Gluconic Acid

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1. Introduction

D-Gluconic acid [*526-95-4*], 1,2,3,4,5-pentahydroxy pentane-1-carboxylic acid, $C_6H_{12}O_7$, M_r 196.16, was discovered in 1870 by HLASIWETZ and HABERMANN during the oxidation of glucose with chlorine. The substance was isolated in the form of its barium and calcium salts. Several authors subsequently reported that gluconic acid could be obtained by treatment of various mono-, di-, and polysaccharides with oxidizing agents such as elemental halogen, copper(II) or hexacyanoferrate(III) salts, or mercury(II) oxide. Depending on the type of sugar and the oxidant employed, byproducts of the reaction include formic acid, glycolic acid, oxalic acid, and carbon dioxide.

As early as 1880 BOUTROUX recognized that gluconic acid was produced, together with acetic acid, by the oxidative action of *Acetobacter aceti* on glucose. This characteristic was also found to be associated with numerous other bacteria [1]. MOLLIARD was the first to report the presence of gluconic acid in cultures of *Sterigmatocystis nigra*, now known as *Aspergillus niger* [2]. The currently preferred method for preparing gluconic acid and its derivatives with the aid of *Aspergillus* strains is based on the work of a number of authors [3]. The catalytic activity of the enzyme glucose oxidase was first described by MÜLLER [4].

During the 1930s anodic oxidation was suggested for the preparation of calcium gluconate [5]; proposals for catalytic oxidation of glucose with the aid of air or oxygen followed somewhat later [6].

Sources. Gluconic acid is a naturally occurring substance in humans and other organisms (cf. Chap. 7); it is found in food products such as wine and honey.

2. Physical Properties

Free D-gluconic acid is difficult to prepare in crystalline form. According to MILSOM and MEERS [7], crystallization of the anhydrous substance is possible below 30 °C, and a monohydrate has been reported to crystallize at 0 – 3 °C with a characteristic crystalline structure, *mp* ca. 85 °C [8].

Anhydrous gluconic acid is a white, odorless, crystalline powder, specific rotation $[\alpha]_D^{20}$ −6.7 ° [9], $[\alpha]_D^{25}$ −5.4 ° [10].

Literature values for the melting point of gluconic acid fall in the range 120 – 131 °C. The spread in values is due to the formation of intramolecular anhydrides whose presence lowers the melting point.

Two *lactones* of gluconic acid are known. These exist both in the solid state and also in aqueous solution, where they are in equilibrium with each other and with the free acid.

This complex equilibrium is also influenced by the dissociation of the free acid, which has a

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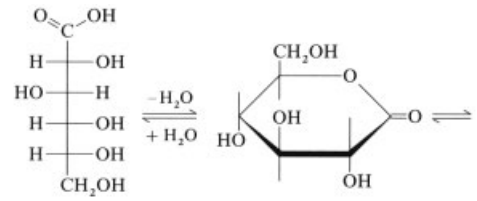
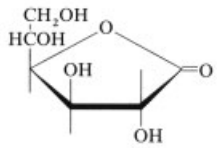
reported [10] dissociation constant K_A at 25 °C of 1.99×10^{-4} and a pK_A of 3.70 (3.62 according to [11]).





Lactone hydrolysis and formation are both first order with respect to hydrogen ion concentration, with rate constants (k and b, respectively) related as follows [12]:

$$k_{1,5}+b_{1,5}=c_{H^+}\cdot 5.5\times 10^{-2}\,s^{-1}$$
$$k_{1,4}+b_{1,4}=c_{H^+}\cdot 4.3\times 10^{-4}\,s^{-1}$$

Thus, equilibrium is established with the 1,5-lactone roughly 100 times faster than with the 1,4-lactone. The following empirical equation describing the kinetics of hydrolysis for the 1,5-lactone in the pH range 0.6 – 8.2 has been reported [12]:

$$k_{1,5}=c_{H^+}\cdot 4.7\times 10^{-2}+c_{OH^-}\cdot 4\times 10^{3}+2.5\times 10^{-4}\,s^{-1}$$

This expression indicates that the OH^- ion strongly catalyzes the opening of the lactone ring, while the H^+ ion is considerably less effective. In the region of neutrality, hydrolysis is quite slow. The rate of hydrolysis over the narrower pH range 3 – 5 is given by

$$-dc_L/dt=kc_L$$

where c_L is the concentration of the 1,5-lactone and $k = 2.3 \times 10^{-4}\,s^{-1}$. The activation energy for the process is reported to be 62.8 kJ/mol [13].

Gluconic acid is quite soluble in water. At 20 °C, commercial 50 % gluconic acid solution has a pH of 1.82 and a density of 1.23 g/cm^3. In this concentration region, solution density increases almost linearly with increasing concentration. The acid is only slightly soluble in ethanol and virtually insoluble in nonpolar solvents.

Storing gluconic acid over a desiccant at room temperature or heating it above 50 °C leads to the formation of lactones. Pyrolysis occurs above 200 °C.

Gluconic acid 1,5-lactone [*90-80-2*] is a white, crystalline substance with a faintly sweet taste, *mp* 153 °C, $[\alpha]_D^{20}$ +66.2 ° [13]. On standing, both the pH and the specific rotation of a lactone solution decrease as a result of hydrolysis, becoming constant only after equilibrium is established. Approximately 90 g of 1,5-lactone dissolves in 100 mL of water at 20 °C; this increases to about 205 g at 50 °C. Only small amounts dissolve in organic solvents. The *1,4-lactone* [*1198-69-2*] crystallizes as fine needles, *mp* 134 – 136 °C, $[\alpha]_D^{20}$ +67.8 ° [13].

3. Chemical Properties

The action of oxidizing agents such as nitric acid or hydrogen peroxide on either the lactones or the calcium salt of gluconic acid leads under mild conditions to mixtures of oxogluconic acids, with the carbonyl groups at the 2- and the 5-positions. 2-Oxo-D-gluconic acid [*669-90-9*] is the principal product of anodic oxidation, oxidation with sodium chlorate in acid solution, or fermentation by species such as *Acetobacter suboxydans, A. xylinum,* or *A. gluconicum.* Treatment with concentrated nitric acid or with N_2O_4 results in formation of glucaric acid, $HOOC(HCOH)_4COOH$ [*87-73-0*].

Hydrogenation of gluconic acid in aqueous solution over a platinum oxide catalyst results in a modest yield of D-glucose, whereas the 1,5-lactone undergoes this reaction in high yield. Refluxing with concentrated hydriodic acid in the presence of red phosphorus causes reduction to hexanoic acid [*142-62-1*].

The six functional groups of gluconic acid can, in principle, react with a variety of reagents, such as alcohols, acids, etc. Nevertheless, the resulting derivatives tend to be stable only if reaction is complete. Partial reaction leads to nonuniform mixtures that are sensitive to hydrolysis; such reactions have little significance.

In contrast, considerable interest exists in the ability of gluconic acid and its alkali salts to form complexes with polyvalent cations; some of these complexes are very stable (see Chap. 5). Nuclear magnetic resonance spectra suggest that complex formation involves both the carboxyl and the hydroxyl groups.

4. Production

For commercial purposes, D-gluconic acid and its salts are prepared exclusively by the oxidation of glucose or glucose-containing raw materials. The oxidation method may be chemical, electrolytic, catalytic, or biochemical.

4.1. Chemical Oxidation

Chemical methods have the disadvantage of limited specificity even under carefully controlled and optimized reaction conditions, which results in unsatisfactory yields (60 – 80 %) and undesirable byproducts. Isolation and purification of the product are correspondingly difficult. Hydrogen peroxide [14], ozone [15], and oxygen [16] have been the oxidants of choice, presumably because of ecological problems associated with other oxidizing agents and their reduction products.

4.2. Electrochemical Oxidation

Similar problems accompany electrochemical procedures, most of which are based on the oxidation potential of halogens. The usual approach is to electrolyze a glucose solution containing bromide (ca. 10 mol % relative to glucose) at a current density of $1 - 20\ A/dm^2$. Carbonates (preferably calcium carbonate) or hydroxides are added to neutralize the resulting acid [17]. Electrical current yields are 80 – 86 %; the product yield based on glucose is 80 – 97 %. The rapid increase in the cost of electricity in recent years has made the electrolytic process noncompetitive.

4.3. Catalytic Oxidation

In contrast, recent developments have led to great improvements in the technique of oxidizing glucose with oxygen or air and serious consideration has been given to adopting this strategy on an industrial scale. Oxidation is carried out, with the aid of an appropriate catalyst, on a glucose solution whose concentration is 1 – 2 mol/L. The pH is held between 8 and 11 (preferably 9 – 10) by continuous addition of alkaline solution.

The catalysts originally employed were finely divided platinum-group metals suspended on activated charcoal, aluminum oxide, or some other carrier [18]. The corresponding reaction mechanism for a Pt – C catalyst has largely been elucidated [19].

The most important observations with respect to the catalytic reaction include a rapid decrease in activity of the catalyst from its initial levels (a decline that cannot be completely reversed despite efforts at reactivation), the need for highly purified glucose solutions, and the formation of various byproducts as a result of insufficient catalyst specificity and the tendency of the substrate to undergo side reactions under the prevailing reaction conditions.

The foregoing disadvantages have been circumvented through a series of developments with respect to the catalysts employed. Thus, platinum-group metals have been found to become more active and more selective if they are doped with lead [20], [21], selenium [22], thallium [21], or bismuth [21], [23]. The preferred carrier is activated charcoal.

The catalyst described in [23] permits conversion of a 2 M glucose solution to gluconic acid with oxygen at $50 \pm 1\ ^\circ C$ and pH 9.5 ± 0.2 (neutralization with 40 % sodium hydroxide solution) in >99.5 % yield within 1 h. The process is also highly selective: product purity (based on sodium gluconate) is >99.5 %. In addition, no special effort need be taken to purify the glucose solution. Loss of catalytic activity is minimal, permitting the catalyst to be recycled many times without an intervening reactivation step.

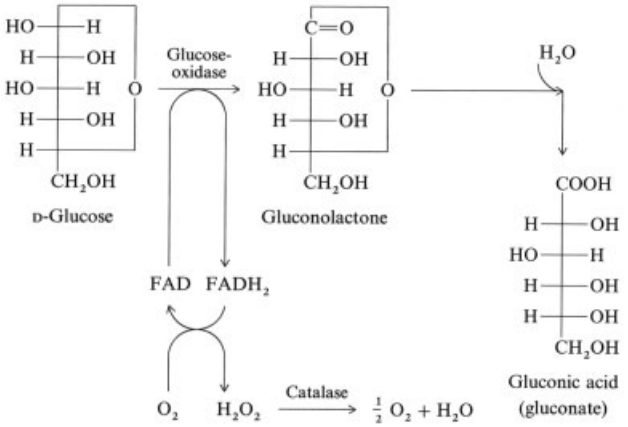
The economic viability of the process depends largely on the activity, selectivity, lifetime, and cost of the catalyst, as well as on the measures required for product purification and the energy demands.

Despite earlier suggestions to the contrary, glucose cannot be oxidized photochemically to gluconic acid [24].

4.4. Methods Involving Biotechnology

The principal organisms employed today for large-scale biological preparation of gluconic acid are *Aspergillus niger* and *Gluconobacter suboxydans.*

Aspergillus Niger Process. The biochemistry of gluconic acid formation by *Aspergillus niger* is illustrated in the following diagram [25], [26]:



where FAD and $FADH_2$ are the oxidized and reduced forms, respectively, of flavine – adenine dinucleotide. β-D-Glucose is oxidized catalytically to glucono-1,5-lactone by the enzyme glucose oxidase (see also → Enzymes). Subsequent hydrolysis of the lactone to gluconic acid is to some extent spontaneous, but the enzyme gluconolactonase also plays a role.

Glucose oxidase (E.C. 1.1.3.4) [*9001-37-0*], M_r 186 000, contains two FAD moieties as prosthetic groups. These are responsible for the abstraction of hydrogen atoms from glucose (to form $FADH_2$), which ultimately combine with oxygen to produce hydrogen peroxide. This is in turn cleaved to water and oxygen by the enzyme catalase. Both glucose oxidase and catalase are present as endoenzymes in the fungal mycelium [27], [28].

In addition to glucose oxidase, *Aspergillus niger* may have other enzymes that also lead to gluconic acid formation, comparable to the glucose dehydrogenase and phosphomonoesterases found in *A. oryzae* [29], [30].

Metabolic investigation has shown fermentation to be a three-stage process [31]: a brief lag phase, followed by an acceleration phase during which glucose oxidase activity rises nearly logarithmically, and finally a stationary phase, which is characterized by a constant rate of gluconic acid formation. The optimum pH for the reaction lies near 5.6 [32].

Production Parameters. Fermentative synthesis of sodium gluconate requires a sterilized substrate; a typical formulation is as follows: glucose 250 – 350 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 – 0.3 g/L, KH_2PO_4 0.2 – 0.3 g/L, and $(NH_4)_2HPO_4$ or urea 0.4 – 0.5 g/L. The substrate may be sterilized either in batches at 110 °C with a residence time of 45 min or continuously under conditions providing several minutes exposure to a temperature of 135 – 150 °C. The sterile medium is then transferred to the fermentation vessel, adjusted to a pH of 4.5 – 5.0, and inoculated with cultured microorganism.

The culture medium contains only about 100 g of glucose per liter, but as much as twice the above amounts of nutrient salts, an increased level of nitrogen compounds, and a growth-stimulating additive consisting of 0.2 – 0.4 g of corn steep powder (a residue obtained by evaporating the water in which maize has been soaked). A lyophilized permanent culture is used to grow the conidia. This culture is first activated with a specific growth medium in culture tubes. After several subcultures have been prepared, the organism is introduced into a culture flask

containing a special medium that encourages the formation of conidia. After an incubation period of 5 – 10 d the conidia are harvested, either in dry form or as an aqueous suspension, and used to inoculate the preculture.

During the production phase, the system is maintained at 30 – 32 °C and pH 5.5 – 6.5. The pH is controlled by continuous neutralization with 30 – 50 % sodium hydroxide solution, the consumption of which is an indication of reaction progress. Depending on the starting concentration of glucose, fermentation continues for a period of 40 –100 h. Under conditions of maximum specific rate of product formation, 20 – 30 mmol of gluconic acid is generated per hour for each gram of biomass (dry weight) [33], [34].

After fermentation is complete, the microorganisms are removed by filtration and washed. The filtrate is decolorized with activated charcoal, subjected to fine filtration, and then either evaporated and crystallized or directly spray-dried.

The fungal mycelium residue may be used for the isolation of glucose oxidase or it may simply be burnt [35].

Various attempts have been made to use mathematical models to optimize the above process [33], [34], [36], [37]. These efforts have shown that both the specific rate of oxygen uptake and the specific rate of formation of gluconic acid increase approximately linearly with increasing concentration of dissolved oxygen or increasing oxygen partial pressure. If the effects of the brief growth phase and retentive metabolism are ignored, the productive phase follows simple Michaelis – Menten kinetics, with K_M 3.6×10^{-4} mol/L at 33 °C and pH 6.6 [34]. At 25 °C and pH 5.6, $K_M = 2.0 \times 10^{-4}$ mol/L [32]. Isolated glucose oxidase under these conditions has K_M 4.8×10^{-4} mol/L.

To ensure economically viable yields of more than 80 %, an adequate oxygen supply (0.1 L of oxygen per liter of solution per minute) must be maintained, and gas distribution within the fermentor must be optimized. The oxygen partial pressure may be increased by conducting fermentation at elevated pressure or by employing air enriched in oxygen [38].

Product concentration in the fermentation solution can be increased if glucose is introduced at a higher concentration and pH control is discontinued near the end of the fermentation [39]. These conditions result in formation of a mixture of sodium gluconate and free gluconic acid, which has a considerably higher solubility than the salt alone. Such a solution (e.g., Naglusol) is suitable for commercial use in technical applications as soon as the mycelium has been removed.

Gluconobacter Suboxydans Process. The *Gluconobacter suboxydans* process is rather different, in that conversion of β-D-glucose to glucono-1,5-lactone occurs through the action of two glucose dehydrogenases: a particulate quinoprotein glucose dehydrogenase and a soluble NADP-dependent glucose dehydrogenase [40], [41].

Gluconobacter suboxydans fermentation is distinguished by a high affinity for oxygen relative to the *Aspergillus* process: simple aeration suffices to ensure a high rate of glucose conversion. In addition, the process displays greater tolerance with respect to acidity, permitting the isolation of free gluconic acid directly from the fermentation solution [42].

Other Methods. Another approach that leads directly to gluconic acid uses acidophilic methylotropic bacteria (*Acetobacter methanolicus*) under nonsterile conditions [43]. Other procedures have been described that involve immobilized enzymes, microorganisms, or both [44–47]. To date, none of these methods is used for commercial production of gluconate, largely because of rapid deactivation of the enzymes or limitations with respect to oxygen diffusion.

Downstream Processing. Ordinary commercial grades of free gluconic acid may be prepared from the corresponding sodium salt either through cation exchange or electrodialysis. Passage of a mixture of sodium gluconate and free gluconic acid over a strong anion-exchange resin results in adsorption of gluconate [48].

Glucono-1,5-lactone crystallizes from a supersaturated aqueous gluconic acid solution between 36 and 57 °C [49]. A two-step continuous crystallization is said to result in increased productivity per unit volume under conditions that are favorable in terms of energy consumption [50]. In the first step, a crystalline suspension (3 – 10 % crystalline material) is produced in a supersaturated gluconic acid solution held at

65 – 75 °C. The second step involves controlled cooling to 40 – 45 °C, followed by a period of crystal maturation at constant temperature. A portion of the resulting crystalline suspension is then recycled to the first step.

According to [51], the 1,5-lactone may also be obtained from an aqueous gluconic acid solution by dehydration involving azeotropic distillation with alcohols, followed by crystallization from the alcohol-containing residue.

Crystallization above 70 °C leads to the 1,4-lactone.

5. Uses

The primary applications of gluconic acid follow from its most important characteristic: it is a weak acid capable of dissolving the oxides, hydroxides, and carbonates of polyvalent cations without attacking metallic or nonmetallic surfaces. The value of this property is further enhanced by the fact that the acid forms water-soluble complexes with such cations. Gluconic acid is thus exceptionally well suited for use in removing calcareous and rust deposits from metals or other surfaces, including beer and milk scale on galvanized iron, magnesium alloys, or stainless steel. The compound is also used in the textile industry together with magnesium salts as a stabilizer for peroxide bleach baths.

Because of its physiological properties, D-gluconic acid has also been used in both the food and beverage and the pharmaceutical industries. Thus, low concentrations (0.02 – 0.1 %) of the substance effect the inversion of sucrose without causing the resulting fructose to undergo further reaction. Trace elements are usually administered in the form of gluconate salts because such compounds are readily absorbed into the body and are well tolerated. Potassium gluconate also has certain pharmaceutical applications and may be dispensed either in anhydrous form or as the monohydrate.

In many applications, gluconic acid 1,5-lactone is a convenient substitute for the free acid. The lactone offers particular advantages in circumstances involving acidic conditions over a long period, e.g., in the preparation of pickled goods and frankfurter-type sausages, or in curing fresh sausage. Another example is its use as a leavening agent in baked goods.

The most common gluconate is sodium gluconate, M_r 218.14, $[\alpha]_D^{25}$ +12.0 ° [10], whose solubility behavior is as follows:

t, °C	0	20	50	80	100
c, g/100 g H_2O	43	60	85	133	160

Sodium gluconate, like the free acid, forms complexes with metal cations. The stability of such complexes often increases considerably with increasing pH [13]. This salt also displays cleansing characteristics with respect to surfaces of widely varying type and structure.

Combinations of sodium gluconate with sodium carbonate or sodium hydroxide solution are useful for removing grease and corrosion from aluminum, rust from steel, oxide coatings from copper and copper alloys, and for etching aluminum. Strongly alkaline gluconate baths permit the removal of zinc coatings from metallic objects, castings, and scrap metal. Such baths are characterized by a high zinc capacity and a correspondingly long life.

Alkaline sodium gluconate solutions at 95 – 100 °C are effective agents for the rapid removal of paint and varnish, without damaging underlying surfaces. Gluconate is also useful in the pretreatment of certain surfaces, e.g., in the galvanic deposition of nickel – cobalt brazing surfaces onto aluminum or in the baths required for preparing smooth, shiny surface platings of nickel, tin, and zinc. In the latter application, gluconate is increasingly used to replace the highly toxic cyanide ion.

Mixtures of gelatin and sodium gluconate are used as sizing agents in the paper industry because they result in a product with increased acid resistance. Textile manufacturers employ gluconate (sometimes in combination with polyphosphates) for desizing polyester or polyamide fabrics and for finishing natural cellulose fibers.

Sodium gluconate is a component of commercial cleansers because of its absolute stability to hydrolysis at high temperature and high pH, as well as its sequestering properties with respect to hardening agents in water. For example, sodium gluconate is used in bottle washing and in cleaning aluminum surfaces (e.g., building facades, aircraft, and containers).

Concrete manufacturers have found sodium gluconate to be a highly effective agent for retarding the curing process. Addition of 0.02 – 0.2 wt % of this substance (relative to cement) produces a very homogeneous concrete with high resistance to water, frost, and cracking. This material is also easily worked and shows increased stability upon setting. Other advantages accompanying the introduction of gluconate include better flow properties for the wet concrete mixture, increased wettability with respect to iron reinforcing structures, and maintenance of plasticity despite reduced water content.

Relative to other organic and inorganic complexing agents, gluconic acid, its lactone, and its salts offer one major advantage: they are all destroyed effectively and quickly by biological wastewater treatment, whether adapted or not. The same applies [52] to the soluble complexes of gluconate with aluminum, copper, iron, and zinc, although biodegradation of the chromium gluconate complex occurs more slowly. Metal ions released during gluconate complex degradation are largely removed from the purified wastewater, either by precipitation or by adsorption on the sludge. This circumstance reduces a major threat that might appear to accompany the use of gluconate as a complexing agent: mobilization or remobilization of heavy metals into surface water [53].

Destruction of heavy-metal gluconate complexes in wastewater may occur not only through biodegradation but also by simple hydroxide precipitation [54], in which iron(III) acts as a precipitant at a pH of 9 – 10; see also [55].

6. Economic Aspects

Annual worldwide production capacity for gluconic acid and its derivatives is estimated to be 60 000 t. The bulk of production (85 %) is in the form of sodium gluconate and other alkali gluconate salts. Present consumption levels are, however, significantly lower, resulting in only 60 – 70 % utilization of existing capacity. Principal manufacturers include: Akzo-Chemie, Amersfoort, Holland; Biochemie Ladenburg, Ladenburg, Federal Republic of Germany; Finnish Sugar Co. Ltd., Espoo, Finland; Fujisawa Pharmaceutical Co., Osaka, Japan; Pfizer, New York, New York; Roquette Frères, Lille, France.

7. Physiology, Product Specifications

D-Gluconic acid and its 1,5-lactone are important intermediates in carbohydrate metabolism. These compounds serve two important functions: (1) they contribute to the synthesis of reduced nicotinamide – adenine dinucleotide phosphate (NADPH), which is required in the biosynthesis of fatty acids and steroids, and (2) they lead to the formation of ribose 5-phosphate, which is used in nucleic acid synthesis [56].

The gluconate salts of sodium [*527-07-1*] calcium [*299-28-5*], copper [*13005-35-1*], iron(II) [*299-29-6*], and manganese [*6485-39-8*] are on the list of GRAS substances [57], [58]. The gluconates of potassium [*299-27-4*], magnesium [*3632-91-5*], and zinc [*4468-02-4*] have not been shown to have teratogenic, mutagenic, or carcinogenic properties [56].

Under the food and beverage law of the Federal Republic of Germany, D-gluconic acid is classified as a foodstuff and not regarded as an additive. Purity standards for gluconate salts are prescribed in the U.S. Food Chemical Codex (FCC III) of 1981, the U.S. Pharmacopeia (1985), and the European Pharmacopeia. Methods of analysis can be found in [32] and [59].

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