SALICYLIC ACID CYCLOSYSTEM COMPLEX

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Contents

General

Enzymatic degradation of starch by means of α-glucosidase or α-amylase normally produces such molecules as glucose, maltose or maltotriose, besides a long series of straight or branched-chain malt-oligomers, known as dextrins. Dextrins are heterogeneous, amorphous, hygroscopic substances that find wide application in food, textile and paper industry. The degradation of starch that produces dextrins is more accurately described as hydrolysis, as the primary product of the broken glucoside bond reacts with one molecule of water.

If the starch is treated instead by the enzyme cyclodextrin glucosyltransferase (CGT), the primary reaction product undergoes an intramolecular reaction without water, to produce the α-1,4-cyclic compounds known as cyclodextrins.

The discovery of cyclodextrins dates back to the last century. Their long history can be subdivided into four basic periods.

- The first reference to cyclodextrins in the literature appeared in 1891. Before then, their physical structure was completely unknown and in no way could their potential uses be imagined. In 1891, Villiers isolated, from a culture medium of Bacillus amylobacter grown in the presence of starch, a small quantity of a crystalline substance that he sought to chemically characterize. Because this substance exhibited properties similar to those of cellulose, Villiers gave it the name "cellulosin".
- Between 1903 and 1911, Schardinger, was studying the heat resistance of certain microorganisms contaminating foodstuffs. He observed that the Villiers' "cellulosin" was formed by the putrefaction of amylaceous products by a bacillus. The bacillus responsible was isolated and named *Bacillus macerans*. It currently represents the main source of the enzyme by which cyclodextrins are industrially produced.
- However, cyclodextrins's structure was clarified only later by Freudenberg and his assistants. Thanks to their studies, scientific interest in cyclodextrins began to grow. There has followed a burst of activity in the literature on the toxicological properties of cyclodextrins and their potential production on an industrial scale.

Interest in cyclodextrins and their application has grown rapidly these past 15 years, above all in the pharmaceutical and food industries. Only a very few years ago did the cosmetic sector become interested in cyclodextrins. But their use has already spread throughout the world. About 750 patents were filed in 1986 alone, covering all fields of application; by June 1987, the total number of patents filed was 2,724. Recent work has studied cyclodextrin derivatives (CD): the various alkylated, hydroalkylated and polymerized α-CD pave the way for new and unexpected application possibilities.

In 1978 Japan became the first nation to develope a highly productive, low cost system to produce cyclodextrins by biotechnology. Besides Japan, the chief producers of cyclodextrins now include the United States, France and Hungary.

In 1988 the cyclodextrin market in the USA was US$50 million. According to the latest estimates carried out by the *Bioprod.Technology*, the potential market in the USA is around US$245 million. There are no data for the whole world, Europe or Italy. However, considering the American data and reflecting that the European and Italian markets generally develop a few years later than the American market, the European market can be expected to grow.

Chemical-physical properties

The cyclodextrins form a family of cyclic oligosaccharides with distinctive subgroups. Three of these groups are particularly well-known and are manufactured industrially, namely: the α-, β and γ-cyclodextrins.

The α-cyclodextrins (also known as the Schardinger α-dextrins, cyclomaltohexose, cyclohexaglucan, cyclohexaamylose, α-CD, ACD and C6A) are made up of a ring formed by 6 glucopyranose units. The β-cyclodextrins (also called as Schardinger β-dextrins, cyclomaltoheptose, cucloheptaglucan, cycloheptamylose, β-CD, BCD, C7A) have 7 glucopyranose units joined together. Lastly, the γ-cyclodextrins (Schardinger γ-dextrins, cyclomaltoctose, cyclo-octoglucane, cyclo-octylamylose, γ-CD, GCD) are made of eight glucopyranose subunits.

Cyclodextrins with less than 6 components per ring cannot be formed for steric reasons. On the other hand, some authors mention the existence of cyclodextrins with rings consisting of more than 8 units. For example, the k-cyclodextrins have a ring of 9 glucose units. However, this and the other larger-ring cyclodextrins have not been sufficiently studied and characterized. The rings of such cyclodextrins, if they really exist, should be very flexible. As the diameter of the cavity increases, it can contain a growing number of water molecules. Complex formation in such a system would not give a significant energy gain. Cyclodextrin cavities that are too large would not be able to hold the enclosed molecules with sufficient force as to prevent them from slipping away.

Sterically, the cyclodextrin molecule is of toroidal form, a small cylinder with a hydrophilic outer surface and a central hydrophobic central cavity of well defined size. The inside is non-polar because the hydroxyl groups, both primary and secondary, face outwards. When cyclodextrins form inclusion complexes, its molecule is of truncated conical shape rather than cylindrical, as the free rotation of the primary hydroxyl groups reduces the effective cavity diameter on the side where complexation takes place.

The approximate dimensions of the cyclodextrins, directly proportional to the number of glucose units, are schematically shown in Figure 1.

The conformation of cyclodextrins in solution is practically identical to their conformation in the crystalline state.

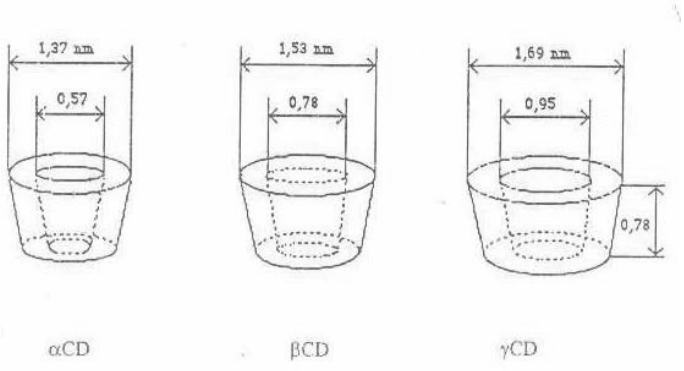

Figure 1: Molecular dimensions of cyclodextrins.

PHYSICAL PROPERTIES

Crystalline structure

The packing of cyclodextrin molecules inside a crystalline lattice can be of two different types: the molecules can be arranged to form either a "cage" (clathrate) structure or a "channel" (canal) structure (Figure 2).

In channel structures, the molecules are aligned one on top of another, just like coins in a roll. The enclosed molecules are, therefore, contained inside "infinite" channels, formed by the linear alignment of the cavities. Such alignment can be either "head to tail" or "head to head" (Figure 3).

In cage structures, the cavity of each cyclodextrin is blocked on both sides by the adjacent cyclodextrin molecules, thereby isolating individual cavities. In this type of crystalline lattice, the cyclodextrins can be arranged in crosswise, block or herringbone (the most frequent conformation in the α–, β-, and γ-cyclodextrins) patterns.

When cyclodextrins are crystallized from water, some water molecules are contained in the cyclodextrin cavities, while others are integral part of the crystalline structure. Cyclodextrins inclusion complexes are formed by substituting suitable enclosed molecules for the included water.

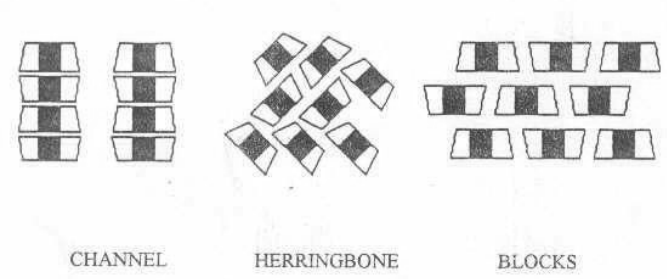

Figure 2: Types of alignment of the cyclodextrin rings in the crystalline lattice.

The same cyclodextrin can exist in various crystalline forms. For example, β-cyclodextrin hydrate has two different crystalline forms: β-cyclodextrin-12-H_2O and β-cyclodextrin-11-H_2O. Both forms have a herringbone lattice structure, with differences due to disordered distribution of the water molecules in the β-cyclodextrin cavity. There are also slight differences in the parameters of the crystalline lattice. The undecahydrate form converts into the more regular dodecahydrate form within a few weeks.

Cyclodextrins do not have a definite melting point, but they start to decompose from 200°C. Their exact thermal properties depend on the water content, crystalline structure, degree of heating and also atmospheric composition.

Properties in solution

Cyclodextrin solubility in water reveals strange behaviour patterns with significant temperature dependance (Table 1). While the solubility of β-cyclodextrin at room temperature is only 1.85 g / 100 ml., the α- and γ-cyclodextrins exhibit appreciably higher solubility: 14.5 and 23.2 g / 100 ml. respectively.

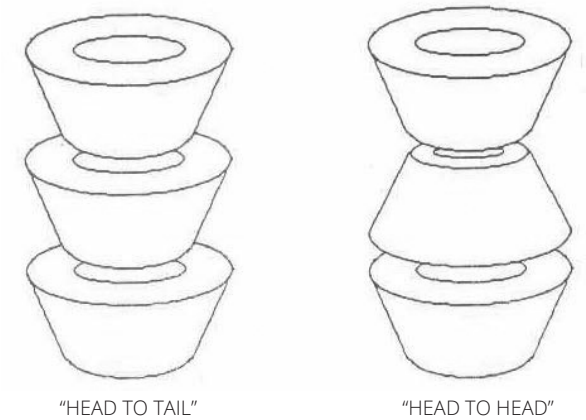

Figure 3: "Head to tail" and "head to head" channel structures.

Cyclodextrin solubility normally decreases in presence of organic molecules owing to the formation of complexes.

In aqueous solution, the cyclodextrin cavities are not empty spaces. The volume of the cavities present in 1 g.of α-cyclodextrin is about 0.1 ml. To keep this much space empty would require 65 kcal / mole; cyclodextrins of larger diameter would require even more energy. Also, if the cavities remain empty, the specific partial volumes of the cyclodextrins in solution would be greater than those of glucose or maltose, which is not the case.

The viscosity of an aqueous cyclodextrin solution does not differ appreciably from that of water. Measured at 25.1 °C, the viscosity of pure water is 8.93 mP, while that of 9.5 x 10^{-4} molar α-cyclodextrin is 8.99 mP and that of 9.98 x 10^{-3} molar α-cyclodestrin is 9.436 mP.

Zurbach (1985) has studied the dielectric properties of cyclodextrins in aqueous solution. According to this author, the relative dielectric constant of the cyclodextrin cavities depends not only on the inside diameter of the cavities but also on the dimensions of the included molecule.

Many of the cyclodextrins' dieletric properties depend on their degree of hydration Conductivity increases with increasing hydration. The conductivity of cyclodextrin hydrates is appreciably greater than that of ice.

t ° C	Solubility (mg CD / g water)		
	α	β	γ
20	90	16.4	185
25	124	18.8	256
30	165	22.8	320
35	204	28.3	390
40	242	34.9	460
45	285	44.0	585
50	347	52.6	–
55	–	60.5	–
60	–	74.9	–
65	–	101.8	–
70	–	120.3	–
75	–	148.0	–
80	–	196.0	–

Table 1: Solubility of cyclodextrins in water at various temperatures, according to Jozwiakowskj and Connors, and Wiedenhof and Lammers.

CHEMICAL PROPERTIES

Cyclodextrins do not possess reducing terminal groups. Generally speaking, they react as non reducing carbohydrates.

The resistance of cyclodextrins to degradation in alkaline solution is similar to that of cellulose.

When the crystalline forms of β- and γ- cyclodextrins are exposed to β-rays, the molecules split mainly at the 1.4-glucoside bond. However, the mechanism is different from that of acidic hydrolysis.

Partial acidic hydrolysis of cyclodextrins produces glucose and a series of acyclic malt-saccharides. This series includes oligosaccharides containing as many glucose units as the original cyclodextrin. The stability of the entire ring against acidic hydrolysis is from 2 to 5 times higher (depending on the temperature and acidity) than that of the acyclic dextrins. Opening the ring by breaking the first glucoside bond is a slower process than the hydrolysis of the acyclic malt-oligosaccharides thus formed.

This can be explained by looking at the hydrolysis of oligo- and polysaccharides. Here, the glucoside bonds of the terminal glucoside units are split more quickly than the bonds between non terminal units. As the cyclodextrins do not contain terminal glucoside units, the activation parameters of the α-1.4-glucoside bonds that form the ring must be different from those of the analogous bonds in the acyclic dextrins.

Cyclodextrin enzymology

ENZYMATIC DEGRADATION OF CYCLODEXTRINS

One of the most remarkable properties of the cyclodextrins is their notable resistance to the enzyme that catalyze starch hydrolysis. In fact, cyclodextrins are totally resistant to β-amylase as they do not contain groups susceptible to attack by this enzyme. The α-amylases, which do not require the presence of free terminal groups, are able to hydrolyze cyclodextrins, although normally only a small percentage, by attacking the molecule from the inside. With few exceptions, the cyclodextrins cannot be fermented and cannot be used by yeasts.

Cyclodextrin-glucosyltransferase

Cyclodextrin-glucosyltransferase or α-1.4-glucane-4-glucosyltransferase, also known as CGT (cyclodextrin-transglucosylase enzyme) is produced by various organisms. This enzyme, besides catalyzing the degradation of starch into cyclodextrin, also catalyzes coupling and other reactions.

Various chemical cyclodextrin syntheses recently published are purely scientific works. Currently, cyclodextrins can only be produced on a commercial scale by the enzymatic degradation of starch.

The origin of the CGT enzyme determines the ratio of the cyclodextrins formed. The proportion between α: β: γ of the cyclodextrins is as follows:

CGT	from *B. macerans*	2.7	:	1.0	:	1.0
	from *B. megaterium*	1.0	:	2.4	:	1.0
	from *Bacillus Sp* No.38-2	1.0	:	11.0	:	1.5

However this ratios are not fixed. Most CGTs initially form α-cyclodextrin; β and γ cyclodextrins have much slower rates of formation. β-cyclodextrin is produced at the expense of α-cyclodextrin during secondary transfer reactions. Consequently, the incubation time determines which cyclodextrin forms the main product. The ratio of the cyclodextrins formed is modified during industrial processes through continuous extraction of the required cyclodextrin.

The main difference between the action of CGT compared with other enzymes that process starch is that CGT's products are not reducing. However, in practice, preparations of raw CGT always contain a certain quantity of other amylotic enzymes, which reduce part of the available substrate and, therefore, the production of cyclodextrins. Hence the raw enzyme must always be purified.

Stored under the correct conditions, CGT is very stable. CGT used in industry can be preserved in solution for months without significant loss of activity.

In 1891, when Villiers first described the preparation of a cyclodextrin by cultivating *Bacillus amylobacter (Clostridium butyricum)* on starch, it was probably an impure culture. In 1903 Schardinger described Bacillus macerans as a source of CGT.

Six bacteria are known to produce extracellular CGT. Of these, four are bacilli.

They are all inducible organisms, which means they produce the enzyme when grown on starch or its derivatives. A list of organisms producing CGT is given in Table 2.

Optimum growing conditions for the chosen bacterial stock should be established for industrial use. The two microorganism stocks most commonly used for industrial CGT production are Bacillus macerans and Alkalophilic bacillus No. 38-2.

	Optimum pH	Opt.t°C	Mol.wt	Isoel.Pt
B. macerans IFO 3490	5.0 – 5.7	55	65000	4.6
B. macerans IMA 1243	6.0	60	14500	
B. macerans ATCC 8514	6.2		193300	
B. macerans CHINOIN	5.9	60	72000	4.45 – 4.65
B. megaterium	5.0 – 5.7	55	66000	6.07 – 6.80
B. stearothermophilus	5.0 – 5.5			
B. circulans	6.0 – 6.5			
Klebsiella pneumoniae	5.2			
Alkalophilic bac.No.38-2	4.5 – 9.0	45 – 50	85 – 88000	5.4
Alkalophilic bac.No.17-1	5.0 – 9.0			
B. ohbensis	5.5			
Micrococcus varians	6.0	50 – 60		

Table 2: Properties of CGT enzymes of various origin.

Cyclodextrin production

Cyclodextrin preparation consists of four main phases:
I – Cultivation of a microorganism producing the CGT enzyme
II – Separation of the enzyme from the culture medium for concentration and purification;
III – Enzymatic degradation of prehydrolyzed starch to a mixture of cyclic and acyclic
dextrins;
IV- Separation of the cyclodextrins for purification and crystallization.
From the vast amount of literature dealing with cyclodextrin production, the following conclusions can be drawn:

- A higher concentration of the substrate (starch) leads to lower running costs but also to less cyclodexstrins. The optimum starch concentration (about 30 %) therefore represents a compromise.
- CGT produces three main types of cyclodextrins (and insignificant quantities of glucosil-cyclodexstrins). The relative percentage of each depends on the degradation time and the enzyme used. The proportions can also be strongly influenced by the reaction conditions, (above all, by the addition of complexing agents, e.g.toluene).
- When inorganic complexing agents are present, a mixture of cyclodextrins is formed; the production of crystalline cyclodextrin is low. Separation procedures to obtain the pure products from such a mixture of cyclodextrins proves uneconomical on an industrial scale.
- Controlled degradation, producing mostly a single cyclodextrin, is only economically feasible when complexing agents are used.
- When an organic complexing agent (e.g. toluene) is used to increase cyclodextrin production, the residual complexing agent content in the refined cyclodextrin should be below the maximum allowable limit, whether that be ppm or even ppb.

Cyclodextrin derivatives

Cyclodextrins can be modified by various procedures:

- Substituting one or more hydrogen atoms in the primary and / or secondary hydroxyl groups (esters, ethers, glucosil -cyclodextrin);
- Substituting one or more primary and / or secondary hydroxyl groups;
- Eliminating the atoms of hydrogen from the group C_5-CH_2OH (C_5COOH);
- Breaking one or more C_2-C_3 bonds through oxidation with periodate.

Many cyclodextrin derivatives are finding application in industry because of the specific properties these modifications can produce.

Cyclodextrin inclusion complexes

Cyclodextrins are able to interact with a variety of ionic and molecular species. The resultant inclusion compounds (Figure 4), combination of a the host molecule and an enclosed molecule, are also known as “host-enclosed” complexes.

A distinctive characteristic of the host molecule is that its binding sites are oriented along the same direction in space. The bond sites on the enclosed molecule, by contrast, diverge. To form an inclusion complexe, the binding sites of the host and an enclosed molecule must be stereoelectrically complementary. The formation of bonds between these converging and diverging sites create a complex of the “host-enclosed” type. The term inclusion (“Einschlussverbindung”) was introduced by Schlenk in 1950.

An included material sits inside the host molecule without significantly modifying its structure. Retention essentially unaltered cavity dimensions and shape is a distinctive characteristic of inclusion complexes.

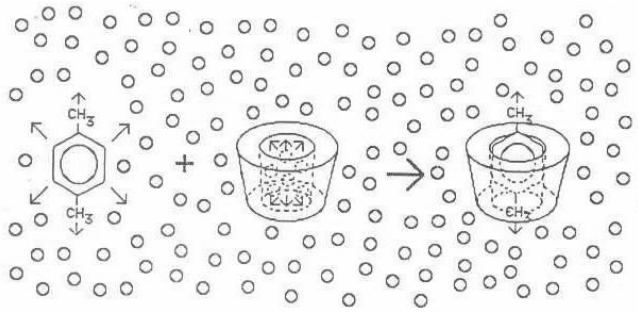

Figure 4: Schematic representation of the formation of a cyclodextrin inclusion complex. p-Xylene is the enclosed molecule, while the circles represent the water molecules.

Cyclodextrin complexes in cosmetics

For some 15 years now, cyclodextrins have been finding wide application in the pharmaceutical and food industries.

Recently, cosmetic manufacturers have begun to use cyclodextrins for the "molecular encapsulation" of active substances solely for cosmetic purposes. Such studies were first conducted in Japan, Hungary and France.

Cyclodextrins have a natural (biological) derivation. They are also innocuous to humans, whether taken internally or applied topically. Particularly attractive to cosmetic formulators is cyclodextrins' capacity to act as "host" molecules for a large variety of substances. There are several advantages to the cyclodextrins' molecular encapsulation, not all of which are present with other, classic encapsulation processes:

- The "enclosed" substance is totally or partially included in the cavity; and thus isolated from the external environment, protecting it from oxidation and degradation.
- Because the inclusion reaction is reversible and the resultant complexes can have a crystalline structure, they are not sensitive to pressure stress.
- Cyclodextrin inclusion inhibits the natural decomposition of the enclosed active substance, thereby boosting its effect over time.
- When certain active ingredients are encapsulated temporarily, they exhibit "controlled release" properties, which translate into improved bioavailability.

Various research has demonstrated an improved or greater activity of the complexed active substance compared with the same substance in the free state. In one of these papers (Zanotti 1990), the activity of a natural tocopherol (δ-tocopherol) was investigated. δ-Tocopherol, an easily oxidized compound, neutralizes free radicals that can initiate chain reactions that form compounds capable of causing skin irreversible harm. By itself, δ-tocopherol is a very active antiradical agent. Complexed with cyclodextrins, it exibits a greater activity than free δ-tocopherol. Moreover, this activity is maintained for a much longer time. On the basis of these results, cyclodextrins are considered suitable not only for skin treatment products but also for makeup, which stays on the face for many hours.

In the near future, an even greater use of cyclodextrins, as stabilizers, emulsifiers and deodorants, can be expected in personal-care products.

Most perfume concentrates, such as rose oil, form complexes with cyclodextrins. Typically citral and citronella form complexes and can be used in any solid preparation, such as powdered detergents. The possible irritant effect of a fragrance, such as eye irritation caused by the scent in a shampoo, can be reduced using β-cyclodextrin. For example, in Japan, there is widespread use of toiletries scented with lemon oil and fluorescein, both complexed with β-cyclodextrin. A solution of β-cyclodextrin in ethanol / water, or the iodo-β-cyclodextrin complex can be used as deodorant for the human body, added to baths or applied as a spray. A cyclodextrin solution can be used as a mouth-refreshing rinse after a highly aromatic foods, such as fish or garlic, have been eaten. The iodo-β-cyclodextrin complex is also suitable for this purpose. A stable anti-plaque toothpaste can be obtained by adding a small quantity of β-cyclodextrins to a normal toothpaste formula.

β-Cyclodextrin can be used in emulsion products, such as face lotions, for their absorbent properties. The oils for these emulsions can include iso-propyl myristate, yellow wax, olive oil and other seed oils, ascorbyl stearate, glyceryl-monostearate, etc. A complexed lipophilic substance is much more water-soluble than when not complexed, allowing a much higher concentration of that substance in an aqueous phase.

If royal jelly is mixed with β-cyclodextrin and water, it can be dried as granules. The physiological activity of this product is not reduced after 100 days of preservation at room tem-

perature. Many other physiologically active substances, such as camomile oil, normally used in cosmetics, can likewise be stabilized by using cyclodextrins.

Absorbent powders, such as bath- and baby-powder, consist of the basic powder, suitable diluents, one or two active ingredients, and / or scents and dyes. The basic absorbent powders or diluents can be inorganic (talc, kaolin, magnesium carbonate, calcium carbonate and zinc oxide) or organic, like starch.

The diluents not only serve to increase the volume or as support, but they should be able to absorb moisture and / or lipids and adhere to the skin. They must not be contaminated by microorganisms; sometimes must be sterile. They should possess well defined rheological properties.

Starches are good absorbents. However, they provide excellent nutrient media for almost all the microorganisms normally found on human skin. Until cyclodextrins became commercially available, there was no known organic substance that could be a good substitute for starch. An important precondition for the use of cyclodextrin complexes in absorbent powders is that these cyclic oligosaccharides do not constitute a growth medium for microorganisms, especially yeasts and fibrous fungi. Cyclodextrins are not only able to substitute for starch but can add useful new properties to absorbent powders.

Most absorbent powders, especially cosmetics, contain fragrances. The volatility and poor solubility of the scents when added to the powders can lead to reduced duration in the long term as well as losses. Samples of absorbent powders preserved in open Petri dishes at room temperature lost most of their fragrance after 6 weeks. Products containing cyclodextrin complexes showed a much better fragrance preservation. When these powders are applied on human skin, the scents are released to give the desired fragrance. Conventional formulations no longer release fragrance 6 weeks after being opened.

A β-cyclodextrin solution applied to human skin has proved an efficient anti-acne agent. The empty" β-cyclodextrins in absorbent powders complex with the polyunsaturated fatty acids in sebum excreted on the skin surface, thereby preventing their oxidation and inhibiting free-radical formation, which also reduces the incidence of infections and inflammations.

The degree of dissolution of a complexed substance is much greater than that of a non-complexed substance. Normally a much higher concentration of dissolved substance in aqueous phase is reached.

Scents complexed in cyclodextrins can be used in solid perfumes, scented sticks, incense and detergents. The cyclodextrins form complexes with the detergent molecules, and can act as anti-foam agents. Their addition to rinse water fixes the last traces of detergent; this anti-foam effect can be used to reduce water consumption.

A considerable part of perfumes used in washing powders is lost during the product manufacturing and storage. When complexed with cyclodextrins, these perfumes must be removed from the detergent molecules and released on contact with water. However, not all detergents produce good enclosed molecules which can be complexed.

The scents are given off much more easily from their complex through the use of active agents having an anionic surface, while oxides of non-ionic aliphatic ethylene and substances derived from quaternary ammonium do not cause sufficient releases. The degree of evaporation of dry complexes depends more on the relative humidity than on the temperature. Under normal conditions of preservation, complexing of cyclodextrins with perfumes brings about a good stabilization when mixed with powered detergent and almost the entire quantity is released on contact with water. Thanks to all these interesting properties, cyclodextrins are therefore expected to become one of the fundamental components in highly efficient and high quality cosmetic products.

Formulation with cyclodextrin complexes (CYCLOSYSTEM COMPLEX®)

Cyclodextrin complexes come in two forms. The α-, β- or γ -cyclodextrins are hydrodispersible powders, in which the guest molecules are incorporated. Cyclodextrins derivatives, especially hydroxypropyl β-cyclodextrins and hydroxyetyl β-cyclodextrins provide liquid, hydrosoluble cyclodextrin complexes.

As their chemical-physical characteristics and inclusion modality differ from the unmodified cyclodextrins, cyclodextrin derivatives have been the object of several studies.

Compared with the unmodified form, cyclodextrin derivatives generally present a substantial increase in water solubility, maintaining the same complexing properties. The increased hydrosolubility makes them particularly suitable for application in various sectors, including cosmetics.

β-cyclodextrin modified by adding alkylic groups becomes particularly soluble in water. Hydroxypropylic and hydroxyethylic groups are generally used. Normally between 3 and 18 molar substitutions are carried out. The polarity of the molecule, and consequently water solubility, increases according to the substitutions number.

Cyclodextrins derivatives (for example hydroxypropyl-β-cyclodextrin) form hydrosoluble complexes with hydrophobic active principles, such as vegetable oils, vitamins, etc., making possible to insert into aqueous products (tonics, gels) active principles which would otherwise not be soluble in water.

The inclusion of a hydrophobic molecule gives the hydroxyalkyl-cyclodestrin complexes an amphypathic nature, because of which they behave in water as phospholipids do: initially disperse forming micelle like structures, with polar groups, made up of cyclodextrin hydroxyalkyl rings, turned outside towards water, and hydrophobic tails, made up of the apolar chains of complexed molecules, turned inside.
Figure 5 schematically illustrates the behaviour in water of Vitamin A complexed with hydroxyalkyl-β-cyclodextrin. An aqueous solution of hydroxyalkyl-cyclodextrin complexes initially present a lactescent aspect which tends to clear up in time.

According to the kind of final product in which the complex has to be included, one form or another will be chosen. In powder products and in emulsions where hydrosolubility of the active principle included is not necessary, the dry, powder-cyclodextrin complexes will be definitely used. In gels or hydroalcoholic tonics, the liquid, hydrosoluble works better. In both cases, the cyclodextrin complexes are added, cold, in the final phases of the production process.

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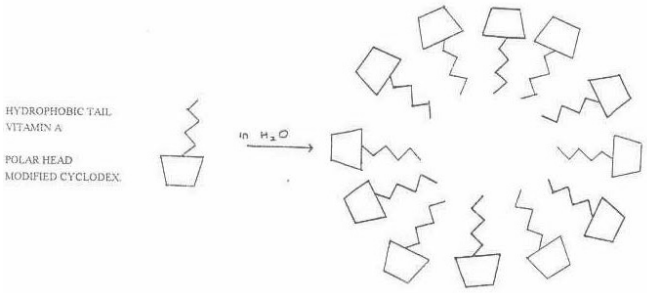


Figure 5: Behaviour in an aqueous solution of a complex between hydroxyalkyl-β-cyclodextrin and Vitamin A.

Product features

SALICYLIC ACID

Salicylic acid (CAS No. 69-72-7) is a beta hydroxy acid. This colorless crystalline organic acid is widely used in organic synthesis and functions as a plant hormone. It is derived from the metabolism of salicin. In addition to being a compound that is chemically similar to but not identical to the active component of aspirin (acetylsalicylic acid), it is probably best known for its use in anti-acne treatments. The salts and esters of salicylic acid are known as salicylates.

Salicylic acid has the formula C6H4(OH)COOH, where the OH group is adjacent to the carboxyl group. It is poorly soluble in water (0.2 g / 100 ml H_2O at 20 °C). Aspirin(acetylsalicylic acid or ASA) can be prepared by the esterification of the phenolic hydroxyl group of salicylic acid.

Salicylic acid is an organic acid biosynthesized from the amino acid phenylalanine.

Sodium salicylate is commercially prepared by treating sodium phenolate (the sodium salt of phenol) with carbon dioxide at high pressure (100 atm) and high temperature (390K) -a method known as the Kolbe-Schmitt reaction. Acidification of the product with sulfuric acid gives salicylic acid:

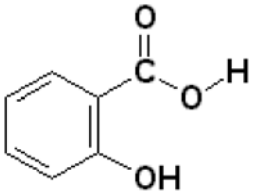


Figure 1: Chemical structure of salicylic acid

It can also be prepared by the hydrolysis of Aspirin (acetylsalicylic acid) or methyl salicylate (Oil of Wintergreen) with a strong acid or base.

Alpha-hydroxyacids are produced by all organisms through glycolysis, the anaerobic degradation of glucose to lactate, and through cellular respiration (cycle of the tricarboxylic acids), characteristic of the aerobic organisms. Both glycolysis and respiration are processes which supply the organisms with the energy necessary for all their functions. Therefore, alpha-hydroxyacids play an important role in the utilisation of energy by living cells.

The main natural sources of salicylic acid are salix.

Applications

Cosmetics

Salicylic acid is used as an exfoliant in various cosmetic formulations, as a moisturiser and anti-wrinkle agent in face and body products. Salicylic acid infact is a key ingredient in many skin-care products for the treatment of acne, psoriasis, calluses, corns, keratosis pilaris, and warts.It works as a keratolytic by causing the cells of the epidermis to shed more readily, preventing pores from clogging up, and allowing room for new cell growth. Because of its effect on skin cells, salicylic acid is used in several shampoos used to treat dandruff. Use of concentrated solutions of salicylic acid may cause hyperpigmentation on unpretreated skin for those with darker skin types (Fitzpatrick phototypes IV, V, VI), as well as with the lack of use of a broad spectrum sunblock.

The United States Food and Drug Administration recommends the use of sun protection when using skincare products containing salicylic acid.

OTC preparations containing salicylic acid used for cleansing of the skin, tonics, creams and astringents, contain 4 – 12 % of salicylic acid partially neutralised.

Dermatology
In dermatology, salicylic acid is the most commonly used substance to induce chemical superficial "peeling" (Murad and Shamban, 1994). For such use, concentrations from 50 to 70 % are used (Jackson 1994a; Dial, 1990). Some authors report the use of salicylic acid in anti-acne treatments, alone (Van Scott and Yu, 1989).

Proposed mechanisms of action

So far, the mechanism of action of the AHA and of salicylic acid in particular has still not been clearly defined. In this regard various hypotheses have in fact been formulated:

1. AHA could control the formation of the corneus layer by reducing cell cohesion between corneocyte of new formation of the deepest layers and the granular layer. It is unlikely that AHA promotes disaggregation of the corneocyte of the mature upper layers of the corneus layer (Van Scott and Yu, 1984; Yu and Van Scott,1994). According to these researchers the influence of the AHA on the cohesion with corneocyte seems to be due to their action on the ionic bonds.
2. The AHA are acids, therefore their pH may induce cutaneous irritation, with clinical (products at high concentration) or subclinical implications (products at low concentration) (Jackson, 1993; Jackson 1994a). Irritation would stimulate the germinative layer of the epidermis, inducing an increase in the epidermic turnover, with accelerated exchange of the corneus layer.
3. Another mechanism proposed is an extension of the theory of cellular proliferation (Jackson, 1993). Exposure to chemical irritants induces a certain edema of the exposed tissues and this could contribute to reducing the signs of ageing (and thin wrinkles).

Side effects, reactions induced after use

Irritations, for example, burning, ambustion, itch, tingling, weeping, in response to external use of AHA containing products, were observed on consumers and on voluntary subjects during laboratory testing (Jackson 1994 a b). Serious irritation, like erythema or edema, were noted occasionally.

The most common side effect following the application of salicylic acid at high concentrations is a persistent erythema. Usually the patients are advised of the possibility of scabs formation and of erythema lasting a week. When severe peeling occurs, the scabs and the erythema can persist for 2 – 3 weeks.

The application of a partially neutralised solution of salicylic acid, composed of 43 % free acid and 5 % ammonium glycolate in a hydroalcoholic base, with pH adjusted to 2.75, can cause burning.

A 70 % solution of salicylic acid induces serious burning of the skin of the eyes. The effect is notably reduced using a 20 % solution.

SALICYLIC ACID 50 %

Cyclosystem complex® in dermocosmetics

The Cyclosystem complex® of salicylic acid 50 % is an inclusion complex for controlled release, in which the host molecule is constituted by β-cyclodextrin, and the guest molecule is salicylic acid, at 50 % concentration.

The controlled release of salicylic acid enclosed in the Cyclosystem complex® determines:

- a greater bio-availability of salicylic acid, with a consequent increase in its exfoliating and cellulal renewal actions;
- a lower concentration of free salicylic acid, with a consequent reduction of its irritation index.

The results of efficacy and irritation tests described in this article show how the inclusion of salicylic acid in cyclodextrins in the Cyclosystem complex® reduces the irritating side effect, increasing at the same time its activity.

The Cyclosystem complex® of salicylic acid 50 % validly substitutes free salicylic acid in all its applications. Its properties make its use particularly advantageous compared to non-complexed salicylic acid. The Cyclosystem complex® of salicylic acid 50 % shows greater efficacy and lower irritation index compared to free salicylic acid at the same concentration and pH. Thanks to this, the Cyclosystem complex® of salicylic acid 50 % gives the same results as free salicylic acid at lower concentrations and less acid pH, reducing drastically the side effects.

Once applied to the skin, the salicylic acid contained in the Cyclosystem complex® of salicylic acid 50 % is released gradually, thanks to the controlled release property of cyclodextrins. The salicylic acid in direct contact with the skin is in part complexed with cyclodextrins and in part in the free form. The concentration of the complexed form (Cyclosystem complex® of salicylic acid) is at equilibrium with the concentration of the free form. This equilibrium is regulated by the availability of free salicylic acid.

Cyclosystem complex® of Salicylic Acid

SALICYLIC ACID

Therefore, while free salicylic acid is adsorbed, the equilibrium shifts towards the right and new salicylic acid is released from the complex with cyclodextrins. The more the skin presents impurities, the greater is the consumption of salicylic acid and the quicker is its release from the Cyclosystem complex®.

This behaviour has two consequences: in the first place the availability of salicylic acid is prolonged in time; in the second place free salicylic acid is present at all times at low concentration. An increased availability corresponds to a greater efficacy of the active principle, while a lower concentration of free salicylic acid present at all times on the skin corresponds a lesser aggressivity of the product and therefore to a reduced irritation index.

Among the benefits for the skin deriving from the use of the Cyclosystem complex® of salicylic acid 50 % we observed a visible reduction in the facial wrinkles, a softer and smoother skin and a better and healthier aspect. These benefits derive from the lighter exfoliating action of salicylic acid, which facilitates the removal of the dead superficial cells of the epidermis.

The Cyclosystem complex® of salicylic acid 50 % is particularly indicated in the formulation of products for thickened, asphyxiated skins and in need of particular cleansing treatments. It restores the normal thickness of the skin, it makes it clear, luminous and smooth without causing any type of irritation and moisturising it at the same time. The Cyclosystem complex® of salicylic acid 50 %, thanks to its exfoliating action, also prepares the skin for subsequent nourising treatments, thus allowing a greater absorption of the active principles contained in the product.

Comparative stability test

AIM OF THE STUDY

The aim of the investigation was to evaluate the long-term stability of salicylic acid, comparing the product under the free form and as Cyclosystem complex, both included in the cosmetic formulation "Whitening Gel with Salicylic acid 50 % Cyclosystem complex and 1 % Glycolic Acid 50 % Cyclosystem complex.

Materials and methods

Test products

A) Whitening Gel with 4 % Salicylic Acid 50 % Cyclosystem complex .

B) The same gel containing 2 % Salicylic Acid in the free form instead of Cyclosystem complex (2 % difference as water)

The gel had the following composition:

Whitening Gel composition	%
Water	88.8*
Salicylic Acid 50 % Cyclosystem complex	4*
Acritamer 940	2.5
Glycerin	3
Irapres F	0.5
Iracide	0.5
Sodium metabisulfite	0.4
EDTA-Na2	0.2
J92-8655 Essence	0.1
20 % NaOH solution	suff. to pH 4.5

*90.8 % water and 2 % free salicylic acid in the formulation B).

Both products were provided by IRA, Istituto Ricerche Applicate- Usmate Velate (MB)-Italy.

pH

The pH of the test products was 4.5.

Method

The stability was investigated by visual observation, evaluating aspect and colour of both products exposed at the same temperature for determined times. Two kinds of tests were carried out: in both cases the evaluation was after 3, 6, 9 and 12 months of temperature treatment.

Thermal Shock (TS)

Each test product was divided into 2 aliquots, assigned to be exposed respectively to the following temperature conditions:

A) 45/4°C at alternate weeks

B) 60/4°C at alternate weeks.

Room Temperature (RT)

Each test product was permanently exposed at 25°C.

Results

Observations at the different times of treatment under thermal shock or room temperature condition are summarized in the following table.

Conclusions

The comparison between the two whitening gels (WG), containing salicylic acid in the free form or as Cyclosystem complex, subjected to different thermal stresses showed that, when included in the Cyclosystem complex, salicylic acid was much more stable than free salicylic acid. This was clearly evidenced by the colour of the gel containing salicylic acid as Cyclosystem complex, which remained perfectly white for 9 months at room temperature and degraded more slowly of the gel containing salicylic acid in the free form when subjected to moderate or extreme thermal stress.

On the contrary, the gel containing salicylic acid in the free form rapidly changed its colour when subjected to extreme thermal shock, becoming darker and darker so much that at 9 months it has gone bad; similarly, it deteriorated faster also at moderate thermal shock and room temperature, presenting a clear beige colour after 6 months at room temperature and a brown colour after 3 months after the 60/4°C temperature treatment.

Temperature treatment	Test product	Time (months)			
		3	6	9	12
45/4°C	WG + 2 % Salicylic.	Beige	Beige	Brown	Gone bad
	WG + 4 % Salicylic. CC	White	Clear Beige	Beige	Brown
60/4°C	WG + 2 % Salicylic.	Brown	Brown	Gone bad	Gone bad
	WG + 4 % Salicylic CC	Clear Beige	Beige	Brown	Gonde bad
25°C	WG + 2 % Salicylic	White	Clear Beige	Beige	Brown
	WG + 4 % Salicylic CC	White	White	White	Beige

How to formulate with CC Salicylic acid 50 %

The use of the Cyclosystem complex® of salicylic acid 50 % is very simple.

It is cold inserted in the final phases of synthesis of the product, whether in emulsion, gel or fluid form.

Following is an examples of formulations containing Cyclosystem complex® of salicylic acid 50 %.

Foot Cream

An essence of wellbeing for tired feet and a single solution for all needs.

A rapidly absorbed soft cream that matches its cooling effect with a bacteriostatic action.

Manufacturing Process:

1) Dissolve A in B at 60 – 70° C.
2) Add the solution obtained in point 1 to C mix then cool quickly.
3) When the temperature falls below 40° C add phase D and turbomix.
4) Add E and turbomix until a homogenous product is obtained.

Phase	Substance	INCI Name EU	%
A	CC Menthol 50 %	Menthol, Cyclodextrin	2
A	FRESCOLAT ML	Menthyl Lactate	1
B	ALCOOL ETILICO 96D	Alcohol Denat.	10
C	WATER DEMIN.	Aqua	80,46
D	COL. C.I. 42090 Sol. al 0,5 %	Aqua, CI 42090	0,04
D	α BISABOLOLO	Bisabolol	1
D	CC Salicylic Acid	Salicylic Acid, Cyclodextrin	2
E	SEPIGEL 305	Polyacrilamide, C13-14 Isoparaffin, Laureth-7	3,5

Analytical methods

DOSAGE OF SALICYLIC ACID

Quantitative determination of salicylic acid by HPLC

HPLC: High Performance Liquid Chromatography

Instruments

Column	Prevail C18, 5 µm, 250*4,6 mm
Mobil phase	CH3CN:H_2O:H3PO4 19:80:1
Gradient	Isocratic
Detector	UV at 254 nm
Flow Rate	1,0 ml/min
Column Temp	Ambient
Retention Time	22.237 min

Procedure

Standard (preparation)

Prepare a solution of salicylic acid at appropriate concentration (amount expected in 1g sample) in methyl alcohol. The solution can be injected without filtration.

Sample (preparation)

Weigh exactly 1g sample and dissolve with methanol in a 100 ml volumetric flask. Shake vigorously for 5 min and filter. Inject the filtered solution.

Analisys

Misure the area of the standard and compare with that of the sample, calculating the relative concentration.

MICROBIOLOGICAL ANALYSIS

Total viable count

(Internal ref. MB001/FU)

The methodology is based on the count of the Colony Forming Units of micro-organisms (CFU) which develop in a suitable cultural medium, incubated at 30+2°C in aerobiosis for 72 hours.

Search for specific micro-organisms

Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans

(Internal ref. MB003, MB004, MB005)

Cell cultures are enriched in a non selective liquid medium, then isolated in a solid selective medium for each type of microorganism and qualitatively tested.

Results

Parameters	Standard	Internal ref.
TVC	≤ 100 C.F.U./g	MB001/FU
Pseudomonas	Absent	MB003
Staphylococcus	Absent	MB004
Candida albicans	Absent	MB005

The complete method is available on request.

Challenge test

The challenge test is a microbiological method which permits to ascertain the efficacy of a preservation system in a cosmetic formulation and therefore to predict the microbiological stability of the cosmetic product.

It is based on the seeding of known quantities of cells of standard microbial strains on the cosmetic formulation. After fixed time intervals, the surviving microbial cells are examined and the results compared.

It is required for the preserving system to be able to drastically reduce, within the first two weeks of preservation, the possible microbial charge of the cosmetic. The observation of the sample at the fourth week shows the possible segregation of microbial strains resistant to the preservative mixture.

The efficacy of the preservative system is considered appropriate if the number of microrganisms decreases during the time.

Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, have not to increase within the first 24 hours. The viable cells concentration has to decrease of 100 fold after 7 days and of 1000 fold after 14 days of incubation. In order to exclude the possible appearance of resistent strains, after 28 days of incubation any microbial increment must occur.

Results

In the following table the results of the challenge test carried out on the product **"SALICYLIC ACID 50 % CYCLOSYSTEM COMPLEX®"** are showed. The data are reported as number of Colony Forming Units (CFU) after the different incubation times. The values are expressed as CFU per gram of material.

Evaluating the present results it must be taken into account that the product contains preservatives and therefore a marked decrease of microorganisms occurs.

Days of incubation	*S. aureus* cfu/g	*P. aeruginosa* cfu/g	*E. coli* cfu/g	*C. albicans* cfu/g	*Penicillium* cfu/g
0	$> 10^6$	$> 10^6$	$> 10^6$	$> 10^6$	$> 10^6$
2	$< 10^5$	$< 10^4$	$< 10^4$	$< 10^5$	$< 10^4$
7	< 10	< 10	< 10	$< 10^2$	$< 10^3$
14	< 10	$< 10^2$	< 10	< 10	$< 10^2$
28	$< 10^2$	< 10	< 10	< 10	< 10

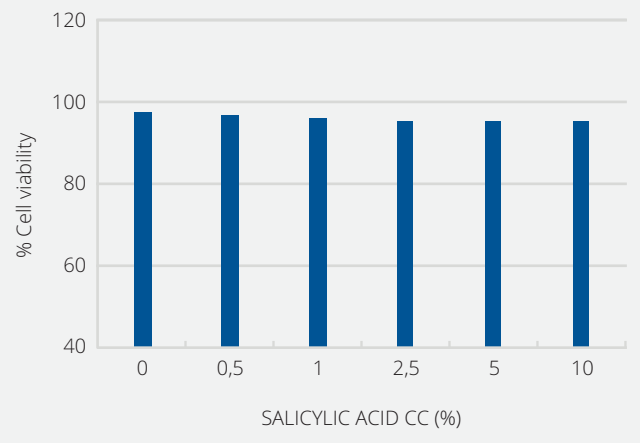
The complete method is available on request.

The present method enables the evaluation of mitochondrial dehydrogenases activity through the transformation of a colourless tetrazolium salt into blue crystals of formazan. Dehydrogenases are enzymes catalyzing the oxidation of various substrates in mitochondria. These reactions are important for the energy production in the cell. The transformation of tetrazolium into formazan is an index of cell viability as it occurs only if mitochondrial dehydrogenases are active, and therefore only if the cell is viable and metabolically active. If a toxic substance is added, it inhibits these enzymes preventing the tetrazolium conversion. The tetrazolium reaction can be followed by measuring spectrophotometrically the stoichiometric production of formazan. Thus, comparing the formazan production before and after the incubation of cells with a test substance, possible toxic effects can be determined. Among the different cell lines suitable for cytotoxicity tests we have chosen a human epithelial line, named Caco-2, isolated from colon. This is a well characterised cell line and one of the most used for tests on human cells. They are quickly reproduced, so that short experimental times are possible.

Results

In the following diagram results of cell viability percentage of Caco-2 cells incubated with SALICYLIC ACID 50 % CYCLOSYSTEM COMPLEX® at concentrations of 0.5, 1, 2.5, 5 and 10 %, are represented.

As can be observed, the **SALICYLIC ACID 50 % CYCLOSYSTEM COMPLEX®** does not present any toxic effect in the explored range.



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