DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 14(5), 733-746 (1988)

INFLUENCE OF CERTAIN ADDITIVES ON THE PHOTOSTABILITY OF PHYSOSTIGMINE SULFATE SOLUTIONS

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ABSTRACT

Certain chemicals belonging to different groups of pharmaceutical adjuvants such as antioxidants, chelating agents, tonicity adjustors, photoprotective agents, buffer salts and preservatives were evaluated as stabilizing agents against photodegradation of physostigmine sulfate solutions in acetate buffer of pH 4.5. Of the materials studied, sodium benzoate appeared to be most effective, followed by potassium acid phthalate and then tartaric acid. Uric acid, sodium thiosulfate and glycerin were less effective. On the other hand, boric acid and sodium metabisulfite demonstrated the least photostabilizing action.

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INTRODUCTION

Physostigmine and its salts are known to be light-sensitive (1). Several antioxidants have been used to prevent or minimize oxidation of physostigmine salts. Ascorbic acid in a concentration of 0.1% has been reported to prevent oxidation of physostigmine hydrobromide for 6 months under normal conditions of storage. However, a pale brown color developed owing to the oxidation of ascorbic acid (2). Sodium metabisulfite has also been recommended by several authors. Swallow (2) used 0.1% sodium metabisulfite which was found to be less effective than 0.1% ascorbic acid. In another report (3), discoloration of physostigmine salicylate on heating was prevented by the addition of 0.1% sodium metabisulfite. In the study by Fletcher and Davies (4), 0.5% of sodium metabisulfite was found to improve the stability of physostigmine sulfate solutions towards gamma irradiations. Physostigmine sulfate eye drops of the B.P.C. (5) contains 0.2% sodium metabisulfite as a stabilizer.

Solutions of physostigmine salts have been reported to be fairly stable if the pH is not above 6 (6). Physostigmine salicylate was found to be most stable in aqueous solution at pH 3.6 at 25° (7). The degradation of physostigmine sulfate on exposure to gamma irradiations was found to be independent of the pH of the solution over the pH range of 1.5-7 (4).

Physostigmine in solution has been reported to undergo decomposition. Its urethane grouping is first lost and a colorless com-

pound termed eseroline is formed. Subsequent oxidation leads to rubreserine, a red quinone, which is later converted to eserine blue or eserine brown (8). The addition of sulfite does not prevent the decomposition, but merely converts the red oxidation products to substances which are colorless in an acid pH (6). Hemsworth and West (9) reported that hydrolysis of physostigmine may occur on sterilization of the eye drops by heating, resulting in the formation of the less active colorless compound, eseroline, before the appearance of the pink oxidation product, rubreserin. Thus, solutions of physostigmine may be colorless, but relatively inactive. All degradation products of physostigmine were reported to be pharmacologically less active than the parent compound (9,10). Hemsworth and West (10) emphasized that lack of coloration of physostigmine does not necessarily indicate full anticholinestrase activity because a colorless product of hydrolysis, eseroline, possesses little or no anticholinestrase activity.

In view of the photosensitivity of physostigmine sulfate solutions and the fact that the degradation products are less pharmacologically active than physostigmine, it appeared worthwhile to investigate some pharmaceutical adjuvants as potential photostabilizing agents for physostigmine sulfate solution in acetate buffer of pH 4.5.

EXPERIMENTAL

Materials: Physostigmine sulfate, sodium acetate, acetic acid, uric acid, tartaric acid, potassium acid phthalate, boric acid, sodium metabisulfite, sodium thiosulfate, sodium benzoate, glycerin, lactic

acid, sodium nitrite and ammonium sulfamate were obtained from commercial sources in pharmaceutical or reagent grade and were used without further purification. Chloroform was obtained in spectrophotometric grade.

Equipment: The following were used: a light-stability cabinet equipped with two 30-inch, 40-watt Sylvania flourescent lamps to serve as the light source; Orion digital pH meter; a Spectronic 20 spectrophotometer.

Exposure to Light: The spectrophotometer tubes containing the solutions to be exposed to light were kept 7 cm from the light source. The light intensity was maintained at 1350 foot-candles.

Procedure: The typical experimental procedure was as follows: Volumes of 0.125% w/v physostigmine sulfate in acetate buffer of pH 4.5 ± 0.05 prepared with and without the various adjuvants were placed in 10 x 100 mm spectrophotometer tubes, covered with parafilm and exposed to the light source. Samples were withdrawn at designated time intervals and assayed for physostigmine content by the stability-indicating method developed by Fletcher and Davies (4) as follows:

A 1-ml sample of physostigmine sulfate solution was pipeted from each tube into a 30-ml separatory funnel. Ten milliliters of 20% lactic acid solution was added followed by 1 ml of 1% sodium nitrite solution. The mixture was shaken for 30 seconds and allowed to stand for 30 minutes. One gram of ammonium sulfamate was then added and allowed to dissolve by shaking. The yellow nitroso-

compound formed was then extracted with 7, 4, 4, 4 and 4 ml aliquots of chloroform. The combined chloroformic extract was collected into a 25-ml volumetric flask and completed to volume with chloroform. The absorbance of the chloroform solution was determined at 417 nm on the Spectronic 20 Spectrophotometer. Each determination was in duplicate, the results averaged and used to calculate the percentage of physostigmine in the sample by reference to a Beer-Lambert plot. The difference between duplicates was usually 0-1.7%.

This stability-indicating method of assay was used to monitor stability of physostigmine sulfate solutions exposed to gamma irradiations or heat (4). Concentrations of the various adjuvants used were 0.2% sodium metabisulfate, 0.05% sodium thiosulfate, 5 mg% uric acid, 20 w/v glycerin, 1% potassium acid phthalate 1% tartaric acid, 2% boric acid and 0.2% sodium benzoate. Because of the poor solubility of uric acid, a 25 mg% solution in glycerin was prepared with the aid of heat.

DISCUSSION OF RESULTS

Effect of Antioxidants:

It can be seen from Figure 1 and Table 1 that the incorporation of 0.2% sodium metabisulfite or 0.05% of sodium thiosulfate into physostigmine sulfate solution, enhanced the photostability of the drug. Sodium thiosulfate appeared to demonstrate a better photostabilizing effect than sodium metabisulfite. The addition of sulfite

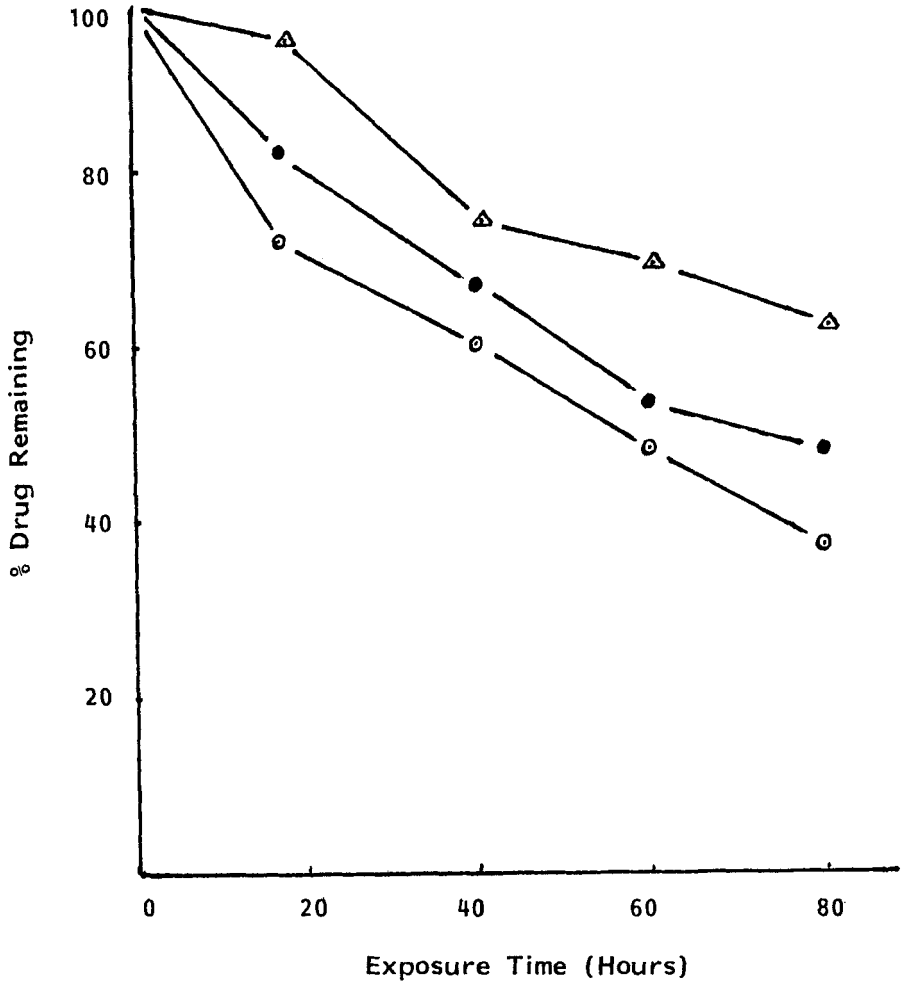

FIGURE 1

Effect of antioxidants on photodegradation of physostigmine sulfate solution. Key: ⊙ solution of physostigmine sulfate, ● solution of physostigmine sulfate + 0.2% sodium metabisulfite, ◬ solution of physostigmine sulfate + 0.05% sodium thiosulfate.

TABLE 1

Effect of Certain Additives on the Photostability of Physostigmine Sulfate in Acetate Buffer of pH 4.5

Solution	% Drug*	Color of Solution*
Drug	38.0	Very dark pinkish brown
Drug + 0.2% sodium metabisulfite	49.3	Yellow
Drug + 0.05% sodium thiosulfate	63.3	Light Pink
Drug + 20% glycerin	61.8	Light pinkish brown
Drug + 1% tartaric acid	70.8	Light yellowish pink
Drug + 2% boric acid	47.0	Very dark pinkish brown
Drug + 1% potassium acid phthalate	83.8	Light pink
Drug + 5 mg% uric acid + 20% glycerin	64.9	Pinkish brown
Drug + 0.2% sodium benzoate	90.6	Light pink

*After exposure to fluorescent light for 80 hours.

has been reported to have no effect on prevention of oxidation of the drug, but to convert the red oxidation products to substances which are colorless at an acid pH (6).

Effect of Chelating Agents:

Chelating agents include substances such as EDTA and its sodium salts, tartaric acid and glycerin (11). EDTA or its disodium salts were found to increase the degradation rate of physo-

stigmine (12). Therefore, it appeared worthwhile to investigate the effect of glycerin and tartaric acid on the photostability of the physostigmine sulfate solution.

Figure 2 and Table 1 shows that the incorporation of 20% w/v glycerin or 1% tartaric acid enhanced the photostability of physostigmine sulfate solution. Tartaric acid appeared to demonstrate a slightly better stabilizing effect than glycerin.

Effect of Tonicity Adjustor and Buffer Salt:

A 2% boric acid solution is a common vehicle for opthalmic solutions. Boric acid is also used as a tonicity adjustor in these solutions. It can be seen from Figure 3 and Table 1 that the incorporation of 2% boric acid into physostigmine sulfate solution produced a relatively low photostabilizing effect. Rae (13) reported that storage in the dark or the addition of 2% boric acid did not prevent the development of color in solutions of physostigmine sulfate.

Potassium acid phthalate is a buffering salt whose 0.05M aqueous solution at 25° has a pH of 4.005 (14). Figure 3 and Table 1 shows that the addition of 1% potassium acid phthalate appreciably enhanced the photostability of physostigmine sulfate solution. The pH of physostigmine sulfate solution containing 1% potassium acid phthalate was found to be 3.85 which is close to the value of 3.6 that has been reported to be desired for maximum stability of solutions of physostigmine salts (5,7). Morch (3) reported that the inclusion of 2% sodium acid citrate was sufficient to buffer solution of

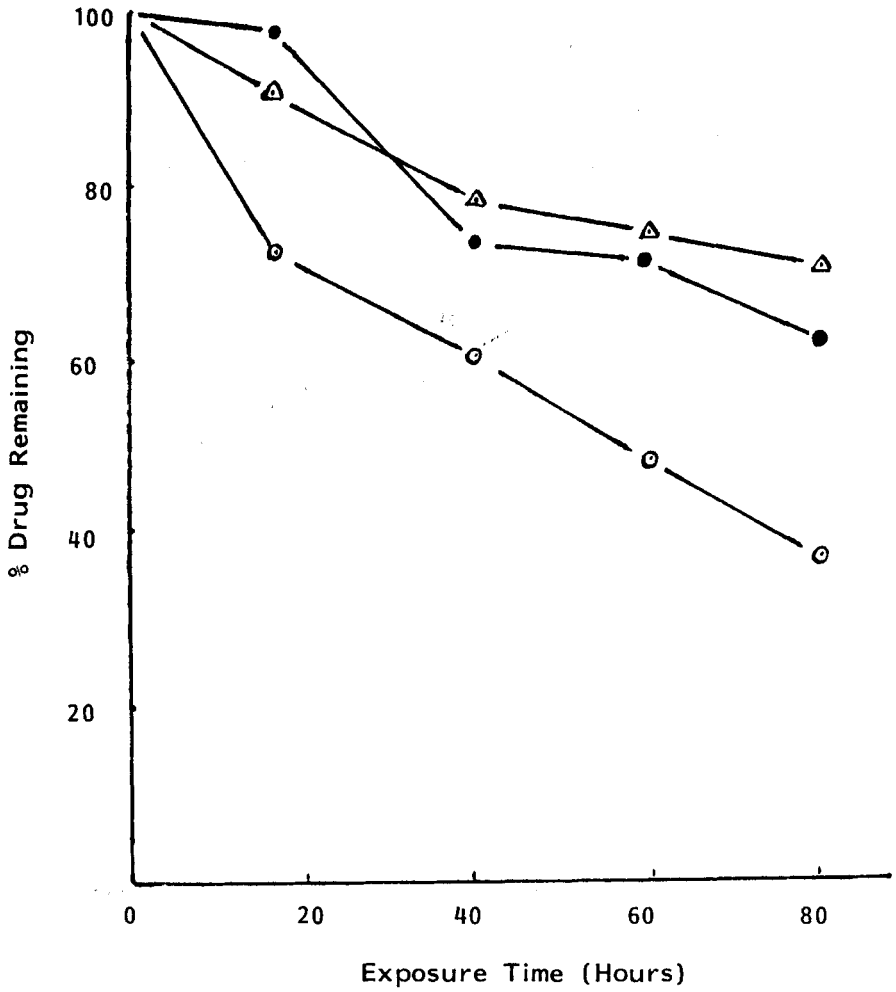

FIGURE 2

Effect of chelating agents on photodegradation of physostigmine sulfate solution. Key: ⊙ solution of physostigmine sulfate, ● solution of physostigmine sulfate + 20 w/v glycercin, ▲ solution of physostigmine sulfate + 1% tartaric acid.

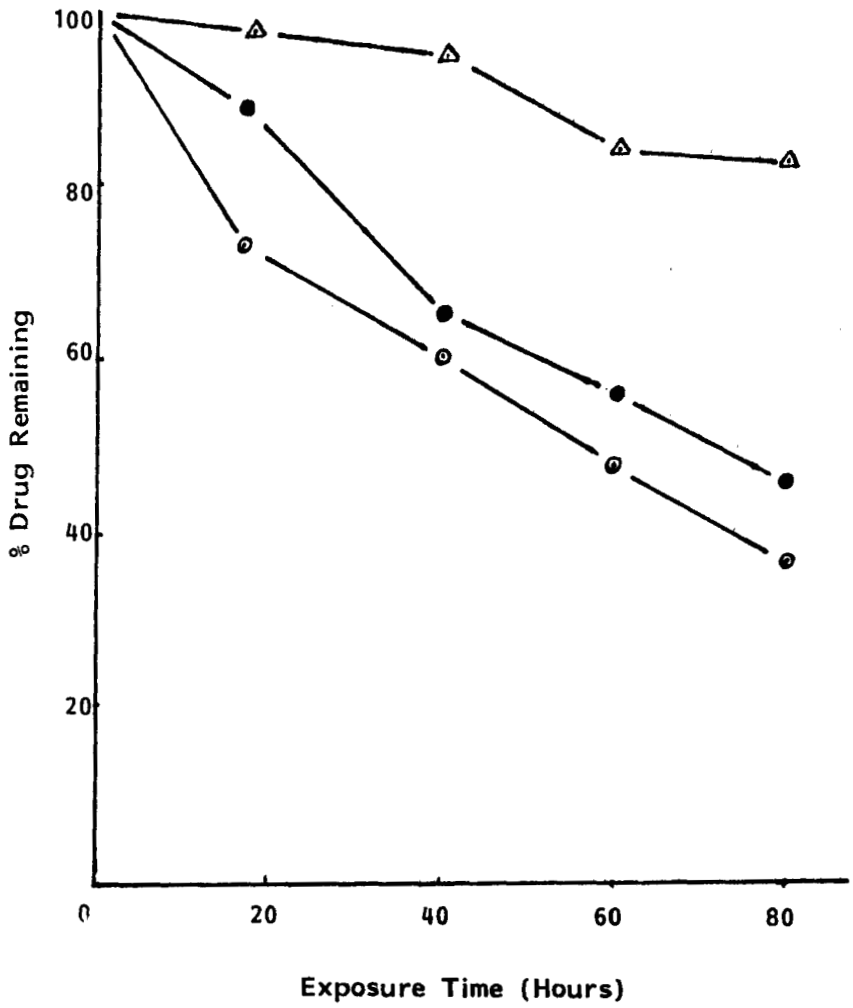

FIGURE 3

Effect of boric acid and potassium acid phthalate on photodegradation of physostigmine sulfate solution. Key: ◐ Solution of physostigmine sulfate, ● Solution of physostigmine sulfate + 2% boric acid ▲ Solution of physostigmine sulfate + 1% potassium acid phthalate.

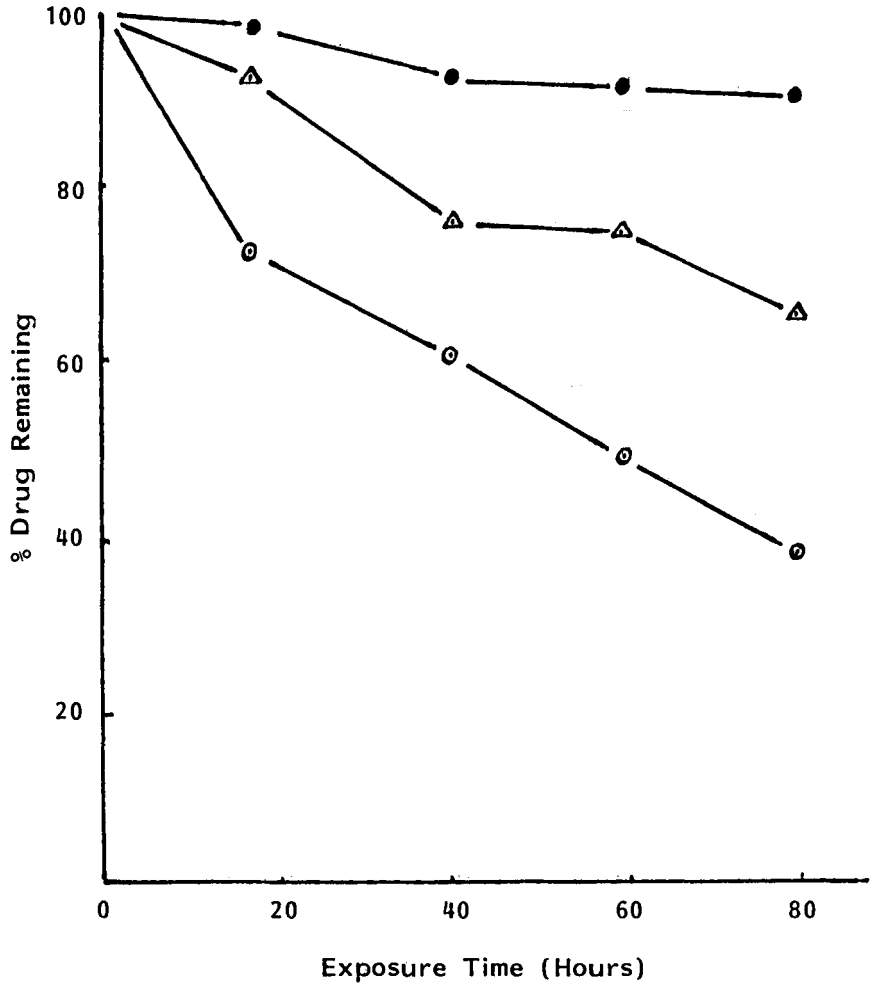

FIGURE 4

Effect of uric acid and sodium benzoate on photodegradation of physostigmine sulfate solution. Key: ⊙ Solution of physostigmine sulfate, ● Solution of physostigmine sulfate + 0.2% sodium benzoate, ▲ Solution of physostigmine sulfate + 5 mg% uric acid.

physostigmine salicylate to pH 5 and such solution showed a loss of 1 to 2% with no discoloration on heating at 100° for 15 minutes.

Effect of Uric Acid and Sodium Benzoate:

Uric acid solution in glycerin has been reported to be an efficient photoprotective agent for solutions of FD&C Blue No. 2 (15, 16). Figure 4 and Table 1 show that the incorporation of 5 mg% uric acid in presence of 20% w/v glycerin as a solvent for uric acid, enhanced the photostability of physostigmine sulfate solution to a slightly greater extent than that produced by 20% w/v of glycerin alone.

Sodium benzoate has been extensively used as a preservative for food and pharmaceutical preparations. Figure 4 and Table 1 show that the incorporation of 0.2% sodium benzoate resulted in a substantial photostabilizing effect for physostigmine sulfate solution. The percentage of physostigmine sulfate remaining in solution after exposure to light for 80 hours was 90.6%, which is the highest value among those obtained for the various adjuvants studied as seen from Table 1. Benzoic acid derivatives such as p-aminobenzoic acid and p-dimethyl aminobenzoate have been used as ultraviolet absorbers and photoprotective agents (17, 18). Therefore, the photostabilizing effect of sodium benzoate could be due to its ultraviolet-absorbing properties. However, further studies are needed before a final conclusion can be made.

CONCLUSIONS

The results of this study show that photodegradation of physostigmine sulfate in acetate buffer of pH 4.5 could be retarded by the incorporation of certain adjuvants. Sodium benzoate appeared to be most effective followed by potassium acid phthalate and then tartaric acid. Uric acid, sodium thiosulfate and glycerin were less effective, while boric acid and sodium metabisulfite demonstrated the least photostabilizing effect.

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