EXAMINATION OF THE AUTOXIDATION OF ASCORBIC ACID

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The reactions of ascorbic acid and its behaviour under various circumstances have been examined by several investigators. Having elucidated and confirmed its structure, many research workers set to tackle the problem of its oxidation, and this was only too natural owing to the susceptibility of this compound to oxidation. Since ascorbic acid has until now been of therapeutical importance only, its oxidation has been studied under physological conditions in a narrow pH range with due regard, in the first place, to the effect of homogeneous catalysts (*e. g.* ions of heavy metals etc.).

In our Institute ascorbic acid has, for some time, been used as a new model in experiments on autoxidation and studied in detail within the whole pH range. Data on its behaviour under physiological conditions have been recorded concerning keeping quality and usefulness in therapeutics and food industry.

Our experiments were conducted to elucidate the dependence of the autoxidation of ascorbic acid from

1. the pH,
2. the temperature and pH,
3. the effect of other added substances.

Ascorbic acid decomposes in aqueous media ; the decomposition is accelerated by alkalis. Its resistance to oxygen is the greatest at pH 5—6. Ascorbic acid is generally believed to be protected by HCN or reducing agents against decomposition. We know of its reversible transformations and its irreversible decomposition. When oxidized, dehydroascorbic acid is formed in the first reversible step. In alkaline media the molecule continues to split [1]. Opinions differ as to the pH range within which the reversible and the irreversible change takes place. BALL [2] found the ascorbic acid—dehydroascorbic acid system to be reversible below pH 5, FRUTON [3] set the limits at pH 5,5—7,5, GHOSH and CHAR [4] put it between pH 2,5—7,5 ; according to STANLEY [5] the rate of the irreversible reaction is lowest at pH 3,5.

The oxidation-reduction potential of ascorbic acid is another widely discussed question ; different values are reported by various authors [1, 2, 4, 6, 7].

The oxidation as related to pH has been studied by a small number of authors only. A few experiments of PREISS and BAUR [8] carried out in ammoniacal media disclosed oxygen uptake of ascorbic acid to be enhanced with increasing pH. As to the quantity of bound oxygen SCHEINKMANN [9] found 1 molecule of ascorbic acid to take up 1 molecule of oxygen and to bind 3 molecules of sodium hydroxide. According to observations made by KUBBI [10].

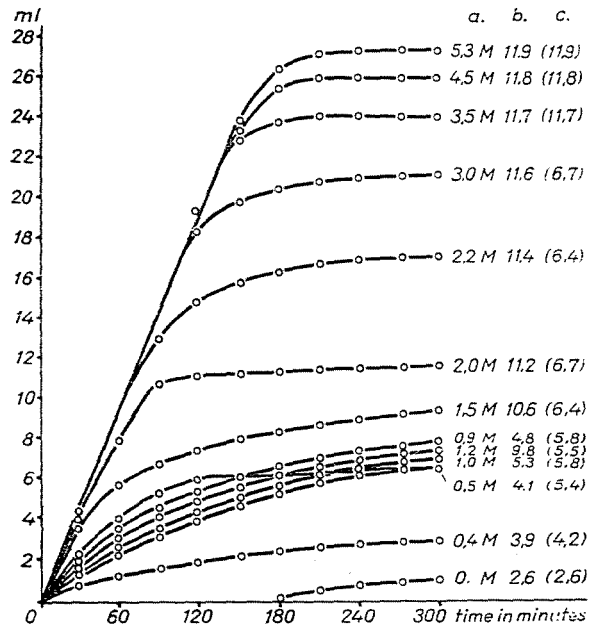


Fig. 1. Oxygen uptake of ascorbic acid at various starting pH, related to time
a) added mols of alkali; b) starting pH; c) final pH

a 2,5% aqueous solution of sodium salt of ascorbic acid is more stable than that of the free acid in the same concentration. Similar data may be found in a few patents dealing with the conservation of ascorbic acid solutions [11].

The decomposition of dehydroascorbic acid, the first oxidation product of ascorbic acid, has also been studied. This was found to be a labile substance, similar to ascorbic acid, to decompose in alkaline media rather quickly as well as in oxygenfree media [14, 15, 16].

According to CARTENS' and MORELLY's experiments in an irreversible decomposition oxalic and threonic acid are formed simultaneously with the conversion of ascorbic acid to dehydroascorbic acid. The quantity of oxalic acid is independent of the concentration and is not proportional to the disappeared ascorbic acid.

GHOSH [12] studied the chemical equilibrium of the cyclic and open chain forms of ascorbic acid and dehydroascorbic acid in relation to pH. The equilibrium constants — unfluenced by pH — are $2{,}5 \cdot 10^{-3}$ for ascorbic acid, $3{,}8 \cdot 10^{-5}$ for dehydroascorbic acid.

In our experiments the effect of pH on the oxygen uptake (18, Fig. 1) has been investigated. The alkali used was NaOH. It is to be remembered that the experiments of other research workers were executed in buffered solutions at constant pH. We have omitted buffers in order

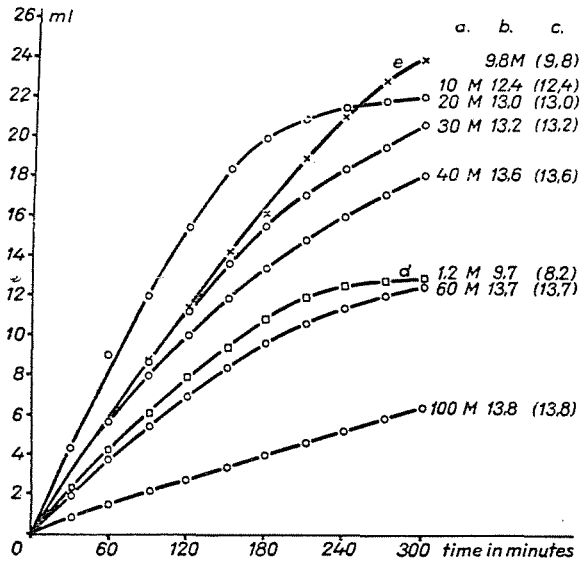


Fig. 2. Oxygen uptake of ascorbic acid related to time and alkali
a) added mols of alkali; *b)* starting pH; *c)* final pH curve *d)* buffered by sodium borate; curve *e)* pH held constant by repeated alkali addition

1. to come closer to natural circumstances;

2. to determine the pH changes permitting a more delicate investigation of the process;

3. to avert the possible disturbing effect of the buffer components;

4. to be able to use solutions of 0,200 g/10 ml concentration, that cannot be buffered effectively, since working with tenfold diluted solutions results in more difficult and less exact determinations.

For the conversion of ascorbic acid to dehydroascorbic acid theoretically 13,6 ml of oxygen are needed, but this is obtainable only in alkaline media containing at least 2 mols of base. The oxygen uptake in the pH range 4,1—10 is quite constant. At a starting pH of 3,9—5,3 it is shifted by 0,3—1,3 pH units towards the neutral, at higher pH by 1—5 units towards the acid side. The increase of pH of the solutions is due to the disappearance of two acid

hydroxyl groups in the conversion of ascorbic acid to dehydroascorbic acid (the first OH is similar to acetic acid, $pK_1 = 4{,}20$, the second the phenol, $pK_2 = 11{,}6$). The decrease of pH is due to different acids appearing during the irreversible reaction in alkaline media. When 3,5 mols of alkali are added the pH does not drop, the excess of alkali being sufficient to neutralize acid products. With 1 mol of alkali added (pH = 5,3) the oxygen uptake will be half of the theoretical, with 2 mols (pH = 11,2) it comes close to the calculated quantity.

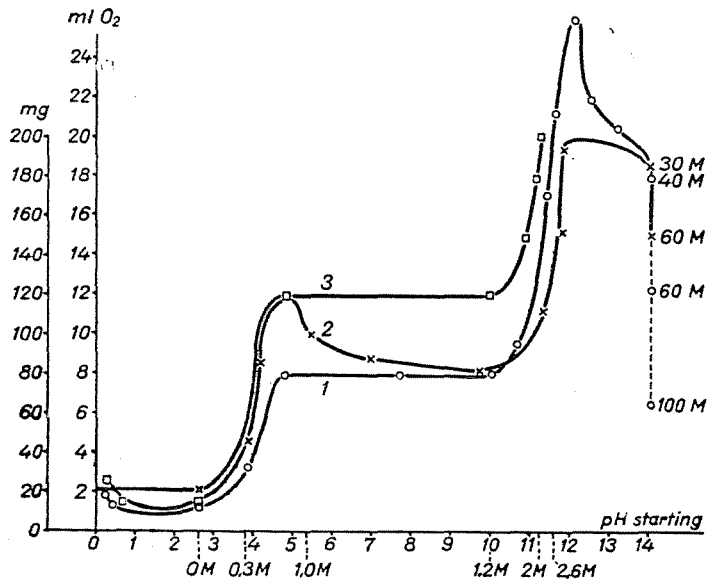


Fig. 3. Effect of oxygen uptake on ascorbic acid. 1. Oxygen taken up in 300 minutes ; 2. actual quantity of oxidized ascorbic acid ; 3. calculated quantity of oxidized ascorbic acid (to dehydroascorbic acid), related to the starting pH

Maximum oxygen uptake — the twofold of the theoretical value — is obtainable with 5 mols of alkali.

With further addition of alkali — over 3 mols — the oxygen uptake is not increased considerably (as opposed to the addition of 1—3 mols). Over 5 mols of added alkali the oxygen uptake decreases and with 100 mols it is as low as with 1 mol (Fig. 2).

As shown in Fig. 1 oxidation comes to a stop after different oxygen uptakes, dependent on the quantity of added alkali. To interpret this phenomenon it is advisable to consider separately the reactions occurring with not more than one molecule of alkali and those occurring with more alkali added. According to our experiments with not more than one molecule of alkali oxidation stops at the degrees of dehydroascorbic acid, i. e. the curves theoretically approach the 13,6 ml upper limit, but in the neutral pH range the reaction rate

is low. As shown in Fig. 1 the reaction did not come to an end, but considerably slowed down after 5 hours. With more than 1 molecule of added alkali, theoretically, there is an excess of base, i. e. oxidation does not stop at the degree of dehydroascorbic acid but proceeds to different levels depending on the excess of alkali and other conditions. That means that it is impossible to denote any theoretical limits to oxygen uptake. Between 1 and 3 molecules this limit is determined by the exhaustion of excess alkali; in the vicinity of neutral pH values the reaction comes to a stop. This is seen in Fig. 2, where curve *e* shows

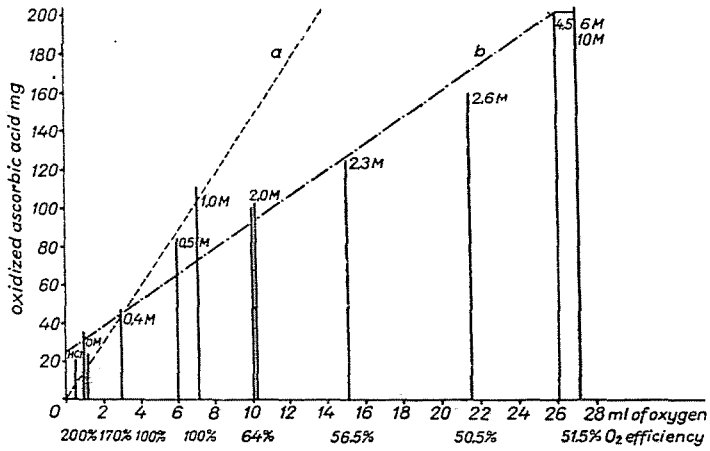


Fig. 4. Ascorbic acid oxidized in 300 minutes, in the presence of added alkali, related to the oxygen uptake

an oxygen uptake 3,5 times greater than the corresponding curve in Fig. 1. The starting pH was held constant by addition of alkali (total addition: 3,2 mols). A similar but slighter effect wa obtained with buffered solution (Fig. 2); this can be interpreted by the insufficient buffer capacity (the pH was not constant, but dropped slower than without added buffer). With 3,5 mols and more a permanent excess of alkali is ensured and this determines the oxygen uptake, as stated above. The small oxygen uptake with extreme (100 mols) excess of alkali can be partly explained by the lesser oxygen absorption in concentrated alkali solutions. (Further comments see below.)

The question of reversibility was studied in detail from various points of view. The oxygen uptake through the whole pH range is shown in Fig. 3 (curve 1). Relying upon these findings we have calculated the amount of ascorbic acid that would have been oxidated if the reaction had proceeded to dehydroascorbic acid only (curve 2). The measured actual ascorbic acid concentration is shown by curve 3. The greater the distance between curves 2 and 3 the more irreversible the reaction. In this way a nearly reversible range between pH 3,8—5 can be concluded.

In order to determine the part of the bound oxygen used for oxidizing ascorbic acid to dehydroascorbic acid, the decrease of reducing capacity related to the oxygen uptake has studied. In Fig. 4 the vertical lines equal the quantities of decomposed ascorbic acid. The dotted line (a) shows the suggested reaction in which oxygen is used only to oxidize ascorbic acid to dehydroascorbic acid. The greater its deviation from line "b" representing measured values, the more oxygen is used for side reactions. It is apparent that only part of the oxygen

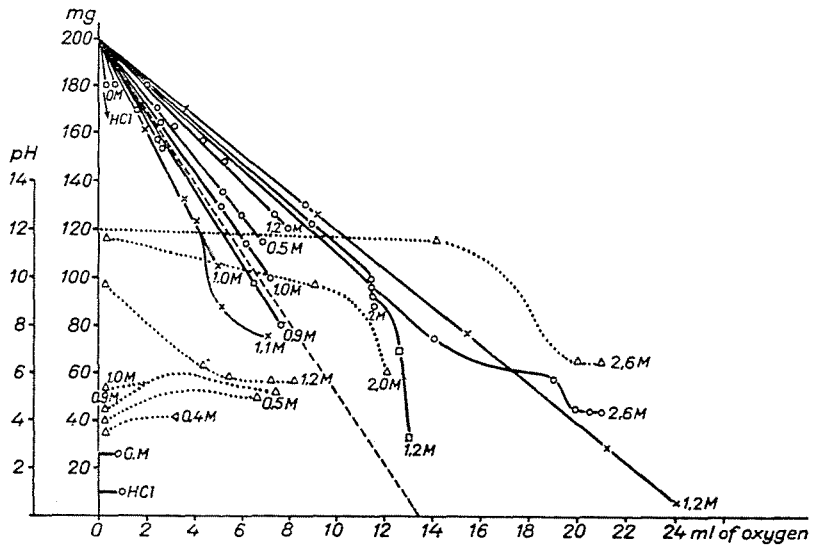


Fig. 5. Consumption of ascorbic acid and changes of pH related to oxygen uptake

is used for the nearly reversible oxidation of ascorbic acid, and this part decreases with increasing alkali excess : the "efficiency" of oxygen decreases with increasing alkali concentration. It is obvious that the reaction up to 1 mol of added alkali is approximately reversible.

Finally the decrease of reducing capacity (ascorbic acid concentration) was examined together with the change in the pH of the solution related to the oxygen uptake (Fig. 5). The dotted line from 200 mg to 13,6 ml represents a reaction in which all oxygen is used to produce dehydroascorbic acid. The greater the deviation of a curve from this line the more irreversible the reaction. The changes in pH are shown by point-lines. The instantaneous drop of the pH of the solution at the beginning of the oxidation proves that the decomposition starts right at the outset in the presence of unoxidized ascorbic acid, that is before all ascorbic acid changes to dehydroascorbic acid.

Hence we can conclude that partly neutralized ascorbic acid solutions are best suited for preserving injections.

We have also investigated the gross reaction rate and found it, in concordance with the literature, to be monomolecular. Fig. 6 shows the rate constant calculated on base of unchanged ascorbic acid concentration. The local maximum at 1 mol of alkali is caused by the reversible reaction. In strongly acid media the reaction rate increases. Experiments were made to elucidate the mechanism of the reaction.

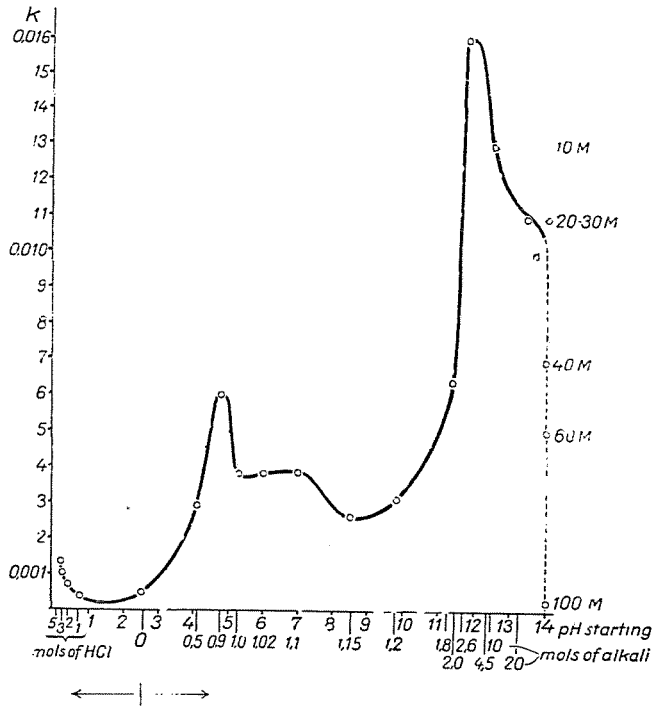


Fig. 6. Change of the rate constant (k) of ascorbic acid related to pH

Experiments performed in nitrogen atmosphere show that in acid or alkaline media ascorbic acid decomposes even in the absence of oxygen, and this decomposition is better catalysed by OH^- ions. This refers to some kind of acid—base catalysis.

Calculated with the equlibrium constants of GHOSH [17] the cyclic form of ascorbic and dehydroascorbic acid in aqueous media is found stable and the open chain form labile. Cyclic and open chain forms are present in identical quantities at pH 9,8 in the case of dehydroascorbic acid, at pH 11,4 in the case of ascorbic acid. Comparing these data with our experiments it appears that the rapid reaction starts at about pH 9,8 at pH 11,4 it accelerates and over this value it becomes very fast. Consequently the open chain forms of both compounds are more labile. The reversible oxidation is probably connected with this phenomenon ; in the neutral pH range the stable cyclic form of the

arising dehydroascorbic acid predominates, resisting further oxidation. The irreversible oxidation, on the other hand becomes very rapid when — with increasing pH — a considerable part of the dehydroascorbic acid and even

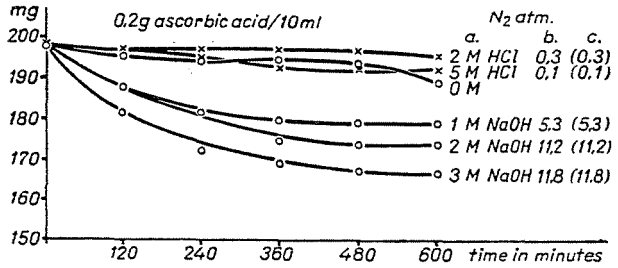


Fig. 7. Decomposition of ascorbic acid in nitrogen atmosphere (without pH changes) at 20° C with different alkali additions, related to time : a) added mols of acid or alkali ; b) starting pH ; c) final pH

more so, when greater part of the primary ascorbic acid assumes the open chain form.

According to the experiments of the rate of decomposition of the anion of ascorbic acid or of some intermediary reaction product presumably controls the reaction. Consequently a proton acceptor is needed. This was studied from another point of view as well. If the anion formed by loss of proton decomposes,

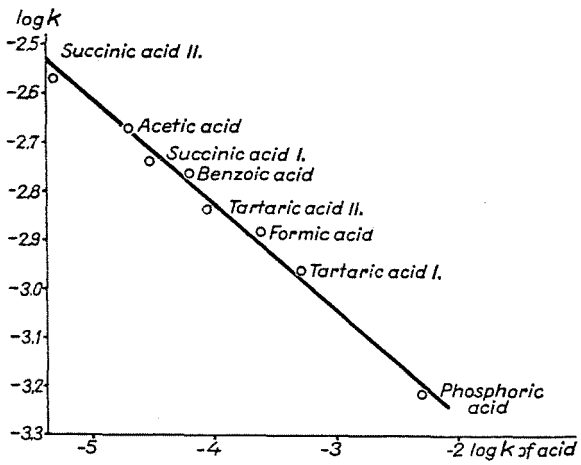


Fig. 8. Changes in the logarithms of gross reaction rates of various acids related to the logaritms of dissociation constants

the process according to the Brönsted theory (1924) is catalyzed by bases. Consequently acids may also act as bases, that is to say the lower the dissociation constant of the acid formed by proton uptake the better proton acceptor a base

will be. In the experiments the autoxidations of ascorbic acid was catalyzed by bases according to Brönsted's theory (Fig. 8). Na. salts of the acids were always used : two equivalents were taken for every molecule of ascorbic acid.

It must be pointed out that sodium benzoate — used in industry as a chemical preservative — accelerates autoxidation.

The danger is enhanced by the fact that — considering the low ascorbic acid content of plants — the concentration of the preservative related to the ascorbic acid contents may be even greater than it was in our experiments.

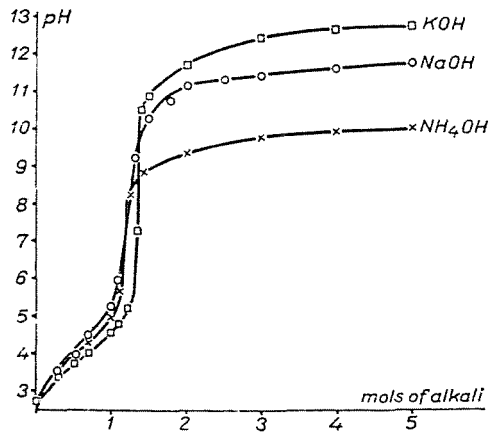


Fig. 9. pH changes of ascorbic acid solutions (0,200 g/10 ml), related to added alkali

For the sake of comparison with another chemical preservative — salicylic acid — tests were made under similar conditions with sodium salicylate : whereby only half quantity of oxygen was taken up. Since the compound used as preservative is free salicylic acid, the catalytic effect is negligible ; this phenomenon justifies the use of salicylic acid.

According to our experiments the course of autoxidation of ascorbic acid differs when — under the same conditions — sodium, potassium or ammonium hydroxide is added as a base [20]. Experiments of this type are not reported in the literature, though it would be very interesting to know different bases used by research workers [8, 9, 21, 22]. To elucidate the question the change in pH of a 0,200 g (10 ml) aqueous ascorbic acid solution — caused by various bases — was determined (Fig. 9). To obtain a given pH, different molar quantities were needed. After the addition of 1 mol of base the deviations naturally grew larger. Owing to the sensitivity of ascorbic acid to alkali, an acceleration of the oxygen uptake was expected at pH 9—10, where the concentration of ammonium hydroxyde is fivefold while the quantity of potassium hydroxyde is the smallest. This was verified by the experiments (Fig. 10). If on the other hand, the relations are viewed as the function of an added quantity of base, ammonium hydroxyde

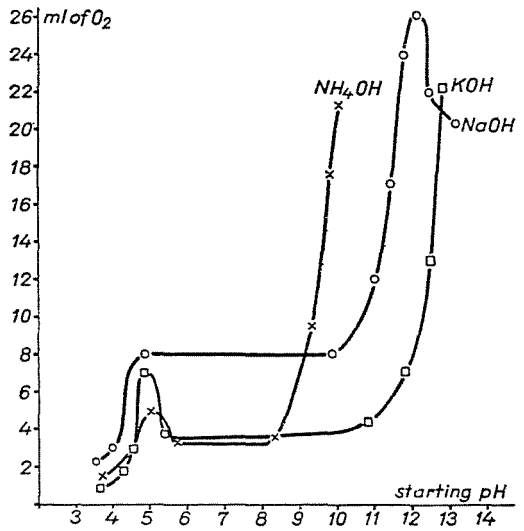


Fig. 10. Oxygen uptake of ascorbic acid in the presence of various alkalis, dependent on the starting pH

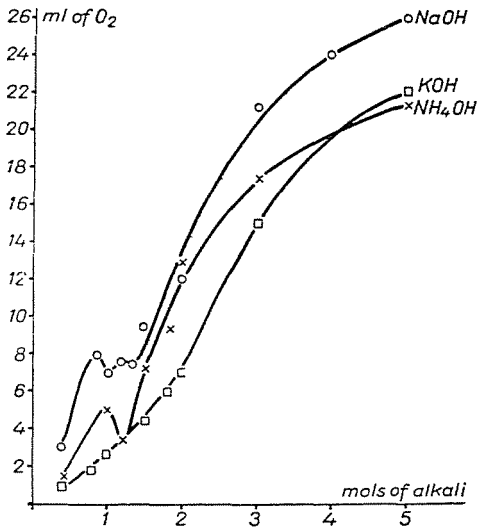


Fig. 11. Oxygen uptake of ascorbic acid related to the quantity of added alkali

does not appear to be in a privileged position. The situation is similar in case of rate constants (Fig. 12 and 13).

The experiments show the type and the molar quantity of the added base and not the pH to be decisive in the delicate examination of autoxidation of ascorbic acid. Thus the conditions of the reaction are not unanimously determined by pH (further explanation see below).

Since data in the literature are incomplete, the elucidation of the effect of temperature besides that of pH seemed important from both theoretical and practical points of view (sterilization and preservation) [23].

According to SABALITSCHKA and PRIEN [24] aqueous ascorbic acid solutions are fairly heat resisting in the absence of oxygen and become even more

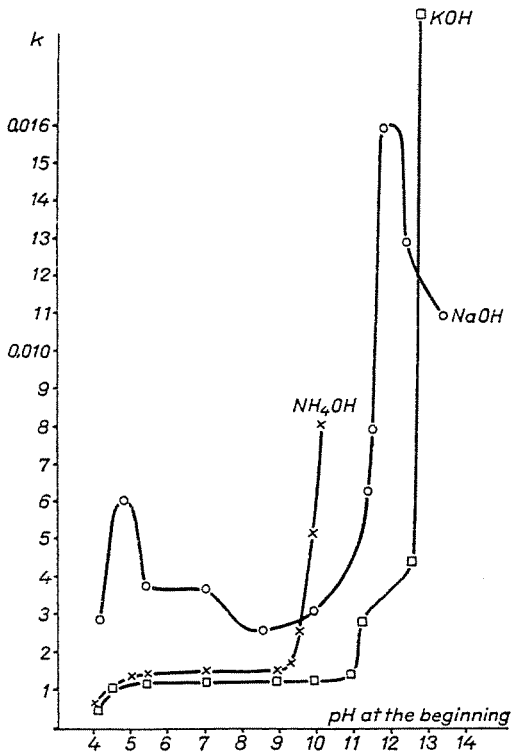


Fig. 12. Rate constant of ascorbic with various alkalis, related to the pH at the beginning

so with increasing concentration. At extremely high temperatures ascorbic acid — like -keto-acids — decomposes with simultaneous CO_2-loss. This was observed by COX and HIRST [25] at 175° C.

According to the unanimous statement of a few scientists who studied it the decomposition is considerably accelerated by the rise in temperature.

MOLL's and WIETERS' [12] experiments show that in 6% aqueous solution appreciable decomposition occurs (50% in 48 hours) at 20° C. The decomposition is accelerated by further the rise of temperature even in a nitrogen atmosphere, and it is enhanced by the presence of acid. PIEN and MEINRATH [26] as well as ENGELHARDT and BUKIN [27] noted considerable decomposition both in

presence and in absence of oxygen at 120 that is 60° C and at pH 8 that is 9, respectively.

In the course of the measurements two difficulties arose, generally, above 60° C. On the one hand, according to experiments performed separately above this temperature, ascorbic acid decomposes with increasing intensity and CO_2

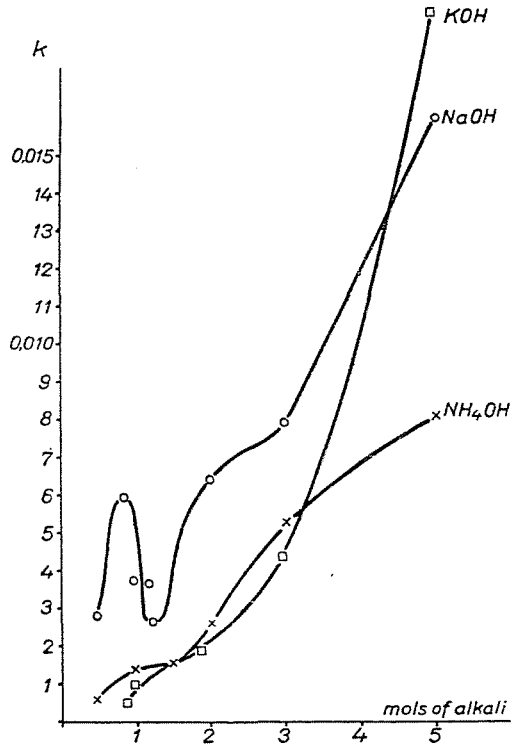
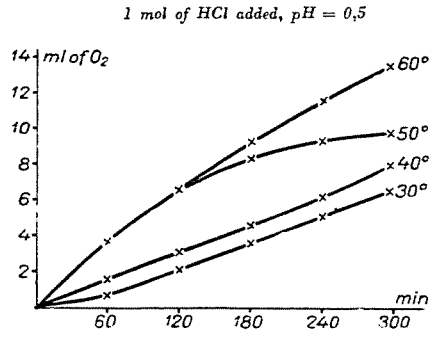


Fig. 13. Rate constant of ascorbic acid with various alkalis, related to the mols of added substance

is developed; on the other hand, the water vapour tension of the solution becomes significant. Both phenomena interfered with the reading of the gas volume above 60° C. Although measurements were performed up to 90° C, data on the oxygen uptake are reliable only up to 60°; above this temperature they may be somewhat higher.

In the acid pH range *(i. e.)* below pH 3 the increase of oxygen uptake due to the rise in temperature is considerably greater than in neutral or alkaline solutions (Figs. 14 and 15). Without addition, the oxygen uptake at 60° is fivefold that at 30° (Fig. 15). The addition of 1 mol of HCl or NaOH produces nearly the same effect. If more than 3 mols of HCl are added, the catalytic effect ceases, moreover, the acid acts definitely as an inhibitor.



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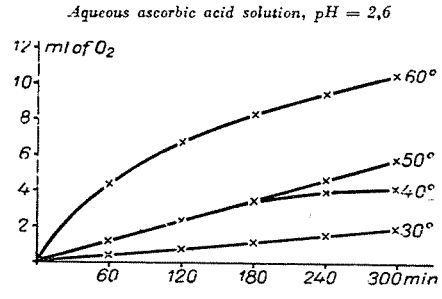

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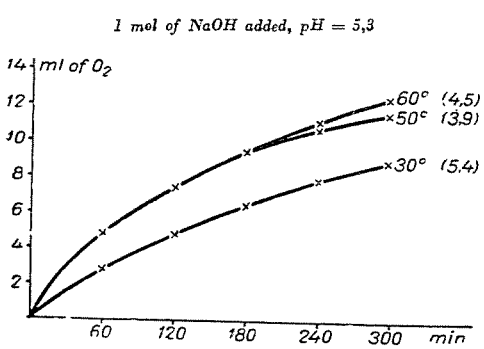

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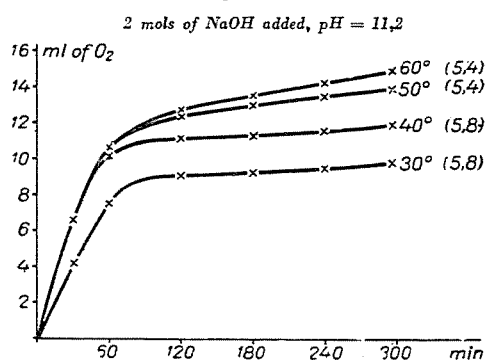

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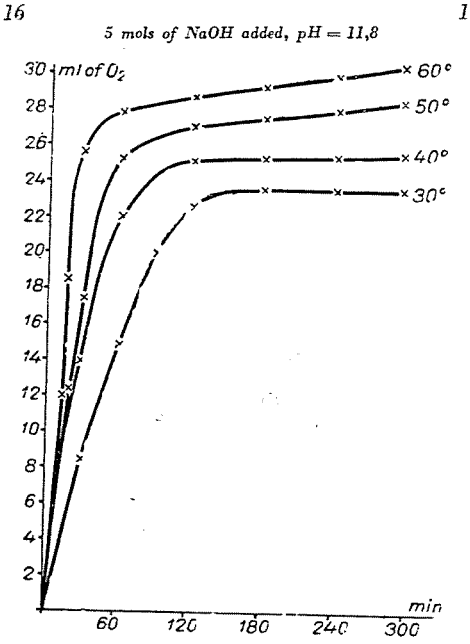

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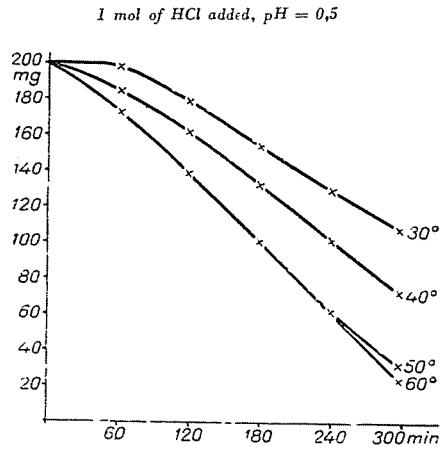
Fig. 14—18. Oxygen uptake of ascorbic acid in the presence of acid or alkali, at different temperatures



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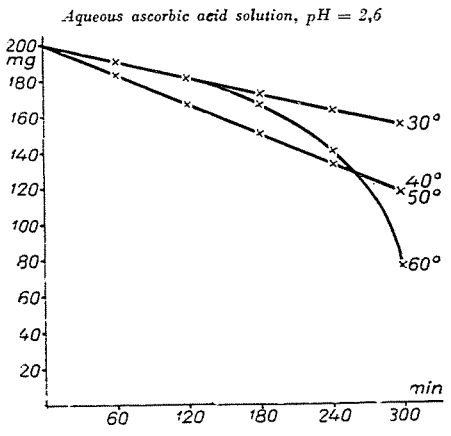

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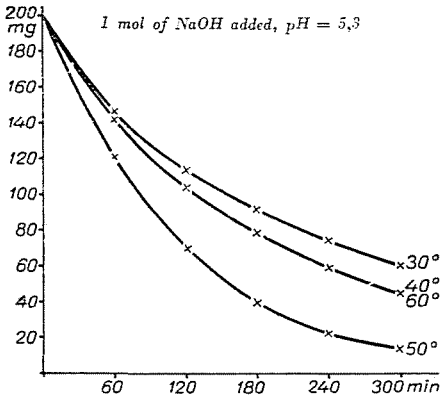

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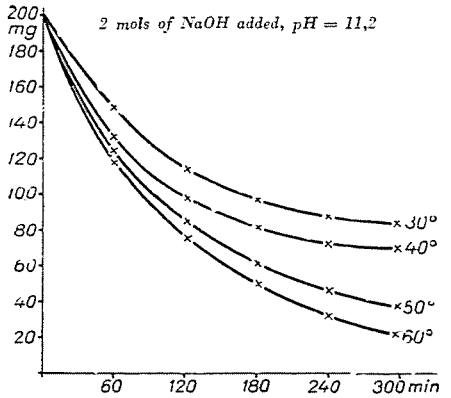

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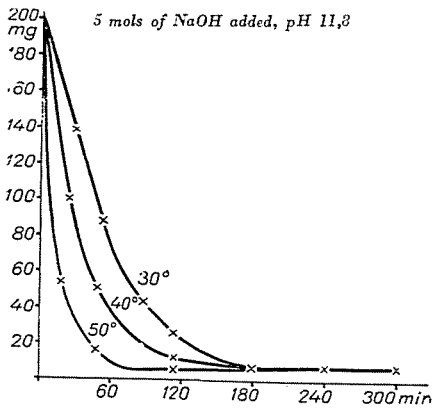

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Fig. 19—23. Decrease of reducing capacity at different temperatures, in the presence of acid or alkali

The rise in temperature causes increased oxygen uptake in neutral and basic media too, but not so rapidly as in acid solutions. E. g. at 60° C the oxygen uptake increases by only 20—30% as compared to 20° C. The greatest oxygen uptake observed was the threefold of the calculated value of the dedydro-ascorbic acid level. At 90° C, 3 mols of NaOH added.

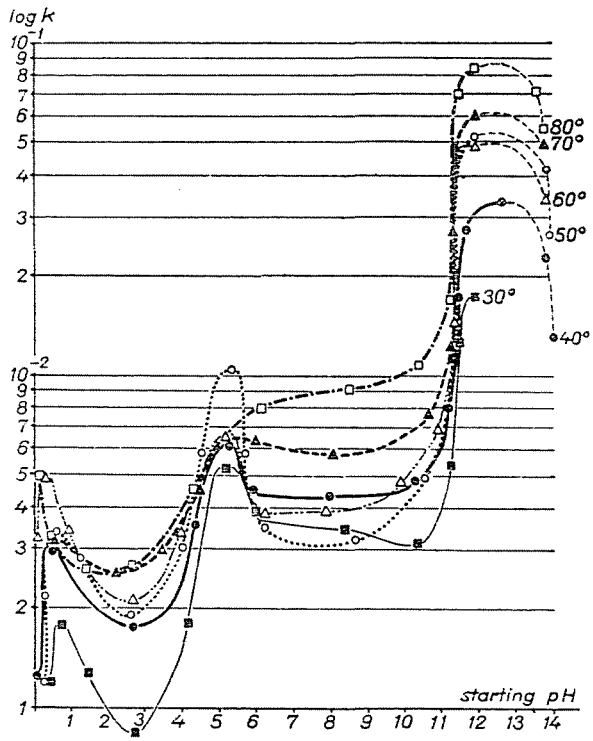

Fig. 24. Logarithm of rate constants at different temperatures

In acid media the greatest conversion was 93%, in 3 hours (50° C, 1 mol of HCl, Fig. 19). All curves are of descending tendency and show no trends towards equlibrium.

At pH 5—7 the measured decrease in reducing capacity up to 60° C is in agreement with values calculated from the oxygen uptake, up to the dehydro-ascorbic acid level (Fig. 21). This observation agrees with the fact known from pharmacology that partly or totally neutralized ascorbic acid solutions keep better during sterilization and storing than solutions of the free acid. The experimental results are shown in Table I.

In basic media the oxygen uptake is considerably greater than expected from the reducing capacity. The deviation increases at higher temperatures

and with increasing excess of alkali (Figs. 22 and 23). At 80° C 97% conversion is obtainable with 2 mols of NaOH, in 3 hours (Fig. 22).

Table I

The effect of NaOH on decrease of reducing capacity of ascorbic acid

Temperature C°	Without addition, pH = 2,6			1 mol of NaOH, pH = 5,2			2 mols of NaOH, pH = 11,2		
	Oxygen uptake* %	Decrease of reducing capacity %	Difference %	Oxygen uptake* %	Decrease of reducing capacity %	Difference %	Oxygen uptake* %	Decrease of reducing capacity %	Difference %
30	18	23	5	62	66	3	74	60	14
40	32	44	12	62	65	3	88	60	28
50	44	54	10	85	83	2	100	82	18
60	77	64	13	85	83	2	102	92	10

* Calculated value to dehydroascorbic acid = 100%.

The rate constant — as related to the pH — shows three maxima up to 60° C, and two above this temperature; close to the addition of 1 mol

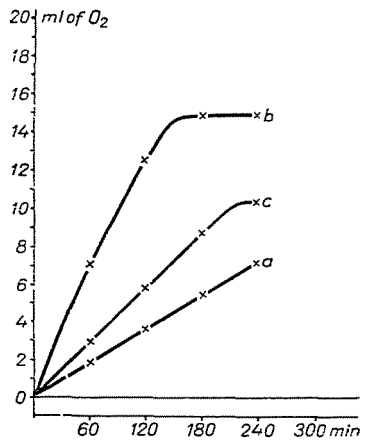

Fig. 25. The effect of substances with large surface on the autoxidation of ascorbic acid. a) same after treatment for 30 minutes in vacuum, b) carbon black of fine particle size, c) carbon black of coarser particle size

of acid and 1 or 2 mols of base respectively. With the rise of temperature the maximum at 1 mol of added NaOH disappears (Fig. 24). The phenomenon may be explained as follows: if more than 1 mol of NaOH is added, the acids formed by irreversible oxidation consume the "catalyst" at lower temperatures and therefore the reaction rate decreases. At higher temperatures this effect is compensated by the increased reaction rate.

In the heterogeneous phase the effect of bone black and different carbon blacks has been studied [28]. These subtances are also able to increase consider-

ably the oxygen uptake of aqueous ascorbic acid solutions (pH = 2,6). BRAGNOLO [29], KUHN and GERHARD [30] examined the effect of bone blacks. According to them and to our own experiments made with carbon black, the "catalytic" effect can be explained by the introduction of oxygen bound on the large surface of these substances ; this is supported also by the fact, that with blacks pretreated in vacuo or with coarser blacks (of smaller surface) the oxygen uptake

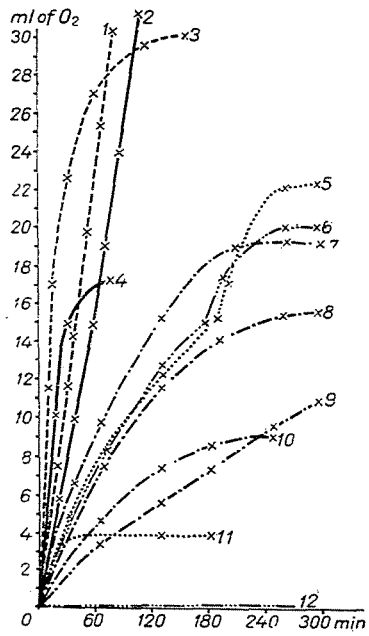

Fig. 26. Effect of hydroquinone, resorcinol and pyrocatechol on the oxygen uptake of ascorbic acid (Hq: hydroquinone, B: blank) 1. 0,1 g Hq 11,3 (9,8), 2. 0,05 Hq 11,3 (9,8), 3. B 11,3 (10,4), 4. B 11,3 (10,9), 5. 0,01 g Hq 11,3 (9,8), 6. 0,05 g of resorcinol 11,3 (9,9), 7. 0,2 g of ascorbic acid 11,3 (9,6), 8. B 11,3 (10,6) (pyrocatechol), 9. 0,05 g of pyrocatechol 11,3 (10), 10. 0,1 g Hq 4,2 (4,8), 11. B 11,3 (10,9), 12. B 11,3 (10,9)

becomes slower (Fig. 25). It has been stated, that by this method a very mild reversible oxidation can be performed when the system takes up in short time 13,6 ml of oxygen (calculated to dehydroascorbic acid) at room temperature in the presence of an appropriate carbon black, without any addition.

Finally the so called carrier effect was examined. It has frequently been observed that the autoxidation of organic substrates is accelerated in the presence of easily oxidizable substances [31]. On all occasions the carrier substance itself becomes oxidized, too. The quantity of oxygen molecules activating 1 mol of carrier is in most cases small. According to the suggestion of FRANKE [32] the autoxidation of the carrier causes a chain reaction. According to the experiments of several Soviet authors, particularly of MEDVEDEV, and to the theoretical

deductions of SEMJONOV [33] the autoxidation processes occuring in the liquid phase, especially the autoxidation of aldehydes, are typical chain reactions, the starting centers of which are intermediary peroxides.

The presence of hydrogen peroxide at the oxydation of ascorbic acid was shown by DEKKER and DICKINSON [34] and others. In our experiments hydrogen peroxide was detected whenever ascorbic acid was present; in tests made with crystalline dehydroascorbic acid no peroxide was detectable. This shows that the formation of peroxide takes place in the first period of oxidation.

The decomposition of hydrogen peroxide at various pH values has been examined by ERDEY [35]; he has found its maximum to be near pH 12, and any further excess of base to inhibit the decomposition of peroxide. According to SCHENCK, VORLÄNDER and DUX [36] in the presence of NaOH the decomposition of hydrogen peroxide takes place at a greater reaction rate than in the case of ammonium hydroxide.

The foregoing statements will help to elucidate our experimental results; at the autoxidation of ascorbic acid peroxide formation is detectable and the reaction considerably accelerates at pH 11—12. The same accounts for the stronger effect of NaOH and the weaker effect of NH_4OH of the same molarity, when comparing NaOH, NH_4OH and KOH.

In our experiments hydroquinone, resorcinol and pyrocatechol were used as carriers. The accelerating effect of hydroquinone is very strong (Fig. 26). It is interesting to note the breaking points on the curves of hydroquinone and resorcinol around 14 ml calculated for the oxidation of ascorbic acid to dehydroascorbic acid. This is followed by a rapid oxidation. Presumably the hydroquinone activated by a little oxygen acts as oxygen donor and its oxidation begins not until the first oxidation level of dehydroascorbic acid is reached; hydroquinone is oxidized easier than dehydroascorbic acid. This suggestion is corroborated by experiments; a hydroquinone solution — run as a blank — becomes brown in the first seconds owing to instantenous oxidation, whilst together with ascorbic acid the solution darkens only after reaching the breaking point of the curve.

* * *

As the result of our experiments the following suggestions can be made on the autoxidation of ascorbic acid:

Autoxidation is accelerated by alkali at various rates in different pH ranges. The effect of alkali is a double one. On the one hand it influences the (presumably anionic) decomposition velocity of the transitory peroxide, on the other hand it determines the equilibrium of the cyclic and open chain forms of ascorbic and dehydroascorbic acid. At neutral pH the decomposition of peroxide is slow and the more stable cyclic forms of ascorbic and dehydroascorbic acid are dominant: the reaction is nearly reversible. In the proximity

of pH = 12 (2 mols of alkali) the decomposition rate of peroxides is at maximum, the process is irreversible. With a greater excess of alkali (more than 5 mols) the reaction rate decreases in consequence of the slowing down of peroxide decomposition. The first (reversible) step of the process seems to be a chain reaction and its starter an active peroxide.

The rise of temperature involves only a nearly proportional acceleration of the reaction through the whole pH range. The picture is made even more complicated by the ability of ascorbic and dehydroascorbic acid to decompose in acid and alkaline media in the absence of oxygen as well. This shows an acid—base catalysis parallel with the oxidation reaction (possibly the hydrolysis of the C_2—C_3 bound?).

Experimental

According to the literature and our own experience the wrong choice of apparatus or concentration may cause several experimental errors. This was duly considered in the developing of the experimental method to be used. The description and use of the apparatus may be found in earlier communications [28, 37], the only deviation being the use of a double walled flask in which water of 20° C was circulated from an ultrathermostate, because reproducibility is influenced by slight temperature changes, even by a draught.

When redistilled water was used, the reading of the buret deviated from the starting point during the experiment by $\pm$ 0,3 ml only.

The hard requirements of the reproducibility of the experiments must be emphasized. The rate of oxygen uptake is influenced — among others — by the dimension of the liquid—gas interface and the rate of stirring. The former is determined by the diameter of the flask, the latter by the r. p. m. of the magnet (the cross section of the flask had nearly the shape of an ellipse, its major axe being 65 mm, the minor axe 40 mm, r. p. m. of the magnet 450, the rods of the stirrer 30 and 22 mm, their diameter 2 mm).

Ascorbic acid was used in 0,200 g/10 ml concentration i. e. 0,00114 mol/10 ml of water, a 0,114 molar solution). The final volume was always 10 ml (in the experiments with alkali addition too). This concentration is the tenfold of that used by other authors (not more 0,01 molar solutions). 0,01 molar or more diluted solutions do not suit the experiment because of the wrong reproducibility and the small oxygen uptake.

The experiments lasted 300 minutes (5 hours). Exceptionally some experiments of low reaction rate were conducted for 600 minutes (10 hours).

The decrease in reducing capacity was determined by titration according to SCHULEK and KOVÁCS [20].

The added alkali was in all experiments sodium hydroxide. The pH changes of the ascorbic acid solution — related to the added NaOH — were

determined by an electrometric pH-meter. For the control of pH we used Lyphan-paper; both methods agreed satisfactorily.

The detection of peroxide was made with lucigenin, according to ERDEY [35]. Maximum deviation of oxygen uptake in repeated experiments was ± 10%. Numerous measurements were performed, but only such results and curves are shown as were several times exactly reproduced.

Summary

Investigations on the autoxidation of ascorbic acid as a function of the pH values showed that this autoxidation changes with each pH region. The course of the process is different when under otherwise quite identical conditions sodium hydroxide or potassium hydroxide or ammonium hydroxyde are applied for alkalinisation. Autoxidation depends also on the temperature, its correlation changing with the pH values.

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