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A novel system for the objective classification of iris colour and its correlation with response to 1% tropicamide

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Summary

Iris colour can provide an enormous amount of information about an individual. In addition to changes with pathological conditions, the colour of the iris can be a particularly useful indicator of how well a person will respond to a topically applied ocular drug. Until recently, classification of iris colour has been subjective, ranging from a basic description ('light' and 'dark') to more detailed grading systems, such as a comparison with preset photographic standards. However, variability within observers and differences in the interpretation between observers can influence the results. Objective techniques, in this respect, possess several advantages. They are able to detect differences in colour that subjective techniques are incapable of and they provide continuous data rather than discrete categories, thus improving the accuracy of drug response predictions. This study assessed iris colour by objective means. Slit-lamp photographs of various coloured irides were taken under standardised conditions. The slides were then scanned into a computer and the colour analysed using a calibrated software package. To establish the optimum colour parameter to be used for predictions of drug response, several parameters were calculated and compared with the subject response to 1% tropicamide (maximum change in pupil size, time to maximum change and total duration of effect). Many parameters had strong correlations with drug response, but the parameters '*z*', '*b*' (the proportion of blue in the image) and '*y*' (the proportion of yellow in the image) were found to exhibit the highest correlations. They also showed better correlations with drug response than did a current iris colour grading system. © 1998 The College of Optometrists. Published by Elsevier Science Ltd

Introduction

The colour of the iris has long been known to depend upon the amount of melanin pigment present in the tissues (Snell and Lemp, 1989). Ocular melanin, synthesised within melanocytes, is believed to have primarily a photo-protective role. Absorption of light protects the retina from overexposure and reduces reflections from the fundus (Sarna, 1992). Melanin is also able to change its molecular form to inactivate potentially harmful free radicals that are photochemically created. In this case, its function is to protect against radiation-induced damage (Ings, 1984). Furthermore, melanin has been considered a protective chemical filter, as it readily absorbs and binds with drugs initially and then slowly releases them in low non-toxic concentrations (Larsson, 1993).

Measurement of iris colour and its changes can be of great importance. The colour of the iris can be affected by conditions such as melanoma (Rootman and Gallagher, 1984), Horner's Syndrome and Fuch's heterochromic iridocyclitis (Imesch *et al.*, 1997). It has also been shown to correlate with diabetic eye disease (Moss *et al.*, 1987), melanoma (Regan *et al.*, 1997) and age-related macular degeneration (Stock *et al.*, 1995). The degree of iris pigmentation has frequently been reported to affect the response charac-

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teritics to a variety of topically applied drugs, such as thymoxamine (Diehl *et al*., 1991), cyclopentolate (Manny *et al*., 1991) and timolol (Laurence *et al*., 1995).

Classification of colour was originally crude, defining the iris as 'light' or 'dark' (Dillon *et al*., 1977) or 'blue' or 'brown' (Lovasik and Kergoat, 1990). Grading systems gradually superceded descriptive methods due to their simplicity, reproducibility and ease of use (for example, Chylack *et al*., 1989). A 5-point numerical grading system whereby the iris is compared with standard photographs (Seddon *et al*., 1990) has been successfully used, as has a similar approach comparing eye colour with a variety (15) of painted glass eyes (Bito *et al*., 1997). However, the accuracy of all these techniques depends upon the reliability of the observer. Colour can be perceived differently between people, which introduces a further source of variation in results. A system that does not involve subjective decision-making will undoubtedly be more repeatable.

Use of objective iris reflectometry to assess iris colour was investigated but interactions between tissue scattering, blood and melanin absorption were complex (Delori *et al*., 1991). Simpler computer analysis of colour has offered a potentially easier method. Colour image processing has been successfully used in the field of dermatology (Herbin *et al*., 1990) and more recently used to monitor age-related macular degeneration (Friberg and Balas, 1996) and assess changes in iris pigmentation (Bee *et al*., 1997).

The present study has addressed the calibration of such a system and established the most appropriate colour parameter to be measured for future predictions of drug response from the colour of the iris.

Methods

Calibration

Initially, the optimum slit-lamp and camera settings for this study were established. Four subjects (mean age 22.5 years), with blue, green, light brown and dark brown irides respectively, were positioned at a Zeiss slit-lamp with a Zeiss 040 photographic attachment and a series of photographs was taken on Kodak Ektachrome 200 film varying the flash intensity, flash time, slit-lamp light intensity, illumination angle, magnification and camera aperture stop. The optimum settings, subjectively determined, were those which produced an evenly illuminated image of one iris quadrant, without overexposure or corneal reflections over the iris area. These settings were used for all future photographs.

The chromaticities (x,y) and luminance (Y) (CIE, 1986) of all 85 caps from the Farnsworth-Munsell 100-Hue Test (Farnsworth, 1943) were then measured using the Minolta Chroma Meter CS-100 with Close-up lens attachment (no. 122) at a distance of approximately 37 cm. The ambient room illuminance was 200–300 lux and the caps were specifically illuminated by a vertical daylight fluorescent tube (Osram T8 triphosphor tube, 36 W, CCT = 6000 K, Ra = 80–89) positioned at 45° and 30 cm from the caps. A white sheet of paper was also measured. Subsequently, all caps and the white paper were mounted and photographed individually at the slit-lamp. The film was developed as a 35 mm slide format using standard Kodak E6 processing.

All slides were processed into a 486 PC computer using the Nikon Coolscan™ 35 mm scanner and viewed through Paint Shop Pro™ (PSP). The area of interest on each image was selected and the average red (R), green (G), blue (B) and intensity values were measured. Values ranged from 0 to 255, where light colours possessed high values and dark colours had low numbers. If R, G and B were all the same, the image appeared to be grey. High frequency noise, which causes local variations in brightness across each slide, was eliminated by viewing the image through a specialist median filter (PSP option) before the RGB and intensity measurements were made.

All *Yxy* values obtained directly from the chromameter (referred to as 'real' values) were converted to X, Y, and Z and then R, G and B values (Hunt, 1987). R, G and B values obtained indirectly from software analysis (referred to as 'slide' values) were compared with the 'real' values and the transformation from 'slide' values to 'real' values was calculated. This then allowed all future 'slide' values to be 'corrected' (converted back to the original 'real' values).

Table 1. Colour parameters calculated in the study

System	Parameters	Reference/Details
1	R, G, B	Hunt, 1987 (p. 45)
2	r, g, b	(proportions of RGB)
3	I1, I2, I3	Ohta *et al*., 1980
4	A, C1, C2	Faugeras, 1979
5	C, M, Y	Poynton, 1995
6	c, m, y	(proportions of CMY)
7	Y, I, Q	Travis, 1991 (p. 78)
8	X, Y, Z	CIE, 1986
9	x, y, z	CIE, 1986 (proportions of XYZ)
10	S, M, L	Travis, 1991 (p. 100)
11	s, m, l	Travis, 1991 (p. 103) (proportions of SML)
12	L*, a*, b*	Hunt, 1987 (p. 197)
13	L*, u*, v*	Travis, 1991 (p. 98); Hunt, 1987 (p. 197)
15	% black	

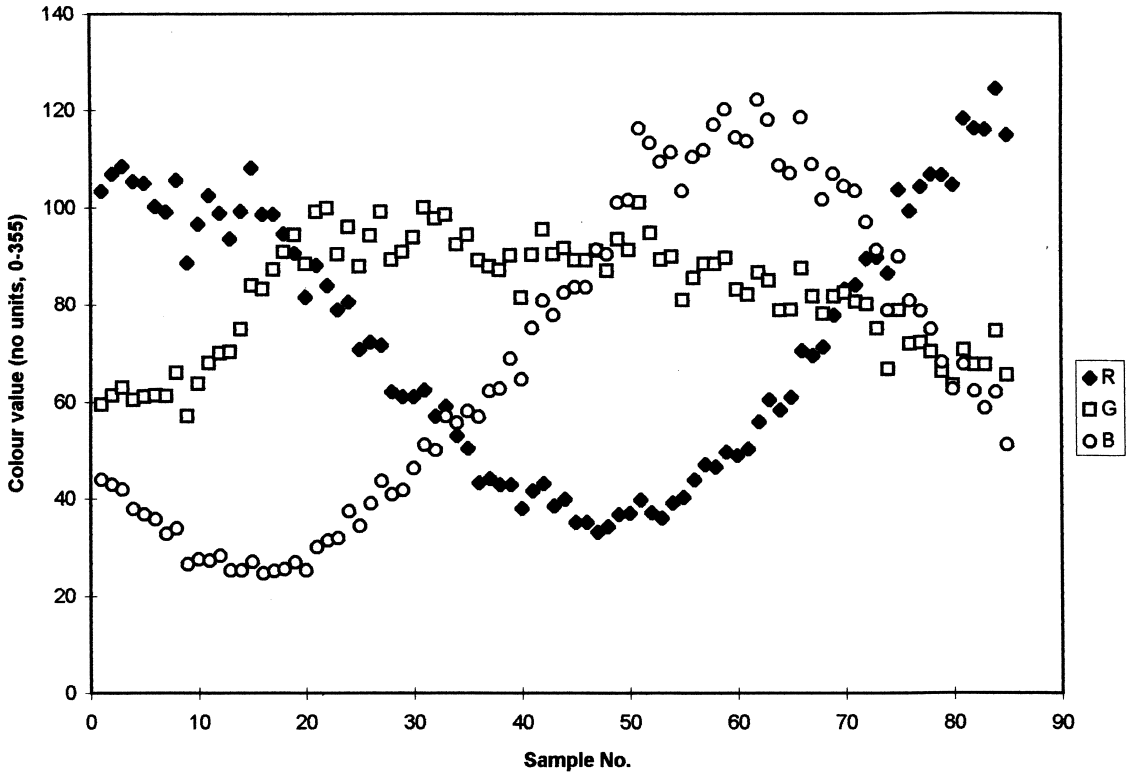

Figure 1. Variation in 'real' R, G and B values for the 100-hue caps.

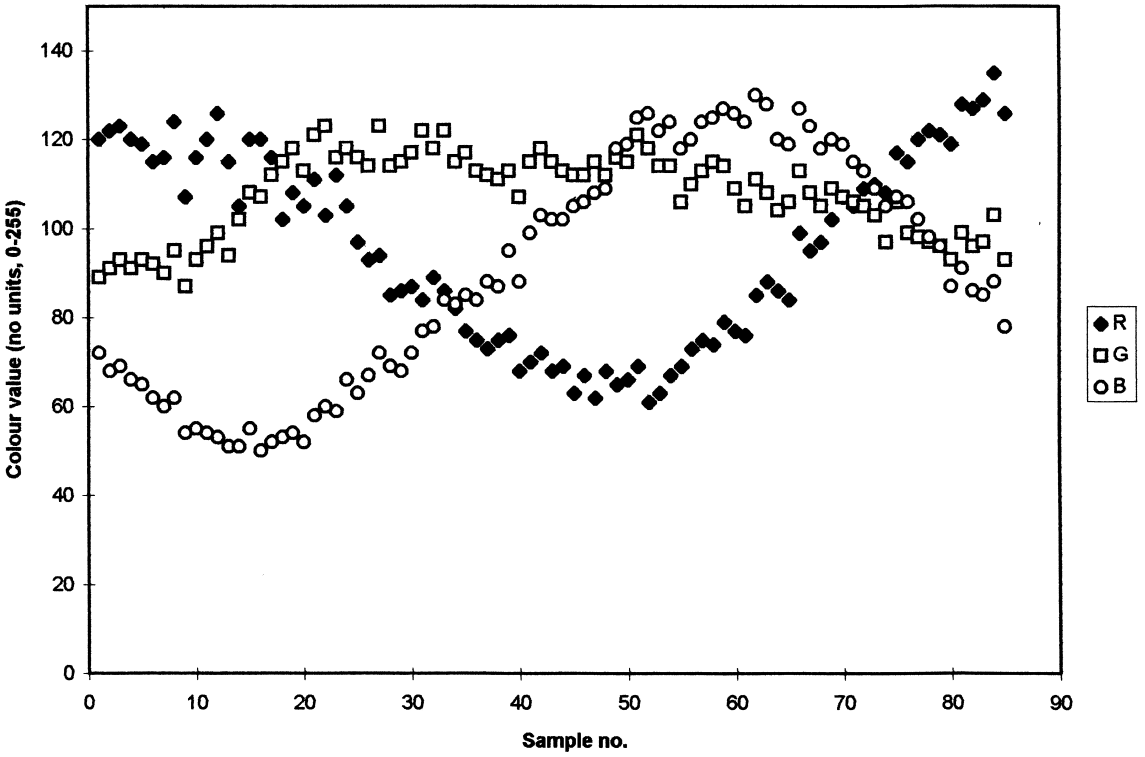

Figure 2. Variation in 'slide' R, G and B values for the 100-hue caps.

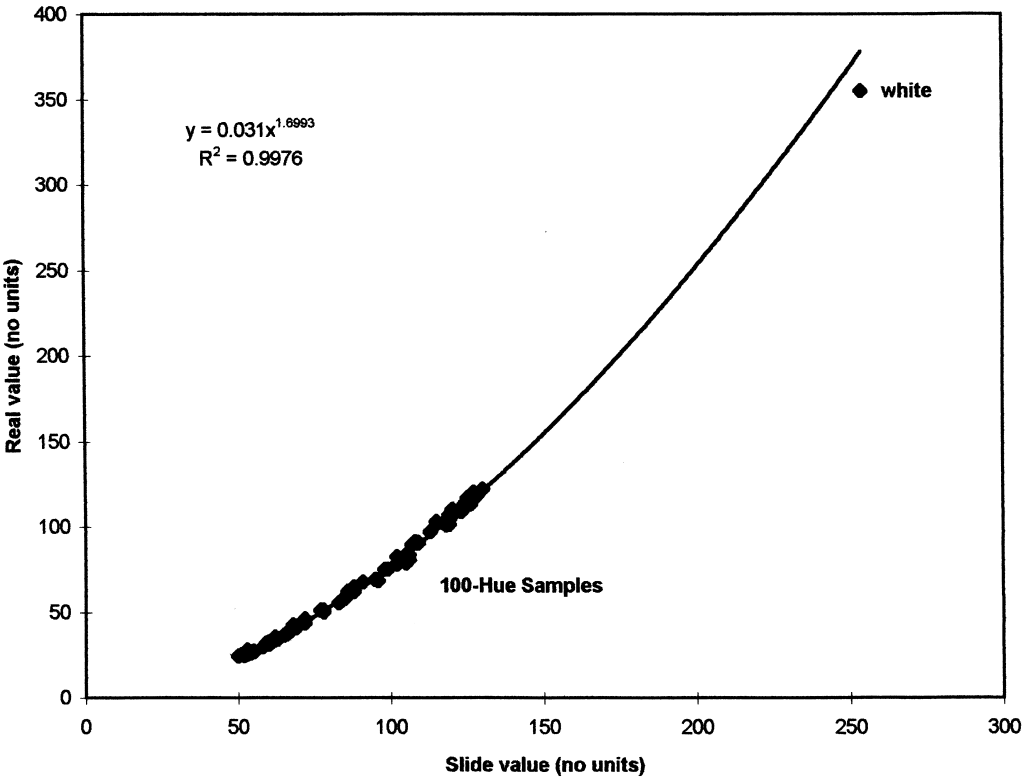

Figure 3. Relationship between 'real' and 'slide' values for the 100-hue caps (only parameter B shown).

Measurement of iris colour

Utilising the established camera and slit-lamp settings, four photographs, one of each iris quadrant, were taken. A broad spectrum of colours was included: 20 subjects from each of the five Seddon categories (Seddon *et al.*, 1990) and four albino volunteers (classified arbitrarily as grade 0) were photographed (104 subjects in total, mean age 21.73 years). All slides were scanned into the computer and the R, G, B and intensity values recorded as described above. 'Slide' values were corrected and a variety of parameters from commonly used colour systems were calculated. These are shown in *Table 1*.

Correlation between iris colour and drug response

The left pupil diameter of all subjects (n = 104, mean age 21.73 years) was measured using a calibrated video slit-lamp, before being dilated with 25 μl 1% tropicamide (measured and instilled using an automated Gilson pipette; approximately equivalent to one drop). The pupil diameter was measured every 3 mins for the first hour following instillation. Thereafter, the subjects were asked to subjectively monitor their pupil size every 15 min until both pupils appeared equal under average room illumination. The maximum % change in pupil diameter, the time to reach maximum effect (T_{max}) and the total duration of effect (from instillation to full recovery, T_{tot}) were calculated and com-

Table 2. Correlations between colour parameters and measurements of response to 1% tropicamide

Parameter	Correlation with max. % pupil change	Correlation with time to maximum effect (T_{max})	Correlation with total duration of effect (T_{tot})
z (sys. 9)	0.968	–0.973	–0.992
b (sys. 2)	0.968	–0.973	–0.991
y (sys. 6)	–0.968	0.973	0.991
Seddon	–0.880	0.895	0.902

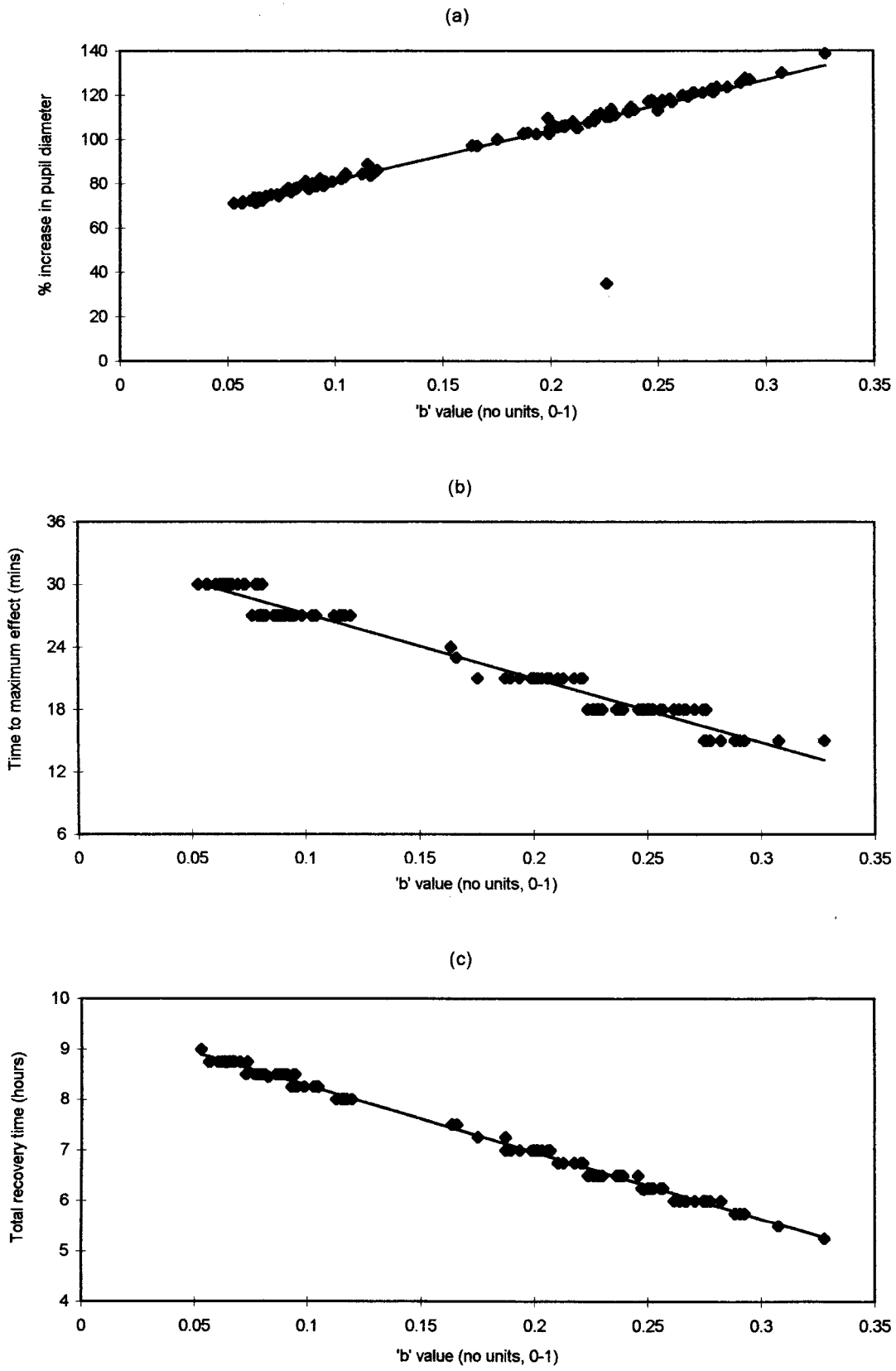

Figure 4. Correlation between drug response to 1% tropicamide and parameter '*b*' (system 2). (a) Maximum % change in pupil diameter, (b) time to reach maximum effect, (c) total time of effect.

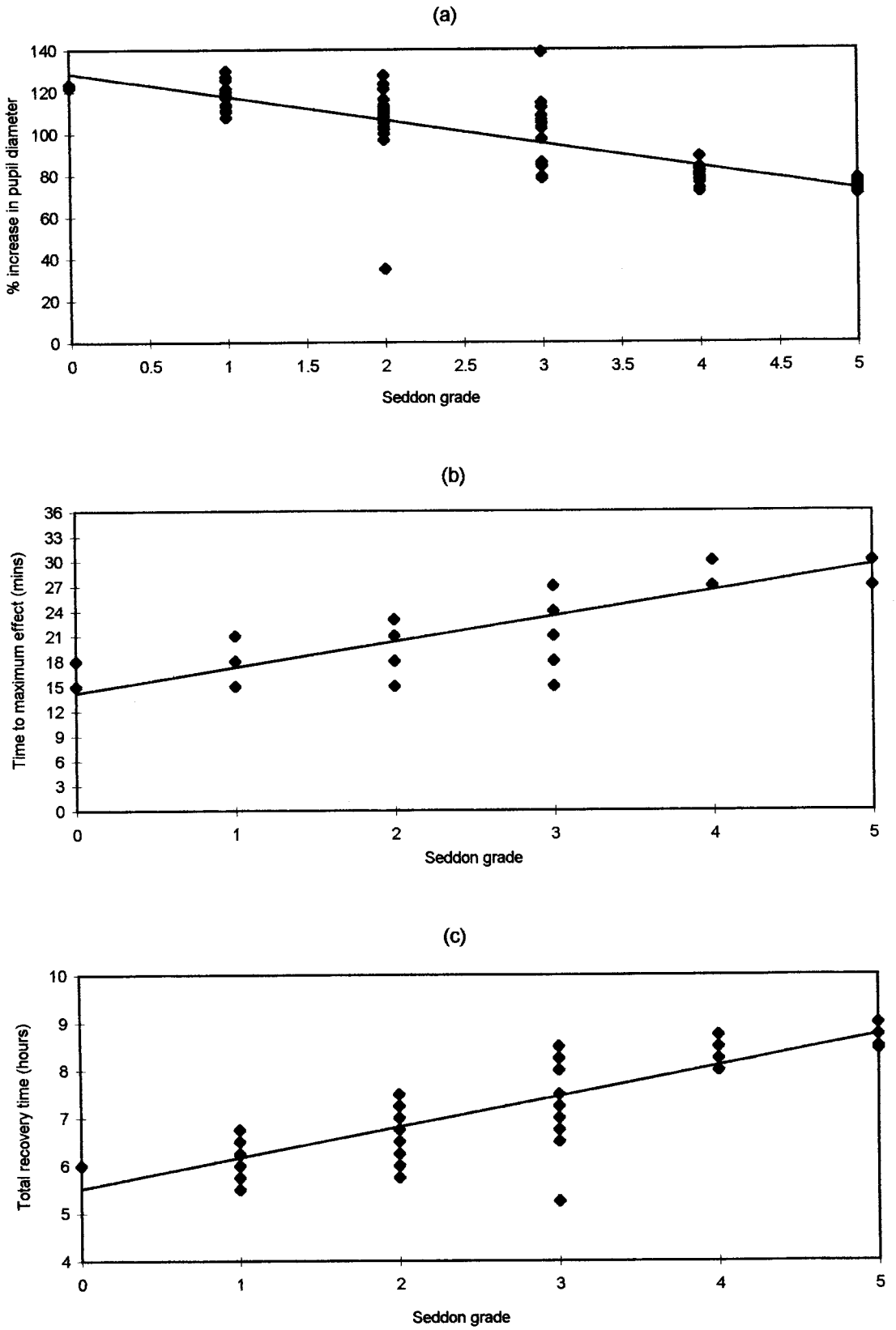

Figure 5. Correlation between drug response to 1% tropicamide and Seddon classification (1990). (a) Maximum % change in pupil diameter, (b) time to reach maximum effect, (c) total time of effect.

pared with previously determined colour parameters. Correlations between each parameter and each response were calculated and tested for significance.

Results

Calibration

The variation in R, G and B ('real') between the different 100-hue caps is shown in *Figure 1* and the photographic alteration in these parameters ('slide') illustrated in *Figure 2*. Comparison of these two data sets enabled the transformation to be calculated, from which future 'slide' values could be converted back to 'real' values (*Figure 3*). If the values for white were excluded from the data set, the transformation equations did not radically change. In summary:

$$\text{R: 'real'} = 0.03\cdot(\text{'slide'})^{1.71}$$
$$\text{G: 'real'} = 0.03\cdot(\text{'slide'})^{1.69}$$
$$\text{B: 'real'} = 0.03\cdot(\text{'slide'})^{1.70}$$

Measurement of iris colour and correlation with drug response

After conversion of all 'slide' values to 'real' values, followed by calculation of each parameter, correlations between the drug responses and iris colour were computed. All correlations (Spearman) were significant ($P < 0.05$) but varied from moderate ($R = -0.42$, system 7, parameter Q correlated with maximum % pupil change) to very strong ($R = -0.99$, system 4, parameter C2 correlated with total time of effect). Parameters z (system 9), b (system 2) and y (system 6) exhibited particularly strong correlations with all three measures of drug response and were not significantly different from each other ($t = 1.0$, $P = 0.42$). See *Table 2* and *Figure 4*. These measurements of iris colour also showed significantly greater correlations with drug response than a currently used subjective grading system ($t = 24.2$, $P < 0.005$; Seddon *et al.*, 1990)—see *Figure 5*. It was not surprising that 'b' and 'y' correlations were equal but opposite, since $b = 1 - y$.

Conclusions

Calibration of a computer and software system for iris colour measurements proved to be relatively straightforward. Comparison of the real colour (RGB) of standards, such as the 100-hue caps (Farnsworth, 1943), with adjusted slide colour measurements allowed changes due to the photographic film, developing, scanning and viewing software to be taken into account. Application of this system for measurements of iris colour was quick and easy. It could be reliably used to monitor iris colour in conditions such as Fuch's heterochromic iridocyclitis, Horner's syndrome and melanoma. It may also provide a good indication of the response to an instilled drug. Although a well-known subjective iris classification system (Seddon *et al.*, 1990) showed good correlations with each drug response for 1% tropicamide, objective measurements of either the 'z', 'b' or 'y' colour value of the iris provided a better indicator of the drug response. Moreover, this may apply for other drugs, but further investigation is required to confirm this.

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