ELSEVIER

International Journal of Pharmaceutics 143 (1996) 93–100

international journal of pharmaceutics

Instrumental evaluation of color of solid dosage forms during stability testing

G. Stark[1,a], J.P. Fawcett[a,*], I.G. Tucker[a], I.L. Weatherall[b]

[a]*School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand*
[b]*School of Consumer and Applied Sciences, University of Otago, P.O. Box 913, Dunedin, New Zealand*

Received 2 May 1996; revised 5 August 1996; accepted 6 August 1996

Abstract

The principles of color measurement established by the Commission International de l'Éclairage (CIE) have been applied to determine color formation during long-term storage and stability testing of four white commercial solid dosage forms. The products tested were preparations of captopril tablets, flucloxacillin sodium capsules, cefoxitin sodium powder for injection and theophylline controlled release tablets in their original packaging. Different batches of medicines were examined for color formation and chemical stability under ambient conditions and a single batch was monitored on accelerated testing. Only flucloxacillin sodium and cefoxitin sodium showed statistically significant degradation of active drug on ambient storage accompanied by color formation (yellowing) in the latter case. On accelerated testing, linear relationships were observed between color formation and drug content for all formulations except theophylline where color formation occurred without significant drug degradation. The rate of color formation obeyed the Arrhenius equation in every case. Color change can be quantified according to the CIE system and may be useful for potency predictions where a causal relationship between color change and drug decomposition can be established. Even where a causal relationship does not exist, patient confidence in a pharmaceutical product may be undermined by perceptible color change. In this situation, the shelf-life could be specified using the CIE colour system.

Keywords: Chemical stability; CIE; CIELAB; Discoloration; Shelf-life; Solid dosage form

1. Introduction

The Food and Drug Administration Guidelines for stability testing of solid state pharmaceuticals includes appearance/color as an important characteristic of stability (FDA, 1987). Unfortunately

* Corresponding author. Tel.: + 64 3 4797290; fax: + 64 3 4797034.

[1] Present address: Kalene Mission Hospital, PO Box 10, Ikelenge, North Western Province, Zambia.

PII S0378-5173(96)04691-1

visual observations and subjective assessments lack precision with respect to the communication of color information (Banker and Andersen, 1986). A way to overcome this deficit, at least for solid surfaces, is to evaluate color and color differences by reflectance spectroscopy. The aim of this study was to apply reflectance spectroscopy based on the color system developed by the Commission International de l'Éclairage (CIE) to measure color changes during long-term storage and stability testing of some solid dosage forms and assess color as an indicator of chemical degradation.

The quantitation of color change in solid state pharmaceuticals during stability testing has received only limited attention. Instrumental evaluation has been used to study the stability of certified dyes in formulated tablets exposed to light (Goodhart et al., 1967) and to examine color change of nystatin powders, ointments and creams during accelerated stability studies (Fairbrother et al., 1980). Quantitation of the color change of nystatin was shown to be useful for assessing its chemical and microbiological stability but a number of drugs are known to undergo color change without a measurable loss of potency, e.g. cephalosporins (AHFS, 1995). More recently color change in cefixime trihydrate powder stored at different temperatures has been measured using the Hunter L-a-b color system (Kitamura et al., 1989) a direct forerunner of the CIE system.

In this study we have examined color development in four different types of solid dosage forms stored under ambient and stress conditions to evaluate the power of reflectance spectroscopy based on the CIE color system to monitor chemical stability. Of particular concern is whether color formation is useful in the assessment of shelf-life.

2. Materials and methods

2.1. Selection and storage of products

Four products were selected for study representing a range of white solid dosage forms and shelf-lives in a range of different types of packaging. The products were Capoten 12.5 mg tablets (captopril), Flucloxin 250 mg capsules (flucloxacillin sodium), Mefoxin 1 g powder for injection (cefoxitin sodium) and Theo-Dur 200 mg CR tablets (theophylline controlled release) in their original manufacturers' packaging. Capoten tablets were strip packaged in sheets of 15 tablets, Flucloxin capsules were in amber glass bottles with polystyrene seals, Mefoxin 1 g injections were in 10 ml glass vials capped with silicon rubber bungs with aluminium crimps and Theo-Dur tablets were in amber glass bottles with plastic press fit lids. The registered shelf-lives of the four products were 3, 1, 2 and 3 years respectively. Batches of various ages within and beyond their registered expiration dates were collected from pharmacies throughout New Zealand to evaluate changes in color on long-term storage under ambient conditions. The mean temperature and relative humidity (RH) of storage were in the ranges 20–23°C and 45–70% RH respectively. Color formation during stability testing under stress conditions was evaluated using containers from a recent batch of each product kept at 70, 55 and 40°C, 50% RH and 40°C, 75% RH for 84 and 168 days.

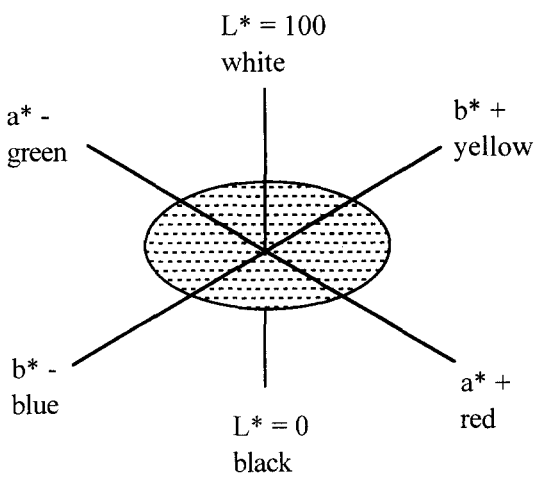

Fig. 1. CIELAB color space diagram.

2.2. Chemical stability

Pure drug samples of captopril, cefoxitin sodium and theophylline monohydrate were AnalaR grade from Sigma Pharmaceuticals (Australia). Captopril disulphide USP and flucloxacillin sodium BP were kindly supplied by Bristol-Myers Squibb (Auckland, NZ) and Smith-Kline Beecham (Auckland, NZ) respectively. All other chemicals were of AnalaR or HPLC grade. Chemical stability of products was assessed using stability indicating HPLC assays for active drug and in the case of captopril, the decomposition product, captopril disulphide, was also quantified. Assays were developed on the basis of published procedures for captopril (Taketomo et al., 1990), cloxacillin sodium (Salem and Alkaysi, 1987), cefoxitin sodium (Stiles et al., 1989; O'Brien et al., 1979) and theophylline (Macheras et al., 1989), and were validated for linearity, precision and stability indicating ability by established methods. Stability of batches stored under ambient conditions was assessed by assay of three samples of powder from 20 tablets/capsules or three bottles of Mefoxin injection. The stability under stress conditions involved assay of a single sample from 5 tablets/capsules or 1 bottle of Mefoxin injection.

2.3. Color measurement

The CIE color system gives an exact numerical specification of human color vision. The system has been described in detail by Weatherall and colleagues in their studies of fruit colors (Weatherall and Lee, 1991) and human skin (Weatherall and Coombs, 1992). The system defines the conditions for perceiving color by (a) specifying the relative spectral energy distributions of various illuminants, known as CIE Standard Illuminants (CIE, 1986), (b) dictating that the modification of an illuminant by interaction with an object be measured with a reflectance spectrocolorimeter conforming to CIE recommendations and (c) quantifying the nature of human color vision in terms of three color matching functions $\bar{x}$, $\bar{y}$ and $\bar{z}$ whose numerical values are available in published tables and are known collectively as a CIE Standard Observer. Three functions are required because color vision has been found to be trichromatic, i.e., a single perceived color results from the effect of three separate stimuli on the visual cortex (Hunter and Harold, 1987).

Colors are measured in terms of their tristimulus values X, Y and Z which combine the illuminant, reflectance and observer data in three summations of the form:

$$X = \sum ER\bar{x} \tag{1}$$

Here E is the relative energy of the chosen illuminant, R is the fraction reflected and $\bar{x}$ ($\bar{y}$ or $\bar{z}$) is the numerical value of the Standard Observer. The tristimulus values are used to calculate the 1976 CIE L^*, a^*, b^* (CIELAB) color space values which enable colors to be regarded as existing in an approximately uniform three dimensional space. Each particular color has a unique location defined by its cartesian coordinates with respect to the L^*, a^* and b^* axes (Fig. 1).

The L^* coordinate serves as the psychometric correlate of perceived lightness and covers a range from white (100) to black (0) along a grey scale. The a^* and b^* coordinates are related to Hering's opponent color theory (McDonald, 1987) and give the locations of the various hues on scales of red-versus-green (a^*) and yellow-versus-blue (b^*). If both a^* and b^* are zero, the color lies on the L^* axis and is termed achromatic. Color difference (ΔE) is calculated using coordinate geometry as the length of the line joining the positions of the two colors:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \tag{2}$$

A change or difference in color corresponding to a value of $\Delta E > 1.5$ can be perceived by the human eye.

A Labscan 6000 (Hunter Associates Inc. USA) scanning reflectance spectrocolorimeter using 0° illumination and 45° viewing geometry with the spectral component excluded was used. A 6 mm diameter viewing port with a 3 mm diameter illuminated area in the horizontal upper surface of the sensor module was used for all tests. The spectrocolorimeter was controlled by an IBM XT microcomputer which performed all color calcula-

Table 1
Color testing and drug content of product batches of various ages stored under ambient conditions (data for L^*, a^*, b^* are means for 3 vials containing powder from 10 tablets/capsules or 3 bottles of Mefoxin injection)

Age (months)[a]	Drug content[b]	L^*	a^*	b^*	ΔE	SD of ΔE
Capoten tablets						
4	100 ± 2	96.2	0.15	2.72		
12	103 ± 1	96.27	0.11	2.72	0.08	0.36
20	102 ± 2	96.15	0.13	2.54	0.19	0.14
27	101 ± 3	96.22	0.13	2.74	0.03	0.24
33	100 ± 3	95.9	0.1	3.25	0.61	0.22
41	101 ± 1	96.01	0.18	2.75	0.19	0.39
50	100 ± 1	96.25	0.13	2.44	0.29	0.10
52	100 ± 1	96.18	0.13	2.43	0.29	0.10
Flucloxin capsules						
3	100 ± 1	96.66	0.01	2.7		
8	100 ± 1	96.60	0.03	3	0.31	0.31
22	100 ± 1	96.80	0.06	2.47	0.27	0.15
29	97 ± 1	96.80	0.21	2.86	0.29	0.08
59	92 ± 1	96.41	0.33	2.8	0.43	0.80
Mefoxin powder for injection						
15	100 ± 1	94.20	0.01	7.9		
18	100 ± 1	93.80	0.10	9.44	1.59	0.20
26	98 ± 2	94.50	0.05	8.5	0.67	0.58
34	99 ± 2	93.90	0.11	8.6	0.77	0.50
90[c]	96 ± 1	91.30	0.93	13.5	6.37	0.60
90[c]	97 ± 1	91.90	0.80	13.1	5.74	0.87
190	87 ± 2	87.00	2.60	19	13.48	0.31
Theo-Dur CR tablets						
8	100 ± 1	95.70	−0.42	2.7		
18	101 ± 1	96.30	−0.09	1.9	1.05	0.35
20	99 ± 1	96.70	−0.14	2.01	1.25	0.24
50	100 ± 1	96.40	−0.19	2.02	1.03	0.24
61	101 ± 1	96.80	−0.16	1.95	1.36	0.28
147	100 ± 1	96.90	−0.22	2.1	1.36	0.28

[a] Age was determined from the date of manufacture (expiry date − registered shelf-life) and the month of analysis.
[b] Percent of initial content ± SD.
[c] Different batches.

tions from digitised spectral data by means of a menu-driven suite of programs supplied with the instrument.

Tabulated data for CIE Illuminant D65 and the CIE 1964 10° Standard Observer were selected under software control and combined with the spectral data at 10 nm intervals to compute the L^*, a^*, b^* values. The mean and variance of the data for each set of three vials were obtained and used to calculate ΔE and the variance of ΔE ($V\Delta E$). The variance of ΔE was calculated from the formula

$$V\Delta E = \left[\left(\frac{(\Delta L^*)^2}{M}\right)V\Delta L^* + \left(\frac{(\Delta a^*)^2}{M}\right)V\Delta a^* + \left(\frac{(\Delta b^*)^2}{M}\right)V\Delta b^*\right] \quad (3)$$

where $M = [(L^*)^2 + (a^*)^2 + (b^*)^2]$. The standard deviation (SD) of ΔE was calculated as the square root of the $V\Delta E$.

For testing of batches on ambient storage, groups of 10 tablets or the contents of 10 capsules were ground in a glass mortar for two minutes.

Table 2
Color testing and drug content of a single batch of each product stored under temperature-stressed and humidity-stressed conditions (data for L^*, a^*, b^* are means for powder from 5 tablets/capsules or 1 bottle of Mefoxin injection)

Test condition	Storage (days)	Drug content	L^*	a^*	b^*	ΔE	SD of ΔE
Capoten tablets							
70°C, 50% RH	84	51	93.52	−0.13	10.85	8.40	0.52
55°C, 50% RH	84	94	95.85	−0.19	4.05	1.41	0.10
40°C, 50% RH	84	101	96.07	0.03	2.81	0.54	0.20
40°C, 75% RH	84	97	96.24	−0.04	2.93	0.75	0.17
70°C, 50% RH	168	23	91.07	0.91	15.35	13.72	0.61
55°C, 50% RH	168	68	95.73	−0.28	5.09	2.46	0.12
40°C, 50% RH	168	97	96.14	0.05	2.88	0.45	0.10
40°C, 75% RH	168	96	96.37	−0.08	2.82	0.28	0.08
Flucloxin capsules							
70°C, 50% RH	84	0	95.00	−1.22	11.79	9.88	0.28
55°C, 50% RH	84	40	96.58	−1.79	7.31	5.44	0.35
40°C, 50% RH	84	88	97.03	−0.18	2.54	0.40	0.10
40°C, 75% RH	84	80	97.13	−0.49	3.28	1.21	0.14
70°C, 50% RH	168	0	94.60	−0.83	14.65	12.24	0.64
55°C, 50% RH	168	3	96.15	−1.78	9.39	7.06	0.45
40°C, 50% RH	168	83	97.17	−0.36	2.99	1.05	0.24
40°C, 75% RH	168	51	96.86	−1.19	5.63	3.34	0.20
Mefoxin powder for injection							
70°C, 50% RH	84	83	80.14	4.73	30.54	26.9	1.7
55°C, 50% RH	84	91	88.14	1.24	18.77	12.3	1.5
40°C, 50% RH	84	100	91.92	0.46	11.06	3.73	0.75
40°C, 75% RH	84	100	91.97	0.22	11.43	3.99	0.90
70°C, 50% RH	168	78	76.98	5.78	31.57	29.2	1.5
55°C, 50% RH	168	90	85.98	2.37	21.28	15.2	1.3
40°C, 50% RH	168	97	91.82	0.54	12.34	4.46	0.98
40°C, 75% RH	168	99	91.87	0.50	12.17	4.3	1.0
Theo-Dur CR tablets							
70°C, 50% RH	84	99	59.46	7.75	24.78	43.50	0.95
55°C, 50% RH	84	100	92.16	0.61	9.05	7.6	1.2
40°C, 50% RH	84	98	96.05	−0.54	3.43	0.93	0.32
40°C, 75% RH	84	101	96.27	−0.49	3.21	0.79	0.41
70°C, 50% RH	168	97	53.69	6.64	20.50	47.1	1.8
55°C, 50% RH	168	100	84.74	2.46	14.08	17.00	0.53
40°C, 50% RH	168	95	95.68	−0.57	4.39	2.41	0.14
40°C, 75% RH	168	99	95.19	−0.75	5.06	3.24	0.24

The powder was then transferred to three 2 ml Cryolok vials (Bioteck, Auckland, NZ) and the mouths covered with a single layer of thin clear polyethylene film (Gladwrap, Auckland, NZ). Each vial was then inverted several times before being placed over the viewing port of the reflectance spectrocolorimeter. Ten readings were performed on each vial with the vial being inverted to mix the sample between each reading. Color of Mefoxin powder for injection was determined using three of the original glass vials after replacing caps and bungs with a layer of polyethylene film. For testing of batches stored under stress conditions, groups of 5 tablets/capsules or a single bottle of Mefoxin powder were treated as above. The latest batch of each product was used as the standard for batches stored under ambient conditions. Samples of the same batch stored at 4°C were used as standards for batches stored under stress conditions.

2.4. Statistics

Data for color are presented as means for L^*, a^*, b^* values and mean ± SD for ΔE values and drug content. Mean concentration–time data for drug content were analysed by linear regression analysis using a validated computer program (Pharmaceutical Statistical Regression, School of Pharmacy, University of Otago). Decomposition was assumed if the slope of the regression line was significantly less than zero (one-tailed t-test). The level of significance was set at $P < 0.05$.

3. Results

The CIELAB color space values and ΔE for batches of various ages stored under ambient conditions together with the content of active drug are shown in Table 1. All except one of the batches had L^* values greater than 90 consistent with white pharmaceuticals. Of the four products, only Mefoxin and Flucloxin showed statistically significant decomposition of active drug and only Mefoxin powder showed a significant increase in ΔE on storage corresponding to a visually perceived yellowing (increase in b^* value) of the powder. There was a correlation between color and age for Mefoxin where linear regression of ΔE versus age (months) gave $y = -0.9 + 0.075x$, $R^2 = 0.98$, $P < 0.001$. Yellowing during stability testing of amorphous and crystalline cefoxitin sodium has been previously observed (Oberholtzer and Brenner, 1979).

The results of color measurements for batches stored under stress conditions are shown in Table 2. Storage at 55°C and above resulted in tablets and powder darkening and taking on a burnt appearance. This is quantified by the increase in positive b^* and decrease in L^* values. For Capoten, the a^* values showed little change. Flucloxin a^* values became negative on storage while for Mefoxin and Theo-Dur, values of a^* became increasingly positive.

Humidity had an effect on color development in Flucloxin capsules and Theo-Dur tablets. For Flucloxin after 84 and 168 days and Theo-Dur after 168 days, ΔE at 40°C, 75% RH, increased significantly more rapidly than at 40°C, 50% RH. In the case of Flucloxin, this was accompanied by greater drug decomposition but in the case of Theo-Dur the color difference at 168 days between 40°C, 50% RH and 40°C, 75% RH was presumably the result of degradation of excipient. The lack of effect of increased humidity on color

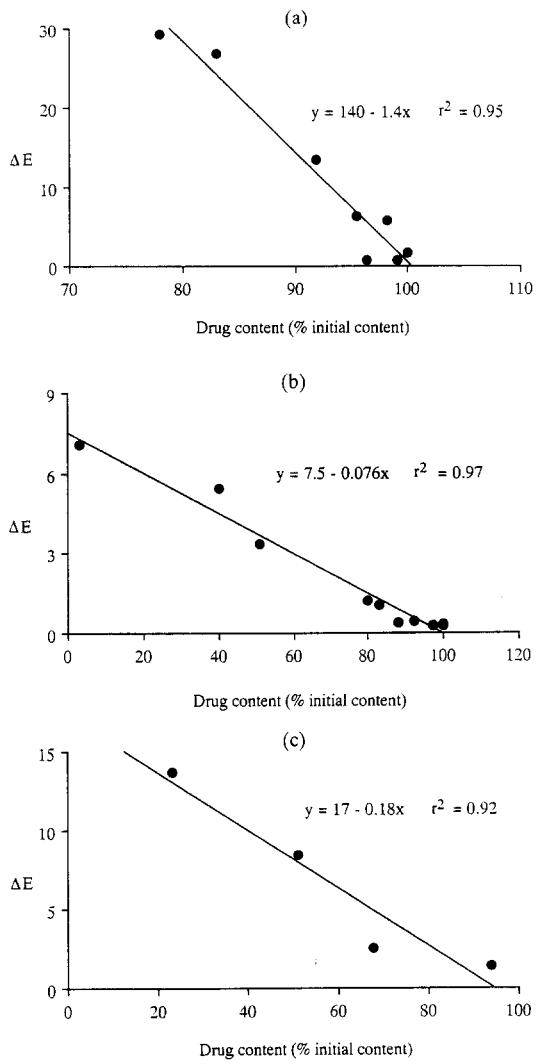

Fig. 2. Correlation of color difference (ΔE) and drug content (% initial content) for (a) Mefoxin, (b) Flucloxin and (c) Capoten formulations. Data for Mefoxin and Flucloxin combine results from different batches on ambient storage and from a single batch on accelerated storage. Data for Capoten are for accelerated storage only.

formation and drug decomposition in Mefoxin and Capoten suggests the packaging of these products is moisture proof.

The relationship between drug content and color change for Mefoxin, Flucloxin and Capoten is shown in Fig. 2. The results show linear relationships between increasing ΔE values and decreasing drug content for all three formulations. For Flucloxin and Mefoxin, results from storage under ambient and stress conditions fit on the same straight line. In the case of Flucloxin at 70°C, color formation continues after all drug has decomposed. As shown in Table 2, formation of color was clearly temperature dependent. Arrhenius plots of $\log(\Delta E/\text{day})$ against the reciprocal of the absolute temperature were reasonably linear ($R^2 > 0.88$) for all four formulations (data not shown) despite the fact that for Theo-Dur, no statistically significant decomposition of active drug occurred.

4. Discussion

Color testing of white commercial solid state pharmaceuticals revealed that in three of the products tested, there was a good correlation between color formation and drug decomposition. The exception was the controlled release formulation of theophylline where color formation occurred during accelerated testing without significant drug decomposition. Even where color change correlates with drug decomposition, this does not necessarily imply a causal relationship. Indeed color may arise due to decomposition of excipients or due to interaction between drug and excipients. Whatever the source of the color change during stability testing of solid dosage forms, the fact that it can be quantified using CIELAB color values suggests color may be useful in assigning shelf-lives.

As for any physical property, the problem in using color as a basis for shelf-life lies in specifying exactly what level of change is acceptable. Given that a value of $\Delta E > 1.5$ is perceptible by the human eye, it seems reasonable to specify a shelf-life based on this degree of color change. Then only Mefoxin powder for injection would expire on storage under ambient conditions in approximate agreement with its registered shelf-life of 2 years. For the other products, color formation is consistent with drug content in predicting shelf-lives much longer than the registered values.

In all four formulations, color formation on aging of these 'white' pharmaceuticals took the form of yellowing or browning. Although the rate of color formation is different for each product, and does not always reflect decomposition of active drug, it appears color change on stress testing obeys the Arrhenius Law. A similar conclusion was reached by Kitamura et al. (1989) for discoloration produced by grinding of cefixime trihydrate powder stored at different temperatures. Thus color change on stress testing may be used to predict shelf-life. It may also be a more sensitive and convenient indication of some inadequacy in a particular batch of a pharmaceutical product.

Recent instrumental developments in the field of color measurement include portable spectrocolorimeters with data storage capacity. Such instruments have the potential to simplify and expand the application of color measurements in the pharmaceutical industry such as in quality control of solid dosage form production. Developments in color difference assessment include an improved formula called CIE94 published by the CIE for use in industrial color difference work (McDonald and Smith, 1995).

Acknowledgements

The authors thank the New Zealand Pharmacy Education Research Foundation for financial support, Mr Paul O'Donnell for technical assistance and Mrs Elizabeth Smart for typing the manuscript.

References

AHFS 95 Drug Information, American Society of Health System Pharmacists Inc., Bethesda, MD, 1995.

Banker, G.S. and Anderson, N.R., Tablets, in Lachman, L., Lieberman, H.A. and Kanig, J.L. (Eds.), *The Theory and Practice of Industrial Pharmacy*, 3rd Edn, Lea and Febiger, Philadelphia, PA, 1986, pp. 293–345.

CIE, *Colorimetric Illuminants (CIE 5001)/ Colorimetric Observers (CIE 5002)*, Publication No. 15.2, Central Bureau of the CIE, Vienna, 1986.

Fairbrother, J.E., Heyes, W.F., Clarke, G. and Wood, P.R., Evaluation of nystatin stability testing using tristimulus colorimetry. *J. Pharm. Sci.*, 69 (1980) 697–700.

FDA, Guidelines for submitting documentation for the stability of human drugs and biologics, FDA, Rockville, MD, 1987.

Goodhart, F.W., Lieberman, H.A., Mody, D.S. and Ninger, F.C., Stability of certified dyes in tablets III. *J. Pharm. Sci.*, 56 (1967) 63–67.

Hunter, R.S. and Harold, R.W., *The Measurement of Appearance*, 2nd Edn, Wiley Interscience, New York, NY, 1987.

Kitamura, S., Miyamae, A., Koda, S. and Morimoto, Y., Effect of grinding on the solid-state stability of cefixime trihydrate. *Int. J. Pharm.*, 56 (1989) 125–134.

Macheras, P., Koupparis, M. and Antimisiaris, S., An in vitro model for exploring CR theophylline–milk fat interactions. *Int. J. Pharm.*, 54 (1989) 123–130.

McDonald, R., *Color Physics from Industry*, Society of Dyers and Colourists, Bradford, UK, 1987.

McDonald, R. and Smith, K.J., CIE94—A new colour-difference formula. *J. Soc. Dyers Colour.*, 111 (1995) 376–379.

Oberholtzer, E.R. and Brenner, G.S., Cefoxitin sodium solution and solid-state chemical stability studies. *J. Pharm. Sci.*, 68 (1979) 863–866.

O'Brien, M.J., Portnoff, J.B. and Cohen, E.M., Cefoxitin sodium compatability with intravenous infusions and additives. *Am. J. Hosp. Pharm.*, 36 (1979) 33–38.

Salem, M.A.S. and Alkaysi, H.N., High performance liquid chromatographic analysis and dissolution of ampicillin and cloxacillin in capsule formulation. *Drug. Dev. Ind. Pharm.*, 13 (1987) 2771–2787.

Stiles, M.L., Tu, Y. and Allen, L.V., Stability of cefazolin sodium, cefoxitin sodium, ceftazidime, and penicillin G sodium in portable pump reservoirs. *Am. J. Hosp. Pharm.*, 46 (1989) 1408–1412.

Taketomo, C.K., Chu, S.A., Cheng, M.H. and Corpuz, R.P., Stability of captopril in powder papers under three storage conditions. *Am. J. Hosp. Pharm.*, 47 (1990) 1799–1801.

Weatherall, I.L. and Coombs, B.D., Skin color measurements in terms of CIELAB color space values. *J. Invest. Dermatol.*, 99 (1992) 468–473.

Weatherall, I.L. and Lee, W.G., Instrumental evaluation of some New Zealand fruit colours using CIELAB values. *N.Z. J. Botany*, 29 (1991) 197–205.