International Journal of Pharmaceutics, 94 (1993) 211–222
Elsevier Science Publishers B.V.

IJP 03214

Assessment of corticosteroid-induced skin blanching: evaluation of the Minolta Chromameter CR200

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(Received 9 December 1992)
(Modified version received 28 January 1993)
(Accepted 8 February 1993)

Key words: Corticosteroid; Skin blanching; Chromameter; Visual assessment; Instrumental measurement

Summary

The major criticism of the human bioassay currently employed for corticosteroid activity is that it requires the use of experienced assessors subjectively to determine and rank the degree of skin pallor (blanching) induced in the skin by the topical application of a formulated product. Recently a number of studies have suggested that the use of tri-stimulus colorimetry (using the Minolta Chromameter CR 200) provides an instrumental and hence objective means of assessing such topical blanching activity. The aim of the present study was to evaluate further the possibility of employing the Chromameter routinely in the bioassay of topical corticosteroids. In one study, employing ten volunteers, the effects of pressure of the measuring device on the skin and the influence of site of application were determined, prior to the application of any active agent. Blanching profiles were determined for clobetasol propionate (0.05%) and betamethasone 0.1% (as valerate) creams under occluded and unoccluded conditions (in a further two studies, each comprising 10 volunteers) using both visual assessment and instrumental measurement. It was established that: (1) application of pressure to the skin induces a measurable change in skin colour and must be avoided to prevent error in instrumental readings, (2) there is a variation in natural skin colour from elbow to wrist, (3) there is a marked but reproducible diurnal variation in skin colour, (4) providing the diurnal variation in skin colour is subtracted from the readings obtained as a result of corticosteroid-induced blanching, then a good correlation exists between the visual and chromameter techniques both for very potent and potent corticosteroids. On the basis of these results, it is recommended that the use of the Chromameter in skin-blanching assays still requires careful development and more validation before its use can be recommended in a bioassay intended to replace the traditional visual assessment method.

Introduction

The observation that visible blanching of the skin often occurs after application of corticosteroid formulations (McKenzie and Stoughton, 1962) and that the degree of blanching can be employed as an index of percutaneous absorption of the active drug has formed the basis of one of the best established human bioassays for assessing topical products.

Since its inception, the bioassay has been both modified and extended by numerous workers (e.g., Barry and Woodford, 1978; Haigh and Kanfer, 1984) and has been shown to be effective in

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discriminating between the potency (Stoughton and Cornell, 1987) and the bioavailability of a variety of corticosteroid formulations (Barry and Woodford, 1974; Martin and Marriott, 1989). Whilst the assay represents an indirect test, a correlation has been established between the degree of blanching and therapeutic potency (Cornell and Stoughton, 1985).

The advantages of the blanching assay are that it is non-invasive, inexpensive, reproducible, the volunteers remain relatively unhindered and, with the use of a suitably portable 'standard' light source, the assessment does not have to be undertaken at a single location. The major criticism of the assay is however, the inherent subjectivity of the visual qualification of the degree of skin pallor (blanching) which requires the use of experienced assessors. The use of ordinal data scales employed in the currently widely-used technique also limits the power of the statistical analyses which can be carried out on the resultant data. In a recent interim guidance report on topical corticosteroids (Shah et al., 1992), the Federal Drug Administration (FDA) suggests that with increasingly sophisticated methods of detecting physical and chemical changes, the use of a human observer to assess the magnitude of physiologic effect is becoming unacceptable.

A number of techniques have been proposed to automate the assay with a view to quantifying the blanching response more objective. Whilst some of these techniques, such as reflectance spectrometry (Zaun and Altmeyer, 1973; Altmeyer and Zaun, 1974; Feather et al., 1982; Ryatt et al., 1983; Conner et al., 1990) have shown promise, other techniques such as laser-Doppler velocimetry (Amantea et al., 1983; Duteil et al., 1990) and thermography (Aiache et al., 1980) have been less successful. Such instrumental methods of assessing the degree of pallor of the skin generally have concomitant disadvantages of utilising expensive, non-portable equipment and cumbersome, time-consuming data collection procedures.

Recently, the technique of tristimulus colorimetry (using the Minolta Chromameter CR200) has been employed with some success to evaluate erythema (Babulak et al., 1986; Westerhof et al., 1986; Seitz and Whitmore, 1988). Waring et al. (1990) first reported the use of the technique to obtain blanching profiles and subsequent studies have enthusiastically recommended its use to provide an objective, precise evaluation of topical corticosteroid potency (Queille-Roussel et al., 1991; Pershing et al., 1992). More recently, it has even been proposed that it forms the basis of an internationally accepted colour measurement standard (Chan and Li Wan Po, 1992a). The FDA realising the need to supplement the vasoconstrictor assay with other methods of determination, is presently advocating the concomitant use of the Chromameter. Further data are undoubtedly required, however, before a full assessment of the validity of this technique can be made.

The present study was undertaken with the intention of evaluating further the effectiveness of the Chromameter CR 200. Application pressure, diurnal and positional variation of skin pallor was investigated and the instrument employed to measure blanching induced by corticosteroid formulations, applied under both occluded and non-occluded regimens.

Methods

Subjects

Three studies were undertaken: each utilised 10 Caucasian volunteers (five male, five female, age range 22–35 years). All volunteers had not been treated with either topical or systemic corticosteroids for at least 2 months prior to the trials. Written informed consent was obtained from each volunteer before the commencement of the investigation and the study protocols were approved by the Ethical Committee, University of Brighton.

Experimental design

Five sites were utilised per forearm and defined using a circular template (40 mm diameter) and marked with permanent ink.

Study 1 was undertaken to characterise the nature of any volunteer, time or positional dependent variation in skin colour. No corticosteroids were applied to the skin, skin colour being deter-

mined using the Chromameter at 2-h intervals (08:00–16:00) over a 5 day period.

In studies 2 and 3, three of the five sites were used as controls, the other two sites being allocated to either clobetasol propionate 0.05% cream (Dermovate, Glaxo Laboratories, Greenford, U.K.) or betamethasone 0.1% (as valerate) cream (Betnovate, Glaxo Laboratories, Greenford, U.K.). The appropriate cream was applied (2 mg/cm^2) to 2 × 2 cm^2 discrete sites outlined with double-sided adhesive Blenderm (3M Health-Care, Loughborough, U.K.) tape, placed centrally within the pre-marked circle. The cream was spread evenly over the site using a solid glass rod (2.2 mm diameter). Control sites and test sites alternated along the arm, although the placement of each corticosteroid to a specific test site was allocated randomly on the 10 volunteers. In study 2 all sites were occluded by placing Melinex S film (ICI plc, Wilton, U.K.) on the Blenderm tape whilst in study 3, the Blenderm tape was removed and the sites left uncovered. In studies 2 and 3, the creams were left in situ for 6 h, after which time the sites were exposed and gently washed with unperfumed soap and water. Skin blanching was then evaluated by both visual and Chromameter assessment at 1, 2, 6, 18, 42, 50, 66, 74, 90 and 98 h after washing the sites.

Visual assessment

Skin blanching was assessed under standardised lighting conditions provided by a Hancocks lightbox fixed approx. 350 mm above the arm. The degree of pallor was estimated using a 0–4 point scale with half point ratings (Table 1). All estimations were made without reference to application charts.

Chromameter assessment

The Chromameter (Model CR 200, Minolta, Milton Keynes, U.K.) is a portable colorimeter which uses a reflected light principle produced by a pulsed xenon arc lamp the response of which is measured by six silicon photocells. It has a range of colour space measurement systems, but for these studies it was operated in the L^* a^* b^* mode, a three-dimensional technique, which closely resembles the human sensitivity of the eye to colour. The measurement system is adapted from the CIE (Commission Internationale de l'Eclairage 1931) x,y chromaticity diagram.

TABLE 1

Scoring scale employed in the blanching assay

Numerical value	Level of blanching
0	normal skin
1	slight blanching of indistinct outline
2	more intense blanching with at least two corners outlined
3	general blanching with a clear outline of the square
4	marked and distinct blanching of high intensity

Results for each test preparation, for all volunteers, were expressed as a percentage of the total possible score (%TPS) at each time point (Barry and Woodford, 1974).

The coordinates which characterise the colour are the brightness factor L^* (black/white 0–100), a^* (red/green +60/−60) and b^* (yellow/blue +60/−60) (Fig. 1). Equal distances in this measurement technique equate approximately to perceived colour differences. Three consecutive readings were taken at each site, at each time point, and the mean values used.

For studies 2 and 3 the chromameter results are expressed as differences from the control which is the mean value obtained from two adjacent untreated sites.

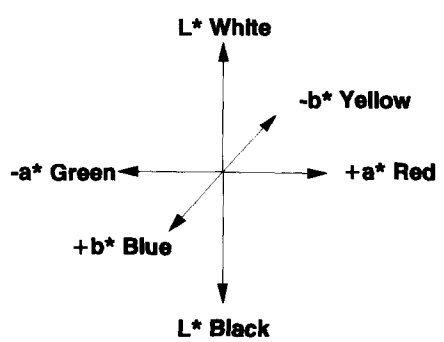

Fig. 1. Three dimensional colour measurement; i.e., colour is expressed in terms of three numerical units: L^* (white-black), a^* (red-green) and b^* (blue-yellow).

Statistics

Statistical analysis was carried out using Instat software, paired Student's *t*-test and linear correlation (Graphpad Software Inc.).

Results and Discussion

This study aimed to assess the feasibility of using the Minolta Chromameter for the measurement of corticosteroid-induced blanching. A preliminary study showed that the colour of the skin was sensitive to the force applied to the measurement head (Fig. 2). Simple application of weights to the head caused changes in the measurement values of all three colour co-ordinates, although the change in the value of the a^* component was most marked. Variation in the applied force is likely therefore to provide erroneous results and although such pressure considerations have been alluded to by other workers (Queille-Roussel et al., 1991; Chan and Li Wan Po, 1992a), the potential problems have not previously been quantified. A stand assembly may be appropriate to support the Chromameter for the measuring process, enabling contact with the skin to be simply controlled, so that the minimum possible pressure is applied to the test site. However, the use of such an assembly would reduce the instrument's portability, an obvious disadvantage when blanching profiles are being monitored over periods up to 96 h, when volunteers are not always available at a single location. In all later studies, measurements using the chromameter were taken whilst ensuring that a light, but complete, contact was made with the skin. Chan and Li Wan Po (1992b) advocate the attachment of a pre-moulded foam casing to the measurement head, so that the colour of sites can be monitored from a focal distance of 1 cm. The mean values obtained for L^* and a^* chromaticity components, collected at all reading times throughout the 5 day study period in study 1, were calculated for the left and right arms of each volunteer (Fig. 3). It is apparent that there is large inter-volunteer variation in skin colour, although inter-arm colour variation was relatively minor with regard to the determined values for both L^* and a^*. It would be anticipated that baseline variation in absolute skin colour could be accommodated, either by expressing any induced changes in colour relative to values obtained at a particular time-point, or alternatively, relative to values obtained from an adjacent untreated site.

Data from study 1 were also analysed in terms of positional variation in skin colour along the arm (Fig. 4). The results obtained indicated al-

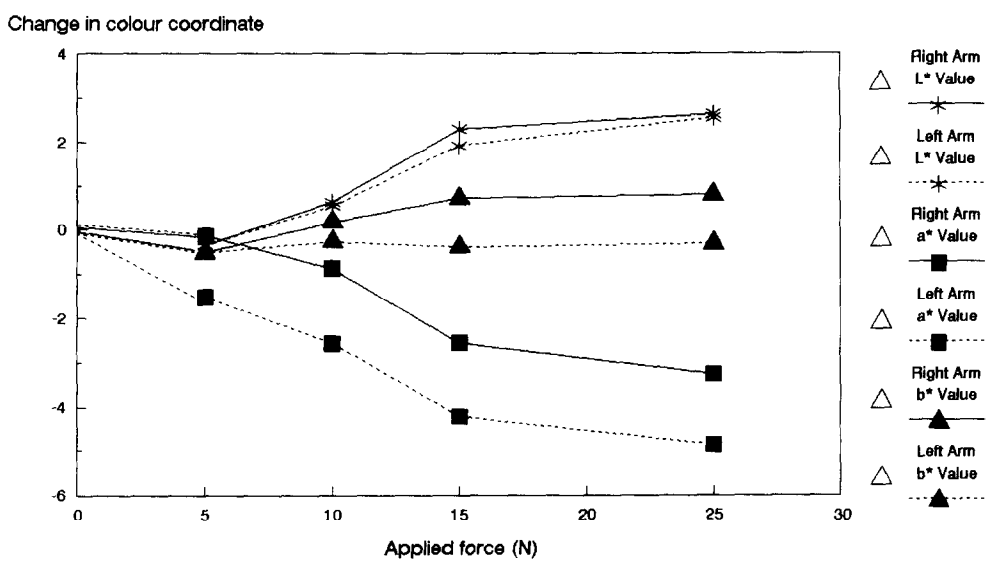

Fig. 2. The effect of application force on chromaticity parameter without corticosteroid application.

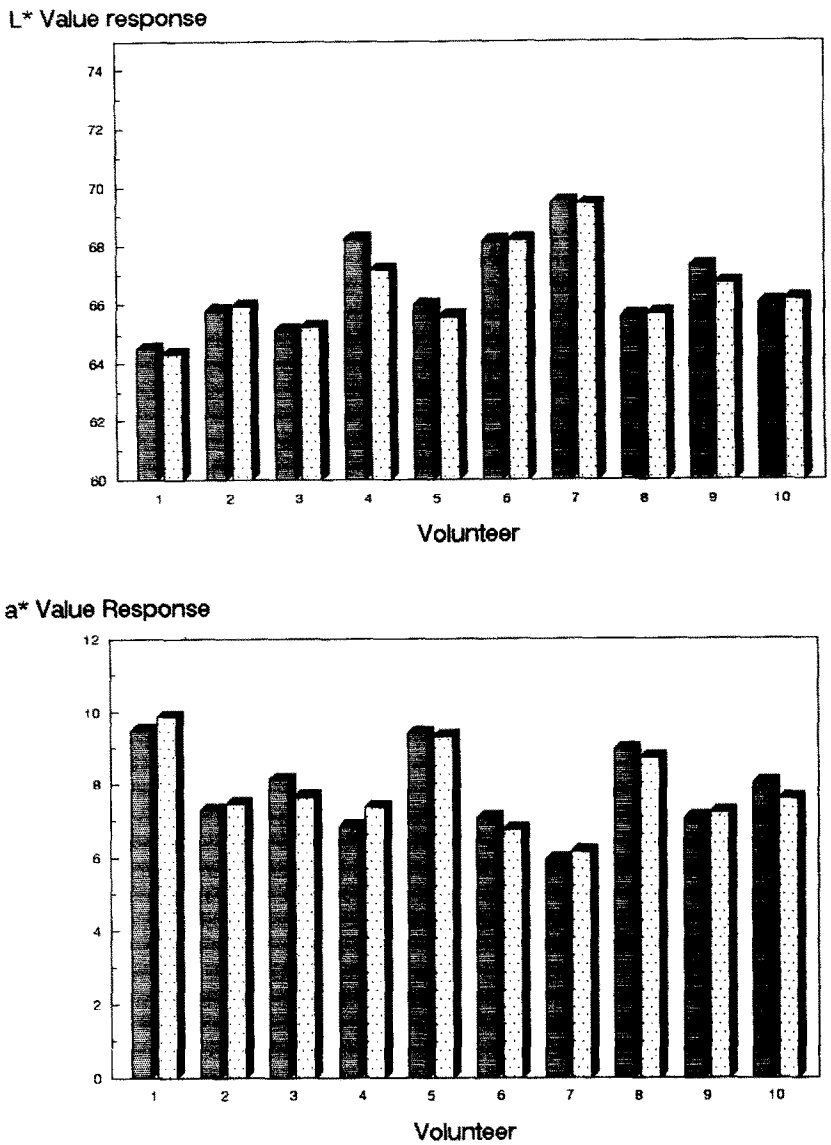

Fig. 3. Intervolunteer skin colour variation measured by the chromameter showing mean L^* and a* components for 10 volunteers, over 5 days without corticosteroid application. Left and right arm values shown for each volunteer.

though the sites more proximal to the wrist were generally whiter (higher L^* value) and less red (lower a^* value) than those close to the elbow. Significant differences were seen for the L^* component for both arms ($p = 0.0001$) but the differences between the elbow and the wrist for the a^* component was seen to significant only for the right arm ($p < 0.05$). When chromaticity values were plotted as a function of time, a distinct diurnal variation in skin colour was evident with L^* values decreasing (becoming darker) and a^* values increasing (becoming redder) during the course of each study day (Fig. 5). This cyclical variation in skin pallor which was clearly reproducible, confirms the observations of Queille-Roussel et al. (1991), and is presumably attributable to a physiological response which may be related to the circadian rhythym for cortisol in

serum. Cortisol has a peak serum level in the early hours and the 8 p.m. level is normally only 50% of the 8 a.m. value (Patel and Lott, 1984). In the visual assessment of blanching, this changing baseline is accommodated automatically by the eye, since the induced blanching is ranked according to the pallor of the skin immediately surrounding the application sites. As a result of these preliminary studies, it is suggested that the study design of any blanching investigations carried out utilising the chromameter, needs to incorporate untreated control sites immediately adjacent to the test sites. Subtraction of the corresponding chromaticity value obtained on the control site from that on the test site could then be employed with confidence, to determine blanching induced by any corticosteroid. This approach is likely to be more valid than simply subtracting the initial chromaticity coordinate value from the subsequently determined test values (Chan and Li Wan Po, 1992a), since allowance for the non-linear baseline can be made more readily. The lack of such stringent control baseline data in the study design, is likely to lead to failure in discriminating corticosteroid-induced effects from diurnal variation in pallor, particularly when low potency corticosteroids are employed.

In studies 2 and 3 the blanching induced by corticosteroids was determined by visual assessment and by the chromameter, the results obtained for the chromaticity coordinates being corrected for diurnal variation in skin colour, as described previously. The resultant blanching profiles, as defined by visual assessment for both the occluded (Fig. 6) and the unoccluded regimens (Fig. 7), concurred with previous studies carried out by ourselves and others (Barry and Woodford, 1974; Martin and Marriott, 1989), maximum blanching being observed 6 h after removal of the corticosteroid cream. All three corrected chromaticity co-ordinates displayed changes that correlated well with the visual as-

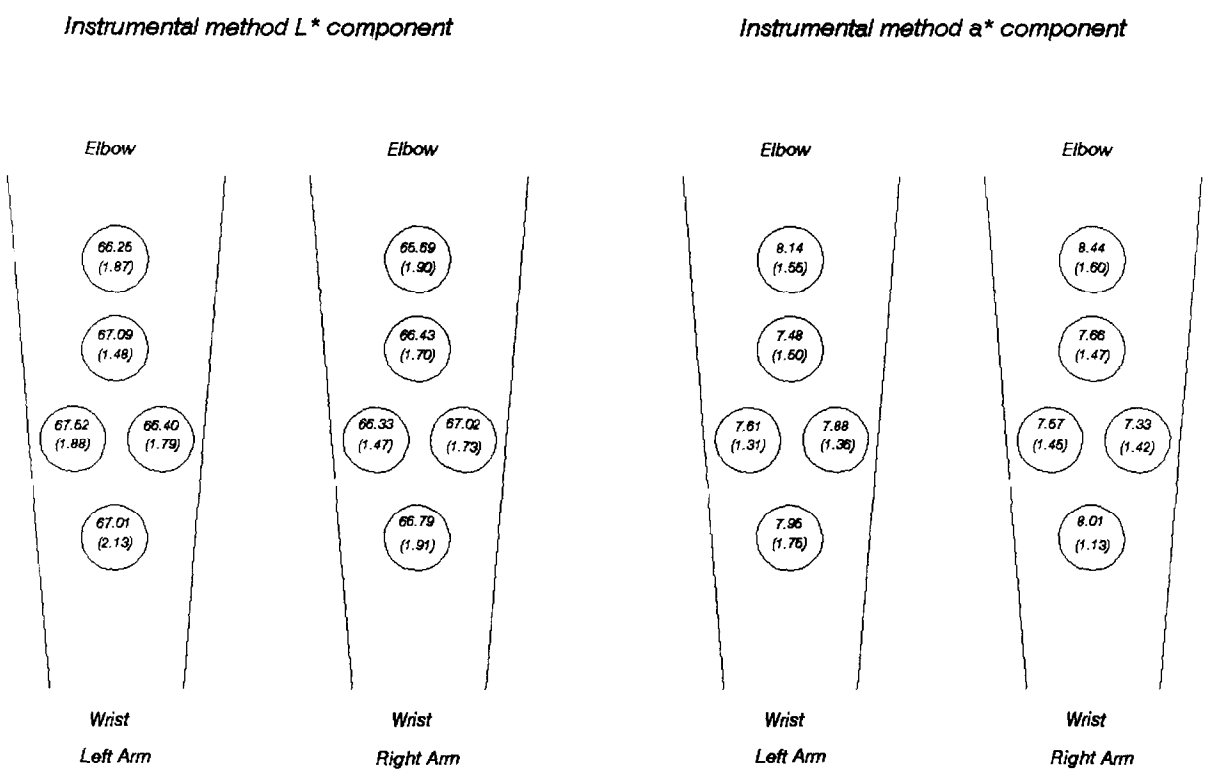

Fig. 4. Mean colour variation ($\pm$S.D., $n = 210$) in skin sites measured by the chromameter showing L^* and a^* components.

sessment, particularly when the corticosteroids were applied under occlusion (Fig. 6). As anticipated from its inherent potency, betamethasone cream, when applied under non-occluded conditions (Fig. 7), induced the least discernable blanching of the applied corticosteroids in the

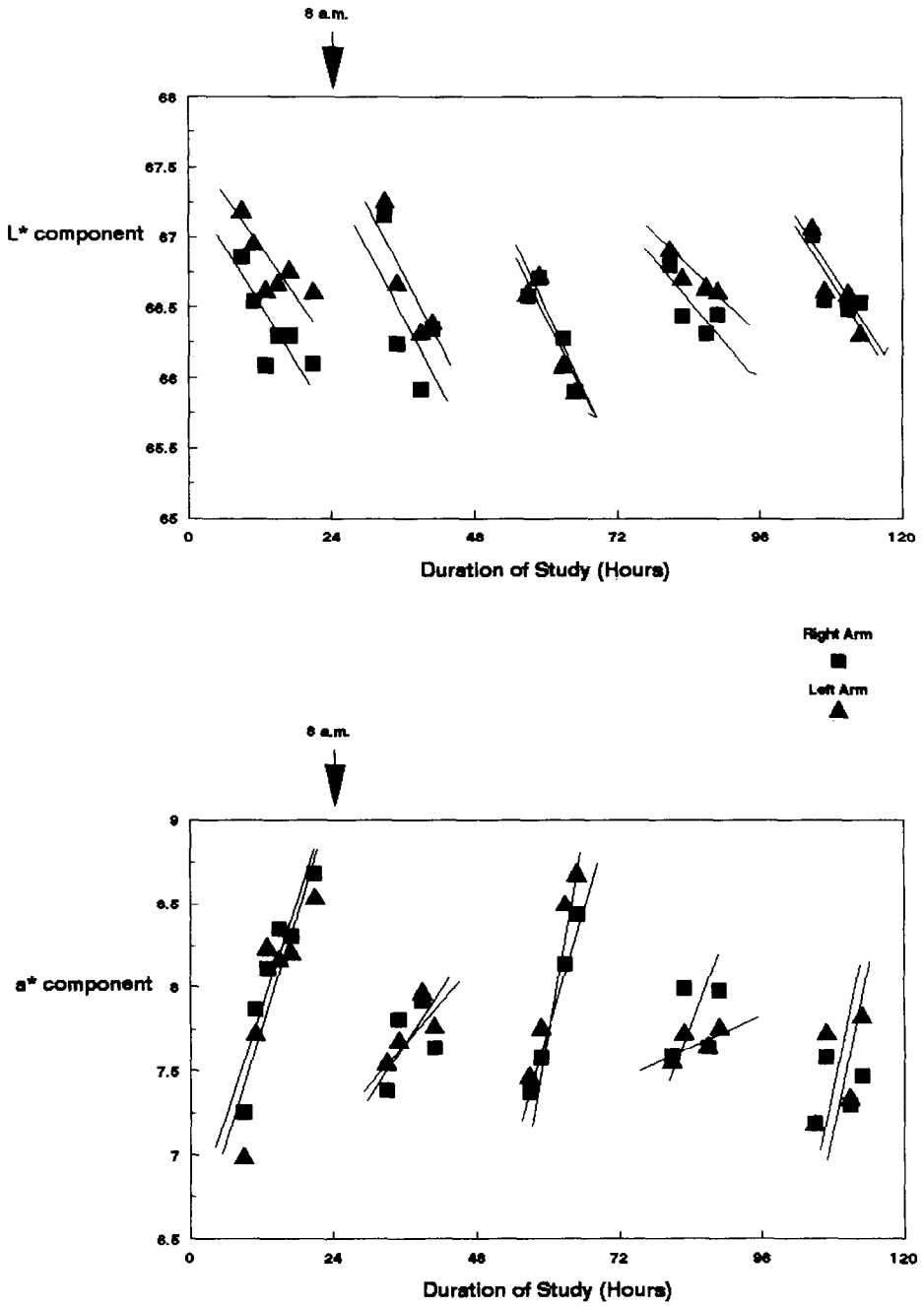

Fig. 5. Effect of time of day on skin colour (L^* and a^* components) without corticosteroid application.

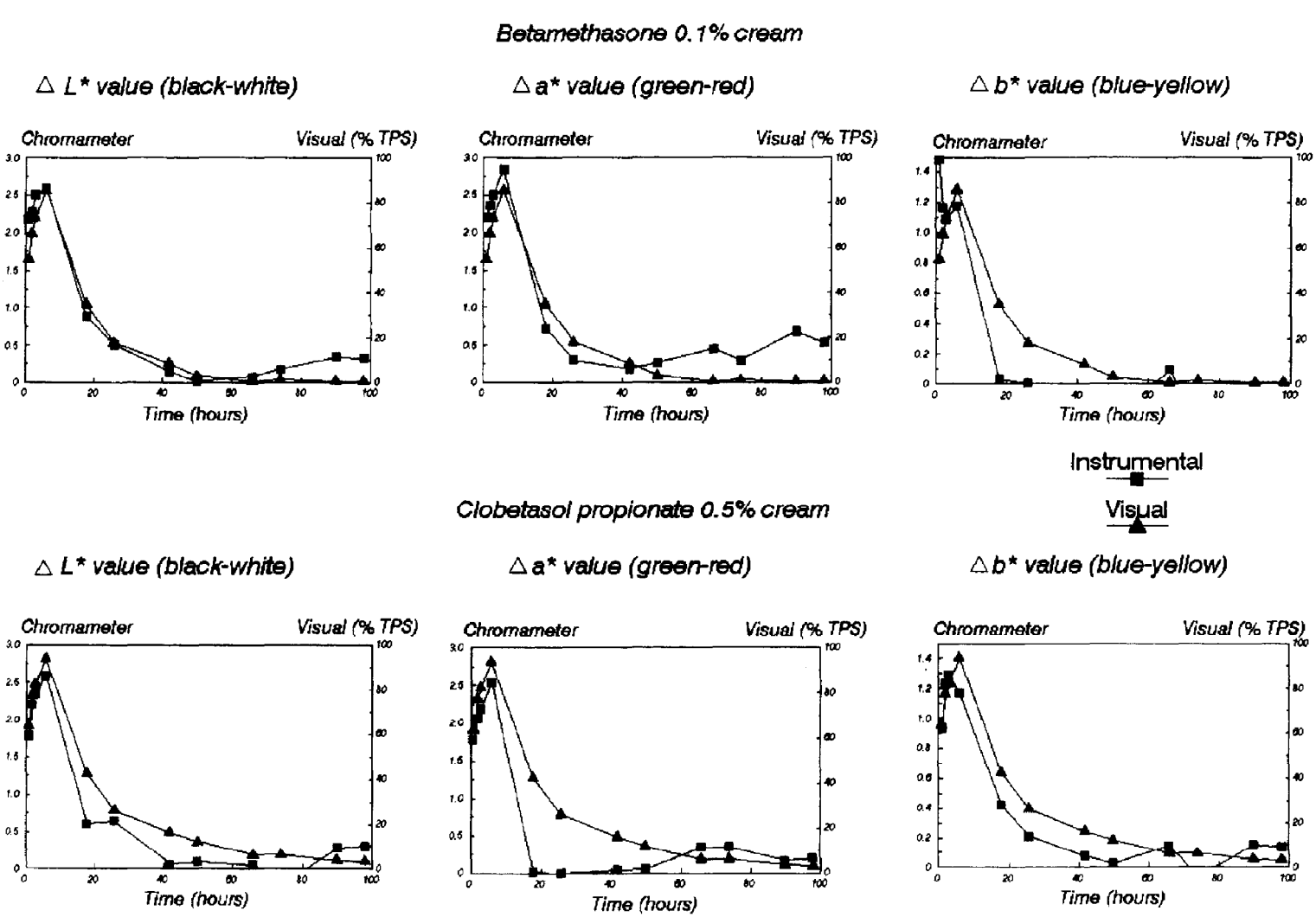

Fig. 6. Time-response profiles: a comparison of the visual and instrumental techniques for the occluded treatments. Chromameter results expressed as the difference from the adjacent control value.

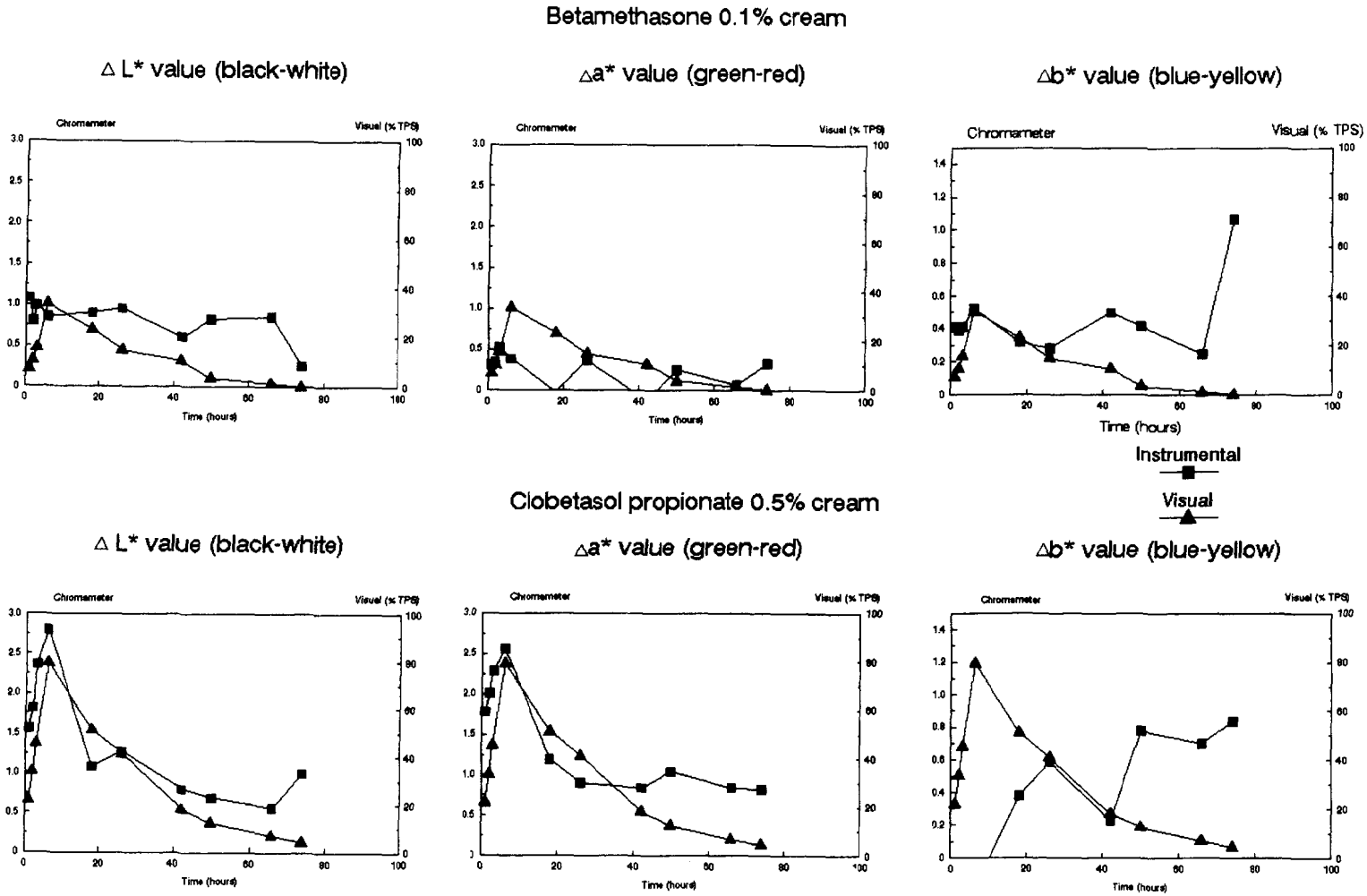

Fig. 7. Time-response profiles, a comparison of the visual and instrumental techniques for the onoccluded treatments. Chromameter results expressed as the difference from the adjacent control value.

current investigations. Correlation between the chromaticity differences and visual assessment was not as high as with other regimens. When all the data from studies 2 and 3 (as depicted in Figs 6 and 7) were combined, then highly significant ($p < 0.001$) correlations were found to exist between %TPS and L^* and a^* chromaticity coordinates (Fig. 8). A poor correlation was found between %TPS and b^* values when the data for both the occluded and non-occluded data were combined. These results support the observations of Wirth (1991), which established for tablet discolouration a similar correlation between subjective visual assessment of colour and chromaticity coordinates.

The chromameter derived profiles for the occluded regimens were found to return to baseline control values over the same time course as the visually obtained %TPS values (Fig. 6). This is in contrast to an earlier study (Chan and Li Wan Po, 1992a) which showed that L^* coordinates for four corticosteroid creams of different potency, including mildly potent hydrocortisone, failed to return to baseline levels within 96 h. Indeed, data are presented showing that the cumulative area under the L^* coordinate curve is still increasing after 96 h as a near linear function of time. This is in marked contrast to the previously visually determined blanching profiles of these corticosteroid preparations, when only the blanching induced by a high potency corticosteroid, such as clobetasol propionate, would be expected to be apparent after this time. Blanching induced by a single occluded application of a hydrocortisone preparation is barely visible after 24 h (Barry and Woodford, 1974, 1976). Whilst a previous study using the Chromameter has been able to discriminate between unoccluded corticosteroid formulations of different potency (Queille-Roussell, 1991), no difference could be discerned between the hydrocortisone preparation and an inactive vehicle on untreated sites. Blanching induced by hydrocortisone cream has been distinguished previously from baseline or control blanching using visual assessment (Barry and Woodford, 1976). Whilst only a limited number of subjects have, as yet, been used in blanching assays involving the use of the chromameter, and hence the sensitivity of the technique still requires further investigation, it would appear that blanching of low intensity is still more readily detected and measured using visual assessment.

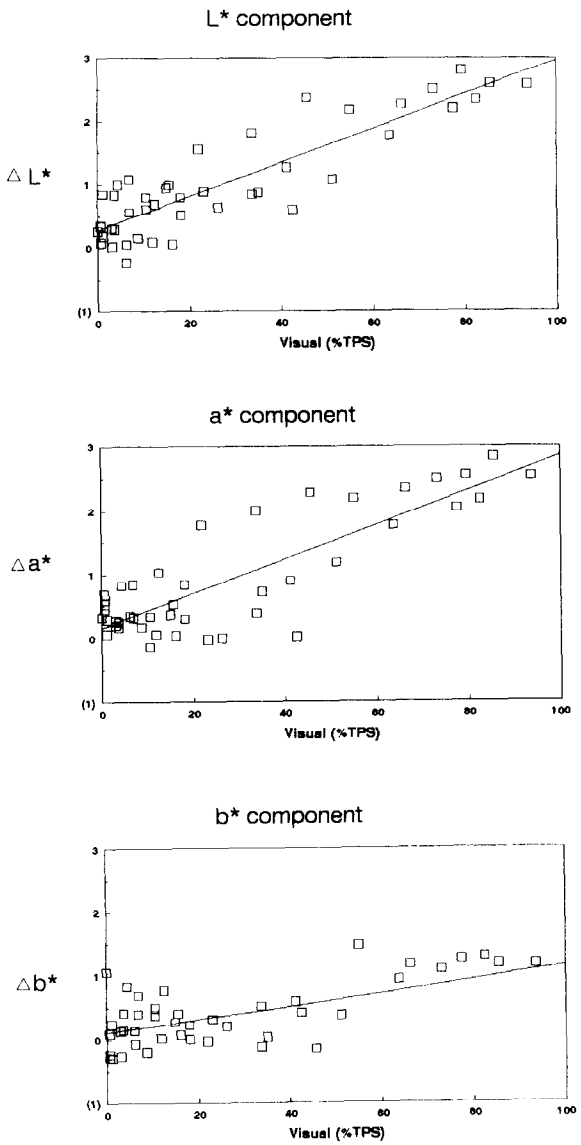

Fig. 8. The correlation between visually assessed blanching and the change in skin colour determined instrumentally. The correlation coefficients were as follows: ΔL^* 0.885 (all data) and 0.9633 (occluded data only); Δa^* 0.843 (all data) and 0.8998 (occluded data only); Δb^* 0.582 (all data) and 0.937 (occluded data only).

Conclusions

On the basis of the results from this investigation, it is recommended that the chromameter should, at present, only be used as a complementary technique to visual assessment for measuring corticosteroid-induced skin blanching. Control sites, adjacent to the treatment sites, should be incorporated within protocols of blanching studies to allow for the diurnal variation in skin colour. This enables the entire blanching profile to be characterised and area under the curve determined. The FDA (Shah et al., 1992) recommends that the blanching assay, undertaken by visually assessing skin pallor, should be supported with chromameter data, although it fails to advocate the instrumented technique alone as providing adequate data for bioequivalence testing. This is in contrast to Chan and Li Wan Po (1992b) who seek to promote the chromameter as an internationally acceptable technique for skin pallor assessment. At present we concur with the viewpoint expressed in the interim guidance issued by the FDA, that the use of the chromameter in skin blanching assays requires careful development and more validation before its use in bioequivalence testing of corticosteroid preparations can be advocated as the sole means of determining biological activity.

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