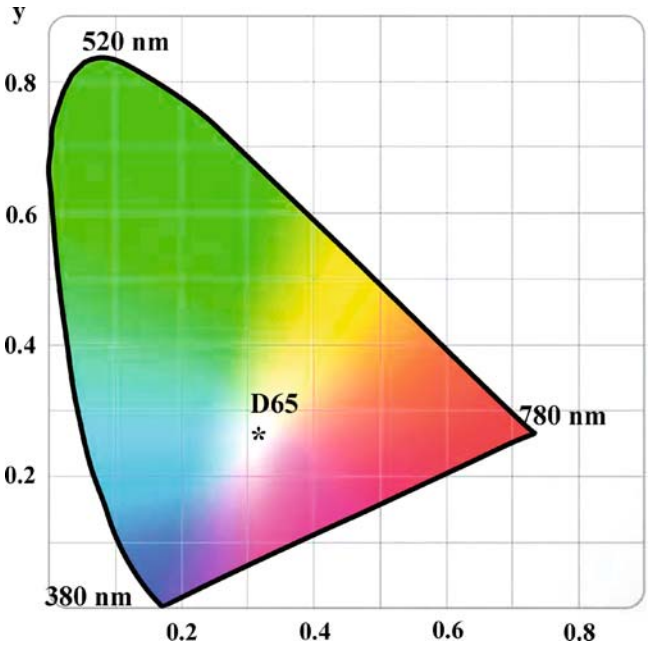

Fig. 2. The CIE XYZ color space chromaticity diagram. The outer curved boundary from 380 to 780 nm through 520 nm is the spectral locus of monochromatic colors. The *straight line* from 380 to 780 nm is a *purple line*. The chromaticity point of illuminant is D65

greenness and yellowness *vs.* blueness. By taking all the above-mentioned points into consideration, a new color space called CIE Lab (color space CIE 1976, DIN 6174) was devised. The CIE Lab color space is very close to human response and device independent (the device dependent spaces are RGB and CMYK used in monitors and printers, respectively). The traditional designation of space is "$L^*a^*b^*$", where the asterisks remind us of the nonlinear nature of three variables. The calculation of CIE Lab from CIE XYZ tristimulus is done as shown below (13)

$$L^* = 116 \cdot Y^* - 16;\ a^* = 500 \cdot (X^* - Y^*);\ b^* = 200 \cdot (Y^* - Z^*)$$

Assuming, $U \in \{X, Y, Z\}$

$$U^* = \begin{cases} \sqrt[3]{\dfrac{U}{U_n}} & \text{for } U/U_n > 0.008856 \\ 7.787\dfrac{U}{U_n} + 0.138 & \text{for } U/U_n \le 0.008856 \end{cases}$$

The subscript n refers to the tristimulus values of the perfect diffuser for the given illuminant and standard observer. The exponent 1/3 in equation for tristimulus values greater than 0.008856 provides the nonlinearity mentioned earlier. For values less than 0.008856, linearity piece is kept so as to avoid difficulty during conversion of Lab to XYZ

Also, L^* designates lightness which is equivalent to luminance like aspect of reflective color. The chrominance is described by two variables a^* and b^* representing red *vs.* green and yellow *vs.* blue axes, respectively. The spatial representation of CIE Lab color space is shown in Fig. 3.

From CIE Lab values of unirradiated and irradiated samples color difference (ΔE^*_{ab}) was calculated using following equation (13)

$$\Delta E^*_{\mathrm{ab}} \approx \left[\left(L^*_1 - L^*_2\right) + \left(a^*_1 - a^*_2\right) + \left(b^*_1 - b^*_2\right)\right]^{1/2}$$

The subscripts 1 and 2 refer to the irradiated and unirradiated samples, respectively.

The ΔE^*_{ab} value is useful for quantitation of color difference and for defining color tolerance limit.

Dominant Wavelength and Excitation Purity

The dominant wavelength of a color correlates in an approximate way with what would be called as the hue of the color as observed under everyday conditions. It can also be defined as the wavelength of the spectrum band, which, when mixed with some specified achromatic stimulus, matches the given color. The derivation of dominant wavelength form the chromaticity coordinate is carried out by drawing a straight line through the point representing the achromatic color D65 and the point S representing the color to be evaluated, and this line is produced in the direction D65 to S to intersect the spectrum locus. The wavelength of the intersection is the required dominant wavelength (λ_{d}) of the given color as depicted in Fig. 4.

The excitation purity of any color possessing a dominant wavelength is an exactly defined ratio of distances in the chromaticity diagram indicating how far the given color is displaced from the achromatic color toward the spectrum color (Fig. 4). Excitation purity is the degree of saturation relative to the most concentrated form.

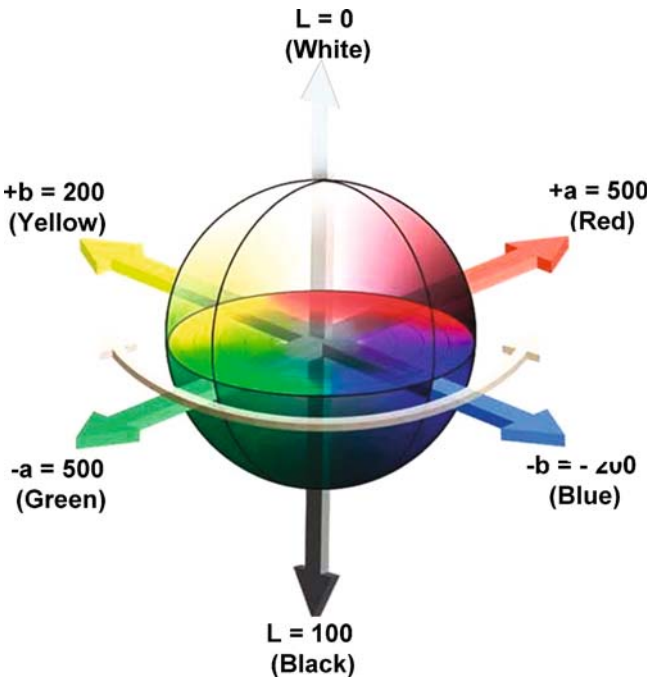

Fig. 3. The cubical CIE Lab color space

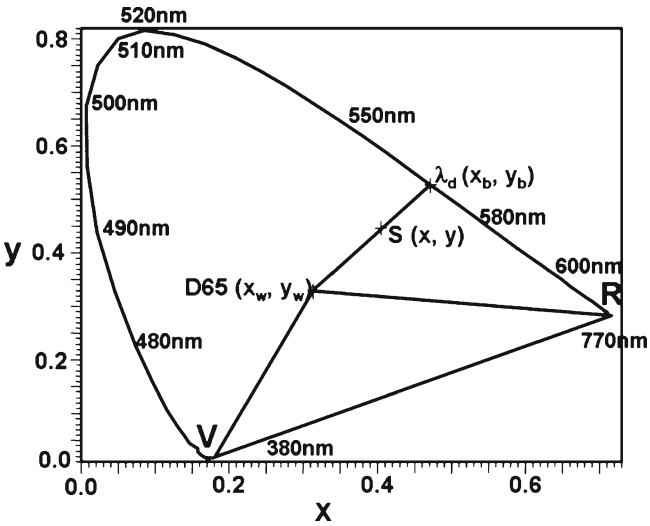

Fig. 4. Derivation of dominant wavelength and excitation purity from CIE chromaticity diagram

The excitation purity calculated from trichromatic values is defined by

$$p_e = \frac{y - y_w}{y_b - y_w} = \frac{x - x_w}{x_b - x_w}$$

Yellowness Index (ASTM Method E313)

The yellowness index shows a degree where the hue leaves white or achromatic color toward yellow. If it takes a negative value, it moves in the blue direction. The yellowness index for 2° observer and D65 illuminant is calculated by following equation

$$YI = [100(1.2985X - 1.1335Z)/Y]$$

The change of yellowness index is expressed by the difference in yellowness indices of two samples.

$$\Delta YI = YI_i - YI_u$$

Where, subscripts i and u denotes irradiated and unirradiated, respectively.

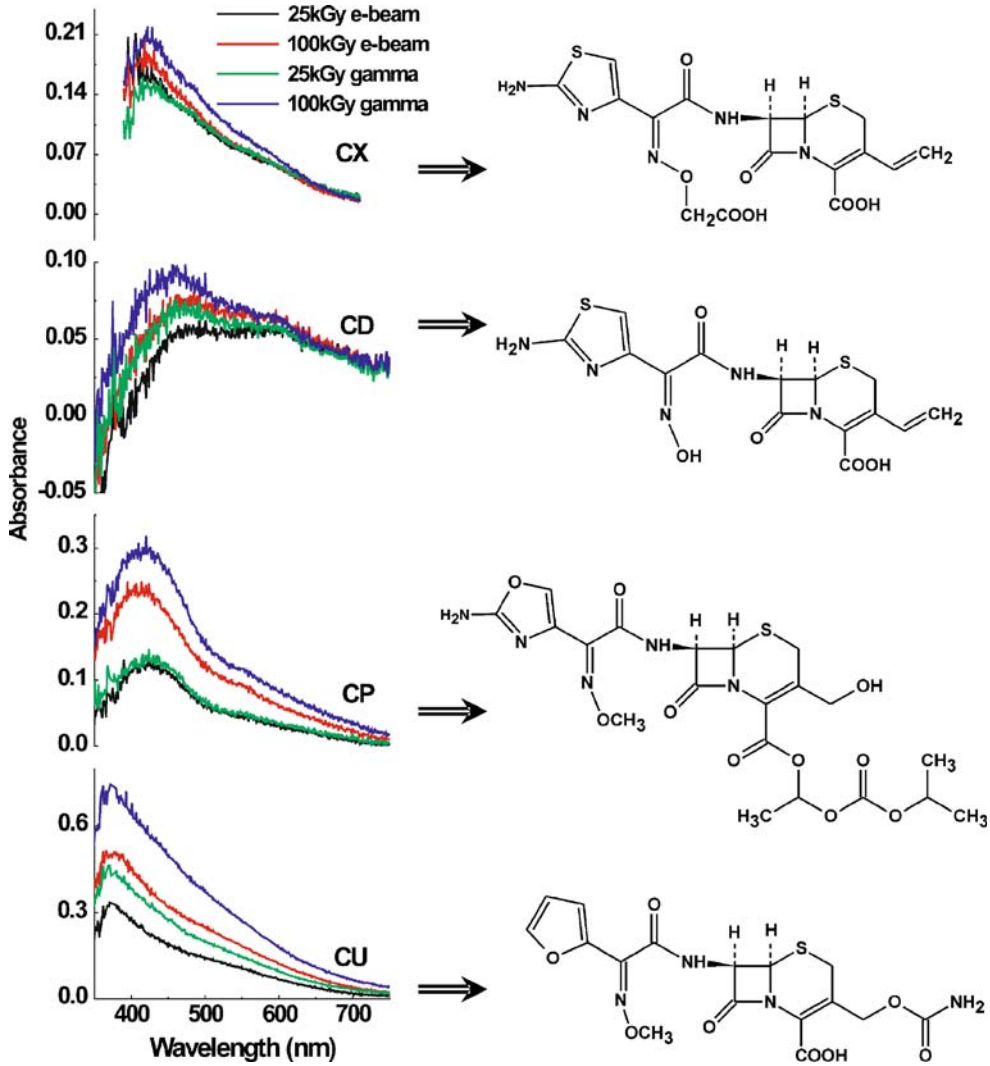

Fig. 5. Difference reflectance spectra of unirradiated and irradiated solid cephalosporins

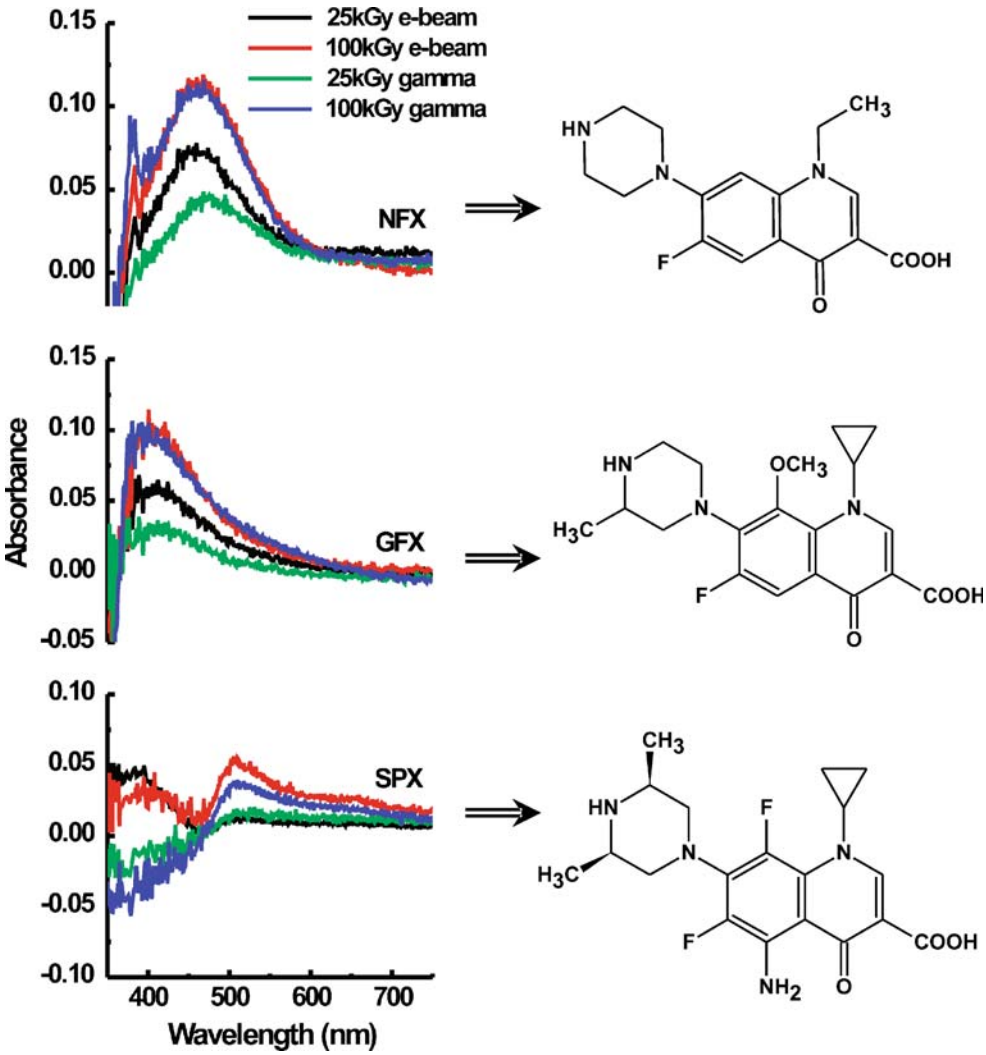

Fig. 6. Difference reflectance spectra of unirradiated and irradiated solid fluoroquinolones

RGB Color Gamut and Color Palette

A large percentage of the visible spectrum can be represented by mixing three basic components of colored light in various proportions. These components are known as the primary colors: red, green, and blue. The primary color space is used in monitor display and the *fill color* options of most of the softwares.

For 2^{O} observer and D65 illuminant, the XYZ to RGB conversion is performed as follows

$$X' = X/100$$
$$Y' = Y/100$$
$$Z' = Z/100$$
$$R' = X' \times 3.2406 + Y' \times (-1.5372) + Z' \times (-0.4986)$$
$$G' = X' \times (-0.9689) + Y' \times 1.8758 + Z' \times 0.0415$$
$$B' = X' \times 0.0557 + Y' \times (-0.2040) + Z' \times 1.0570$$
$$U' \in \{R', G', B'\}$$
$$\text{if } (U' > 0.0031308), U' = 1.055 \times \left(U'^{\wedge}(1/2.4)\right) - 0.055$$
$$\text{else } U' = 12.92 \times U'$$
$$U = U' \times 255$$

The RGB values were calculated from XYZ tristimulus values to display the color palette on screen for audience. The color given in the palette may differ from the original color of the compound, as RGB color space is not equivalent to human perception. But, for the sake of simplifying discussion for readers depicting color by RGB, color palette is also sufficient

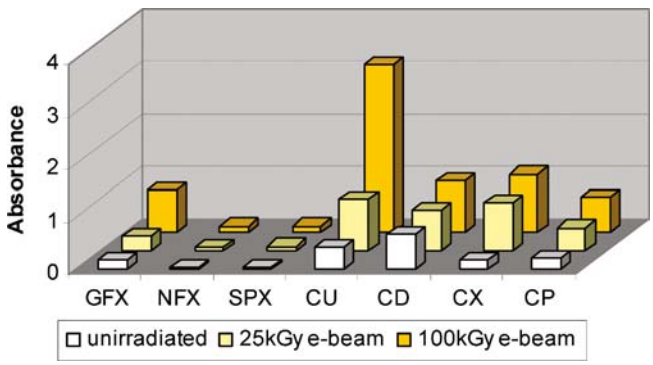

Fig. 7. Absorbance of solution of e-beam irradiated solid drug samples at 450 nm. The *plot* shows absorbance as a function of dose

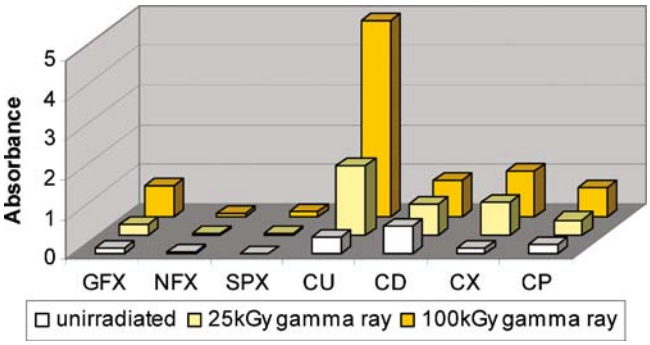

Fig. 8. Absorbance of solution of gamma irradiated solid drug samples at 450 nm. The *plot* shows absorbance as a function of dose

RESULTS

The difference reflectance spectrum was obtained by subtracting spectrum of unirradiated sample from spectrum of irradiated sample. The difference reflectance spectra of fluoroquinolones and cephalosporins are shown in Figs. 6 and 5, respectively. In the difference reflectance spectra of irradiated drug samples, absorbance bands had emerged in the visible region and the intensity of the absorption peak increased with increase in dose from 25 to 100 kGy for both gamma and e-beam irradiation. The fluoroquinolones showed slight increase in absorbance in visible region at 25 kGy dose, and at 100 kGy, the absorbance increase became significant. It can be seen in Fig. 6 that, for fluoroquinolones, e-beam was more damaging than gamma radiation. Among cephalosporins, CD has slight increase in absorbance, whereas CX, CP, and CU had significant increase in absorbance even at 25 kGy dose which further increased at 100 kGy dose. In the case of cephalosporins, gamma radiation was found to be more damaging than e-beam. Thus, from the difference reflectance spectrum distinction can be made between gamma and e-beam-irradiated samples.

In the transmittance spectra of the solution of irradiated solid drug samples, the absorbance band appeared in the visible region, and the absorbance increased with increase in dose form 25 to 100 kGy. The plot of absorbance at 450 nm against the dose is shown for e-beam and gamma-irradiated samples in Figs. 7 and 8, respectively. It was found that the quantum of discoloration, as assessed by increase in absorbance at 450 nm, by electron beam and gamma radiation, was nearly equal at a particular dose. This did not agree with reflectance results and the visual observation. Thus, this method, although can be used to distinguish an unirradiated sample from an irradiated one, cannot differentiate between gamma and e-beam-irradiated samples.

The complete set of color parameters determined (spectral 1.7 software) are shown in Table I for cefixime. For the sake of brevity, XYZ tristimulus, CIE Lab, and RGB values are not given for other compounds, since they are only useful for calculation of other color parameters and do not have any relevance in further discussion. The color parameters for remaining cephalosporins and fluoroquinolones are given in Tables II and III, respectively. The color parameters were determined to quantitate color difference and to define color tolerance limit.

The calculated color difference (ΔE^*_{ab}) between CIE Lab values of irradiated and unirradiated samples was found to be very sensitive parameter, with which assessment of small difference in color that was not even visually perceptible can be done. The investigation of color difference values indicated that for fluoroquinolones, e-beam was more damaging than gamma radiation, whereas for cephalosporins, gamma radiation was found to be more damaging than e-beam. These results are in conformation with the results obtained by studying diffuse reflectance spectra. In case of ΔYI, which is difference of yellowness indices of irradiated and unirradiated samples,

Table I. Color Parameters for Unirradiated and Irradiated Solid Cefixime (CX) Computed Using 2° Observer and D65 Illuminant

Color parameter	Notation	Unirradiated	25kGy e-beam	100kGy e-beam	25kGy gamma	100kGy gamma
	X	75.66 (0.84)	66.38 (0.42)	66.21 (0.27)	66.10 (0.39)	64.85 (0.18)
Tristimulus values	Y	80.21 (0.67)	69.83 (0.50)	69.57 (0.31)	69.48 (0.44)	67.91 (0.13)
	Z	78.51 (0.16)	61.04 (0.06)	59.82 (0.09)	60.80 (0.23)	56.91 (0.32)
	L*	91.78 (0.18)	86.91 (0.22)	86.78 (0.14)	86.74 (0.20)	85.96 (0.06)
CIE Lab values	a*	-1.18 (0.01)	0.99 (0.09)	0.19 (0.03)	0.14 (0.02)	0.68 (0.01)
	b*	6.49 (0.24)	12.53 (0.33)	13.42 (0.16)	12.45 (0.14)	14.70 (0.18)
Color difference	ΔE^*_{ab}		1.18 (0.03)	1.65 (0.02)	1.12 (0.02)	2.13 (0.02)
Dominant wavelength	(nm)	573.37 (0.03)	576.20 (0.05)	576.42 (0.01)	576.38 (0.03)	576.94 (0.05)
Excitation purity	pe	0.065 (0.001)	0.137 (0.004)	0.147 (0.001)	0.136 (0.001)	0.163 (0.002)
Yellowness index	YI	11.54 (0.11)	24.33 (0.23)	26.12 (0.18)	24.35 (0.16)	29.00 (0.19)
ΔYI	YI_i - YI_u		12.79 (0.26)	14.58 (0.21)	12.81 (0.19)	17.46 (0.23)
	R	234.6 (3.4)	227.7 (1.7)	228.3 (1.2)	227.4 (1.5)	227.7 (0.7)
RGB values	G	231.6 (1.4)	216.8 (1.6)	216.2 (1.1)	216.2 (1.4)	213.5 (0.3)
	B	219.0 (0.3)	194.0 (0.5)	192.0 (0.2)	193.7 (0.6)	187.4 (1.1)
Color palette	(RGB)					

Means and (SDs) for three replicates are given

Table II. Color Parameters for Solid Cephalosporins for Determination of Color Tolerance Limit

Color parameter	Notation	Unirradiated	25kGy e-beam	100kGy e-beam	25kGy gamma	100kGy gamma
Cefdinir (CD)						
Color difference	ΔE^*_{ab}	-	0.22 (0.05)	0.68 (0.04)	0.43 (0.04)	1.19 (0.04)
Dominant wavelength	(nm)	578.11 (0.04)	578.17 (0.03)	578.09 (0.01)	578.22 (0.02)	577.95 (0.07)
Excitation purity	pe	0.148 (0.006)	0.155 (0.005)	0.164 (0.001)	0.163 (0.002)	0.182 (0.001)
Yellowness index	YI	27.01 (0.44)	28.30 (0.35)	29.66 (0.13)	29.60 (0.29)	32.55 (0.37)
ΔYI	$YI_i - YI_u$		1.29 (0.56)	2.65 (0.46)	2.59 (0.53)	5.54 (0.58)
Color palette	(RGB)					
Cefpodoxime (CP)						
Color difference	ΔE^*_{ab}	-	2.03 (0.03)	3.35 (0.02)	2.01 (0.03)	3.91 (0.03)
Dominant wavelength	(nm)	572.36 (0.02)	574.46 (0.01)	575.76 (0.01)	574.37 (0.02)	575.66 (0.02)
Excitation purity	pe	0.051 (0.006)	0.125 (0.004)	0.179 (0.004)	0.129 (0.005)	0.214 (0.005)
Yellowness index	YI	8.96 (0.65)	21.71 (0.38)	30.90 (0.44)	22.37 (0.49)	36.29 (0.49)
ΔYI	$YI_i - YI_u$	-	12.75 (0.76)	21.94 (0.79)	13.41 (0.82)	27.33 (0.82)
Color palette	(RGB)					
Cefuroxime (CU)						
Color difference	ΔE^*_{ab}	-	3.89 (0.05)	5.91 (0.06)	5.23 (0.04)	8.66 (0.05)
Dominant wavelength	(nm)	570.52 (0.01)	577.81 (0.08)	578.84 (0.06)	577.86 (0.06)	579.37 (0.06)
Excitation purity	pe	0.026 (0.006)	0.157 (0.008)	0.242 (0.008)	0.212 (0.006)	0.349 (0.006)
Yellowness index	YI	4.43 (0.71)	28.48 (0.86)	42.80 (0.88)	37.41 (0.69)	59.64 (0.65)
ΔYI	$YI_i - YI_u$	-	24.05 (1.11)	38.37 (1.13)	32.98 (0.99)	55.21 (0.96)
Color palette	(RGB)					

Means and (SDs) for three replicates are given

Table III. Color Parameters for Solid Fluoroquinolones for Determination of Color Tolerance Limit

Color parameter	Notation	Unirradiated	25kGy e-beam	100kGy e-beam	25kGy gamma	100kGy gamma
Gatifloxacin (GFX)						
Color difference	ΔE^*_{ab}	-	0.93 (0.03)	1.83 (0.03)	1.29 (0.05)	2.15 (0.05)
Dominant wavelength	(nm)	572.86 (0.01)	573.12 (0.06)	573.09 (0.04)	573.30 (0.12)	574.54 (0.15)
Excitation purity	pe	0.038 (0.004)	0.060 (0.004)	0.095 (0.004)	0.075 (0.002)	0.096 (0.007)
Yellowness index	YI	6.78 (0.09)	10.61 (0.19)	16.49 (0.14)	13.14 (0.10)	16.92 (0.13)
ΔYI	$YI_i - YI_u$	-	3.83 (0.22)	9.71 (0.17)	6.36 (0.13)	10.14 (0.16)
Color palette	(RGB)					
Norfloxacin (NFX)						
Color difference	ΔE^*_{ab}	-	1.64 (0.02)	3.88 (0.03)	1.04 (0.03)	3.51 (0.03)
Dominant wavelength	(nm)	570.84 (0.03)	572.29 (0.05)	574.19 (0.08)	573.05 (0.04)	573.58 (0.10)
Excitation purity	pe	0.049 (0.001)	0.110 (0.002)	0.155 (0.001)	0.084 (0.003)	0.151 (0.002)
Yellowness index	YI	8.43 (0.16)	18.38 (0.19)	26.45 (0.16)	14.57 (0.38)	25.47 (0.34)
ΔYI	$YI_i - YI_u$	-	9.95 (0.25)	18.02 (0.22)	6.14 (0.41)	17.04 (0.37)
Color palette	(RGB)					
Sparfloxacin (SPX)						
Color difference	ΔE^*_{ab}	-	-0.69 (0.02)	-0.64 (0.03)	-0.73 (0.02)	-0.82 (0.03)
Dominant wavelength	(nm)	568.71 (0.04)	569.04 (0.04)	569.95 (0.06)	568.96 (0.04)	569.71 (0.01)
Excitation purity	pe	0.247 (0.002)	0.246 (0.003)	0.249 (0.006)	0.239 (0.004)	0.237 (0.005)
Yellowness index	YI	35.88 (0.23)	36.00 (0.36)	37.06 (0.72)	35.05 (0.47)	35.37 (0.56)
ΔYI	$YI_i - YI_u$	-	0.12 (0.43)	1.18 (0.75)	-0.83 (0.52)	-0.51 (0.61)
Color palette	(RGB)					

Means and (SDs) for three replicates are given

similar trends were observed. The basis for selection of ΔYI for assessment of discoloration was that the observed color change for most of the studied fluoroquinolones and cephalosporins was from white to off-white to sand to brownish. The tolerance limits proposed for ΔE^*_{ab} and ΔYI are ±2 and ±10 U, respectively, because, within these limits, the change in color is not conspicuous as can be seen in the color palette (Tables I, II and III). NFX, GFX, SPX, and CD at 25 kGy dose fall within this limit, whereas CU, CP, and CX were outliers.

The dominant wavelength for all compounds had shifted to higher values indicating change in hue. The shift of 1 nm did not result in much discoloration, as can be seen in the case of NFX 25 kGy e-beam and NFX 25 kGy gamma-irradiated samples (Table III). Sometimes, 2-nm shift was also found to be acceptable, but in such cases, the increase in excitation purity should be moderate. For example, (a) the dominant wavelength increase between NFX 25 kGy e-beam, and NFX 100 kGy e-beam samples was 1.90 nm, and the corresponding excitation purity difference was 0.045, as a result, there was no striking perceptible difference between them; (b) the dominant wavelength increase between CU 25 kGy gamma and CU 100 kGy gamma-irradiated samples was 1.49 nm, and excitation purity difference was 0.137 here ,although the dominant wavelength increase was less but due to large difference in excitation purity, the perceptible color difference was found to be significant.

DISCUSSION

The discoloration of solids on irradiation can be attributed to free radical species trapped in solid matrix and/or molecular radiolytic species (3). The study of difference reflectance spectra revealed that the fluoroquinolones were more susceptible to damage by e-beam, whereas for cephalosporins, gamma ray was more damaging. The plausible explanation for this behavior can be that the radiation chemical yield of two color-rendering species, i.e., free radical species and molecular radiolytic product is dependent on the nature of the compound and dose-rate of ionizing radiation (e-beam has higher dose rate than gamma radiation).

In case of CX, CP, and CU significant absorbance increase was obtained even at 25 kGy dose. The reason for such a significant increase of absorption in visible region can only be due to unsaturated chromophore systems in the radiolytic products of these compounds.

The increase in absorbance at 450 nm obtained in solution of irradiated samples may be attributed to molecular radiolytic products formed during irradiation of solids and from radical reactions (inter-radical, radical-oxygen, and radical-solvent) occurring upon dissolution (7). The absorbance at 450 nm of e-beam and gamma-irradiated samples was nearly same. Therefore, distinction between e-beam and gamma irradiated was not observable in solutions of irradiated samples. Thus, reflectance measurement has clear advantage over transmittance measurement of solution in assessing discoloration of irradiated drugs.

Among color parameters, ΔE^*_{ab} and ΔYI were found to be suitable for defining color difference. The proposed color tolerance limits for ΔE^*_{ab} and ΔYI are ±2 and ±10 U, respectively, because, in this range, the perceptible color difference between irradiated and unirradiated sample was not significant. All fluoroquinolones at 25 kGy dose were within this range. The radio-resistance of fluoroquinolones can be attributed to the aromatic character of quinolone moiety (14). In case of cephalosporins, CU, CP, and CX were outliers and, hence, radiosensitive, whereas CD was found to be radio-resistant. From the structure of cephalosporins, it seems that the lactam ring might be responsible for their radio-sensitivity. The radio-resistance of CD, however, could not be explained.

As dominant wavelength values should always be accompanied with excitation purity, therefore, defining color tolerance limit with this parameter would be cumbersome.

CONCLUSIONS

The color parameters ΔE^*_{ab} and ΔYI obtained from color analysis of diffuse reflectance spectrum can be reliably used for assessing discoloration of solid fluoroquinolones and cephalosporins. The distinction between gamma and e-beam-irradiated samples can be done by color parameters derived from reflectance spectrum, which was not possible by measuring absorbance of solution at 450 nm. All fluoroquinolones were found to be radio-resistant, whereas in case of cephalosporins, only CD was radio-resistant.

The proposed tolerance limits are not restricted for these antibiotics only, but its use can be extended to study the effect of ionizing radiations on other drugs also.

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REFERENCES

1. The European agency for the Evaluation of Medicinal Products. Decision tree for the selection of sterilization methods (CPMP/ QWP/ 054/ 98 corr). EMEA committee for proprietary medicinal products, London, 5 April 2000.
2. G. O. Mercanoğlu, A. Y. Özer, S. Colak, M. Korkmaz, M. Özalp, M. Ekizoğlu, N. Barbarin, and B. Tilquin. Radiosterilization of sulfonamides I: determination of the effects of gamma irradiation on solid sulfonamides. *Rad. Phy. Chem.* **69**:511–520 (2004).
3. A. S. Crucq, C. Slegers, V. Deridder, and B. Tilquin. Radiosensitivity study of cefazolin sodium. *Talanta*. **52**:873–877 (2000).
4. M. Gibella, and B. Tilquin. Detection of the radiolysis of solid ampicillin by UV spectroscopy. *Analusis*. **27**:657–662 (1999).
5. B. Marciniec, Z. Plotkowiak, L. Wachowski, M. Kozak, and M. Popielarz-Brzezińska. Analytical study of beta irradiated antibiotics in the solid state. *J. Therm. Anal. Calorim.* **68**:423–436 (2002).
6. A. S. Crucq, V. Deridder, and B. Tilquin. Radiostability of pharmaceuticals under different irradiation conditions. *Rad. Phy. Chem.* **72**:355–361 (2005).
7. L. Varshney, and P.B. Dodke. Radiation effect studies on anticancer drugs, cyclophosphamide and doxorubicin for radiation sterilization. *Rad. Phy. Chem.* **71**:1103–1111 (2004).

8. British pharmacopoeia, Appendix IIB and Appendix XVIII, 2004.
9. A. Tücks, and H. P. Beck. The photochromic effect of bismuth vanadate pigments. Part I: Synthesis, characterization and lightfastness of pigment coatings. *J. Solid State Chem.* **178**:1145–1156 (2005).
10. G. Paschos. Fast color texture recognition using chromaticity moments. *Pattern Recogn. Lett.* **21**:837–841 (2000).
11. S. A. Nouh, N. Ibrahim El-Tayeb, A. F. Said, M. M. Radwan, and S. A. El-Fiki. Structural and optical studies of electron beam-irradiated Makrofol nuclear track detector. *Radiat. Meas.* **42**:8–13 (2007).
12. G. Wyszecki, and W. S. Stiles. *Color Science, Concepts and Methods: Quantitative Data and Formulas*, Wiley, New York, 1967.
13. K. Nassau. *Color for Science, Art and Technology*, Elsevier, New York, 1998.
14. J. E. Williard. Radiation chemistry of organic solids. In I. Farhataziz, and Michael A. J. Rodgers (eds.), *Radiation Chemistry: Principles and Applications*, VCH, New York, 1987, pp. 410.