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The effect of antioxidants on the hydrolytic and oxidative degradation of sulphacetamide in aqueous solutions

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The effect of heat and light stresses on the degradation of sulphacetamide solutions at pH 7, in the presence and absence of the antioxidants sodium metabisulphite and sodium edetate, have been investigated. Sodium metabisulphite accelerates the hydrolytic degradation of sulphacetamide to sulphanilamide, whereas sodium edetate does not change the rate. The absorption peak causing colour development due to heat stress is shown to differ from that due to light stress. The development due to both stimuli has been measured quantitatively with time. Sulphanilamide present in heat-degraded solutions is shown to be insignificant in the development of colour due to heat stress, but to have a major role in colour formation under light stress.

The rate of oxidation of solutions of pharmaceuticals is generally dependent on the presence of oxygen, and the process is accelerated by heat, light and the presence of some metal ions. Stabilization relies either on the complexing of catalytic metal ions by chelating agents such as ethylenediamine tetra acetic acid (EDTA) or sodium edetate, or on the inclusion of a substance more readily oxidized than the drug, e.g. sodium metabisulphite.

Where drugs are also subject to degradation by other routes such as hydrolysis, the addition of antioxidants may produce adverse effects (Kokoski, 1958, Higuchi & Schroeter, 1959).

An example of a drug which degrades in aqueous solution by hydrolysis and oxidation is sulphacetamide sodium. Eye drops of this drug (B.P.C.) contain sodium metabisulphite which has been reported to decrease the rate of development of a yellow colour which normally forms on storage (Whittet, 1949, Anderson & Maudson, 1963 a, b), although the latter workers found a concommitant increase in rate of disappearance of sulphacetamide.

This work was initiated to investigate quantitatively the effect of sodium edetate and sodium metabisulphite on the degradation of sulphacetamide sodium solutions.

EXPERIMENTAL

Materials. Sulphacetamide sodium B.P. (mp. 183°) was recrystallized twice from ethanol, sulphanilamide B.P.C. (mp. 163·5°) was recrystallized twice from water. Sodium metabisulphite, B.P. (B.D.H.) and sodium edetate (May and Baker) were used without further purification. Oxygen and oxygen free nitrogen (white spot grade) were obtained from British Oxygen Company. All other chemicals and reagent solutions were as described elsewhere (Tansey, 1969).

Buffer solutions. In all experiments solutions were adjusted to pH 7 ± 0·05 with McIlvaine's citric acid, disodium hydrogen phosphate buffer.

pH Measurement. A Radiometer type 27 meter fitted with a Radiometer type C glass-calomel electrode system, standardized with 0·25M potassium dihydrogen phosphate and 0·25M disodium hydrogen phosphate (Bates, 1957), was used.

Oxygen tension measurement. Oxygen tensions were measured using a Radiometer type PHA 927b gas monitor fitted with a Clark type oxygen electrode, standardized with 0·1M borax containing 1% w/v sodium metabisulphite.

Heating procedure. Solutions, buffered at pH 7·0, were equilibrated with oxygen or nitrogen as required and sealed under the appropriate gaseous atmosphere, by fusion in glass ampoules (Tansey 1969). These were maintained at the required temperature ±0·5° for different graded periods of time. On removal the ampoules were chilled in crushed ice and stored at 2° until required.

Exposure to light. Solutions of sulphonamide were aspirated with oxygen to a maximum oxygen tension and transferred to stoppered 1 cm quartz cuvettes and the air space above the solutions filled with oxygen. Five of these cells were placed between two fluorescent tubes (Atlas 15W Northlight) at a distance of 5 cm from each tube to the optical surface of the cell. No precautions were taken to control the temperature in the cells as this remained constant at 27° ± 1·5°. The dose rate was determined using a thermopile (Hilger Watts No. FT.17.1) in combination with a millivoltammeter (Keithley Inst. 150B) which had been calibrated by reference to a potassium ferrioxalate actinometer (Hatchard & Parker, 1956). The dose rate was $2{\cdot}69 \times 10^{-5}$J mm^{-2} s^{-1} (coefficient of variation of ± 1·3%). In one series of experiments the Northlight tubes were replaced by Phillips T.U. 15W low pressure tubes, emitting chiefly at 254 nm.

Assay procedures

Assay for sodium metabisulphite. Sulphacetamide was separated from its degradation products by a thin-layer chromatographic technique using silica gel G as adsorbent and a running solvent consisting of acetone–methanol–diethylamine (9: 1: 1). The areas of silica gel containing the sulphacetamide sodium were removed and extracted with methanolic hydrochloric acid, and the sulphacetamide assayed colorimetrically at 536 nm following a modified Bratton-Marshall reaction (Tansey, 1969). Experiments with formulated mixtures of sulphacetamide and sulphanilamide in different ratios showed that adequate separation was obtained and that all the sulphacetamide could be recovered with a coefficient of variation on ten replicate samples of 1·4%.)

Assay for sodium metabisulphite. As sodium metabisulphite is readily oxidized in aqueous solution all batches prepared were assayed. When an ampoule was opened, an aliquot (1 ml) was immediately transferred to iodine solution (25 ml) contained in a stoppered conical flask and assayed in the standard manner (coefficient of variation < 2%).

Determination of coloured degradation products. An absorption spectrum of sulphonamide solutions, exposed to heat, light, or both sequentially, was obtained in 1 cm quartz cuvettes using a Perkin-Elmer recording ultraviolet and visible spectrophotometer. Quantitative values of absorbance at specific wavelengths were subsequently measured using a Unicam SP500 ultraviolet and visible spectrophotometer.

Loss of sulphacetamide in solution on heating

Treatment of results. In the presence or absence of antioxidants, the thermal degradation of 1% solutions of sulphacetamide sodium followed apparent first order kinetics. Heated solutions were assayed for both sulphacetamide and sulphanilamide under all conditions of test, and in each case the results suggested that sulphacetamide loss can be accounted for by formation of sulphanilamide. The percentage residual concentration of sulphacetamide was calculated, with respect to an unheated sample, for each period of heating. Values for the apparent first order rate constant (k) were obtained from log % concentration—time data by means of a least squares regression analysis, which gave values of k, its associated standard error and the correlation coefficient.

The effect of 1% *sodium metabisulphite.* Two anoxic solutions of sulphacetamide sodium were prepared, 1% sodium metabisulphite was added to one of them and both were sealed under nitrogen into ampoules (Tansey 1969). Both were heated at 120° for varying times, removed and assayed. The appropriate log % residual concentration-time plots are shown in Fig. 1, the calculated values for the rate constants being $10{\cdot}05 \times 10^{-2}$ and $2{\cdot}79 \times 10^{-2}\ h^{-1}$ for solutions in the presence and absence respectively of sodium metabisulphite.

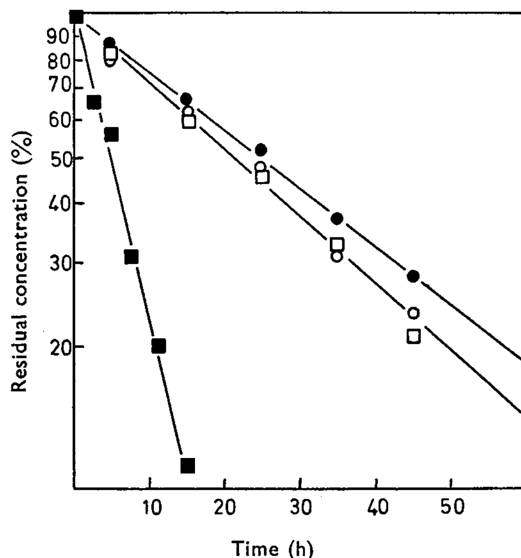

Fig. 1. Plots of percentage residual concentration against time of heating at 120° (h) for 1% solutions of sulphacetamide sodium at pH 7·0. ● Heated anoxically. ■ Heated anoxically in the presence of 1% sodium metabisulphite. ○ Heated after saturation with oxygen. □ Heated after saturation with oxygen in the presence of 1% sodium metabisulphite.

The effect of oxidized sodium metabisulphite was determined by heating solutions that had been equilibrated with and sealed under oxygen. The residual concentration of unoxidized sodium metabisulphite was less than 0·02% w/v. These results are also shown in Fig. 1, the calculated values for the rate constant are $3{\cdot}46 \times 10^{-2}$ and $3{\cdot}23 \times 10^{-2}\ h^{-1}$ for solutions in the presence and absence of oxidized sodium metabisulphite respectively.

It is evident that sodium metabisulphite has an accelerating effect on the rate of degradation of sulphacetamide in the absence of oxygen. The rate increase, due to a nominal 1% concentration (0·65% by assay) can be represented by $k_1/k_2 = 10{\cdot}05/2{\cdot}79$ a factor of 3·6. This effect is dependent on the retention of the integrity of the antioxidant molecule as, after oxidation by atmospheric oxygen, the rate of degradation is not significantly different from the rate in the absence of antioxidant (t calc. = 1·41, at $P = 0{\cdot}05$, t tabulated = 2·31). The presence of oxygen increases the rate of degradation of sulphacetamide, this effect is independent of the presence of oxidized sodium metabisulphite, the values of t being 4·12 and 3·16 respectively, for heating in the presence and absence of antioxidant (t tabulated at $P = 0{\cdot}05$ is 2·31). This increase, though significant, is small and may be due to oxidative degradation which does not occur in deoxygenated solutions.

The effect of different concentrations of sodium metabisulphite. Oxygen free solutions of sulphacetamide sodium were prepared containing different concentrations of sodium metabisulphite, the effective concentrations being determined by assay. Over the range examined an increase in the concentration of antioxidant increased the rate of degradation of sulphacetamide sodium. A plot of log k against concentration was linear with a correlation coefficient of 0·9983.

A direct relation between the rate constant and concentration of sodium metabisulphite would indicate that the accelerating effect is due to specific ion catalysis and that this was not observed may indicate a more complex accelerating mechanism. Although it has been reported that metabisulphite accelerates ephedrine degradation by chemical interaction (Schroeter, Higuchi & Schuler, 1958), this was not the mechanism responsible in the present case since no fall in the sodium metabisulphite concentration was observed during the course of the reaction (Schroeter, 1961).

The effect of sodium metabisulphite at different temperatures. Samples of sulphacetamide were heated in the presence of a nominal 0·5% w/v of sodium metabisulphite (0·46% by assay). On different occasions samples were heated at 100, 110 120 and 140° and the results used to obtain rate constants (k). These were then fitted by a least squares analysis according to the Arrhenius equation: $k = Ae^{-Ea/RT}$ to obtain values for the activation energy (Ea) and frequency factor (A) of 19·54 k cal mol^{-1} ($8{\cdot}181 \times 10^4$J mol^{-1}) and $6{\cdot}029 \times 10^9$ h^{-1} respectively. These compare with values of 22·90 k cal mol^{-1} ($9{\cdot}588 \times 10^4$J mol^{-1}) and $1{\cdot}76 \times 10^{11}$ h^{-1} obtained for sulphacetamide in McIlvaine buffer at pH 7·4 in the absence of sodium metabisulphite (unpublished observations). These also show that the rate of degradation of sulphacetamide is independent of pH over the range of 5 to 9, and so the two sets of results may be compared directly. These findings are not inconsistent with the action of metabisulphite being that of a specific ion catalyst, although as is clear from the concentration data the mechanisms involved do not appear to be open to a simple description.

The accelerating effect of sodium metabisulphite on hydrolysis rates has been reported by Fletcher & Norton (1963) and Anderson & Maudson (1963a) who observed that crystals of sulphanilamide were deposited, after exposure to high temperatures, from concentrated solutions of the drug that contained antioxidant. An unsuccessful attempt was made by Clarke (1967a) to relate the effect quantitatively to the concentration of sodium metabisulphite. Forse (1967) suggests that the effect of metabisulphite is temperature dependent, the hydrolysis being accelerated

at 116° but not at lower temperatures. In all those studies sulphanilamide had to be formed in sufficient concentration to precipitate at room temperature before an effect could be noted and this is probably the reason for the lack of correlation and difficulty of interpretation of the published work.

This report shows that the action of metabisulphite at all concentrations and temperatures studied is an accelerating one, and that this effect can be described by simple kinetic expressions. The results obtained should enable accurate predictions to be made of the stability of solutions of the drug in the presence or absence of antioxidant under normal storage conditions.

Effect of sodium edetate. Solutions of sulphacetamide sodium and 0·5% sodium edetate were heated, on separate occasions, in oxygen-free and in oxygenated solutions; the values of the first order rate constants obtained were $2{\cdot}98 \times 10^{-2}$ and $2{\cdot}99 \times 10^{-2}\,h^{-1}$ respectively. These compare with a value of $2{\cdot}79 \times 10^{-2}$ and $3{\cdot}23 \times 10^{-2}\,h^{-1}$ obtained for degradation in the absence of edetate in oxygen-free and oxygenated solutions. The value of the rate constants obtained with edetate is independent of the presence of oxygen ($t = 0{\cdot}06$, t at $P = 0{\cdot}05 = 2{\cdot}31$) and the value for oxygen-free solutions is indistinguishable from the anoxic value of $2{\cdot}79 \times 10^{-2}\,h^{-1}$ obtained in the absence of edetate ($t = 1{\cdot}70$, t tabulated $= 2{\cdot}31$). The presence of edetate however does serve to protect sulphacetamide sodium against the accelerating effect of oxygen the value of k being reduced from 3·23 to $2{\cdot}99 \times 10^{-2}\,h^{-1}$, and the value of t being 2·40 compared to the tabulated value of 2·31.

Thus it appears that the oxygen acceleration is dependent on the presence of metal ions which the sodium edetate neutralizes, probably by chelation. As its protective action will not be abolished by oxidation, and as it has no significant accelerating effect on the rate of degradation, it would appear that solutions of sulphacetamide sodium would be better preserved by sodium edetate than by sodium metabisulphite.

Colour development in sulphacetamide solutions

The development of colour in sulphacetamide solutions when exposed to a variety of stresses has been assumed to be due to oxidative breakdown. Various workers have attempted to assess the role of oxidation by absorbance measurements (Anderson & Maudson, 1963a; Clark, 1967b; Mital & Gupta, 1968; Pandula, Racz & Pajor, 1969). With the exception of Pandula & others, who used 336 nm corresponding to the wavelength of maximum absorption of their reported oxidation breakdown products, viz. azobenzene 4,4′-disulphonamide and azoxybenzene 4,4′-disulphonamide at alkaline pH, no rationale is given for the choice of the specific wavelengths used which include 370, 420 and 520 nm.

Initial experiments were made to qualitatively assess the effect of heat (120°), ultraviolet, natural, and artificial daylight on the degradation of sulphacetamide. All solutions were equilibrated with oxygen, and after degradation the absorption spectra of the solutions were obtained. These are shown in Figs 2 and 4 and indicate that the main breakdown products are different for each stress. With the ultraviolet source, colour development (brownish yellow) is quickest and this is accompanied by a general shift in the absorption curve to the visible region, with non-specific absorption extending beyond 500 nm (curve a, Fig. 4). The effect of the daylight tubes is quite different (curve c, Fig. 4), since a clearly defined maximum occurs at 450 nm in addition to a general increased absorption below 400 nm. For compara-

tive purposes samples were also stored in natural daylight (curve b, Fig. 4) which gave a similar result to the artificial daylight tubes. Thus in all subsequent experiments the light source was the Northlight tubes as these produce conditions closest to natural storage. Under the influence of heat alone a pale yellow colour was produced. This is due to an absorption peak at 365 nm (curve a, Fig. 2), there being no peak at 450 nm. as was produced by visible light. However there appears to be a shoulder in the region of 336 nm which is interesting in view of the work of Pandula & others (1969). Thus it is apparent that the coloured breakdown products produced under the influence of heat and light are different.

On the basis of these results the wavelengths chosen for quantitative measurements were 336, 365 and 450 nm.

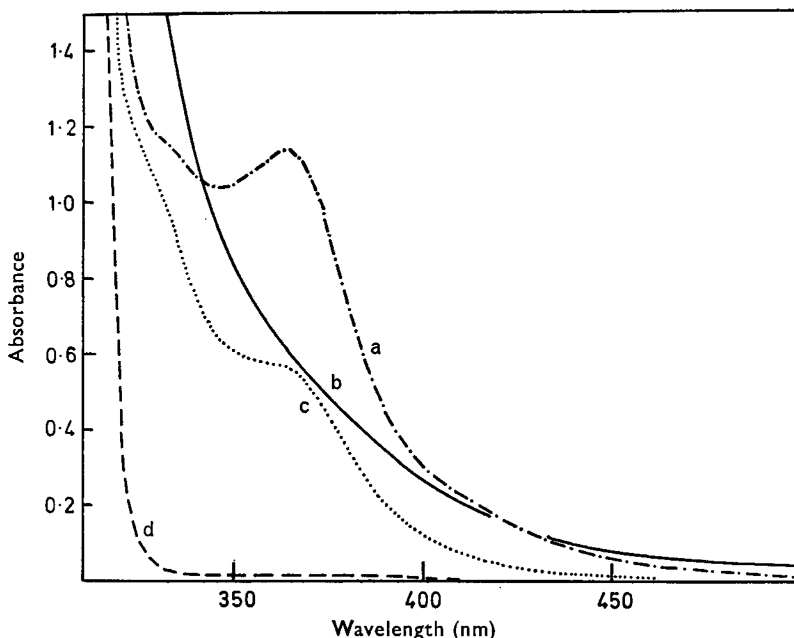

FIG. 2. Visible absorption spectra for sulphacetamide sodium and sulphanilamide solutions at pH 7·0 after heating at 120° for 36 h. (a) 0·5% sulphacetamide sodium, saturated with oxygen. (b) 0·5% sulphacetamide sodium, saturated with oxygen and with the addition of 1% sodium edetate. (c) 0·5% sulphacetamide sodium saturated with oxygen and the addition of 1% sodium metabisulphite. (d) Common spectrum for (i) 0·5% sulphacetamide sodium heated anoxically; (ii) 0·338% sulphanilamide heated either anoxically or after saturation with oxygen.

Colour development due to heat at 120°. Sulphacetamide solutions show no colour development when heated in the absence of oxygen, the absorption spectrum remaining close to curve d, Fig. 2. When heated under oxygen, however, sulphacetamide solutions soon develop a pale yellow colour and give the absorption spectrum shown in curve a, Fig. 2. The changes in absorbance with time at 120° for the three wavelengths chosen are given in Fig. 3, the increase at 450 nm is non-specific and is not due to the development of an absorbance peak. Heated solutions containing an equivalent amount of sulphanilamide, show no colour development upon heating in either oxygen or nitrogen, and no peaks in the absorption spectrum (curve d, Fig. 2), merely a slight general shift towards the visible. This indicates that the sulphanilamide, present as a result of hydrolytic degradation, plays no part in the formation of

colour in heated sulphacetamide solutions that have not been exposed to light. Indeed, the increasing amount of sulphanilamide being formed may be the reason for the apparent decreasing rate of absorbance increase at 336 and 365 nm (Fig. 3).

The effect of sodium metabisulphite and sodium edetate on colour development is also shown in Fig. 2, curves c and b respectively. Sodium metabisulphite reduces the magnitude of the absorption peaks without altering the general shape of the absorption spectrum. The action of the antioxidant in this instance is probably an indirect

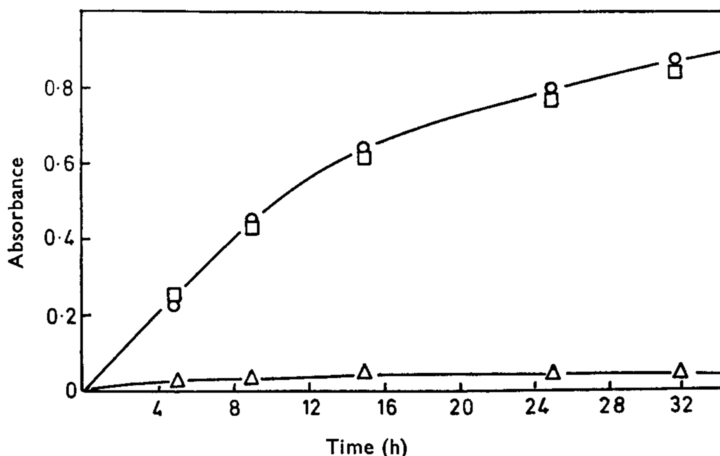

FIG. 3. Plots of absorbance against heating time at 120° (h) for 1% sulphacetamide sodium solutions at pH 7·0 measured at 336 nm (○), 365 nm (□), 450 nm (△).

one, as it acts by reducing the concentration of oxygen in the system. Sodium edetate exerts a profound effect on the general shape of the absorption curves obtained under these conditions. The removal of the 365 nm peak, and the marked reduction in general absorbance at 336 and 450 nm suggests that reactions catalysed by metal ions are largely responsible for the oxidative degradation of sulphacetamide. With the fact that edetate does not accelerate the degradation rate of sulphacetamide this would suggest that sodium edetate and not sodium metabisulphite should be added to solutions of sulphacetamide that are heat sterilized.

Colour development due to artificial daylight. After exposure to artificial daylight, unheated solutions of both sulphacetamide and sulphanilamide gave similar absorption spectra with a definite peak at 450 nm (curve c, Fig. 4). The peak height was achieved with sulphanilamide in 17 h compared with 30 h for sulphacetamide (Fig. 6).

The better to simulate normal storage conditions, 0·5% solutions of sulphacetamide were heated in the absence of oxygen at 120° for different periods before exposure to light under oxygen. These heated solutions remained colourless even on prolonged storage in natural daylight until opened, when a brown colour rapidly developed. The rate of absorbance increase at 450 nm of these heated solutions is shown in Fig. 5. The rate of colour development is dependent on time of heating up to 15 h, which is the time necessary to form 33% sulphanilamide, calculated as total sulphonamide. Longer periods of heating result in colour development data indistinguishable from sulphanilamide (0·338% w/v, equivalent to 0·5% sulphacetamide). Figs 5 and 6 show that a relation between rate of colour development and sulphanilamide concentration exists, although this is clearer in formulated

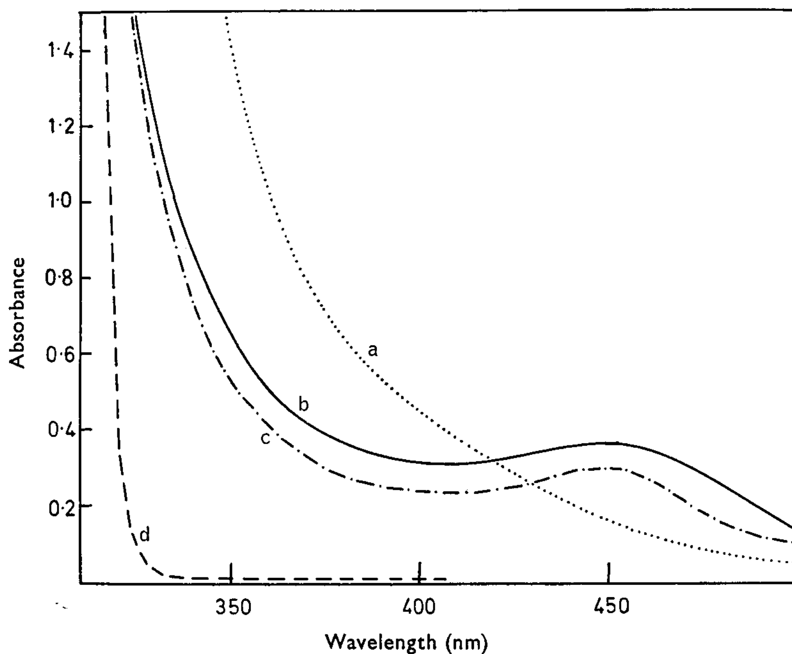

FIG. 4. Visible absorption spectra for 0·5% sulphacetamide sodium solutions at pH 7·0 after saturation with oxygen and exposure to light (unheated). (a) Ultraviolet light (14 h). (b) Diffuse natural daylight (40 days). *(c) Artificial daylight (30 h). (d) No light exposure.

* Curve "c" is also the spectrum of 0·338% sulphanilamide under the same conditions after 17 h.

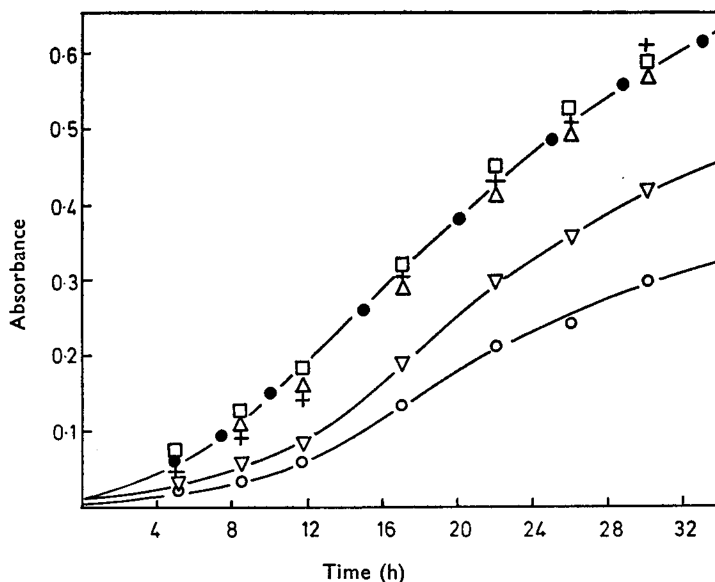

FIG. 5. Plot of absorbance, measured at 450 nm against time of exposure to artificial daylight (h) for 0·338% solutions of sulphanilamide and 0·5% sulphacetamide sodium at pH 7·0 heated anoxically at 120° for varying periods, and then saturated with oxygen. Sulphacetamide: Time of heating (h); zero (○), 5 (▽), 15 (+), 25 (□), 35 (△). Sulphanilamide: Times of heating as above, (●) represents mean values.

mixtures (Fig. 6) than in heated solutions (Fig. 5). The lag periods as illustrated in Figs 5 and 6 vary widely and such batch to batch variation has been noticed on a number of occasions; the explanation is as yet unknown. Clarke (1967b) measuring colour development at 420 nm ascribed the effect to a variation in metal ion content although this has been disputed by Forse (1967) with sulphanilamide.

Two general conclusions can be drawn; first, that colour development in sulphacetamide solutions proceeds by two distinct pathways dependent upon the degradation stimulus—heat giving a clear lemon colour, and an absorption peak at 365 nm, light in presence of oxygen giving a brown colour with an absorption peak at 450 nm.

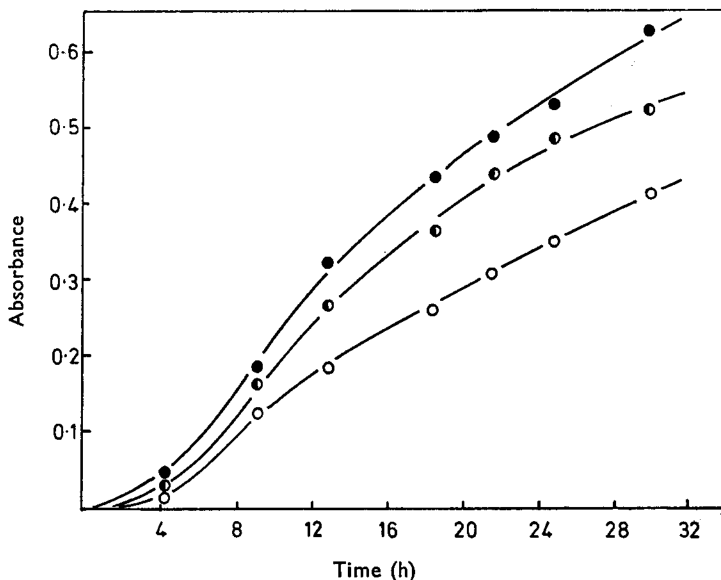

FIG. 6. Plot of absorbance, measured at 450 nm against time of exposure to artificial daylight (h) for unheated solutions of sulphacetamide and sulphanilamide at pH 7·0. ○ 0·5% sulphacetamide sodium. ● 0·33% sulphanilamide. ◐ 0·25% sulphacetamide sodium, 0·169% sulphanilamide.

Second, the sulphanilamide present as a result of hydrolysis in heated solutions plays no part in colour formation due to heat, but is significant in increasing the rate of colour development due to light.

As yet the oxidative breakdown products produced by heat and light have not been identified. We do not, however, believe that they are azobenzene 4,4′-disulphonamide (I) and azoxybenzene-4,4′-disulphonamide (II) which have been postulated both by Clarke (1965) and Pandula & others (1969) to be responsible, at least in part for the colour development. We prepared I (orange crystals) and II (yellow crystals) by the method of Seikel (1940) and authenticated their structure by nmr, infrared and mass spectroscopy. Ultraviolet spectra in aqueous 0·1N sodium hydroxide showed peaks at 232, 335, 437 nm for I and 220, 336 nm for II. However, after shaking I and II for several days in buffer solution at pH 7·0, at room temperature, followed by filtration through a millipore membrane, no detectable absorbance was recorded in either the visible or ultraviolet regions of the spectrum, indicating virtual insolubility in water at this pH. These compounds were found to be soluble only in strongly alkaline solution.

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