CHAPTER III

RESULTS

1. Experiments to find the suitable method to evaluate the color formation of sulfacetamide sodium due to light stress.

1.1 Absorption spectra of various components before and after light exposure.

Figure 2 illustrates the absorption spectra of freshly prepared sulfacetamide sodium (10 mcg/ml) and sulfanilamide (0.8 mg/ml) between 200-600 nm. It shows that both sulfacetamide and sulfanilamide have absorption peak between 200-300 nm and show no peak at all between 300-600 nm.

Figure 3 illustrates the absorption spectra of antioxidants, 0.1% sodium metabisulfite and 0.1% sodium thiosulfate and chelating agent, 0.05% disodium EDTA, which are the ingredients used in this experiment.

The freshly prepared solutions and the solutions after exposure to artificial daylight light for 3 days show the same pattern of the absorption spectra. The absorption peaks occur only between 200-300 nm and no peak occurs between 300-600 nm range.

Figure 4 illustrates the visible absorption spectra between 300-600 nm of 0.5% sulfacetamide sodium solution after exposure to artificial daylight light for 1, 3, 5, 7 and 8 days. It shows that an absorption peak occurs at 450 nm. The height of the peak varies to the light exposure time.

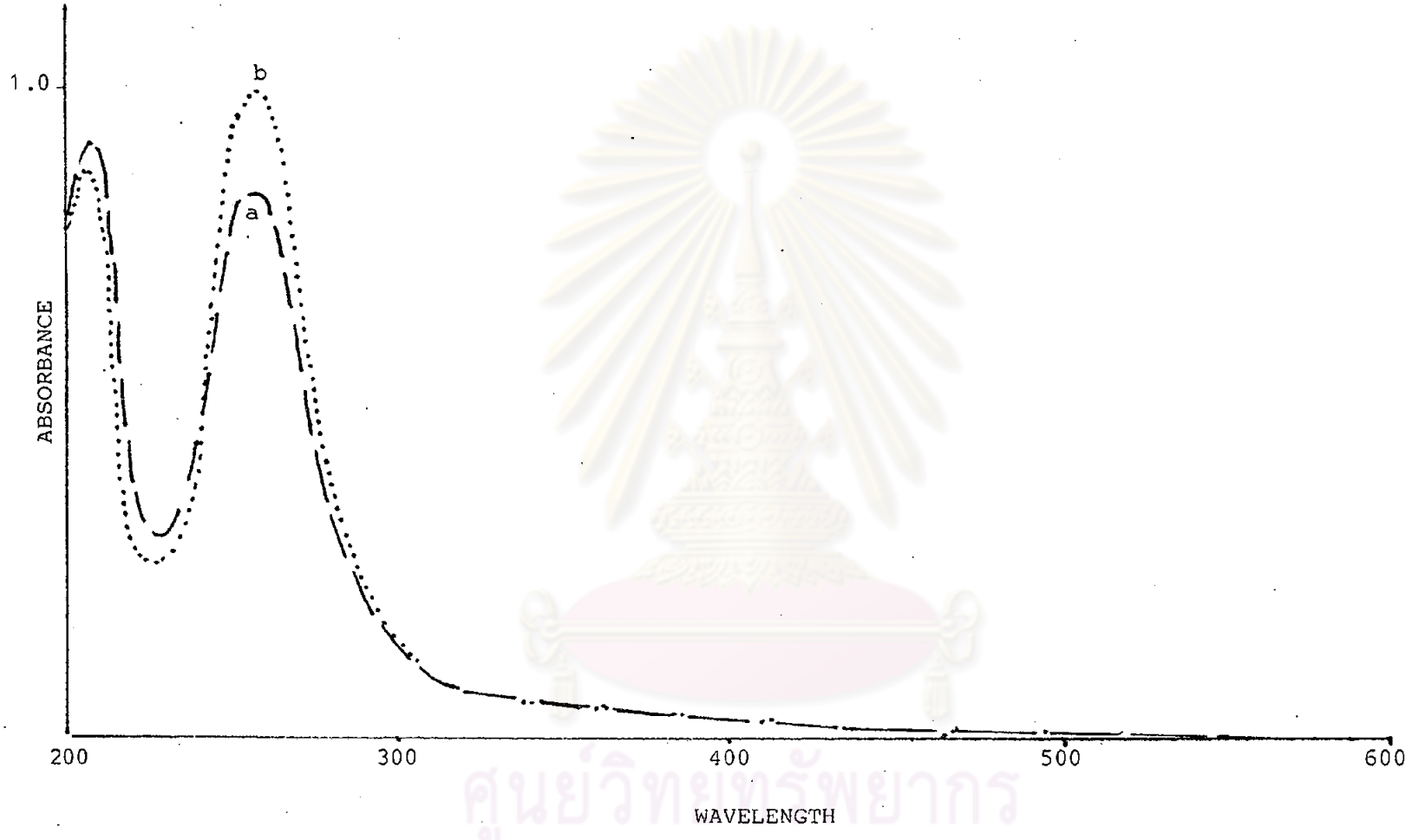

Fig. 3 Absorption spectra between 200-600 nm of 10 mcg/ml sulfacetamide sodium (a), 0.8 mg/ml sulfanilamide (b).

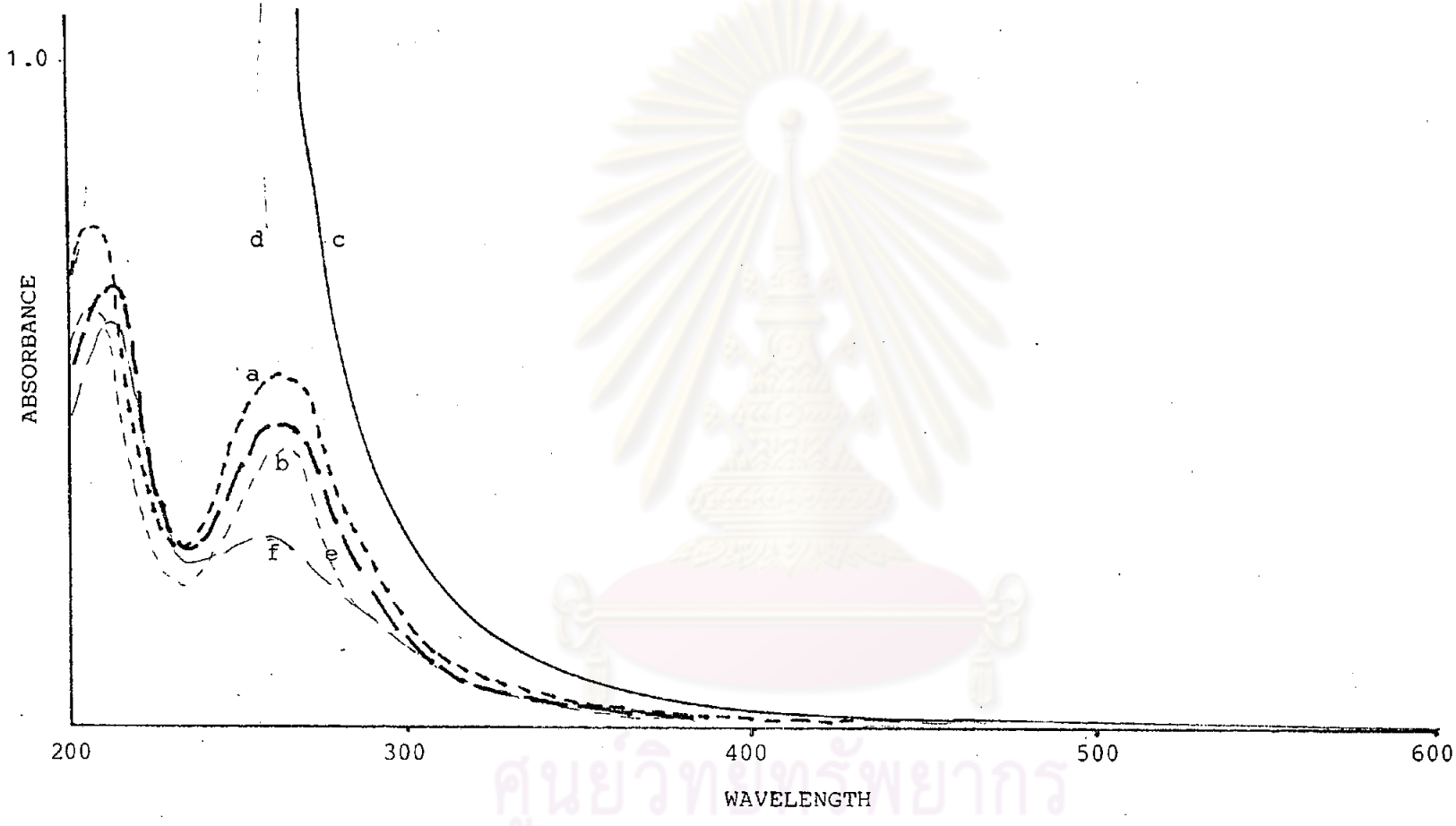

Fig. 3 Absorption spectra of 0.1% sodium metabisulfite (a) and after exposure to artificial daylight for 3 days (b); 0.1% sodium thiosulfate (c) and after exposure to artificial daylight for 3 days (d); 0.05% disodium EDTA (e) and after exposure to artificial daylight for 3 days (f), at 200-600 nm.

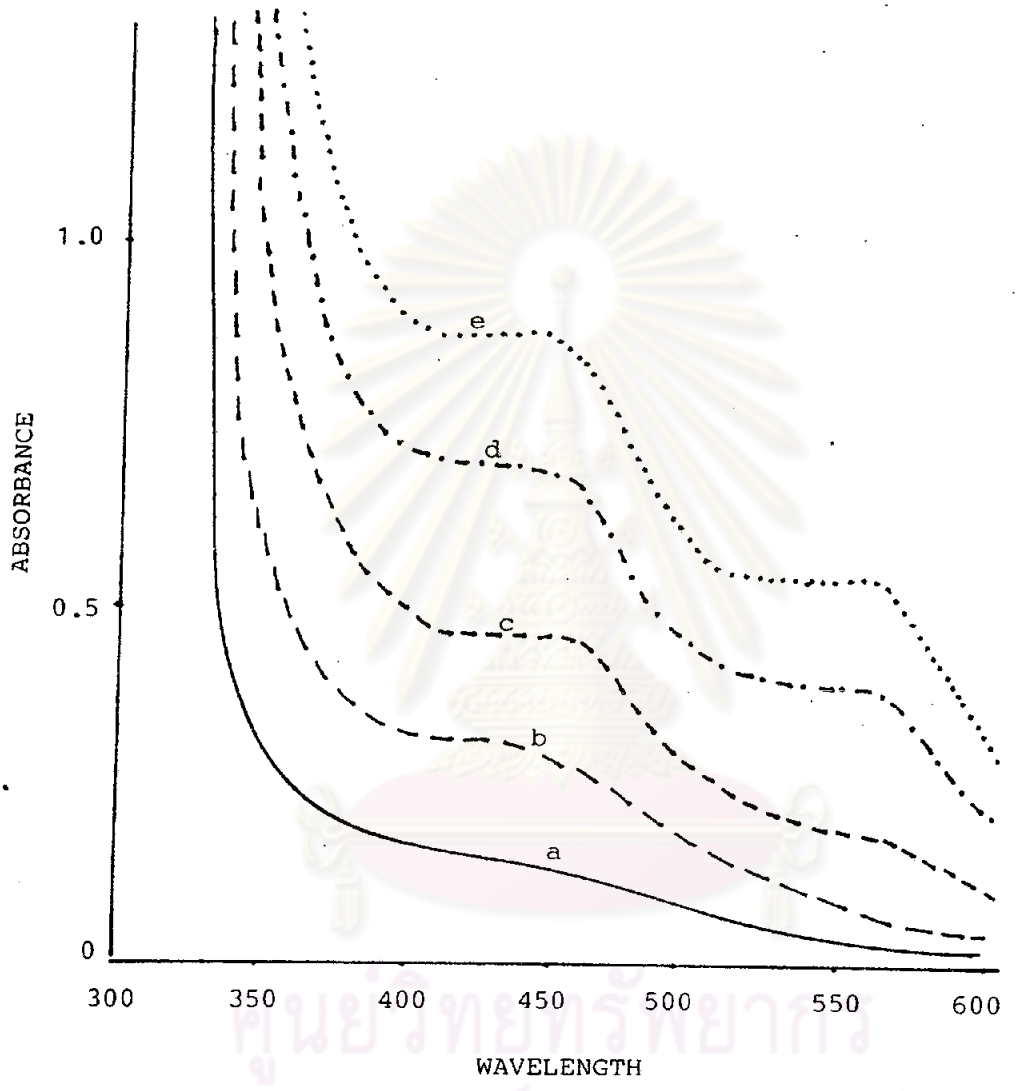

Fig. 4 Visible absorption spectra of 0.5% sulfacetamide sodium solution after exposure to artificial daylight light for 1 day (a), 3 days (b), 5 days (c), 7 days (d) and 8 days (e).

1.2 Comparison of color and potency of sulfacetamide sodium solution with standard caramel solutions.

Standard caramel color solutions were prepared and the shade or intensity of the color is assigned in letter codes as illustrated in table 1. The height of the absorption peaks illustrated in figure 3 is correlated with the intensity of caramel color solutions. However, the potency of sulfacetamide sodium does not change significantly even-though the solutions are darken in color, as shown in table 2.

1.3 Comparison of the effects of sunlight and artificial daylight light.

Figure 5 illustrates the effect of exposure to artificial daylight light (fluorecent, 40 W.) for 96 hours and natural sunlight for 48 hours. The absorbance peak of 0.5% sulfacetamide sodium solution in visible region at 300-600 nm is in the same pattern under exposure to both types of radiation, which means that the results obtained by using artificial daylight light can be compared with the results obtained under natural sunlight. The only different is the light intensities.

1.4 Effect of pH and buffers on the absorption spectra and color formation of sulfacetamide sodium solution.

Figure 6 illustrates the visible absorption spectra between 300-600 nm of 0.5% sulfanilamide sodium solution after exposure to artificial daylight light for 3 days. It shows that the buffered solution of pH 8 shows an absorption peak at 450 nm but the solution of pH 11 shows no peak at 450 nm.

Table 1 shows the concentrations of standard caramel solution, the shade of color and the code used in this experiment.

% W/V of color	color	code
0	clear	A
0.01	very light yellow	B
0.02	light yellow	C
0.04	yellow	D
0.06	light yellowish brown	E
0.08	yellowist brown	F
0.1	brown	G
0.2	reddish brown	H
0.4	darker reddish brown	I
0.6	darker reddish brown	J
0.8	darker reddish brown	K
1	darker reddish brown	L

Note: The shade of the color in between each code will be assigned as the following example.

G^- = slightly lighter than brown

G^+ = slightly darker than brown

G/H = color between brown and reddish brown

Table 2 The color formation compared with standard color and the percentage of labelled amount of 0.5%sulfacetamide sodium solution after exposure to artificial daylight light for 1, 3, 5, 7 and 8 days.

Days	Color	% Label amount
0	A	101.5
1	A	101.3
3	B^+	100.8
5	D^+	99.7
7	E	99.5
8	F^+	99.6

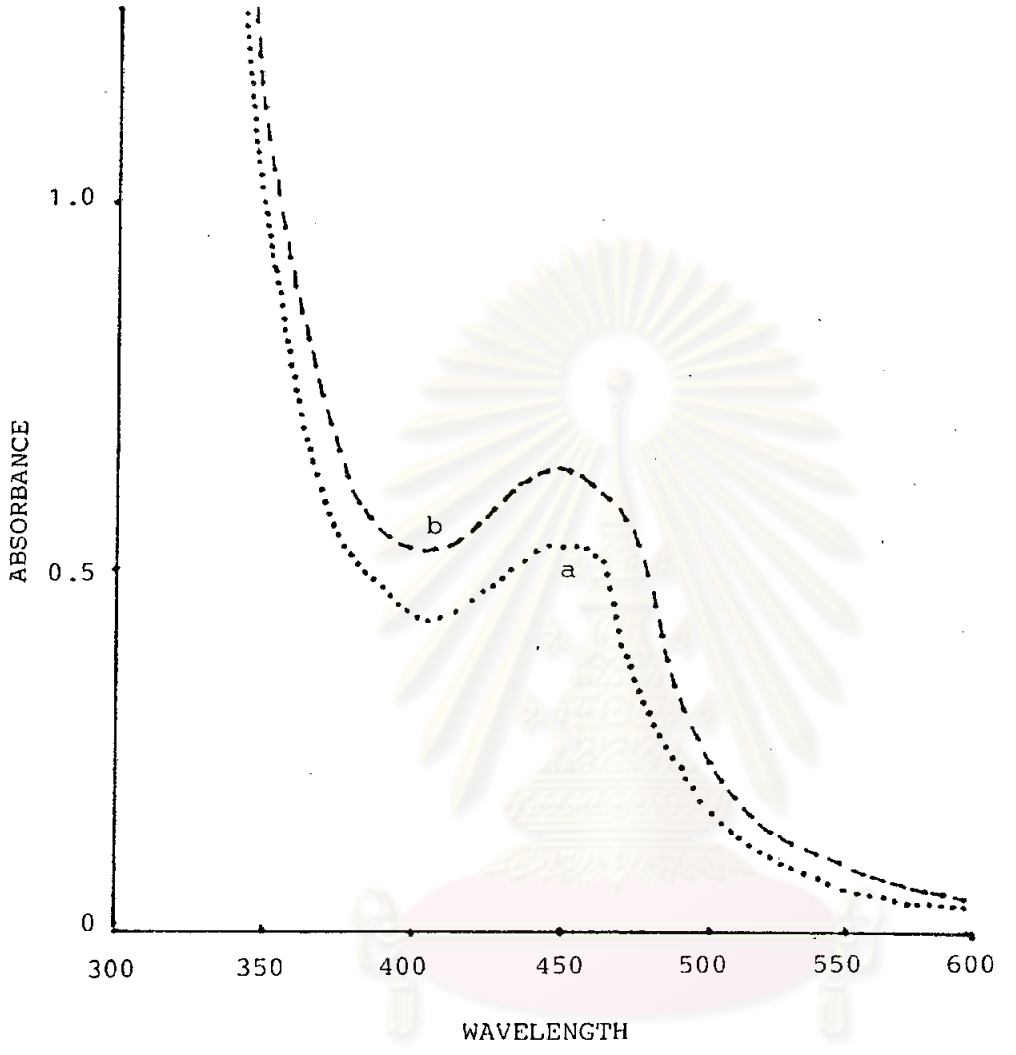

Fig. 5 Visible absorption spectra of 0.5% sulfacetamide sodium solutions with 0.1% sodium metabisulfite and 0.05% disodium EDTA after exposure to artificial daylight light for 96 hours (a) and to natural sunlight for 48 hours (b).

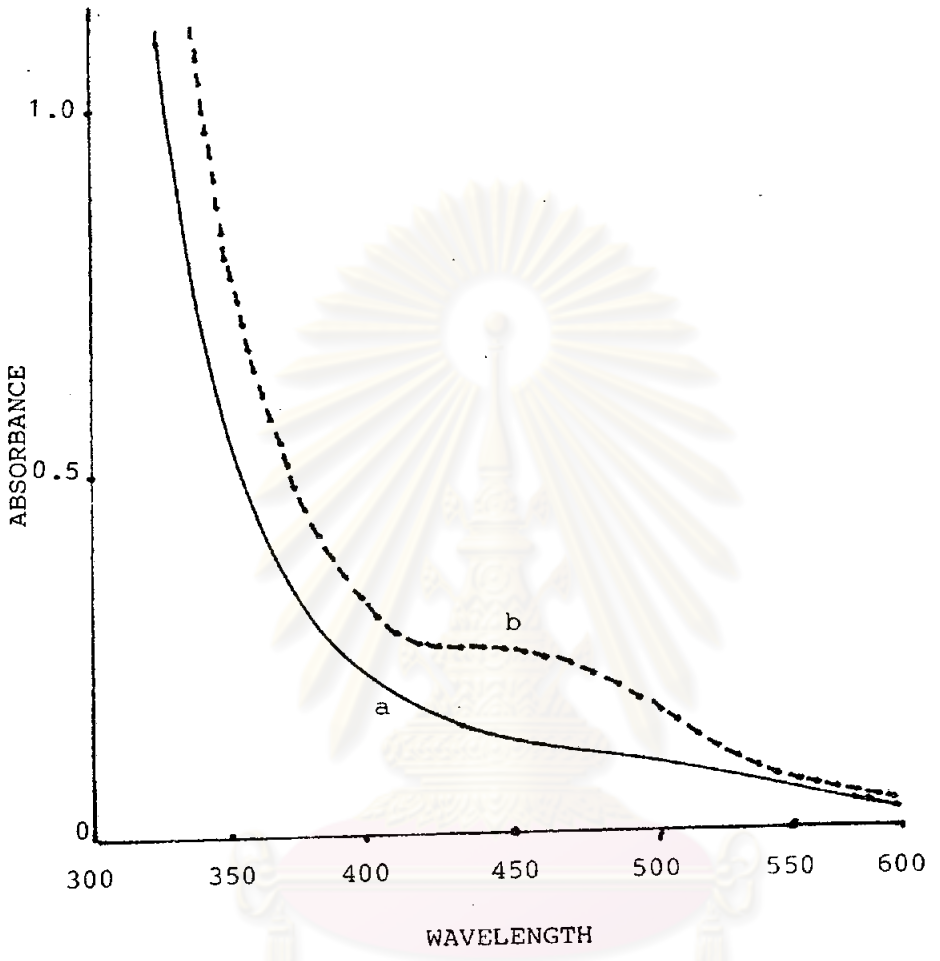

Fig. 6 Visible absorption spectra of 0.5% sulfanilamide sodium solution, pH 11 (a) and in phosphate buffer, pH 8 (b) after exposure to artificial daylight light for 3 days.

Figure 7 illustrates the visible absorption spectra at 300-600 nm, of sulfacetamide sodium solutions at concentrations of 0.5, 1, 5 and 10%, after exposure to artificial daylight light for 6 days. It shows the absorbtion peak at 450 nm clearly in 0.5 and 1% sulfacetamide sodium solutions but 5% solution shows unresolved peak at 450 nm while no visible peak of 10% solution. The pH of 0.5, 1, 5 and 10% sulfacetamide sodium solutions are 6.4, 6.6, 9.0 and 9.1.

The color of each solution after exposure to the light for 2-6 days, compare with standard color solutions is shown in table 3.

Buffers were used in preparing 10% sulfacetamide sodium solutions by using opthalmic phosphate buffers (30), pH 7.0, 7.4 and 8.0. The unbuffered solution possessed pH of 9.1. Visible absorption spectra were measured from 300-600 nm which show that at pH 9.1 sulfacetamide sodium solution is more stable than at more acidic pHs as shown in figure 8 and table 4.

2. Experiments to find the most effective antioxidant in retarding of color formation of sulfacetamide sodium solution and to find the most proper formulation.

Figure 9 illustrates the absorbance at 450 nm of solutions used in figure 8 at 1, 2, 3, 4 and 5 days which shows clearly that the rate of decomposition of sulfacetamide is dependent on the pH of the solution at any time. It also shows that the higher the concentration of phosphate buffer used, the faster change in color is observed.

In table 5 shows the pH of sulfacetamide sodium solutions at concentrations of 0.5, 5 and 10% with various amount of antioxidants and

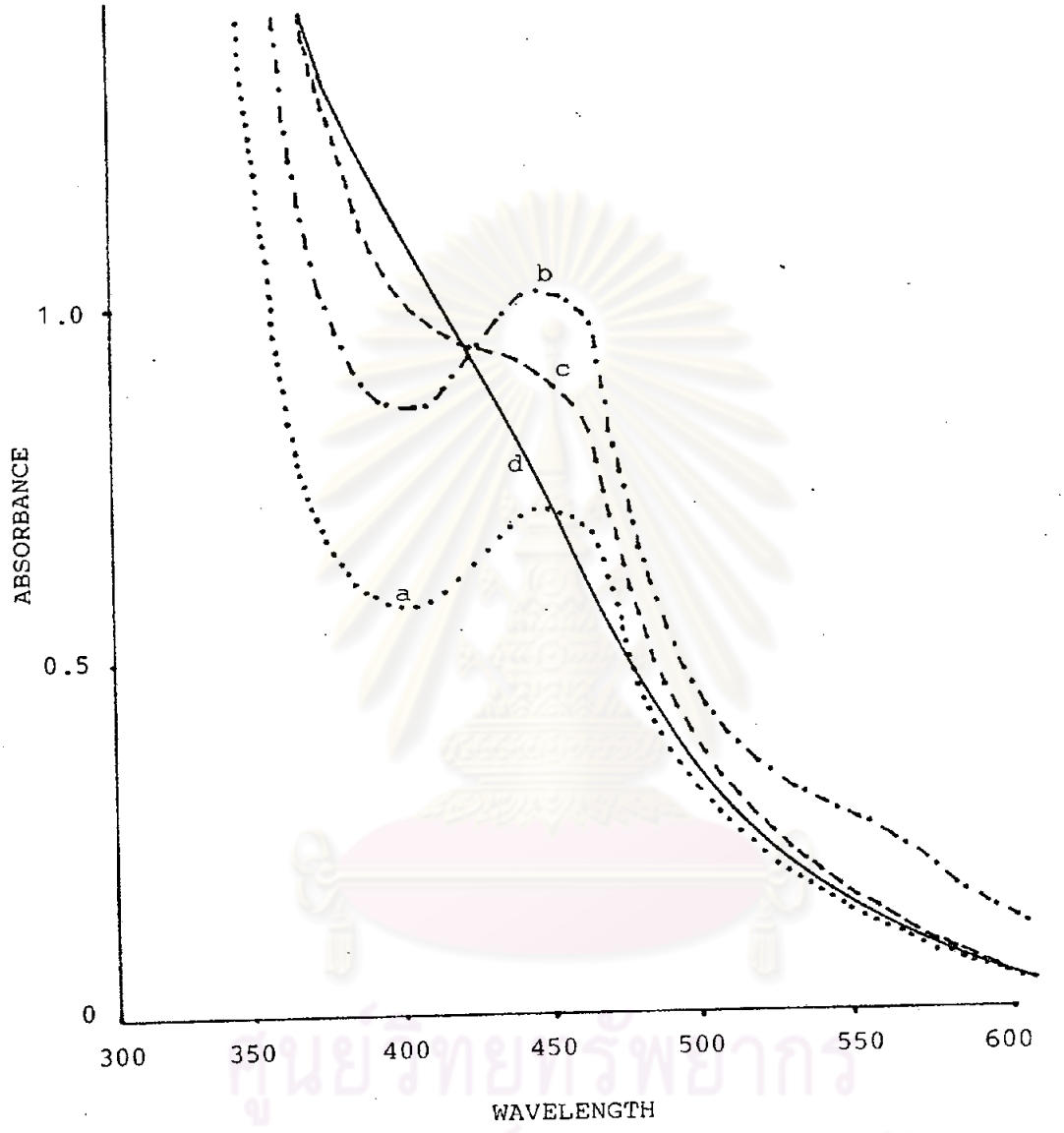

Fig. 7 Visible absorption spectra of sulfacetamide sodium solutions at concentrations of 0.5% (a), 1% (b), 5% (c), and 10% (d), after exposure to artificial daylight light for 6 days.

Table 3 Color formation of sulfacetamide sodium solution 0.5, 1, 5 and 10% compared with standard color solution after exposed to artificial daylight 2, 4 and 6 days

Days / % Sulfa.	2	4	6
0.5	B	C	D^{+}
1	B^{+}	C^{+}	E^{-}
5	B	C	D^{+}
10	B	C	D

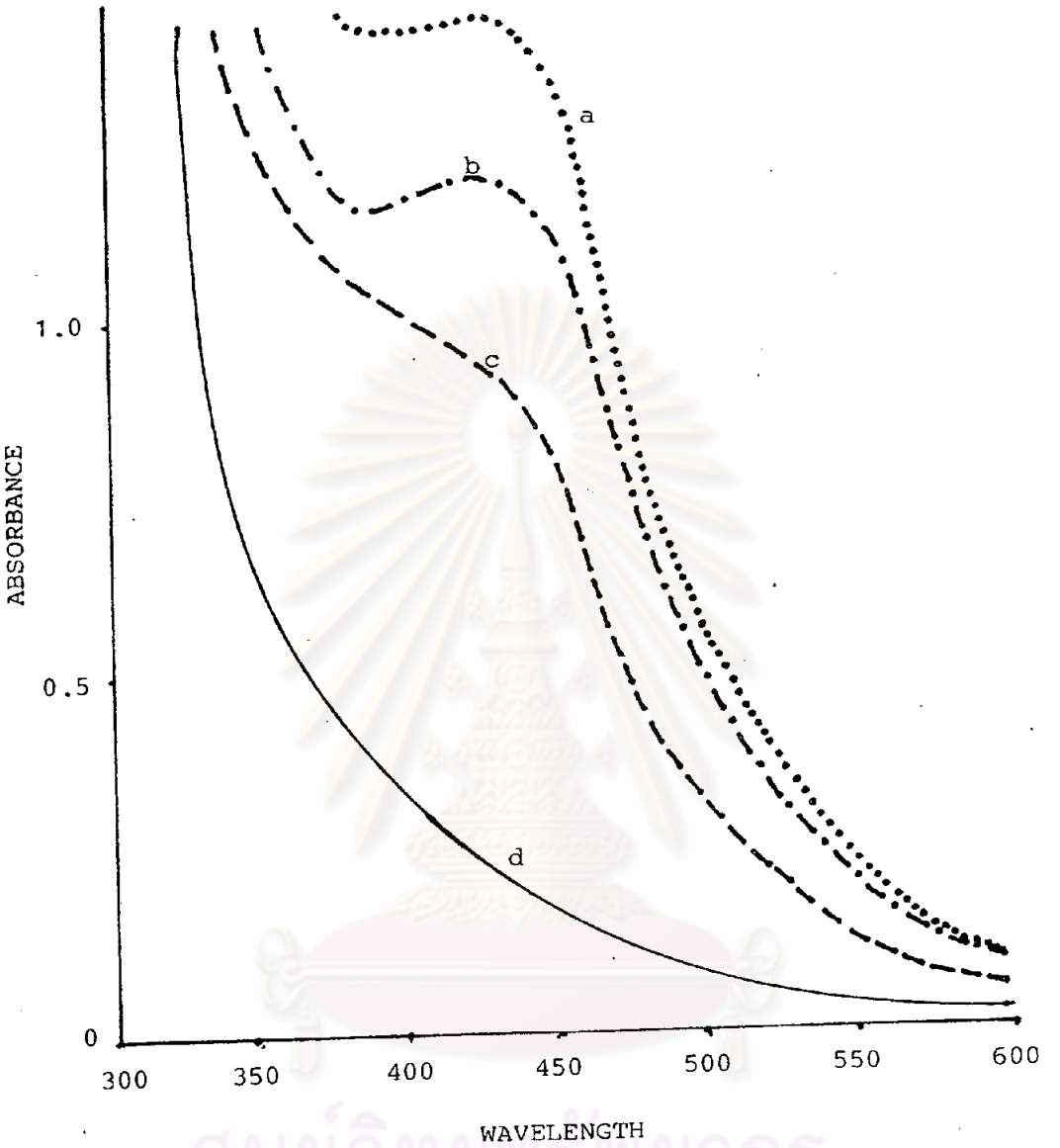

Fig. 8 Visible absorption spectra for 10% sulfacetamide sodium, buffered at pH 7 (a), pH 7.4 (b), pH 8.0 (c) and unbuffered solution pH 9.1 (d), after exposure to artificial daylight light for 3 days.

Table 4 The color formation and absorbance measured at 450 nm after 1, 2, 3, 4, 5 days of exposure to artificial daylight light of 10% sulfacetamide 10% solution pH 9.1 and solutions buffered at pH 7, 7.4 and 8.

pH \ day	1	2	3	4	5
7	C 0.7	E 1.09	F 0.52	G 1.9	G^+ > 2
7.4	C 0.63	E 1.04	F 1.45	G 1.83	G^+ > 2
8	B 0.35	C 0.61	D 0.88	F 1.16	F 1.5
9.1	A^+ 0.14	B 0.3	B^+ 0.44	C^- 0.58	D^+ 0.98

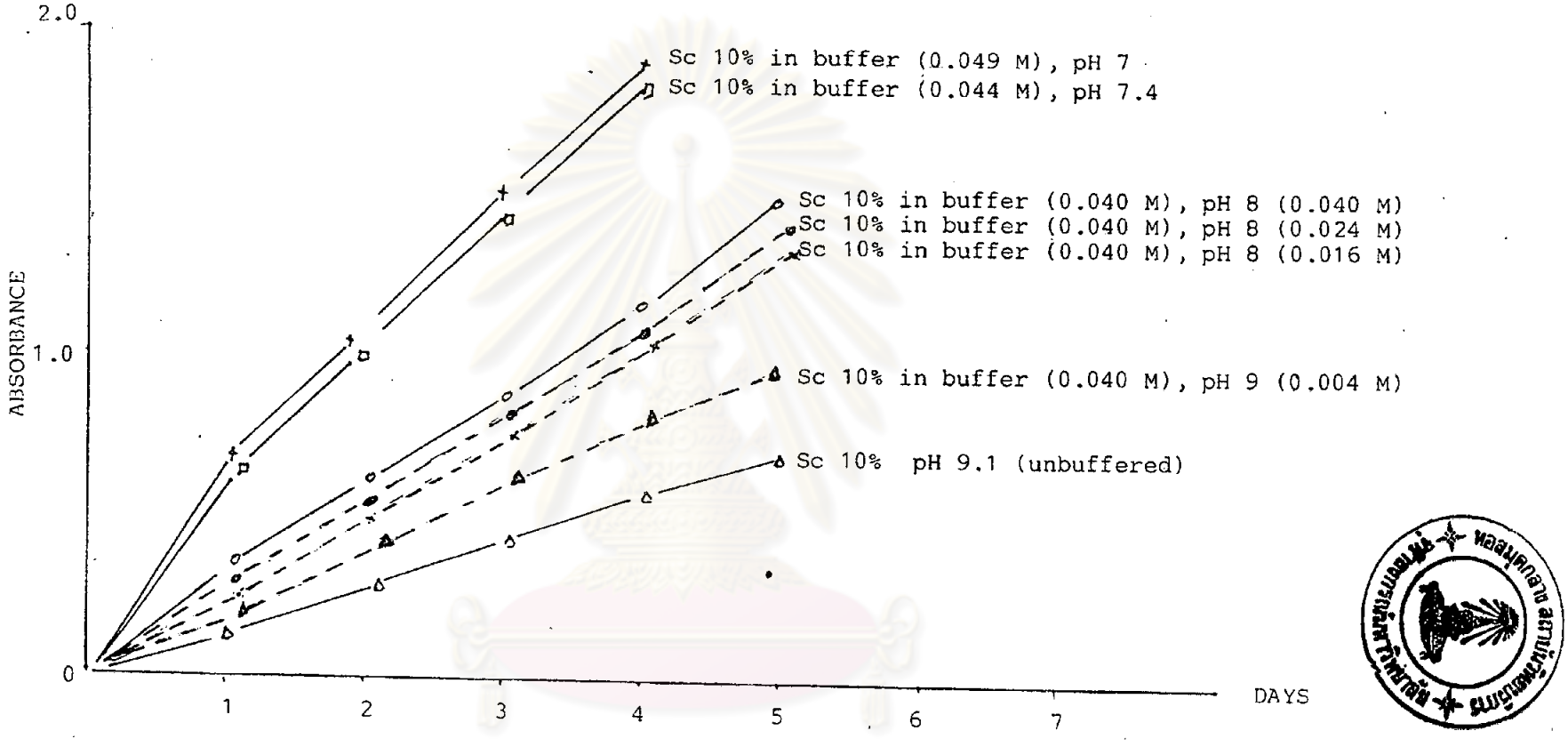

Fig. 9 Absorbance at 450 nm plotted at different times after exposure to artificial daylight light, of 10% sulfacetamide solution buffered at different pH and at different concentration of buffer at pH 8.0.

Table 5 pH or sulfacetamide sodium soultions (Sc) at concentration of 0.5, 5 and 10% with and without antioxidants and/or disodium EDTA at different concentrations.

% Sc	% Antioxidant		% of disodium EDTA			
	Sod. Metabisulfite	Sod. Thiosulfate	0	0.01	0.05	0.1
0.5	-	-	6.40	6.40	6.40	6.40
	0.1	-	6.40	6.40	6.40	6.20
	0.3	-	6.50	6.50	6.30	6.15
	0.5	-	6.20	6.20	6.20	6.10
	-	0.1	6.70	6.70	6.60	6.30
	-	0.3	6.80	6.80	6.50	6.30
	-	0.5	6.80	6.80	6.50	6.30
5	-	-	9.00	8.20	7.60	7.30
	0.1	-	8.50	7.50	7.30	7.10
	0.3	-	8.30	7.30	7.20	7.00
	0.5	-	8.00	7.20	7.10	7.00
	-	0.1	9.00	8.20	7.60	7.30
	-	0.3	9.00	8.20	7.60	7.30
	-	0.5	9.20	8.50	8.00	7.50
10	-	-	9.10	8.50	7.90	7.20
	0.1	-	8.10	7.95	7.65	7.50
	0.3	-	7.50	7.50	7.40	7.35
	0.5	-	7.25	7.20	7.20	7.10
	-	0.1	9.20	8.50	8.00	7.70
	-	0.3	9.50	8.50	8.00	7.70
	-	0.5	9.70	8.80	8.15	7.70

chelating agent. It can be seen that pH of 0.5% sulfacetamide sodium solutions with and without antioxidants and chelating agent falls in the range 6.1 to 6.8. While 5% solutions are in the pH range of 7 to 9.0 and 10% solutions are in the pH range of 7.5 to 9.7.

The pH of 0.5% sulfacetamide sodium solutions is within a narrower range. Therefore they were selected for experiments to find the effeciency of antioxidants and chelating agent on the retardation of the color formation of sulfacetamide sodium solution. This will roughly eliminate the influence of pH and buffer.

Solutions of sulfacetamide sodium (0.5%) were prepared with antioxidants (sodium thiosulfate and sodium metabisulfite) and chelating agent (disodium EDTA) according to table 5. Absorbance of the solutions at 450 nm were measured after exposure to artificial daylight light for 1, 2, 3, 4, 5, 6, and 7 days. The results are shown in figure 10, as the plot of absorbance versus times of exposure to light, of 0.5% sulfacetamide sodium solutions with antioxidants.

Figure 11 shows the absorbance at 450 nm of 0.5% sulfacetamide solutions with various concentration of antioxidants combined with 0.05% disodium EDTA, at times after exposure to light.

It is clearly seen from figures 10 and 11 that sodium thiosulfate has better effect on retarding the color formation of solution comparing with pure sulfacetamide sodium solution. Sodium metabisulfite has some retardation effect at the beginning (about 3 days). The color formation increases very rapidly after 3 days of exposure to light and even faster than the color formation of pure sulfacetamide sodium solution at the

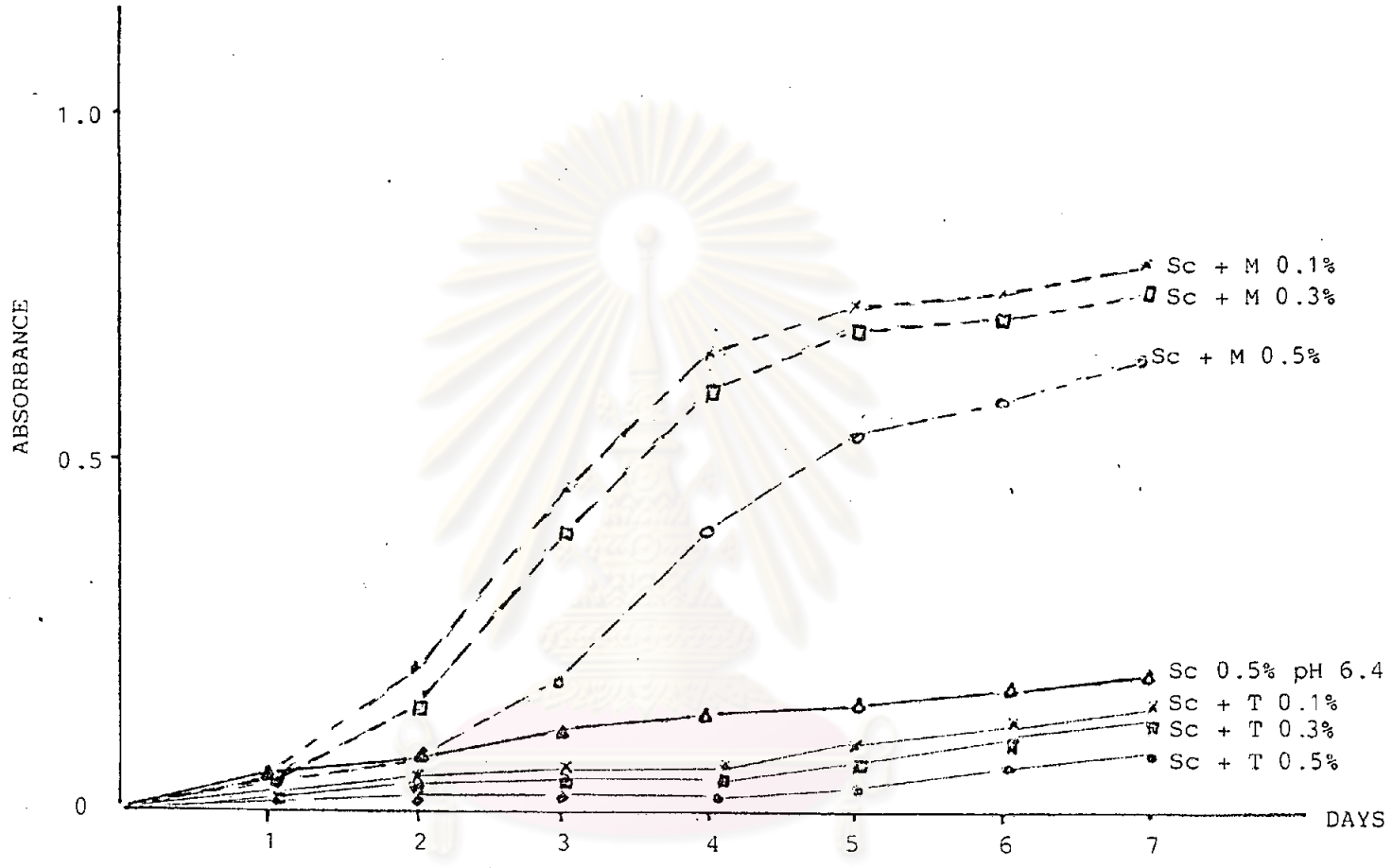

Fig. 10 Absorbance at 450 nm plotted at different times after exposure to artificial daylight light, of 0.5% sulfacetamide sodium solutions (Sc), with various concentrations of antioxidants, sodium metabisulfite (M) and sodium thiosulfate (T).

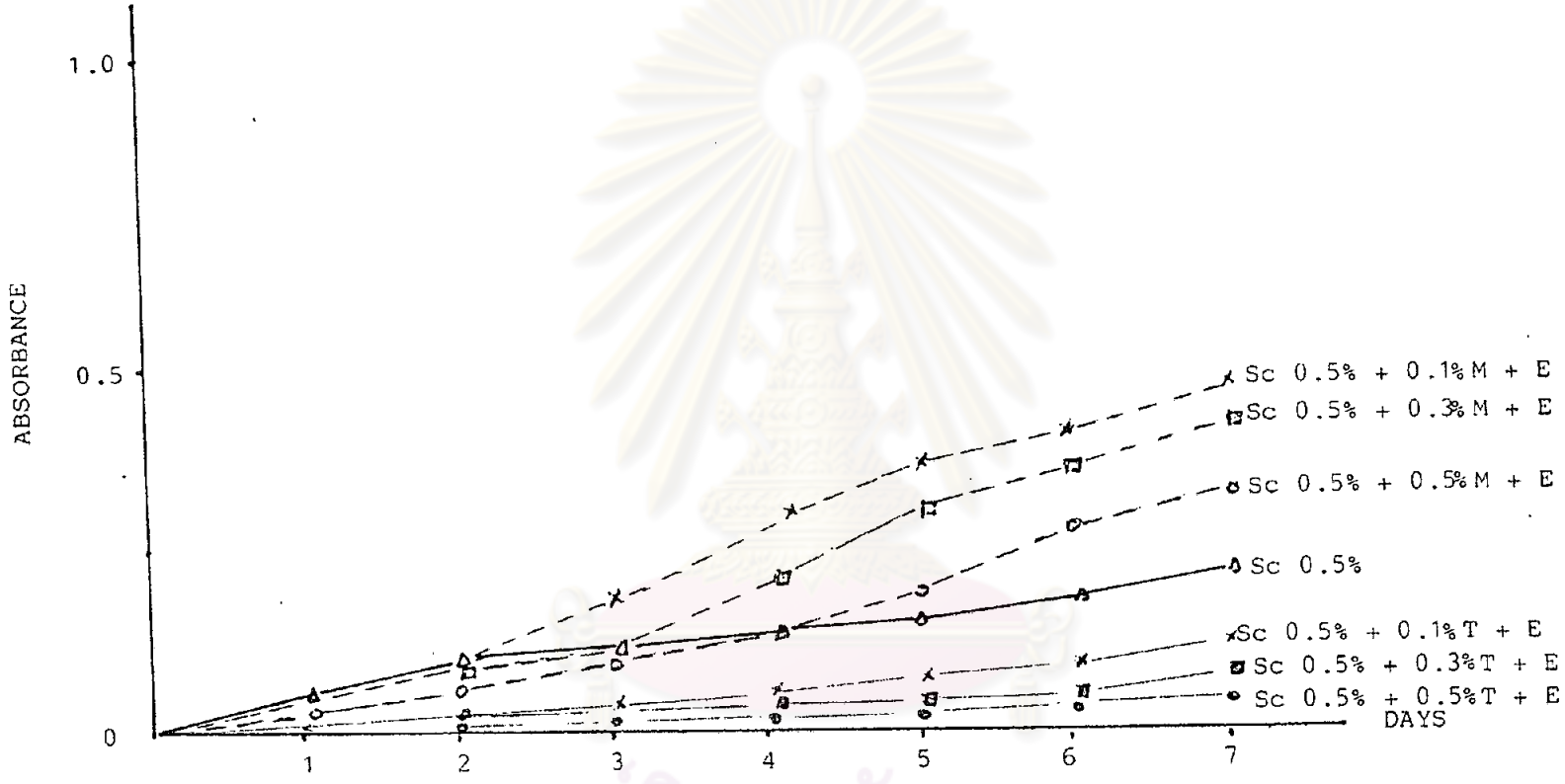

Fig. 11 Absorbance at 450 nm plotted at different times after exposure to artificial daylight light of 0.5% sulfacetamide sodium solutions (Sc), with various concentrations of antioxidants, sodium metabisulfite (M), sodium thiosulfate (T) and 0.05% disodium EDTA (E).

same duration. The same pattern is observed either with or without added disodium EDTA.

Disodium EDTA clearly shows the increase in retardation effect on the color formation when it is used in combination with antioxidants. The retardation effect on the color formation of sulfacetamide sodium solution by antioxidants also varies to the amount of antioxidants used.

Figure 12 shows that 0.5% sulfacetamide sodium with 0.1% sodium thiosulfate or with 0.1% sodium metabisulfite, with or without 0.05% disodium EDTA buffered at pH 7.4 behave in the same pattern as unbuffered solution (fig. 10 and 11), but the decomposition under light stress is faster when phosphate buffer is used.

Figure 13 indicates that the absorbances of 10% sulfacetamide sodium solutions buffered at pH 7.4 with various concentration of antioxidants change in the same pattern as 0.5% sulfacetamide sodium solutions. The addition of chelating agent, disodium EDTA, shows no effect on the color formation at all.

From Figure 11, 12, 13 it can be summarized that sodium thiosulfate can retard the color formation of sulfacetamide sodium solution much better than sodium metabisulphite. The effect is increasing when larger amount of antioxidants is used. The chelating agent(disodium EDTA) has a synergist effect with antioxidants.

3. The study to find the best formulation of 10% sulfacetamide sodium solution.

Sulfacetamide sodium solutions (10%) were prepared by using antioxidants (sodium metabisulfite and sodium thiosulfate) at concentrations of 0.1, 0.2, 0.3%.

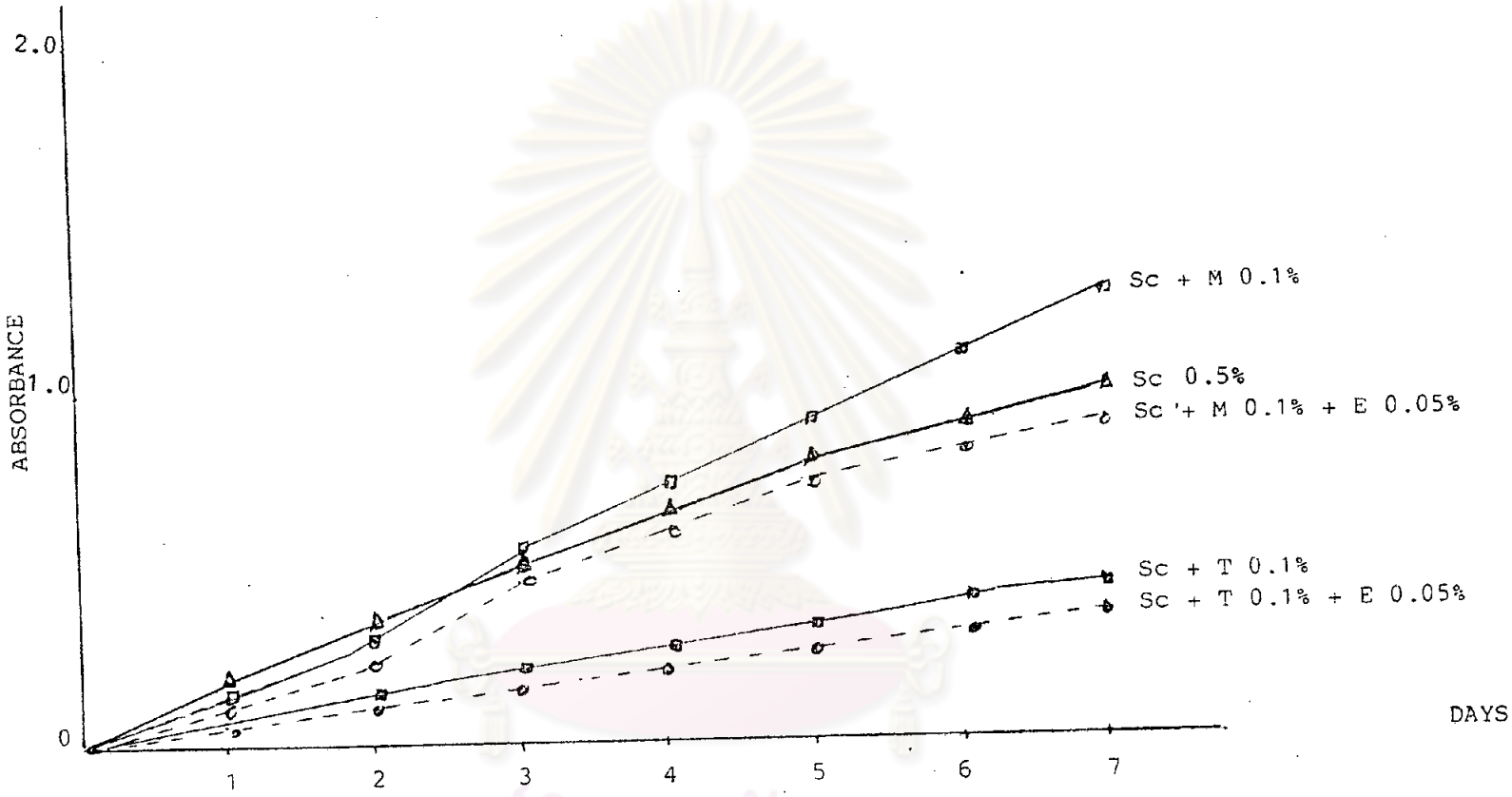

Fig. 12 Absorbance at 450 nm plotted at different times after exposure to artificial daylight light of 0.5% sulfacetamide sodium (Sc), with 0.1% antioxidants, sodium metabisulfite (M) and sodium thiosulfate (T) with and without 0.05% disodium EDTA in buffer at pH 7.4.

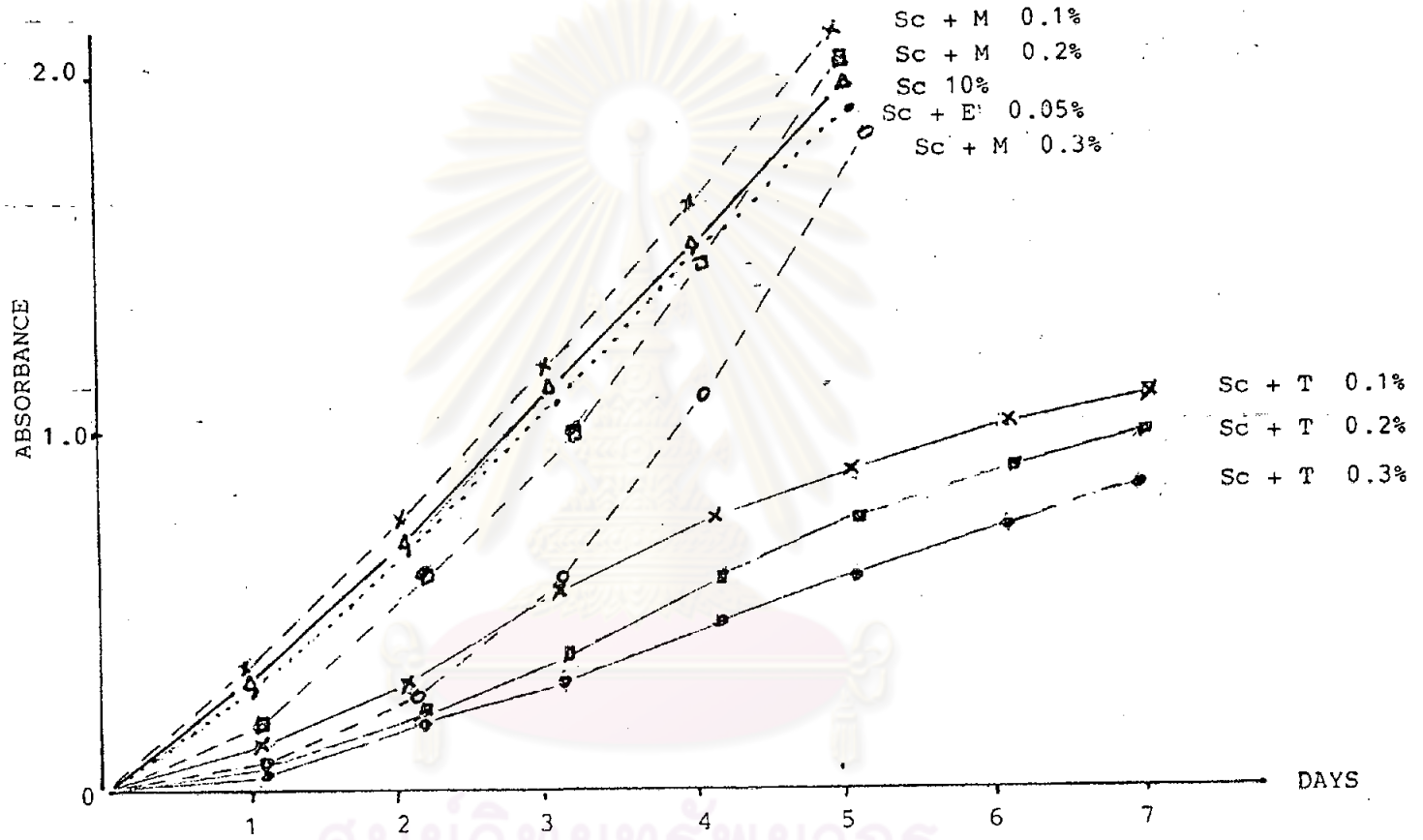

Fig. 13 Absorbance at 450 nm plotted at different time expose to artificial daylight light of 10% sulfacetamide sodium solutions (Sc) with various concentrations antioxidants, sodium metabisulfite (M), sodium thiosulfate (T) and with chelating agent 0.05% disodium EDTA (E) in buffer at pH 7.4.

The absorbances of the solutions measured at 450 nm are plotted with time as shown in fig. 14. It shows that sodium metabisulfite must be over 0.2% in order to be able to retard the color formation of sulfacetamide sodium solution.

While sodium thiosulfate has very good retardation effect on color formation at all concentrations used.

Disodium EDTA alone shows no effect on the color formation of sulfacetamide sodium solution.

Figure 15 shows the effect of using disodium EDTA in combination with antioxidants. It indicates that disodium EDTA can increase the retardation effect on color formation of sulfacetamide sodium solution futher. The higher the amount used the less color formation occurs.

Sulfacetamide sodium solutions(10%) with antioxidants sodium metabisulfite and sodium thiosulfate at concentrations of 0.1, 0.2 and 0.3%, in combination with disodium EDTA at concentrations of 0, 0.01, 0.05 and 0.10% were exposed to artificial daylight light for 7 days. The color formed after light exposure and pH of solutions before and after light exposure is shown in table 6.

Figure 16 shows the effect of using disodium EDTA at different concentrations in combination with 0.1% antioxidants, sodium metabisulfite and sodium thiosulfate. It shows that disodium EDTA can lower the color formation rate of sulfacetamide sodium solution.

Table 7 comparing the color formation and absorbance at 450 nm of two formula of 10% sulfacetamide sodium solutions with 0.1% antioxidants in combination with 0.05% disodium EDTA filled in clear ampoules and amber

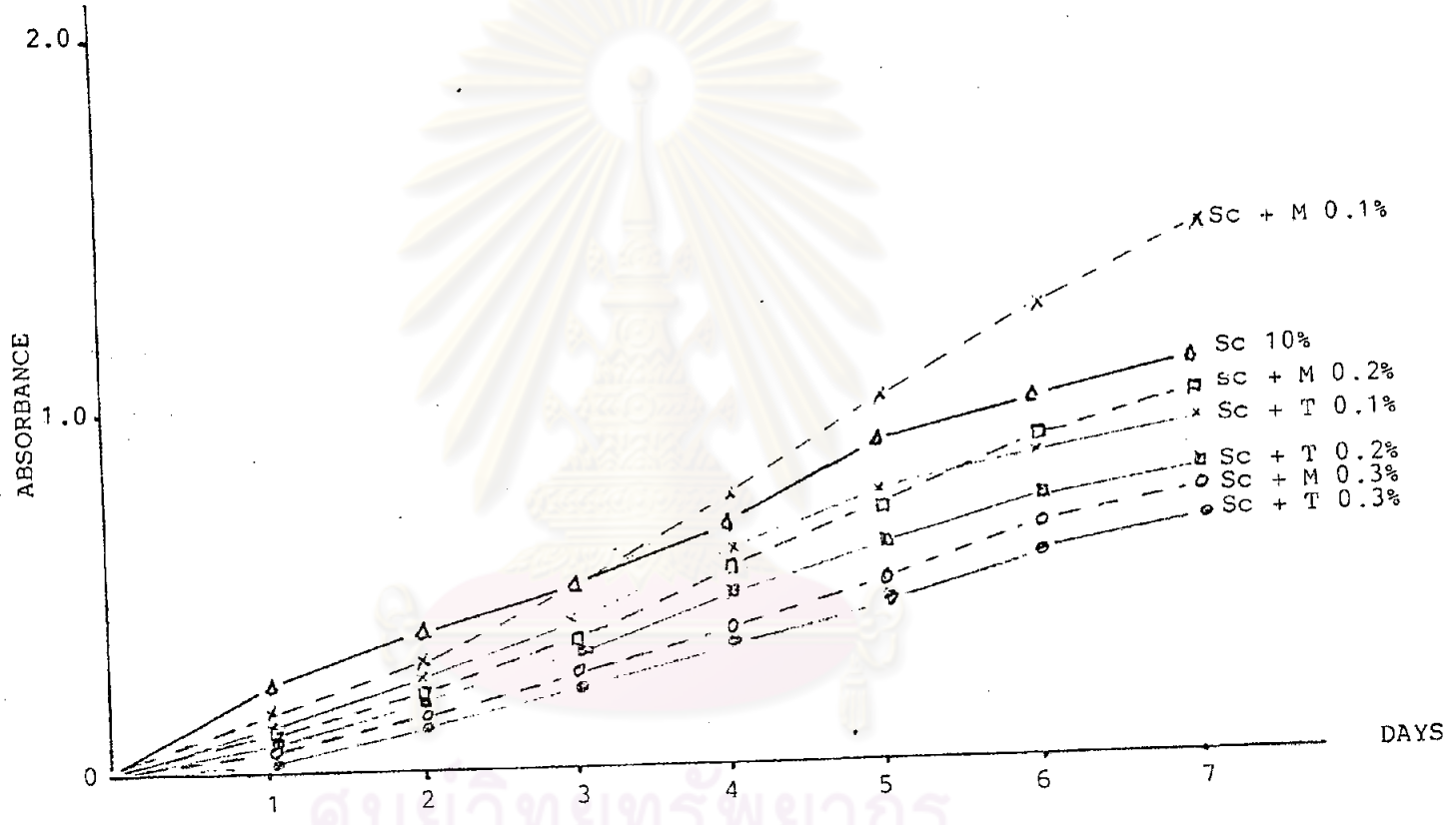

Fig. 14 Absorbance at 450 nm plotted at different times after exposure to artificial daylight light of 10% sulfacetamide sodium solutions with various concentrations of antioxidants, sodium metabisulfite (M) and sodium thiosulfate (T), without buffer.

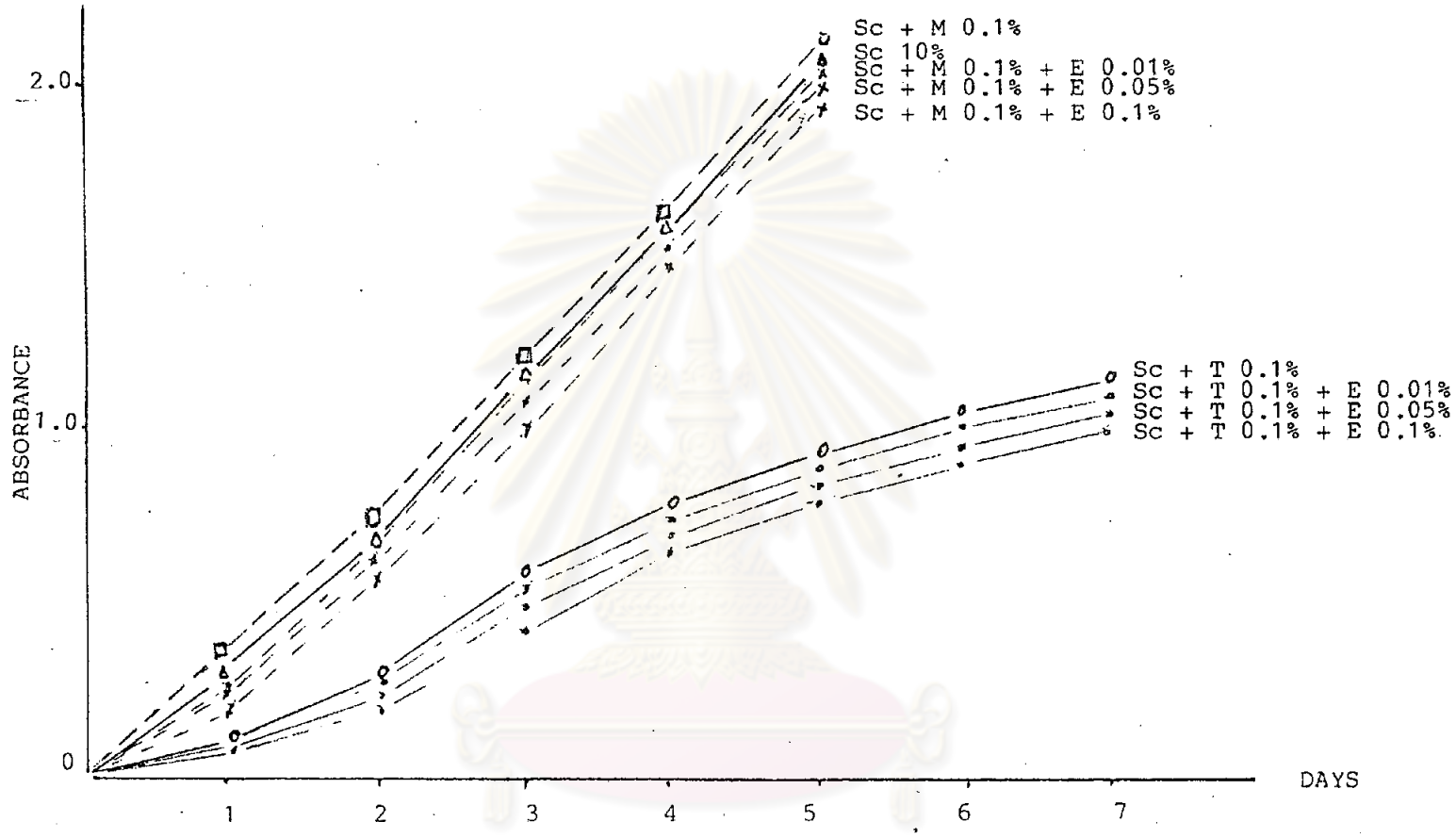

Fig. 15 Absorbance at 450 nm plotted at different times after exposure to artificial daylights light of 10% sulfacetamide sodium solutions with 0.1% antioxidants, sodium metabisulfite (M) and sodium thiosulfate (T) and various concentrations of disodium EDTA (E), 0.01, 0.05 and 0.10% in buffer at pH 7.4.

Table 6 The color compared with standard color after 7 days exposed to artificial daylight light and pH initial and after 7 days of sulfacetamide solution 10% solution.

	% Sodium Metabisulfite				% Sodium Thiosulfate		
% Di sod. EDTA	.3	.2	.1	-	.3	.2	.1
0.1	F 7.1 - 7.0	G^- 7.3 - 7.25	I 7.5 - 7.5	H 7.8 - 7.6	D 7.7 - 7.7	E 7.7 - 7.65	F^- 7.7 - 7.7
0.05	F 7.2 - 7.1	H 7.35 - 7.25	I 7.65 - 7.6	H 7.9 - 7.8	D 8.15 - 8.0	E 7.95 - 7.90	F 8.0 - 7.9
0.01	F^+ 7.2 - 7.2	H 7.5 - 7.35	I^- 7.95 - 7.7	G^- 8.5 - 8.2	D^+ 8.8 - 8.0	E^+ 9.0 - 8.0	F 8.5 - 7.9
-	F 7.25 - 7.2	H 7.5 - 7.4	G 8.1 - 7.8	F 9.1 - 8.4	D 9.7 - 8.45	E 9.5 - 8.0	F^- 9.2 - 7.85

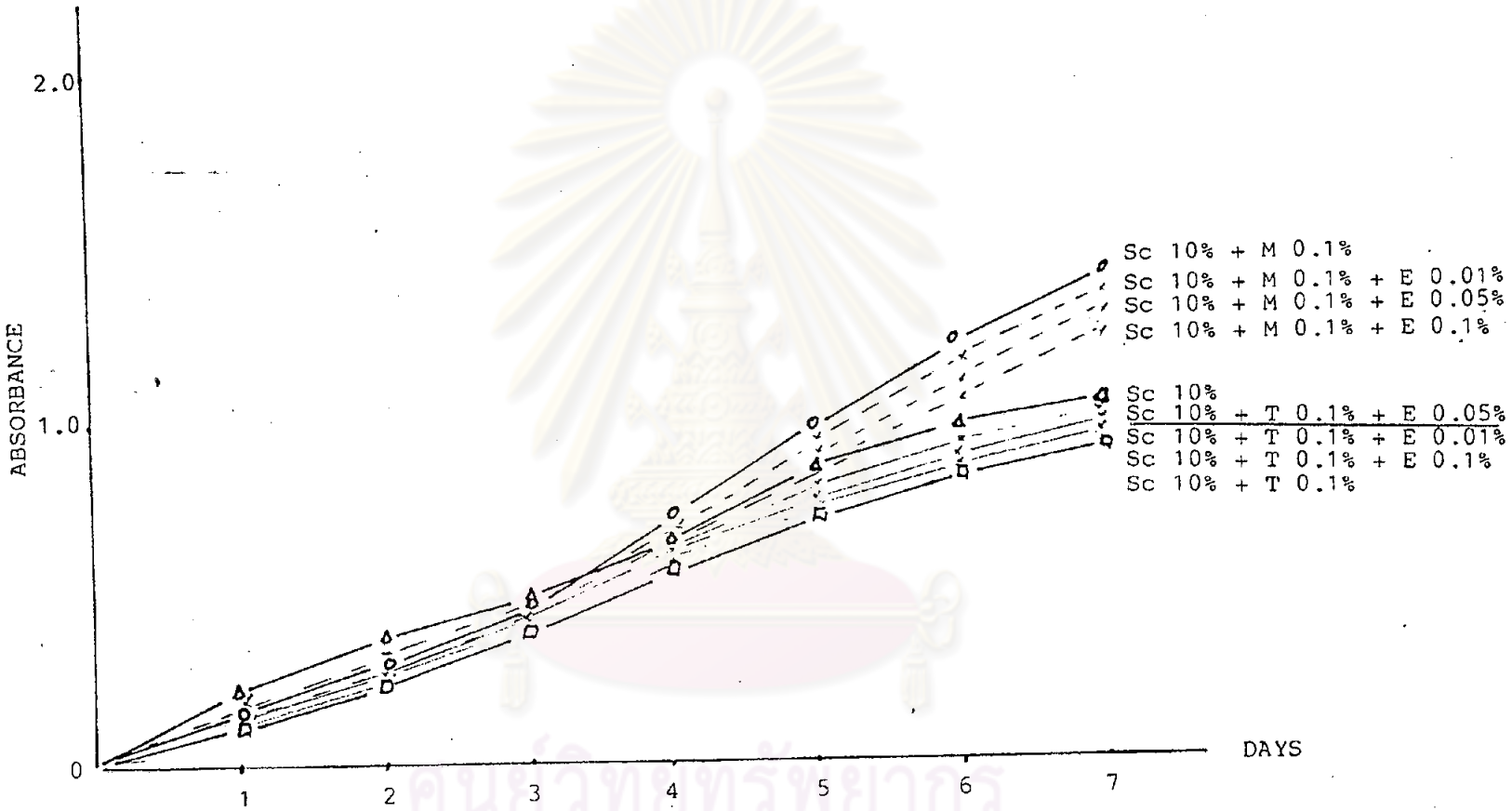

Fig. 16. Absorbance at 450 nm plotted at different times after exposure to artificial daylight light of 10% sulfacetamide sodium solutions with 0.10% antioxidants, sodium metabisulfite (M) and sodium thiosulfate (T) and various concentrations of disodium EDTA.

Table 7 Comparing the color formation and absorbance at 450 nm of two sulfacetamide sodium (Sc) solution 10% filled in clear ampoules and amber ampoules after expose to the artificial daylight for 6 days.

Solution SM: Sc + Sodium Metabisulphite 0.1%
+ Di sodium EDTA 0.05%

ST: Sc + Sodium Thiosulphite 0.1%
+ Di sodium EDTA 0.05%

Sol.	days / ampoule	1	2	3	4	5	6
SM	clear	B .25	C .57	E .90	E^{+} 1.05	F 1.18	G 1.30
	amber	A .01	A .03	B .34	C .55	D .70	E .95
ST	clear	B^{-} .16	B^{+} .35	C .52	D^{-} .65	D^{+} .82	E .91
	amber	A .00	A .01	A .03	A .05	A .06	A^{-} .07

ampoules after expose to the artificial daylight light for 6 days. It shows that amber glass can prevent the light exposure of the solution and so the color formation can be reduced very clearly especially when using sodium thiosulfate as an antioxidant.