

## Research Papers

### Viscosity and stability relations of the system ascorbic acid: water: polysorbate 20

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The viscosity of dispersions of ascorbic acid in solutions of polysorbate 20 has been determined and found to be Newtonian at all concentrations studied. The course of the oxidation in polysorbate 20 appeared to be by the normal chain reaction, but the rate at which it occurred was modified by a number of factors. Incorporation within the micelle appeared to be responsible for an increase in oxidation rate, but the high viscosity of concentrated polysorbate 20 solutions, which would affect the diffusion of oxygen to the reaction site caused a much larger reduction in oxidation rate.

**M**ANY pharmaceuticals have been prepared in recent years in which the medicament has been solubilised by non-ionic surface-active agents. Aqueous preparations of oil-soluble vitamins have been particularly popular and increased stability to oxidation has been claimed.

Previously this department has reported the oxidation of model relatively water insoluble substances in surface-active agents (Nixon, 1958; Mitchell, 1960; Swarbrick, 1963). It is possible to include the water-soluble vitamin ascorbic acid in this type of preparation and we now describe its oxidative behaviour in the model system ascorbic acid: water: polysorbate 20.

### Experimental

*Ascorbic acid.* Assay (iodometric) 99%. M.p. 190-192°.  $[\alpha]_D^{20}$  2% in water + 22°. pH of 2% in water 2.5.

*Polysorbate 20* (Tween, Honeywill-Atlas Ltd). This material complied with the manufacturer's specification dated October, 1956.

*Copper sulphate.* Analar. Used at  $1 \times 10^{-4}M$   $CuSO_4 \cdot 5H_2O$  as a catalyst.

*Buffer solution,* pH 3.4.  $Na_2HPO_4$ /citric acid (McIlvaine, 1921).

*Determination of solubility.* The solubility of the ascorbic acid was determined by equilibration in glass-stoppered flasks immersed in a water-bath at  $25^\circ \pm 0.1^\circ$ . The end-point was taken as the average between an under- and over-saturated dispersion. Because of the viscosity of high concentrations of polysorbate 20 it was necessary to warm the flask to  $60^\circ$  to speed the equilibration period. This did not affect the quantity solubilised. In all instances the excess ascorbic acid separated out as crystalline material.

*Determination of viscosity.* This was measured using a Ferranti-Shirley cone and plate viscometer fitted with an automatic flow curve recorder. The viscosity was measured at  $25^\circ$  using either a 4 cm (angle

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20'. 26'') or 7 cm (angle 20'. 25'') cone. Flow curves were determined as the shear rate was continuously increased from zero up to 1800  $\text{sec}^{-1}$  and then decreased to zero again. The samples were also subjected to recycling.

*Measurement of oxygen uptake.* The oxidation of the systems was followed by means of a Warburg constant volume respirometer at 25° as previously described (Carless & Nixon, 1957). The oxygen uptake of polysorbate 20 in water was also measured and subtracted from the total uptake as a correction.

*Chromatography of oxidised ascorbic acid solutions.* The lower layer of a butanol:glacial acetic acid:water system (40:10:50) was used to develop the chromatogram. The Whatman No. 1 filter paper was equilibrated for 24 hr with the upper layer of the mixture before development. The spots were made visible with ammoniacal silver nitrate and the chromatograms were also examined under ultraviolet light.

## Results

The presence of polysorbate 20 did not cause any large increase in the solubility of ascorbic acid (line AB, Fig. 1) and at high surface-active agent concentrations the solubility fell until in the polysorbate 20 itself only 5% w/w of ascorbic acid was soluble. None of the dispersions

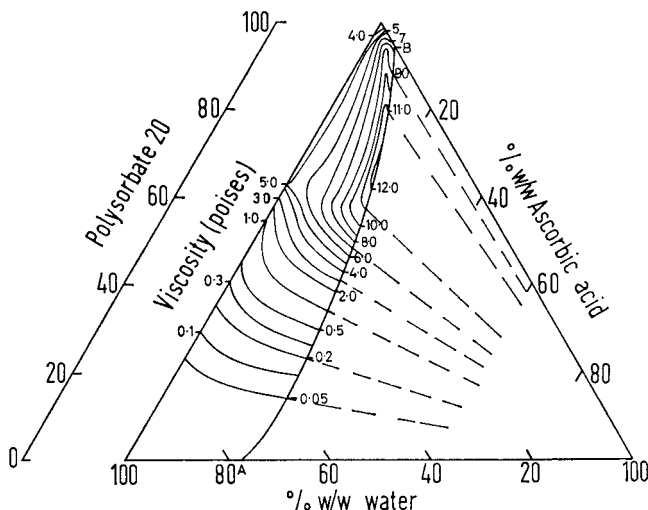


FIG. 1. Viscosity and solubility relationships in the system polysorbate 20/ascorbic acid/water. Line A-B is the solubility curve of ascorbic acid. ——— Viscosity contours of one phase system. - - - Viscosity contours of equilibrium liquid in contact with excess ascorbic acid.

showed birefringence when examined under polarised light, indicating the absence of liquid crystals.

The viscosity of all the dispersions was Newtonian and did not vary on recycling.

The rather complicated viscosity map, produced by connecting similar viscosities within both the solubilised and solubilised plus excess solid regions, is shown superimposed on the solubility curve in Fig. 1.

In the binary system polysorbate 20:water there was a very slow increase in viscosity to 30% w/w polysorbate 20 after which the increase was extremely rapid and reached a maximum of 5.2 poises at 62.8% w/w polysorbate 20. The viscosity then fell gradually and polysorbate 20 itself had a viscosity of 3.81 poises.

The initial addition of ascorbic acid to any polysorbate 20:water system caused an increase in viscosity. The subsequent behaviour on further addition of ascorbic acid depended on the starting concentration of polysorbate 20. At concentrations up to about 60% w/w polysorbate 20 a point was reached where further addition of ascorbic acid caused little or no change in the viscosity. This occurred after the addition of 10–12% w/w of ascorbic acid. From 60–90% w/w polysorbate 20 the viscosity of the dispersions continued to increase with addition of ascorbic acid until the solubility limit was reached. In the heterogeneous region of Fig. 1, the viscosity of solutions on the same tie line was, as expected, found to be constant. This proved a useful check on solubility data which would otherwise have been difficult to determine. For initial concentrations of polysorbate 20 in excess of 90% w/w a third behaviour pattern was observed. Here the initial increase of ascorbic acid caused a rapid increase in viscosity but on further addition the contours turned back upon themselves and the result was a slight fall of viscosity.

The viscosity of saturated solutions of ascorbic acid in polysorbate 20:water exhibited a similar form to the binary polysorbate 20:water. The peak viscosity was 12.3 poises at 68% w/w polysorbate 20.

The catalysed oxidation of ascorbic acid in water at pH 3.4 and 6.0 was a first order reaction. The rate was approximately twice as fast at the higher pH but both showed a rapid increase in oxidation rate at ascorbic acid concentrations of less than 8% w/w (Table 1).

TABLE 1. OXIDATION OF AQUEOUS ASCORBIC ACID

Concentration of ascorbic acid % w/w	Oxidation rate (ml/kg/hr)	
	pH 3.4	pH 6.0
2.7	7,500	13,600
5.95	3,300	5,900
8.1	1,950	3,400
11.9	1,650	2,900
16.2	1,500	2,800

Catalyst:  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

The induction period of ascorbic acid in polysorbate 20 was much more extended than in water alone, and after the induction period a rise to an approximately steady oxidation rate occurred. The period of declining oxidation rate was also extended.

The oxidation of ascorbic acid in polysorbate 20 is slightly complicated due to the slow uptake of oxygen by the polysorbate 20 itself. This occurs more rapidly at acid pH, and at high polysorbate 20 concentrations

ASCORBIC ACID: WATER: POLYSORBATE 20

could form a significant proportion of the total oxygen uptake. Fig. 2 shows the uptake of oxygen by polysorbate 20 and it can be seen that although increasing viscosity did initially cause a fall in oxidation rate, at high polysorbate 20 concentrations this had increased again and was now in excess of dispersions with low viscosity. The method of increasing gas:liquid transfer by increasing the shaking rate did not cause any noticeable increase in this oxygen uptake.

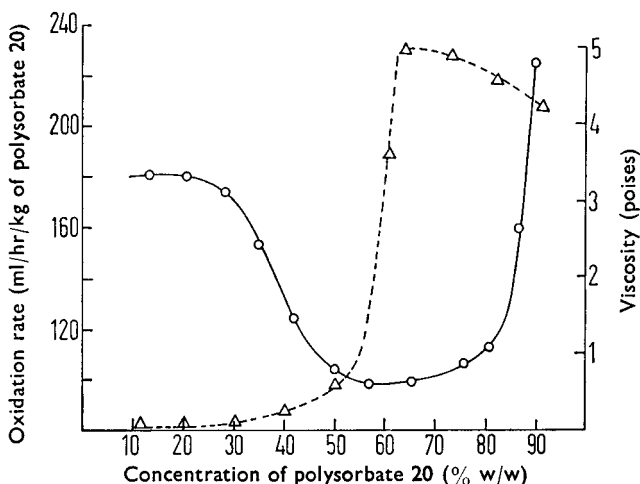


FIG. 2. The oxidation of polysorbate 20. Temperature 25°. Catalyst  $1 \times 10^{-4}M$   $CuSO_4 \cdot 5H_2O$ . pH 3.4. — Oxidation rate. - - - Viscosity.

Before studying the oxidation in relation to the viscosity solubilisation diagram (Fig. 1) the effect of catalyst and pH was determined. Saturated solutions in 15 and 30% w/w polysorbate 20 were used. With both polysorbate concentrations the rate of oxidation increased rapidly with

TABLE 2. EFFECT OF COPPER CATALYST ON THE RATE OF OXIDATION OF SATURATED SOLUTIONS OF ASCORBIC ACID

Polysorbate concentration % w/w	Copper sulphate M	Steady oxidation rate ml/kg/hr
15	0	15
	$1 \times 10^{-5}$	130
	$5 \times 10^{-5}$	480
	$1 \times 10^{-4}$	670
	$5 \times 10^{-4}$	1,100
30	0	15
	$1 \times 10^{-5}$	220
	$5 \times 10^{-5}$	580
	$1 \times 10^{-4}$	750
	$5 \times 10^{-4}$	1,240

increasing catalyst concentration, although no linearity was found (Table 2). An increase in pH also caused an increased oxidation rate except in the region pH 5.6 to 7.2 where a plateau existed (Fig. 3).

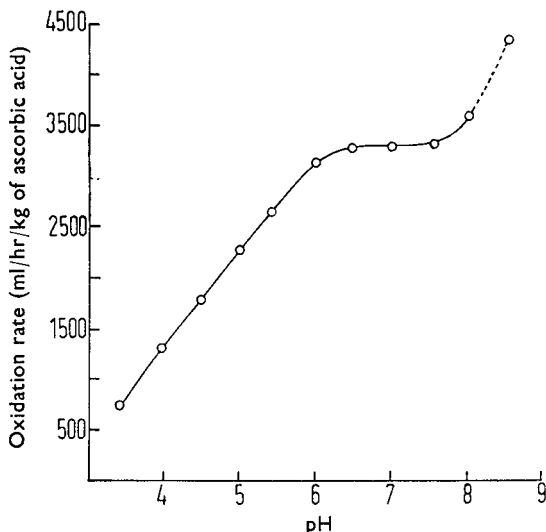


FIG. 3. The effect of pH on the oxidation of ascorbic acid in polysorbate 20. Polysorbate concentration 30% w/w Temperature 25°. Catalyst  $1 \times 10^{-4}M$   $CuSO_4 \cdot 5H_2O$ .

The oxidation of ascorbic acid-saturated dispersions, both catalysed and uncatalysed, was studied at pH 3-4. This pH was adopted to prevent the catalysed oxygen uptake rate becoming too fast to measure. The large increase in the viscosity of concentrated polysorbate 20 dispersions had a negligible effect on the oxidation rate of the uncatalysed reaction. As the concentration of polysorbate increased, the oxidation rate rose slightly, although there was a sharp fall in rate at polysorbate 20 concentrations greater than 90% w/w (Fig. 4).

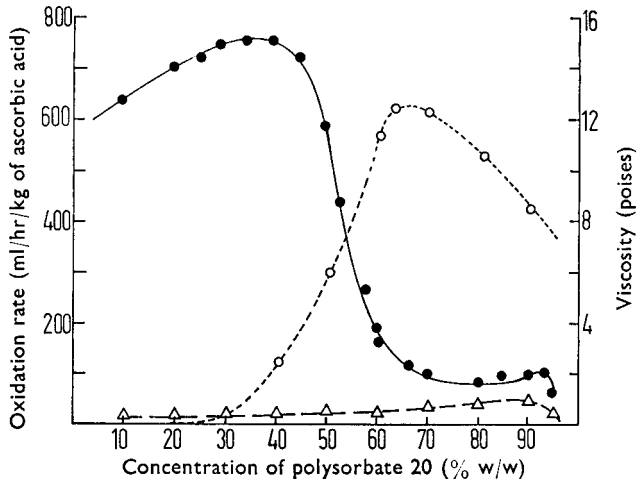


FIG. 4. The oxidation of saturated solutions of ascorbic acid in polysorbate 20. Temperature 25°. pH 3-4. ——— Catalyst  $1 \times 10^{-4}M$   $CuSO_4 \cdot 5H_2O$ . — — — Uncatalysed. - - - - Viscosity.

ASCORBIC ACID : WATER : POLYSORBATE 20

The oxidation of the catalysed systems differed considerably. At lower polysorbate concentrations, where the viscosity remained almost unchanged, the rate of oxidation rose steadily, but once the viscosity started to increase, the oxidation rate fell precipitously and reached a minimum at around 75% w/w polysorbate 20. Thus, even after the maximum viscosity was passed, the oxidation rate showed a further decrease. There was little further change in oxidation rate, although the trend was slightly upward until dispersions in pure polysorbate were reached, when a further sudden fall in oxidation rate occurred. None of these results showed significant variation on increasing the rate of agitation.

The presence of even small traces of polysorbate 20 caused a considerable fall in the oxidation rate of the more saturated solutions of ascorbic acid when compared with the rate of similar aqueous solutions. This suggested that the incorporation of ascorbic acid in the polysorbate, and the consequent depletion of the water pseudophase, was resulting in a measure of protection. At very low polysorbate concentrations the protection may be due to the normally found effect of surface-active agents on the diffusion of oxygen into solutions (Downing, Melbourne & Bruce, 1957). To study this effect, a concentration of 32% w/w polysorbate 20 was used; this being the highest concentration possible before the viscosity commenced its rapid increase, and also because it possessed the highest oxidation rate for a saturated solution (Table 3). Conversely, if the concentration of ascorbic acid was constant and the polysorbate 20 concentration varied, then increased incorporation of the acid in the polysorbate would be expected to confer some degree of protection on the ascorbic acid as is also illustrated in Table 3.

TABLE 3. OXIDATION RATE OF ASCORBIC ACID IN POLYSORBATE 20: EFFECT OF SATURATION LEVEL

Polysorbate 20 32% w/w with ascorbic acid % saturation	Rate of oxidation ml/kg/hr	Ascorbic acid 20% in polysorbate 20 % w/w	Rate of oxidation ml/kg/hr
9.9	12,350	0	1,450
26.2	2,579	10	1,360
57.8	1,124	25	1,100
100.0	763	35	850

Catalyst:  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

Because of the small quantities of material involved and the uncertain interference of the polysorbate 20 with most assays, the quantitative formation of the oxidation products was not followed. However, a number of chromatograms of the oxidising material were made. The polysorbate 20 tended to follow the solvent front, but three spots were observable with  $R_f$  values in the ranges (a) 0.08–0.09; (b) 0.33–0.37; (c) 0.53–0.59. The spot (b) was faint in all instances. In one system (ascorbic acid 10% w/w, polysorbate 20 30% w/w) a fourth spot was detected under ultraviolet light,  $R_f$  value 0.80. This was not identified and was probably due to impurity. The spots a, b and c are considered to correspond respectively to diketogulonic acid, ascorbic acid and dehydro-ascorbic acid. The slight variation from the  $R_f$  values of Mapson &

Partridge (1949) we consider is caused by the polysorbate 20 increasing the hydrophilic property of the material. The large tail of the spots was also due to this cause.

## Discussion

Mulley (1961) studied the phase equilibria of systems containing non-ionic surface-active agents and suggested a general form of ternary phase diagram. In the present work solid material separated out once the solubility limit was reached and, therefore, only the left-hand portion of the diagram would apply. No evidence was found of an anisotropic liquid crystalline phase, birefringence and non-Newtonian viscosity being absent. The polysorbate 20 was probably too hydrophilic to allow for its formation. The transfer would appear to be direct from an  $S_1$  to an  $S_2$  type micellar distribution (Windsor, 1954). There was no evidence to show when this commenced, nor the relative proportions of each micellar type at a given concentration. The alternative is that a weak gel structure may exist, but no evidence for this was found since the flow curves were Newtonian and not shear dependent.

The increase in viscosity would appear to be associated with the formation of  $S_2$  type micelles, and the two forms must coexist in dynamic equilibrium over most of the diagram (Fig. 1). At the two extremes, one or other will predominate, the  $S_1$  being the main type at low polysorbate 20 concentrations. Even so, it cannot be assumed that equal quantities of the two micellar entities exist at the highest viscosity although it is probably the antagonism of the two types which causes the viscosity pattern.

If the viscosity contours are examined, it is seen that the addition of ascorbic acid causes very large increases in viscosity in the region where Mulley (1961) predicted the presence of a liquid crystalline phase. As the ascorbic acid would tend to be solubilised towards the outside of the  $S_1$  type micelle rather than in the hydrocarbon interior, the bulk of the micelle would be considerably increased and in consequence the relative density of packing of the micellar pseudophase.

In the  $S_2$  type micelle the ascorbic acid would be towards the centre and the increase in bulk would not be so great, therefore a slight fall in viscosity would result as the  $S_2$  type began to predominate. This coupled with the mutual antagonism of the two micellar types would appear to account for the complex viscosity map.

The mechanism for the oxidation of ascorbic acid in polysorbate 20 dispersions, as shown by the chromatograms, did not appear to be abnormal. However, solubilisation in the polysorbate, the effect of pH and the changes in viscosity did modify the rate at which this oxidation took place. At pH values above 7.5, where the plateau region had been passed and secondary ionisation had commenced, the oxidation rate was too fast to be conveniently studied by the methods available, so that pH 6, corresponding to complete primary ionisation, and pH 3.4 were used. This meant that only one grouping was being attacked and therefore the mechanism of oxidation was simpler.

Unlike previous studies, the part played by solubilisation in preventing oxidation appeared to be small. In previous studies the materials were far less water soluble and therefore at equilibrium the bulk would be in the micelle. In the present instance the solubility in the polysorbate 20 was low when compared with the water solubility and it was in the water where most of the oxidation appeared to take place. Except where the saturation of the ascorbic acid solution was in the region of 10% or less, two effects were apparent. Immediately small amounts of polysorbate were present, the oxidation rate fell. This could not be due to solubilisation and it is suggested that the fall was caused by a reduction in the rate of diffusion of oxygen into the system, similar to the effect of detergents on the aeration of sewage effluents (Downing, Melbourne & Bruce, 1957). Once micelles formed, the close proximity of the solubilised molecules facilitated the continuance of the oxidation chain reaction and until the viscosity began to increase rapidly the rate of oxidation rose. It was in this region of low viscosity that the previous studies were made.

In the region of high viscosity it is probable that the much slower diffusion of oxygen to the site of oxidation is the cause of the very low oxidation rate. However, an increased shaking rate, which in the event of undersaturation with oxygen would normally increase this, produced no effect. Even so, from the shape of the oxygen uptake curve, it appeared probable that this was the explanation. In support of this the uncatalysed oxidation showed no drop over the same region and the oxidation rate even rose slightly as the viscosity increased, showing that here the system contained an optimum amount of oxygen and that gas exchange was fast enough to prevent depletion of the system.

Once the  $S_2$  type micellar system predominated and the viscosity began to fall again, the systems showed little further change in oxidation rate until almost pure polysorbate 20 was reached. In this region the water, where the bulk of the oxidation appeared to take place, was enclosed as a discontinuous pseudophase inside the polysorbate 20 micelle and any ascorbic acid dissolved in it was protected from the oxygen by the polysorbate itself. Once all the water was eliminated, the rate of oxidation in the polysorbate itself showed a further sharp fall which suggested that the presence of water even in small quantities inside the micelles allowed the easier formation of the initiating and propagating free radicals.

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