the four compounds when tested on bovine liver was also true for mouse brain and liver. Compound III, the most active of the four, demonstrated twice the activity in mouse brain as in bovine liver.

Substitution of a methyl group on the indolic nitrogen of II did not give a 34-fold increase in the inhibition of MAO from either mouse brain or mouse liver as it did with MAO from bovine liver. When the 9-H compound (II) and the 9-methyl compound (I) are compared in the two latter cases, I is only 2- and 2.6-fold more active than II, respectively. An additional example indicating species variation of inhibitory activity can be seen in VII. Fluorine substitution on the 6-position of I does not seem to affect the binding of VII on either mouse liver or brain MAO, while with bovine MAO this substituent causes a twelvefold decrease in inhibitory activity.

## CONCLUSION

The methyl group on the indolic nitrogen (N-9) was found to facilitate the penetration of tetrahydro-β-carbolines into the brain. Judging from the low solubility of II in heptane, this group would seem to increase the solubility of this compound in lipids. Halogen substitution increased the amount of compound in the brain by lowering the pKa, thus providing more uncharged compound for passage into the brain. Compound III, substituted by the 8-chlorine atom and the 9-methyl group, was shown to be the best inhibitor of mouse brain MAO; its activity was even twofold greater than that previously reported using bovine liver as the enzyme source (3). This compound was also demonstrated to enter the brain not only to a greater extent, but also at a faster rate.

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# Interaction of Sodium Erythrosin and Polyvinylpyrrolidone


**JON C. ANDERSON and GERARD M. BOYCE**



**Abstract** □ Sodium erythrosin and polyvinylpyrrolidone (PVP) will form a complex in aqueous solution. The effects of pH and PVP concentration have been investigated and a spectrophotometric method developed for the quantitation of the dye in oxymix, a PVP-containing pharmaceutical system. It has also been determined that PVP improves the color stability of an erythrosin solution over a broad pH range.



**Keyphrases** □ Polyvinylpyrrolidone, interaction—sodium erythrosin □ Spectrophotometry—quantitation of dye □ Stability—erythrosin solutions □ pH effect—erythrosin and erythrosin-PVP solutions


Numerous investigations have been conducted on the binding of polyvinylpyrrolidone (PVP) with various pharmaceutical agents and azo dyes in aqueous solution (1–8). A recent publication described the complex formation between sodium fluorescein, a phthalein dye, and this polymer (9).

In these laboratories, while investigating the chemistry of the excipients in oxymix,[1] Tarlin (10) observed that an association occurred between PVP and the 2,4,-

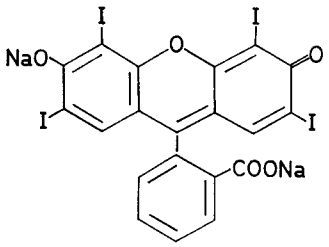


**Figure 1**—*Sodium erythrosin.*

5,7-tetraiodo derivative of sodium fluorescein, sodium erythrosin (Fig. 1). The complexation was indicated by a bathochromic shift of the $\lambda_{max.}$ of erythrosin.

In this communication, the effects of pH and PVP concentration on the complex are reported. In addition, a spectrophotometric method for the quantitation of erythrosin in oxymix is described.

## EXPERIMENTAL

**Materials**—Polyvinylpyrrolidone,[2] average molecular weight 25,000; sodium erythrosin,[3] pharmaceutical grade; oxymix (gran-

[1] Ascoxal (Gum-ox, Ascumist), marketed for oral hygiene by Astra Läkemedel, Södertälje, Sweden.

[2] Kollidon, Badische Anilin und Soda Fabrik AG, Ludwigshafen am Rhein, Germany.

[3] Saturnus, Malmö, Sweden.

Table I—Effect of PVP Concentration on the Absorbance of Erythrosin Solutions[a]

| PVP Concn., % | pH 3 $\lambda_{max.}$, mμ | pH 3 Absorbance | pH 5 $\lambda_{max.}$, mμ | pH 5 Absorbance | pH 6.6 $\lambda_{max.}$, mμ | pH 6.6 Absorbance | pH 11 $\lambda_{max.}$, mμ | pH 11 Absorbance |
|---|---|---|---|---|---|---|---|---|
| 0.00 | 534 | 0.20 | 527 | 1.24 | 527 | 1.29 | 527 | 1.30 |
| 0.0075 | 548 | 0.58 | 540 | 0.92 | 531 | 1.14 | 530 | 1.15 |
| 0.0375 | 547 | 0.83 | 543 | 1.09 | 536 | 1.13 | 536 | 1.14 |
| 0.0750 | 547 | 0.99 | 543 | 1.12 | 538 | 1.18 | 538 | 1.19 |
| 0.750 | 547 | 1.15 | 543 | 1.24 | 538 | 1.27 | 538 | 1.26 |
| 1.125 | 547 | 1.16 | 543 | 1.24 | 538 | 1.27 | 538 | 1.27 |
| 7.500 | 547 | 1.19 | 543 | 1.25 | 538 | 1.30 | 538 | 1.28 |
| 10.00 | 547 | 1.21 | 543 | 1.25 | — | — | 538 | 1.28 |

[a] Erythrosin concentration = $3 \times 10^{-5}$ *M*.

ulate), quantitative composition (%): ascorbic acid 33.33, sodium percarbonate 19.67, cupric sulfate 0.07, polyvinylpyrrolidone 7.67, erythrosin 0.003, tartaric acid 16.00, sodium bicarbonate 21.51, mannitol 1.27, menthol 0.08, saccharin, 0.40 Oxymix control: same as above, excluding erythrosin.

**Solutions**—Erythrosin stock solution: a standard stock solution of sodium erythrosin was prepared by weighing accurately 100 mg. of the dye and diluting to 1 l. with distilled water. Buffer solutions,[4] pH 1, HCl; pH 2, KCl-HCl; pH 3, 4, 5, acid phthalate; pH 6, 7, 8, 11, phosphate; pH 12, NaOH. (The ionic strength of all buffer solutions was adjusted to 0.1 with sodium chloride.)

**Apparatus**—Absorbance values were determined using a spectrophotometer (Perkin Elmer model 202) equipped with matched 1.001-cm. quartz cells.

**Effects of pH and PVP Concentration**—Preliminary experiments were conducted to examine and compare the spectral absorbance values of a $3 \times 10^{-5}$ *M* solution of sodium erythrosin with a solution containing equimolar[5] concentrations of erythrosin and PVP over a pH range of 1–12. The curves are shown in Figs. 2 and 3.

Additional studies were performed to determine the effect of PVP concentration on the complex. While maintaining the erythrosin solution at $3 \times 10^{-5}$ *M*, the polymer concentration was varied from 0 to 10% w/v (0 to $4 \times 10^{-3}$ *M*), absorbance values being obtained at pH 3, 5, 6.6, and 11. Data are shown in Table I.

**Assay Procedure**—Accurately weigh approximately 2.40 g. of the oxymix granulate and transfer it quantitatively to a 100-ml. beaker. Add 8 ml. of distilled water and allow the mixture to react spontaneously for 30 sec. Stir the preparation magnetically for 15 min. and transfer it along with subsequent rinsings to a 10-ml. volumetric flask. Dilute to volume with distilled water and mix thoroughly. Transfer the contents to a 10-ml. graduated centrifuge tube and centrifuge for 5 min. at 2000 r.p.m. Decant the supernatant and determine its absorbance at 538 mμ using an oxymix control, similarly treated, in the reference beam. The concentration of erythrosin is determined directly from a Beer's law standard curve of erythrosin in oxymix. Solutions for the standard curve are prepared by adding accurately measured quantities of the erythrosin stock solution to samples of the oxymix control and treating these mixtures in the manner as described above.

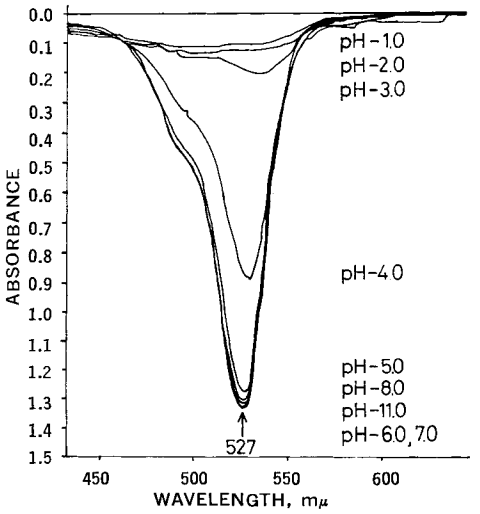


**Figure 2**—*Effect of pH on the absorption spectrum of sodium erythrosin.*

## RESULTS AND DISCUSSION

The effect of pH on both erythrosin and erythrosin-PVP solutions is depicted in Figs. 2 and 3. The data indicate that (*a*) the erythrosin molecule exists in more than one form and (*b*) an association occurs in solution between it and the polymer, PVP.

Erythrosin solutions are quite unstable at pH levels less than 3. Freshly prepared solutions do not absorb in the visible range and precipitate within 1 to 2 min. As the pH increases, stability increases concomitantly and the $\lambda_{max.}$ is shifted slightly to shorter wavelengths, stabilizing at 527 mμ, pH 6, where maximal absorbance values are attained. The shift observed between pH 4 and 6 is assumed to be due to the ionization of the carboxyl group (pKa 4.95) (11).

The pH profile of the solutions containing PVP closely resembles that of the erythrosin *per se*, stable absorption being reached at approximately pH 6. At all pH levels, however, the $\lambda_{max.}$ of erythrosin is shifted to longer wavelengths in the presence of the polymer. Such bathochromic shifts were not reported by Phares (9) while investigating solutions containing equimolar concentrations of sodium fluorescein and PVP.

On addition of PVP, stability of the erythrosin solution appears to be enhanced in the acid range. Marked improvement is observed

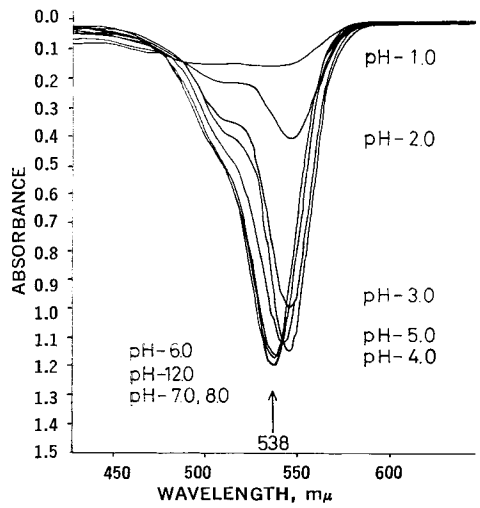


**Figure 3**—*Effect of pH on the absorption spectrum of equimolar sodium erythrosin–PVP.*

[4] USP XVII.

[5] Molar concentration of PVP calculated using average molecular weight of 25,000; thus, a $3 \times 10^{-5}$ *M* solution has a concentration of 0.075 g./100 ml.

**Table II**— Stability of Erythrosin Solutions

| pH | Assay Intervals, days: 0 Absorbance | 2 Absorbance | 6 Absorbance | Degradation, % |
|---|---|---|---|---|
| 5 | 1.28 | 1.04 | 0.40 | 68 |
| 6 | 1.33 | 1.09 | 0.46 | 65 |
| 7 | 1.33 | 1.12 | 0.47 | 64 |
| 8 | 1.31 | 1.12 | 0.49 | 63 |

**Table III**—Stability of Erythrosin–PVP Solutions

| pH | Assay Intervals, days: 0 Absorbance | 2 Absorbance | 6 Absorbance | Degradation, % |
|---|---|---|---|---|
| 5 | 1.19 | 1.13 | 1.02 | 14 |
| 6 | 1.23 | 1.15 | 1.02 | 17 |
| 7 | 1.24 | 1.15 | 1.02 | 18 |
| 8 | 1.26 | 1.18 | 1.03 | 19 |

at pH 2 and 3, indicated by the absence of precipitation as well as by increased absorbance.

The data (Table I) show that at pH 5 and greater, on addition of small amounts of PVP (*e.g.*, 0.0075%), an initial decrease of absorbance results. As the PVP concentration is increased, absorbance also increases, ultimately reaching a maximum at a concentration of $\geq$0.75%. The changes in the absorption spectrum, on admixture of PVP, can most probably be attributed to weak interaction, *e.g.*, van der Waals forces or charge transfer as suggested by Frank (8) and Phares (9), respectively. It should be pointed out that noticeable differences were observed at pH 3, possibly due to the labile nature of erythrosin at this level.

The data do suggest that one should be able to quantitate for erythrosin in a PVP-erythrosin containing solution of known pH in which a minimum molar ratio of 10:1[6] exists. Oxymix, a redox system containing PVP and erythrosin, was selected to test this hypothesis.

**Analytical Studies**—Reference to the oxymix formulation shows that the molar ratio of PVP to erythrosin is satisfactory; therefore, maximal absorbance values for erythrosin should be obtained.

The granulate, when treated in the manner previously described, should give a solution with a concentration of 8 mcg./ml. of erythrosin. A standard curve was, therefore, prepared from oxymix control solutions of erythrosin containing 5, 8, 10, 12 mcg./ml. The solutions were scanned in the visible and were found to have a $\lambda_{max.}$ of 538 m$\mu$ at pH 6.6, the pH of the oxymix solution after 15 min. of stirring. A plot of absorbance *versus* concentration was found to be linear and to pass through the origin.

**Stability of Erythrosin Solutions**—It was previously mentioned that the stability of an erythrosin solution was increased at acid pH's in the presence of PVP. As a result of this observation, a study was conducted to investigate and compare the stability of an aqueous erythrosin solution (about $3 \times 10^{-5}$ *M*) with one containing 6% PVP. The study was carried out for 6 days over a pH range of 5 to 8; the solutions were stored at room temperature in the light and assayed at appropriate intervals spectrophotometrically. The data are shown in Tables II and III.

The results indicate that PVP did improve the color stability of erythrosin at all pH levels. Approximately 65% degradation was observed with the erythrosin solutions as compared with 17% for the PVP-containing systems. It should be pointed out, however, that although the polymer increased the stability due to some binding mechanism, the color that resulted from the complex formation did differ somewhat from a solution of the dye *per se*.

[6] A 0.75% solution of PVP has a molar concentration of PVP of $3 \times 10^{-4}$ *M*; the erythrosin solution is $3 \times 10^{-5}$ *M*.

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