Method Accuracy—Table I shows the amounts of I added and those recovered in the synthetic samples containing different ratios of placebo plaster base. The average recovery of I, covering the range of 400–2700 μg of I/sample, was 100.6 ± 1.6%. The accuracy of the method was maintained even when the I concentration was below the formula's theoretical level.

Advantages of Direct Solid Analysis—Because the plaster base samples were directly analyzed without preseparation such as extractions and filtrations, mechanical loss of I was avoided. The proposed method is superior to the methyl ester–methyl ether and trimethylsilyl GLC methods because multiple derivatives of I may be formed upon derivatization (11–14), which may lead to errors. With this method, interferences from solvents and derivatizing agents were totally eliminated.

Since all nonvolatile components in the plaster base were retained in the aluminum capsule and kept from contacting the column, there was essentially no contamination of the packing material. This undoubtedly contributed to the reproducible performance of the column, which has been used to analyze more than 1000 samples but shows no sign of deterioration.

No significant interference is caused by the other volatile ingredients for analysis of I using this method. Figure 1 shows a typical chromatogram (recorded in log scale of the digital integrator) for the analysis of I in a keratolytic plaster, illustrating the separation of I from the other impurities and the general elution characteristics of I.

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Stability of Phenylbutazone in Presence of Pharmaceutical Colors

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Abstract □ The degradation of phenylbutazone was studied in the presence of lakes suitable for coloring the sugar coats of phenylbutazone tablets. The drug was degraded, in light, in the presence of erythrosine sodium. The degradation probably proceeds *via* singlet oxygen generated by the light-excited dye. The degradation may be important in some quality control procedures and can lead, for example, to unusual results in dissolution rate testing.

Keyphrases □ Phenylbutazone—stability in presence of various pharmaceutical colors, effect of light □ Stability—phenylbutazone in presence of various pharmaceutical colors, effect of light □ Colors, various pharmaceutical—effect on stability of phenylbutazone, effect of light □ Antirheumatic agents—phenylbutazone, stability in presence of various pharmaceutical colors, effect of light

Colors are used in pharmaceutical dosage forms for their aesthetic appeal and as an aid to identification. Coated tablets are frequently colored, and lakes are becoming increasingly popular for this purpose because they offer advantages in the speed of application and cover uniformity. The stability of pharmaceutical dyes and lakes was assessed (1, 2), but little attention has been paid to the possible interactions between colors and drugs.

Amaranth (FD&C Red No. 2 lake), erythrosine sodium (FD&C Red No. 3 lake), tartrazine (FD&C Yellow No. 5 lake), and FD&C Yellow No. 6 lake may be used in combination to give a color suitable for incorporation in the sugar coats of phenylbutazone tablets. This report is concerned with the interaction between these lakes and phenylbutazone.

EXPERIMENTAL

Materials—Amaranth lake[1], erythrosine sodium lake[1], tartrazine lake[1], and FD&C Yellow No. 6 lake[1] (sunset yellow) were obtained as separate powders and as a mixture in syrup[1]. Phenylbutazone[2] was of BP quality, and soluble erythrosine sodium[3] and methylene blue[3] were of reagent grade.

Irradiation of Samples—Suspensions or solutions of the materials in phosphate buffer (pH 7.4) were prepared and stored in subdued light. The test mixtures were exposed, in 1-cm quartz cells, to unfiltered light from a 300-w projector bulb situated 50 cm from the cell. Measurements of the liquid temperature during runs indicated no heating effect by the light beam. Due to the fine particle size of the lakes, sedimentation problems did not arise during the experiment.

[1] Colorcon Ltd., Orpington, England.
[2] Thomas Kerfoot Ltd., Ashton under Lyne, England.
[3] B.D.H. Ltd., Poole, England.

Table I—Rate Constants for Degradation of 0.001% Phenylbutazone under Various Conditions

	Concentration, mg/liter													
	None	None	Lake Mixture, 9.00	Lake Mixture, 9.00	Erythrosine Sodium Lake, 1.23[a]	Erythrosine Sodium Lake, 2.45[a]	Erythrosine Sodium Lake, 4.90[a]	Erythrosine Sodium Lake, 9.80[a]	Erythrosine Sodium Lake, 19.60[a]	Soluble Erythrosine Sodium, 7.84	Amaranth Lake, 2.48	Tartrazine Lake, 2.45	FD&C Yellow No. 6 Lake, 2.51
Conditions	Light	Dark	Light	Dark	Light	Light	Light	Light	Light	Light	Light	Light	Light
Rate constant, min^{-1}	~0	~0	0.016	~0	0.017	0.019	0.091	0.155	0.195	0.062	~0	~0	~0

[a] Dye content = 40%.

At various intervals, the cell was removed from the beam, and the phenylbutazone concentration was measured spectrophotometrically at 264 nm. Corrections were applied for absorbance due to the lake. Interference from the degradation products of phenylbutazone was accounted for by measuring absorbance at the absorption peak of the degradation product (235 nm) and applying the simultaneous equations suggested by Beckstead *et al.* (3).

Identity of Degradation Products—Identification of the degradation products was accomplished by TLC using the method of Awang *et al.* (4). The use of silica gel–kieselguhr plates buffered at pH 6 prevented on-plate oxidation. As a further precaution, the plates were dried under nitrogen.

Dissolution Rates—Dissolution rates were measured by the method of Barrett and Fell (5). Coated phenylbutazone tablets were allowed to dissolve in 1 liter of phosphate buffer (pH 7.4) maintained at 37° and stirred at 70 rpm with a three-blade stirrer. Samples were removed at suitable intervals and filtered through membrane filters[4] (0.45 μm) mounted in special adaptors[4], and the phenylbutazone concentration was determined spectrophotometrically. The filtration procedure removed the majority of the lake.

RESULTS AND DISCUSSION

The concentration of phenylbutazone in solution (0.001% w/v) was monitored on exposure to light and to light in the presence of the lake mixture (0.0009% w/v). Rate constants for the degradation of phenylbutazone under these conditions were calculated from semilogarithmic plots derived from the original data shown in Fig. 1 (Table I). Comparative data for the changes of phenylbutazone concentration in the dark and in the dark in the presence of the lake mixture also are shown in Table I.

The rate constants indicate that phenylbutazone was stable in solution on illumination and in the dark and in the dark when mixed with the lakes. However, illumination of the phenylbutazone plus the lake mixture led to rapid phenylbutazone decomposition.

The effect of the individual lakes in approximately the same concentration as in the lake mixture on the degradation of phenybutazone was examined, and the resulting rate constants are given in Table I. Only erythrosine sodium lake caused measurable degradation of the phenylbutazone, the rate increasing with increasing lake concentration (Fig. 2 and Table I). This effect was also found with a solution of erythrosine sodium (0.00078% w/v), but a slower rate of degradation was noted than with an equivalent lake concentration (Table I), possibly due to a surface catalytic effect.

Only one degradation product could be isolated on TLC (4) from the phenylbutazone–erythrosine sodium mixture after illumination. It was identified as 1,2-diphenyl-4-*n*-butyl-4-hydroxypyrazolidine-3,5-dione

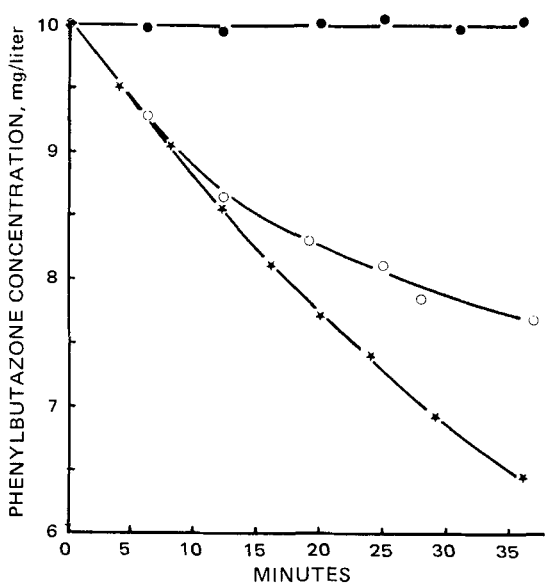

Figure 1—*Effect of the lake mixture on the degradation of 0.001% phenylbutazone solution under various conditions. Key: ★, phenylbutazone plus lake mixture in the light; ○, phenylbutazone plus lake mixture in the light after bubbling with nitrogen prior to irradiation; and ●, phenylbutazone plus lake mixture in the dark. Similar plots were obtained for phenylbutazone alone in the light and phenylbutazone plus other lake mixture components in the light.*

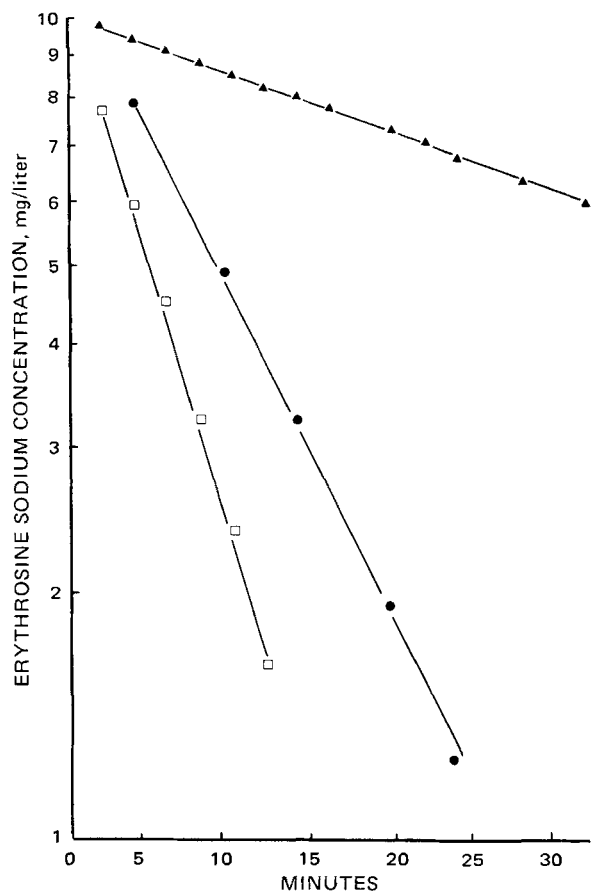

Figure 2—*Effect of erythrosine sodium lake concentration on the degradation of 0.001% phenylbutazone solution exposed to unfiltered light. Key: ▲, 1.23 mg/liter; ●, 4.90 mg/liter; and □, 9.80 mg/liter.*

[4] Millipore filters in Swinnex adaptors, Millipore Ltd., London, England.

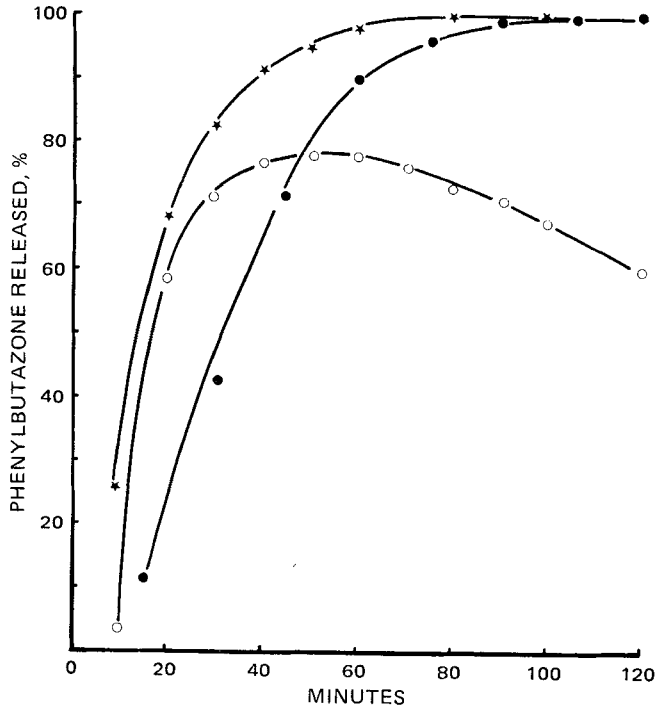

Figure 3—*Release rate of phenylbutazone from coated tablets containing erythrosine sodium in the light (○) and dark (★) or ponceau 4R in the light and dark (●).*

by comparison of R_f values on TLC with an authentic sample. This product is a known degradation product of phenylbutazone and is formed by oxidation (4). Illumination of phenylbutazone solution in the presence of the lake mixture after flushing the liquid with nitrogen markedly decreased the degradation rate of phenylbutazone (Fig. 1). This finding suggests that a mechanism involving oxygen is involved in the dye-catalyzed degradation of phenylbutazone on illumination.

Reactions involving dyes as photosensitizers are often mediated by singlet oxygen (6). A possible mechanism is suggested in Scheme I, where D = dye and R = oxidizable material. This mechanism involves the excitation of the dye into its triplet state by the light. The dye in the triplet state may return to the ground state with release of energy or may transfer the energy to ground-state triplet oxygen, generating singlet oxygen. The singlet oxygen may return to the ground state or attack oxidizable material present, in this case, phenylbutazone.

$$D + h\nu \rightarrow {}^3D^*$$
$$^3D^* \rightarrow D + h\nu'$$
$$^3D^* + {}^3O_2 \rightarrow D + {}^1O_2^*$$
$$^1O_2^* \rightarrow {}^3O_2$$
$$R + {}^1O_2^* \rightarrow RO_2$$

Scheme I

Further support for the suggestion that this scheme operates during the degradation of phenylbutazone by erythrosine sodium came from experiments in which methylene blue (10^{-7} *M*), a known generator of singlet oxygen, was substituted for erythrosine sodium and exposed to identical conditions. The phenylbutazone was degraded to the same degradation product as was produced with erythrosine sodium (first-order rate constant = 0.0282 min^{-1}).

The importance of this reaction lies in procedures where the drug, in solution, is brought into contact with erythrosine sodium in the presence of light, as in quality control procedures such as assays or dissolution rate testing. Figure 3 shows the results from the dissolution rate testing of coated phenylbutazone tablets. In one case, the color in the sugar coating contained erythrosine sodium; in the other, the erythrosine sodium had been replaced by ponceau 4R and the proportions of the other lakes had been altered to obtain an identical color. The tablets were not prepared from the same batch of cores, but comparison can be made between the types of dissolution profiles rather than absolute values.

The testing was carried out under normal laboratory lighting conditions, consisting of natural light from windows and artificial light from fluorescent fittings, and in the dark. When the tablet coat contained erythrosine sodium, the concentration of phenylbutazone in the beaker fell after a certain time due to degradation in the light in the manner described. Equivalent tests run in the dark showed no degradation, and the concentration of phenylbutazone rose to 100%. For tablets with the reformulated color, the drug dissolved and reached 100% in solution both in the dark and in the light, indicating that no degradation had occurred.

If tests are carried out in the light on tablets containing erythrosine sodium in the sugar coat and samples are taken only before the phenylbutazone concentration starts to fall, a falsely low dissolution rate will be indicated due to the competing reactions of dissolution and degradation.

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