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Grégorio Crini
Sophie Fourmentin
Eric Lichtfouse *Editors*

The History of Cyclodextrins

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Preface

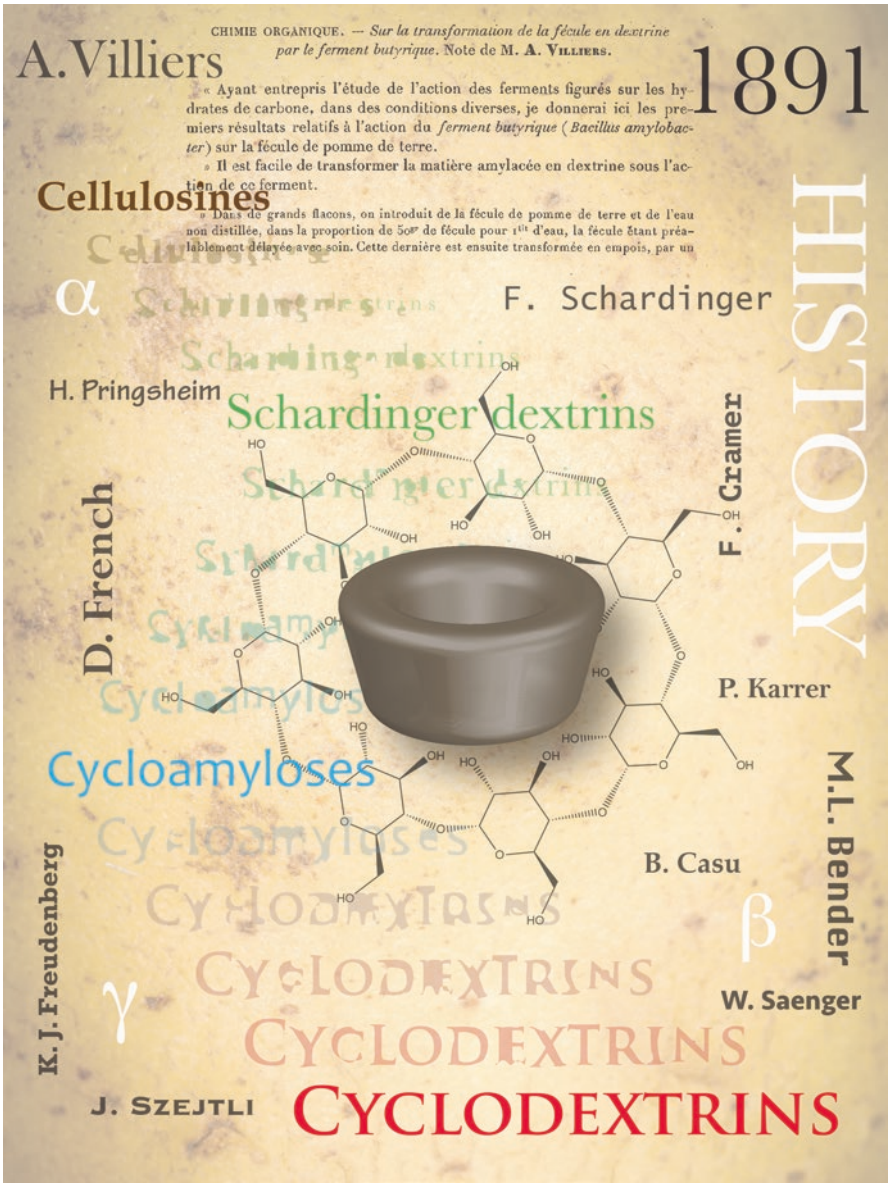
The Schardinger dextrins will continue to serve, delight, teach, and intrigue the carbohydrate chemist for many years to come.

(Professor Dexter French, 1957)

Although discovered about 130 years ago in France by the pharmacochemist Antoine Villiers, cyclodextrins are cage-like compounds that still fascinate researchers. Cyclodextrins are produced by enzymatic degradation of starch. Cyclodextrins are among the most remarkable macrocyclic molecules of major theoretical and practical impacts in chemistry, biology, biochemistry, health science and agriculture. Cyclodextrin investigations have broken frontiers between many different disciplines and, as a result, actual scientists work together to disclose new concepts and applications. The unique feature of cyclodextrins is their ability to form inclusion complexes with various molecules by host–guest interactions, which are at the origin of many applications in almost all industrial sectors.

This book, entitled *Cyclodextrin History,* is the third volume on cyclodextrins published in the series Environmental Chemistry for a Sustainable World. Written by 36 international contributors from 11 countries who are leading experts in the cyclodextrin field, the 3 volumes focus on the developments, research trends, methods and innovations related to the use of cyclodextrins for both fundamental research and applied technology. The first volume explains cyclodextrin fundamentals, synthesis and characterization[1]. The second volume focuses on cyclodextrin applications in medicine, food, environment and liquid crystals[2].

This book presents the history of cyclodextrins. In addition, the book contains invited chapters from senior scientists who have made a major contribution to cyclodextrin knowledge. The first chapter by Nadia Morin-Crini et al. outlines the historical milestones of the discovery, exploration, development and practical applications of cyclodextrins. The next two chapters review the achievements of two prestigious researchers: Professors József Szejtli and Benito Casu. Chapter 2 by Grégorio Crini et al. presents the scientific and industrial work of Professor József Szejtli, considered as the ‘godfather of cyclodextrins’. Chapter 3 by Giangiacomo



Torri et al. pays tribute to Professor Benito Casu, one of the pioneers in the dissemination of the cyclodextrin knowledge. Then, Éva Fenyvesi et al. describe the history of cyclodextrin production in Hungary in Chap. 4. Chapter 5 by Bastien Léger et al. reviews metal nanoparticles and cyclodextrin for catalytic applications. Cyclodextrin-based polymers for food and pharmaceutical applications are then described by

Max Petitjean et al. in Chap. 6. In Chap. 7, Abhishek Pandey explains how cyclodextrins and nanomaterials can be used in drug delivery systems. The last chapter by Grégorio Crini reviews the work carried out on water-insoluble cyclodextrin-epichlorohydrin polymers over the past 30 years at the Chrono-environment Laboratory in Besançon, France.

The editors extend their thanks to all authors who contributed to this book for their efforts in producing timely and high-quality chapters. The creation of this book would not have been possible without the assistance of several friends deserving acknowledgement. They have helped us by choosing contributors, reviewing chapters, and in many other ways. Finally, we would like to thank the staff at Springer Nature for their highly professional editing.

Besançon, France — Grégorio Crini
Dunkerque, France — Sophie Fourmentin
Aix-en-Provence, France — Eric Lichtfouse

References

Fourmentin et al (eds) (2018) Cyclodextrin fundamentals, reactivity and analysis. In: Environmental chemistry for a sustainable world, vol 16. Springer, 255p. https://doi.org/10.1007/978-3-319-76159-6

Fourmentin et al (eds) (2018) Cyclodextrin applications in medicine, food, environment and liquid crystals. In: Environmental chemistry for a sustainable world, vol 17. Springer, 240p. https://doi.org/10.1007/978-3-319-76162-6

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Chapter 1
History of Cyclodextrins

Nadia Morin-Crini, Sophie Fourmentin, Éva Fenyvesi, Eric Lichtfouse, Giangiacomo Torri, Marc Fourmentin, and Grégorio Crini

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G. Crini et al. (eds.), *The History of Cyclodextrins*, Environmental Chemistry for a Sustainable World 52, https://doi.org/10.1007/978-3-030-49308-0_1

Abstract Cyclodextrins are cyclic oligosaccharides obtained by enzymatic degradation of starch. They are remarkable macrocyclic molecules that have led major theoretical and practical advances in chemistry, biology, biochemistry, health science, and agriculture. Their molecular structure is composed of a hydrophobic cavity that can encapsulate other substances to form inclusion complexes through host-guest interactions. This unique feature is at the origin of many applications. Cyclodextrins and their derivatives have a wide variety of practical applications in almost all sectors of the industry, including pharmacy, medicine, foods, cosmetics, chromatography, catalysis, biotechnology, and the textile industry.

Villiers published the first reference to cyclodextrins in 1891. Since the beginning of the twentieth century, major researchers, such as Schardinger, Pringsheim, Karrer, Freudenberg, French, Cramer, Casu, Bender, Saenger, Nagai, Szejtli, and Pitha, have paved the history of the cyclodextrins. Several time periods have marked their history. After their discovery and characterization from 1891 to 1911, there has been a period of doubt and disagreement from 1911 to 1935. Then, the 1935–1950 exploration period was marked by structural results on the "Schardinger dextrins." In 1949, Cramer introduced the cyclodextrin-based nomenclature. Research between 1950 and 1970, the period of maturation, focused on conformations and spectroscopic data of cyclodextrins and their inclusion complexes, with applications in catalysis and as enzyme models. Finally, the period of use has been ongoing since 1970 and has seen cyclodextrins find many industrial applications. Cyclodextrins have then found many industrial applications, initially in the pharmaceutical and food sectors. In 1984, the first chromatographic columns were commercialized. At that time, many cyclodextrin-based catalysts were developed for biomimetic

chemistry and other applications such as artificial enzymes. Currently, more than 2000 publications on cyclodextrins are published each year.

In this chapter, we present a historical overview of the discovery, development, and applications of cyclodextrins.

Keywords History · Schardinger dextrins · Discovery · Production · Separation · Native cyclodextrins · Development · Inclusion complexes · Applications

1.1 Introduction

Figure 1.1 shows that cyclodextrins occur in many daily products such as an ibuprofen tablet, a nonsteroidal anti-inflammatory drug, a whooping cough vaccine, a curative antidote, a hair loss solution, a stop smoking aid, toothpastes, shampoo, colognes, a deodorant toilet, razors, a turmeric-based food supplement, a butter, a mayonnaise, fish sausages, modified steaks, a horseradish powder, mustard sauces, a sweetener, honey, a cinnamon extract, green tea without bitterness, vanilla coffee, clarified fruit juices, chewing gums, chromatographic columns, biopesticides, catalysts, tubular materials, a curtain, cosmetotextiles, an ink, a detergent, a bioflocculant for swimming pool, or a bioadsorbent for water treatment.

Fig. 1.1 Commercial products containing cyclodextrins in our daily lives

Cyclodextrins are cyclic oligomers obtained from the enzymatic degradation of starch. They are one of the most remarkable macrocyclic molecules with significant impacts in our daily lives. Cyclodextrins have a particular structure composed of a hydrophobic cavity that can encapsulate other substances to form inclusion complexes through host-guest interactions. This characteristic feature is at the origin of many applications. Today, all industrial sectors are concerned, e.g., pharmaceuticals, cosmetics, food, hygiene and toiletries, biotechnology, medical, radiology, agrochemistry, catalysis, packaging, textile industry, nanotechnology, and soil and water treatment.

The French pharmacist Villiers published the first reference to cyclodextrins in 1891 (Villiers 1891a, b, c, d). Since the beginning of the twentieth century, Schardinger, Pringsheim, Karrer, Freudenberg, French, Cramer, Casu, Bender, Saenger, Nagai, Szejtli, and Pitha have marked the history of cyclodextrins (Szejtli 1998; Loftsson and Duchêne 2007; Kurkov and Loftsson 2013; Crini 2014; Morin-Crini et al. 2015; Crini et al. 2018).

The first important period on the history of cyclodextrins, from 1891 to 1911, covers their discovery by Villiers, and their characterization and chemistry by Schardinger (Thoma and Stewart 1965; Caesar 1968; Clarke et al. 1988; Szejtli 1998). In 1891, Villiers discovered a crystalline dextrin from the *Bacillus amylobacter* digest of potato starch, which he named *cellulosine*. At the beginning of the last century, Schardinger also observed the formation of two crystallized products during his investigations of food spoilage, which he called crystallized dextrin-α and crystallized dextrin-β. Schardinger gave the first detailed description of the preparation and separation of these two dextrins. He was also the first to isolate the strain of bacteria responsible for dextrin formation, i.e., *Bacillus macerans*. However, from 1911 to 1935 came a period of doubt and disagreement, in particular between the groups of Pringsheim and Karrer, although they published numerous studies on the composition, properties, and chemistry of the crystallized dextrins (Crini 2014).

It was not until the mid-1930s that research on dextrins developed again. The exploration period from 1935 to 1950 was marked by the numerous results obtained by Freudenberg and French on the structure of the "Schardinger dextrin" molecules. In 1935, Freudenberg was the first to develop a relatively simple method for the obtention and purification of the two Schardinger dextrins. Freudenberg also suggested in 1936 a cyclic structure for α-dextrin and β-dextrin, which was confirmed in 1938. In the 1940s, French proposed that Schardinger dextrins be called cycloamyloses and described new protocols for the preparation of cycloamyloses with high purity. In 1942, Hudson discovered the enzyme in *Bacillus macerans* responsible for the conversion of starch into dextrins, and the same year, French published the exact molecular weights of the cyclohexaamylose and cycloheptaamylose, i.e., α-dextrin and β-dextrin, respectively. In 1948, Freudenberg discovered γ-dextrin or cyclooctaamylose, and 1 year later, Cramer, his PhD student, introduced the cyclodextrin-based nomenclature. From 1950 onward, this terminology was increasingly used although the nomenclature of cyclodextrins remained a subject of debate until the end of the 1990s.

The period between 1950 and 1970, known as the period of maturation of notions, focused on inclusion complexes with Cramer's work in the foreground. At the beginning of the 1950s, French finally demonstrated the chemical cyclic structures of cycloamyloses. In 1953, Cramer gave the basis for supramolecular catalysis involving cyclodextrins, and the same year, with Freudenberg and Plieninger, he published the first patent concerning the applications of cyclodextrins in pharmaceutical formulations. In 1956, Cramer introduced and detailed the notion of an *inclusion complex*. From that time on, the interest in cyclodextrins increased. During the maturation period, the works of Casu on the conformation and spectroscopic characterization of cyclodextrins were acknowledged to have brought an important contribution. At the same time, much attention was also focused on their use for catalysis and as enzyme models, and one name stands out in particular in the enzymology and catalysis by cyclodextrins: Bender (Crini 2014). Nonetheless, until the mid-1970s, the three main native cyclodextrins, i.e., α-, β-, and γ-cyclodextrins, available only in small quantities, were long considered as just laboratory curiosities (Thoma and Stewart 1965; Caesar 1968; Kainuma 1984; Clarke et al. 1988). In the way of industrial development, the three main obstacles were their price, e.g., in 1975 1 kg of β-cyclodextrin had a price of about 1500 $ (Szejtli 1982a), their presumed toxicity (French 1957a), and the lack of sufficient knowledge of these substances (Szejtli 1982a). In addition, very few researchers were convinced of the industrial potential of cyclodextrins.

The 1970s were marked by two important events: firstly, several manufacturers started to produce and to commercialize cyclodextrins; at that time, due to improvements in the production of cyclodextrins, their prices have dropped significantly. Secondly, the first toxicological studies had established that β-cyclodextrin administrated orally was a harmless substance. As a result, this has led to spectacular progress. From then on, the period of use began and cyclodextrins found many industrial applications. During this period of utilization, four names stand out: Saenger, Szejtli, Nagai, and Pitha. In the mid-1970s, pharmaceutical and food applications started to appear and rapidly gained ground, especially in Japan (Hamada et al. 1975; Szejtli 1977; Pitha et al. 1983; Uekama and Otagiri 1987; Frömming and Szejtli 1994). In 1980, Saenger published the first comprehensive review about the potential industrial applications of cyclodextrins (Saenger 1980). The first International Cyclodextrin Symposium organized by Szejtli took place in Budapest in 1981, and 1 year later, he wrote the first comprehensive cyclodextrin book (Szejtli 1982a). At that time, many interesting catalysts based on cyclodextrins were also constructed for biomimetic chemistry and other processes of interest such as artificial enzymes (Breslow 1979; Breslow and Dong 1998). Both from an academic and industrial point of view, the number of communications then started to increase exponentially, as did the filing of patents.

In the mid-1980s, cyclodextrins were produced in large quantities and commercialized at a reasonable price, i.e., 10–15 $/kg (Szejtli 1982a). Other industrial applications have become possible. In 1984, the first chromatographic columns were marketed (Armstrong 1984; Ward and Armstrong 1986, 1988; Armstrong and Jin 1989). Since then, an increasing interest in cyclodextrins and their possible

applications has existed (Duchêne 1987, 1991; Szejtli 1988). An abundant scientific literature has built up since the 1980s. Currently, every year, more than 2000 publications, including articles and book chapters, are devoted to cyclodextrins (Cyclodextrin News, CycloLab Ltd., Hungary). Nowadays, these molecules still fascinate researchers and industrials.

The objective of this chapter is to describe historical landmarks of the discovery, exploration, and utilization of cyclodextrins. We also present some highlights of their early industrial applications. To this end, an extensive list of data from about 500 original publications has been compiled. Although this historical chapter cannot hope to be exhaustive, it does highlight the work of those researchers who have contributed to the knowledge of cyclodextrins throughout the 129 years of its history.

1.2 Discovery and First Chemical Studies of Cyclodextrins

1.2.1 Discovery: 1891–1911

During experiments on the degradation and reduction of carbohydrates under the action of ferments, Antoine Villiers, a French pharmacist and chemist, was the first to observe in 1891 the formation of unwanted crystals with particular properties, i.e., the formation of cyclodextrins. Among various Villiers' biographies, those by French (1957a), Thoma and Stewart (1965), Caesar (1968), Szejtli (1998), Loftsson and Duchêne (2007), Kurkov and Loftsson (2013), Crini (2014), and Morin-Crini et al. (2015) deserve particular mention.

Studying the degradation and reduction of carbohydrates, Villiers showed how easy it was to transform starch to yield "novel crystalline dextrins" with particular properties under the action of ferments. He first obtained a small amount of crystalline dextrins from digests of *Bacillus amylobacter*, i.e., *Clostridium butyricum*, on potato starch under certain conditions (Villiers 1891a, b): 50 g potato starch in 1 L of water at 100 °C subsequently seeded with *Bacillus amylobacter* and incubated for several days in an oven at 40 °C. Villiers presented his results to the French *Académie des Sciences* in February 1891 (Fig. 1.2). At that time, the dextrins, previously discovered in 1821, were the degradation products and/or the intermediate decomposition products of starch through heating. For Villiers, his dextrins were degradation products of starch. When purified by fractional precipitation, the crystals presented very different optical rotation properties and were difficult to hydrolyze any further. Iodine stains red those dextrins that had a high optical activity, and the intensity of the stain decreased with the optical activity. The butyric ferment caused the transformation of the starch directly into dextrin without the involvement of intermediates such as diastases secreted by the ferment. Later, Villiers considered his dextrins as the intermediate decomposition products of starch (Villiers 1891b). Villiers also obtained *un curieux sous-produit*, i.e., a curious by-product, in small

CHIMIE ORGANIQUE. — *Sur la transformation de la fécule en dextrine par le ferment butyrique*. Note de M. **A. Villiers**.

« Ayant entrepris l'étude de l'action des ferments figurés sur les hydrates de carbone, dans des conditions diverses, je donnerai ici les premiers résultats relatifs à l'action du *ferment butyrique* (*Bacillus amylobacter*) sur la fécule de pomme de terre.

» Il est facile de transformer la matière amylacée en dextrine sous l'action de ce ferment.

Fig. 1.2 Extract of the first proceedings of the French *Académie des Sciences* of February 1891 where Villiers described the action of the butyric ferment *Bacillus amylobacter* on potato starch

CHIMIE ORGANIQUE. — *Sur la fermentation de la fécule par l'action du ferment butyrique*. Note de M. **A. Villiers**.

« J'ai montré dernièrement (*Comptes rendus*, février 1891, p. 435) que la fécule de pomme de terre peut, dans des conditions déterminées, fermenter sous l'action du *Bacillus amylobacter*, les produits principaux de cette fermentation étant constitués par des dextrines.

» Il se forme en même temps, mais en très petite quantité, soit environ 3^{gr} pour 1000 de fécule, un hydrate de carbone qui se sépare en beaux cristaux radiés, au bout de quelques semaines, dans l'alcool ayant servi à la précipitation des dextrines. Ces cristaux renferment de l'eau et de l'alcool de cristallisation, la proportion de ce dernier étant très faible, environ 4 pour 100. Au contact de l'air, ils deviennent opaques, en perdant de l'alcool et absorbant de l'eau, sans que leur poids varie d'une manière notable. En les dissolvant dans une assez grande quantité d'eau chaude, on obtient, par refroidissement, de petits cristaux brillants, inaltérables à l'air, dont la composition est représentée par un multiple de la formule

$$C^{12}H^{10}O^{10} + 3HO.$$

Fig. 1.3 Extract of the second proceedings of the French *Académie des Sciences* of June 1891 where Villiers described the chemical composition of two novel crystalline dextrins which he named *cellulosines*

quantities after several weeks of incubation: 3 g of this carbohydrate was obtained as crystals after bacterial digestion of 1000 g of starch. This new substance was found in the alcohol that was used for the precipitation of dextrins (Villiers 1891b).

In a second proceedings of the French *Académie des Sciences* of June 1891 (Fig. 1.3), Villiers described the chemical composition of the novel highly crystalline dextrin having a composition between that of starch and that of dextrin (Villiers 1891c). In air, the crystals, containing water and alcohol of crystallization (the proportion of the latter is rather small, about 4%), became opaque. They lose alcohol

and absorbed water without any change in weight. After purification in large amount of hot water, Villiers obtained small brilliant crystals, most probably β-cyclodextrin, and determined the chemical composition of this crystalline carbohydrate. He gave the first empirical formula: $[(C_6H_{10}O_5)_2 + 3H_2O]$. Its solubility in water at room temperature was low but raised with temperature. The white crystals with a very slight sweetness showed extremely high optical activity, much higher than those of certain dextrins formed under the action of the butyric ferment. Villiers then considered this novel substance as an isomer of starch (Villiers 1891c, d). By manipulating the experimental conditions, Villiers obtained two distinct crystalline dextrins, most probably α-cyclodextrin and β-cyclodextrin, having a composition represented by a multiple of the formula $[(C_6H_{10}O_5) + 3H_2O]$. Villiers noted again that the white crystals with a very slight sweetness showed extremely high optical activity. Pursuing his experiments, he observed that the two dextrins, always considered as isomers of starch, were almost insoluble in water, soluble in alcohol, non-fermentable, and acid resistant, and they could also be converted into ethers under the action of acid chlorides. Villiers finally concluded that the properties of these two particular dextrins were very clearly different from those of the various saccharides and polysaccharides known at the time and proposed the name of *cellulosines* due to the similarities with cellulose, e.g., with "regard to difficulty of acid hydrolysis" (Villiers 1891c, d).

At the beginning of the 1900s, Heinrich Robert Koch, a famous German physician and microbiologist, who received a Nobel Prize in 1905, remained unconvinced by Villiers' conclusions (Crini 2014). In Koch's opinion, Villiers used "primitive bacteriological techniques and probably impure cultures." This was also pointed out by Schardinger (1904). Later, French (1957a) indicated that "Villiers used impure cultures but his digests contained sufficient *Bacillus macerans* to account for the small amount of crystalline dextrin obtained."

The recognition to cyclodextrins is attributed to Franz Schardinger, an Austrian chemist and bacteriologist. Schardinger is the first Great Scientist who has left its mark on the history of these oligosaccharides. He is considered the "Founding Father" of cyclodextrin (Szejtli 1982a; Crini 2014).

At the beginning of the last century, Schardinger also observed the formation of dextrins during his investigations of resistant microorganisms that can lead to food poisoning (Fig. 1.4). Like other researchers at that time, Schardinger studied these dextrins with the expectation that they would shed some light on the synthesis and degradation of starch. In 1903, Schardinger discovered that a type of extremely heat-resistant microorganism was able to dissolve starch and form crystalline by-products (Schardinger 1903a), remarkably similar to *cellulosines* reported by Villiers. Using the iodine test, Schardinger distinguished two types of *krystallisiertes dextrins* which he called crystallized dextrin A and crystallized dextrin B. The B form resembled Villiers' cellulosine. Indeed, the chemical behavior and the physical constants given by Schardinger for his substance agree very well with those of the dextrin previously described by Villiers. Schardinger found that it was possible to isolate pure fractions with a maximum yield of 30% crystallized dextrins from starch, the main form obtained being always dextrin B. *Krystallisiertes dextrins*

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Untersuchung der Nahrungs- und Genußmittel,
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Heft 19. 1. Oktober 1903. 6. Jahrgang.

Über thermophile Bakterien aus verschiedenen Speisen und Milch,
sowie über einige Umsetzungsprodukte derselben in kohlenhydrathaltigen Nährlösungen, darunter krystallisierte Polysaccharide (Dextrine) aus Stärke.

Von

Franz Schardinger.

Mitteilung aus der K. K. Allgemeinen Untersuchungsanstalt für Lebensmittel in Wien.

Im vergangenen Jahre hatte die hiesige Anstalt die Frage der Zulässigkeit des Genusses längere Zeit hindurch warm aufbewahrter Speisen zu prüfen, wobei sich im Verlaufe der Untersuchung beachtenswerte mikrobiologische Funde ergaben, über die im nachstehenden eingehender berichtet werden soll. Auf Grund der Forschungsergebnisse über thermophile Bakterien konnte es keinem Zweifel unterliegen, daß bei der in Betracht kommenden Temperatur zwischen 50—60° bakterielles Leben überhaupt möglich ist, es war also zunächst festzustellen, ob in den Speisen derartige Keime vorhanden und welcher Art die von ihnen veranlassten Zersetzungen sind, soweit eine Feststellung in letzter Beziehung derzeit möglich ist.

Fig. 1.4 First page of the article published by Schardinger on dextrins in 1903

were first considered as the degradation products of starch through heating (Schardinger 1903a). Schardinger also managed to isolate the strain of bacteria responsible for the degradation of starch – he called it *strain II* (Schardinger 1903b). He observed that this heat-resistant organism had considerable starch-fermenting power. When sub-cultured on starch, *strain II* broke down starch, giving an alcohol-insoluble "soluble starch" together with crystallized dextrin A (fine hexagonal plates) and crystallized dextrin B (stout prismatic crystals). Schardinger also observed that with time, the activity of the *strain II* microorganism decreased. Indeed, he was unsuccessful in maintaining a culture of *strain II* which had the characteristic starch-degrading activity.

In 1904, Schardinger isolated a new microorganism, considered as "an accidental contaminant," which he first called *Rottebacillus I* owing to its action on potato starch, i.e., it produced acetone and ethyl alcohol by fermentation of carbohydrate media (Schardinger 1904). The name *Rottebacillus I* was used to express the fact

Bacillus macerans, ein Aceton bildender Rottebacillus.

[Mitteilung aus der k. k. Untersuchungsanstalt für Lebensmittel in Wien.]

Von **Franz Schardinger.**

Ueber die durch den genannten Mikroben bewirkte Acetongärung wurde bereits in No. 8 der Wiener klinischen Wochenschrift, Jahrg. 1904 berichtet. Die Ergebnisse fortgesetzter Studien über diese Gärung und die Verrottung pflanzlicher Gebilde sollen im nachfolgenden einem weiteren Leserkreise unterbreitet werden.

Fig. 1.5 Abstract of the article published in the journal *Wiener Klinische Wochenschrift* by Schardinger on *Bacillus macerans* in 1904

the microorganism was able to form both acetone and ethyl alcohol. Several months later, Schardinger used the Latin term *Bacillus macerans* to name his microbe, i.e., *macerare*, to rot (Fig. 1.5). This bacillus was able to give the same crystalline dextrins as before, which he designated as *krystallisiertes polysaccharides*, i.e., crystallized polysaccharides, considered then as the intermediate decomposition products of starch (Schardinger 1904). Using the characteristic reaction that starch derivatives show with iodine, Schardinger proposed a distinction between a "crystallized amylose" and a "crystallized amylodextrin." The yields obtained were tenfold those reported by Villiers. To explain this result, Schardinger suggested that, in the conditions of sterilization described by Villiers, the bacillus used was "probably not pure" (Schardinger 1904). One year later, Schardinger was also the first to observe that different starchy substrates differed in their behavior with *Bacillus macerans*, especially in the yields obtained (Schardinger 1905).

1.2.2 The Foundation of the Cyclodextrin Chemistry

Schardinger is acknowledged as being the first to lay down the basis of the cyclodextrin chemistry (French 1957a; Thoma and Stewart 1965; Szejtli 1998). Indeed, he was the first researcher to describe the fundamental properties of *cellulosines*, to introduce the terms crystallized α-dextrin and crystallized B-dextrin, to isolate the microorganism able to synthesize the enzyme that catalyzes the degradation of starch into crystallized dextrins, to hypothesize that the crystallized substances were cyclic "polysaccharides," and also to suggest their ability to form complexes.

Between 1905 and 1911, Schardinger made several important observations (Schardinger 1903a, b, 1904, 1905, 1909, 1911). He observed that *cellulosines* were often formed in starch-based media containing putrefying microorganisms. The formation of the two crystallized dextrins depended on the type of bacteria digesting starch. The distinction between the two forms was always made through their ability to form complexes of different colors with iodine. Schardinger also studied the chemistry of the two dextrins, pointing out their lack of reducing power and hydrolysis to reducing sugar. Dextrins were non-reducing to copper reagents and

non-fermentable by yeast. Schardinger also reported their behavior in the presence of alcohols, chloroform, ether, and iodine solution. He used the complexes with these solvents as a means of precipitation of dextrins (Schardinger 1911). This was the first indication of the ability of dextrins to form "inclusion" complexes (Crini 2014). Finally, Schardinger proposed empirical formulae of dextrins. However, he did not propose a structure for his crystallized dextrins and also did not attempt their molecular-weight determinations. It will take another 20 years before the cyclic nature of Schardinger dextrins will be recognized. Professor Schardinger decided to stop his research into dextrins in 1911, and as a conclusion he wrote: "I realize that still very many questions remain unsolved; the answer to these I must leave to another, who, owing to more favorable external conditions, can deal with the subject more intensively."

In the 24 years following Schardinger's final paper (Schardinger 1911), the field of research on crystallized dextrins was dominated by the groups of Pringsheim and Karrer. Pringsheim is recognized as the first researcher to have published prolifically on dextrins. However, the works were repetitive, marred by frequently contradictory results and by even hot debate between the two groups (French 1957a; Szejtli 1998; Crini 2014; Morin-Crini et al. 2015).

1.3 Historical Landmarks in the Exploration of Cyclodextrins: From 1911 to 1970

1.3.1 Nomenclature

In 1891, cyclodextrin was initially called *cellulosine* by Villiers because he assumed that the novel crystalline substance, obtained from digests of *Bacillus amylobacter*, was *une sorte de cellulose*, i.e., a kind of cellulose (Crini 2014).

In 1903, Schardinger reported the formation of two *krystallisiertes dextrins* during his investigations of food spoilage, which he called crystallized dextrin A and crystallized dextrin B, because most of their properties were similar to the already known partial degradation products of starch, i.e., the dextrins (Schardinger 1903a, b). One year later, the *krystallisiertes dextrins*, considered as the intermediate crystallized decomposition products/by-products of starch, were designated by the term *krystallisiertes polysaccharides*, i.e., crystallized polysaccharides (Schardinger 1904). Pursuing his investigations on the structure of starch, Schardinger then introduced a distinction between a *crystallized amylose* for dextrin A and a *crystallized amylodextrin* for dextrin B, because, for him, there was an analogy between his dextrins and amylose and amylodextrin, especially with respect to their iodine color reactions (Schardinger 1905, 1907). Finally, Schardinger considered that these names were inappropriate and thus decided to rename it *crystallized dextrin*-α and *crystallized dextrin*-β (Schardinger 1911).

In the mid-1910s, the German chemist and biochemist Hans Pringsheim used the name of *krystallisiertes polyamylosen*, i.e., crystallized polyamyloses, distinguishing two series, the α-series of dextrins containing 2n D-glucose units per molecule and the β-series containing 3n D-glucose units per molecule. Four substances, i.e., α-diamylose, α-tetraamylose, α-hexaamylose, and α-octaamylose, were included in the α-series of dextrins, while the β-series only contained two substances, i.e., β-triamylose and β-hexaamylose. Indeed, for Pringsheim, the Schardinger dextrins arose through the bacterial depolymerization of starch to the fundamental units: the amylose fraction being broken down into the α-series of dextrins, i.e., polyamyloses, and the amylopectin fraction being degraded to the β-series (French 1957a; Thoma and Stewart 1965; Caesar 1968; Szejtli 1998; Morin-Crini et al. 2015). Pringsheim also used the terms of α-amylosan, α-allo-amylosan, and α-iso-amylosan and β-amylosan, β-allo-amylosan, and β-iso-amylosan for α-dextrin and β-dextrin, respectively (Crini 2014). At the same time, the Swiss chemist Paul Karrer also introduced the notion of series of crystallized dextrins. Like Pringsheim, Karrer was convinced that the α-series of dextrins was composed of at least four distinct substances differing in molecular size. However, he disagreed with the subdivision of the β-series into triamylose and hexaamylose. For Karrer, these two products were identical. In addition, Karrer regarded maltose as the fundamental unit of the whole of the starch molecule, while Pringsheim considered the polyamyloses as the basic units of the starch molecule (French 1957a; Thoma and Stewart 1965; Caesar 1968; Szejtli 1998; Morin-Crini et al. 2015). Like Pringsheim, Karrer also used the amylosan-based terminology (Crini 2014).

In the 1920s, as a tribute of the pioneering work of Schardinger, the German chemist Karl Johann Freudenberg called them "Schardinger dextrins" and referred to these compounds as α-dextrin and β-dextrin (French 1957a; Thoma and Stewart 1965; Caesar 1968; Szejtli 1998; Loftsson and Duchêne 2007; Kurkov and Loftsson 2013; Morin-Crini et al. 2015) and later as pentaosan and hexaosan, respectively (Crini 2014). For many years, cyclodextrins were called "Schardinger dextrins" in his honor, almost up to the 1970s, or also sometimes simply as dextrins (Szejtli 1998). Schardinger dextrins were subsequently named "cycloamyloses" by the American chemist Dexter French in 1942 (French and Rundle 1942), "cycloglucanes" by Freudenberg in 1943 (Freudenberg 1943), and finally "cyclodextrins" in 1949 by the German chemist Friedrich Cramer, a pupil of Freudenberg (Cramer 1949). The model of "cycloamyloses" was constructed from glucopyranose units in the boat conformation. For French, α-dextrin, β-dextrin, and γ-dextrin must be called cyclohexaamylose, cycloheptaamylose, and cyclooctaamylose, respectively, the Greek prefix to the "amylose" corresponding to the degree of polymerization, i.e., indicating the number of glucose units in the ring (French and Rundle 1942). However, at that time, Freudenberg claimed that "this new nomenclature was inappropriate and ambiguous" (Freudenberg 1943). Again, in 1947, Freudenberg wrote: "It appears to be premature to rename the α-dextrin cyclohexa-amylose and the β-dextrin cycloheptа-amylose" (Freudenberg et al. 1947a, b). In 1943, Freudenberg proposed the cycloglucane-based nomenclature, e.g., cyclohexaglucane α(1→4), cycloheptaglucane α(1→4), and cyclooctaglucane α(1→4) for α-dextrin, β-dextrin,

and γ-dextrin, respectively (Freudenberg 1943). During the mid-1940s, there was another system in current use (Crini 2014). In the alternate system, the number of residues in the cyclic polymer was indicated by prefixing a Greek letter to the series name. Since the smallest known cycloamylose was a hexamer, it was assigned the prefix α. The cyclic heptatose, octaose, etc. were referred to, respectively, as β, γ, etc. The first system introduced by French was however preferred because it was more descriptive of the structures.

At the end of the 1940s, Cramer first proposed the cyclo-based nomenclature for the nomenclature of the Schardinger dextrins, e.g., (6-ose)-cyclo, (7-ose)-cyclo, and (8-ose)-cyclo for α-, β-, and γ-dextrins, respectively. For the first time in 1949, Cramer introduced the term cyclodextrin. This name was included in the title of his PhD dissertation entitled *Die Cyclodextrine aus Stärke* (Cramer 1949). For Cramer, the term of cyclodextrin must be used to refer to cyclic oligosaccharides made up of 6, 7, or 8 units of D-glucose joined by α-(1 → 4) linkages termed α-, β-, and γ-cyclodextrin, respectively. Because of its relative brevity, the term cyclodextrin was soon accepted, but the nomenclature of cyclodextrins remained a subject of debate until the end of the 1990s (Szejtli 1998; Loftsson and Duchêne 2007; Kurkov and Loftsson 2013; Crini 2014; Morin-Crini et al. 2015). Indeed, at that time, several laboratories proposed clarifications of the nomenclature of cyclodextrins because the term cyclodextrin only specified the nature of the sugars but did not give any information on the bonding between them. Thus, the name cyclomaltohexaose was suggested in 1997. This name is composed of first the term cyclo followed by a term indicating the type of linkage, i.e., malto for glucose unit bound by α-(1→4) linkages, and the number of sugar units with the ending ose, i.e., hexa for 6 or hepta for 7. This final term, present in cyclomaltohexaose, implies a free anomeric center, which is not present in cyclodextrins. Both the terms cyclodextrins and cyclomaltooligosaccharides were used (Crini 2014).

Other nomenclatures have also been proposed. For instance, α-cyclodextrin was named cyclohexakis-(1→4)-α-D-glycosyl or cyclo-α-(1→4)-glucohexaoside. The term of the glycosyl residue is preceded by the type of linkage between brackets, which in turn is preceded by the term cyclo plus an indication of the number, i.e., cyclohexakis, etc. The literature uses all of these nomenclatures. Nevertheless, the cyclodextrin-based nomenclature is still the most widely used in literature today. The nomenclature for large-ring cyclodextrins, i.e., LR-CDs with a degree of polymerization between 9 and >100, is more simple: each molecule is designated by an abbreviation CDn where *n* indicates the number of glucose units in the macrocycle, e.g., CD14 (boat-like structure) composed of 14 glucose units (Morin-Crini et al. 2015; Assaf et al. 2016; Sonnendecker and Zimmermann 2019a, b; Sonnendecker et al. 2018, 2019).

1.3.2 Native Cyclodextrins

Schardinger recognized only dextrin-α and dextrin-β, while Freudenberg obtained γ-dextrin in 1948, although previously regarded by him as a cyclic heptasaccharide (Freudenberg and Cramer 1948). Two years later, Freudenberg elucidated the structure of γ-dextrin (Freudenberg and Cramer 1950). The same year, using partial acid hydrolysis and enzyme digestion followed by X-ray measurements and paper chromatography, French also elucidated the structure of γ-dextrin, first named *cycloöctaamylose* and later cyclooctaamylose (French et al. 1950b). This dextrin was composed of eight glucose residues symmetrically arranged in a ring and linked together by α-1,4-glucosidic bonds. In the late 1950s, French and co-workers had established the molecular weight, the exact chemical structure, the dimensions, and the types of bonding in the three cycloamyloses, cyclohexaamylose, cycloheptaamylose, and cyclooctaamylose, i.e., α-dextrin, β-dextrin, and γ-dextrin, respectively (French and McIntire 1950; Norberg and French 1950; French et al. 1950a, b).

In 1948, the first indications of the existence of higher homologues of dextrins were published by Freudenberg and his young student Cramer (Freudenberg and Cramer 1948). Two years later, French also suggested the possible existence of cycloamyloses containing more than 8 glycosyl units (Norberg and French 1950; French et al. 1950b). The same year, Akiya and co-workers claimed the "discovery of new series of cyclic oligosaccharides" similar to the Schardinger dextrins, containing more than 8 glucose units (Akiya and Watanabe 1950a, b, c; Akiya and Okui 1951). Later, Caesar (1968) reported that these "new" compounds were the α- and β-dextrins. In fact, the existence of larger homologues of cycloamyloses was clearly demonstrated a decade later by French. In 1957, French discovered delta-dextrin or δ-dextrin and epsilon-dextrin or ε-dextrin, containing 9 and 10 units of glucose, respectively (French 1957a, b). He proved their existence using radioautography and chromatography measurements. However, French elucidated their structures only in 1965 (French et al. 1965). At that time, French also wrote: "there is no obvious reason why the series should stop here" (French 1957a), suggesting the existence of cycloamyloses with 11 and 12 units of glucose, i.e., ξ-dextrin or zeta-dextrin and η-dextrin or eta-dextrin, respectively. In the beginning of the 1960s, French continued to study cycloamyloses with a larger ring. His objective was to develop a fractionation method for isolation of larger homologues of cycloamyloses after extensive β-amylase digestion to hydrolyze maltooligosaccharides. In 1961, the existence of cycloamyloses with 11 and 12 units of glucose is confirmed using radioautography (Pulley and French 1961), and 4 years later, he was the first to propose a fractionation method for their isolation (French et al. 1965). The structure and the dimensions of ξ-dextrin and η-dextrin are reported. French finally introduced the notion of Schardinger dextrin series, "a Schardinger dextrin family" (French et al. 1965). The same year, Thoma and Stewart (1965) also published similar results, and the discovery of ξ-dextrin and η-dextrin is attributed to them (Caesar 1968; Szejtli 1998; Loftsson and Duchêne 2007).

French's results had for many years been regarded as dubious since they were not able to experimentally distinguish the large cyclodextrins from branched derivatives. As late as 1988, Szejtli expressed his doubts, in his monograph *Cyclodextrin Technology*, to whether cyclodextrins larger than γ-cyclodextrin exist (Szejtli 1988). In fact, higher cyclic cyclodextrins than the three native cyclodextrins, reported in the 1960s, were probably so-called branched derivatives such as branched diglucosyl-cyclodextrins. When a section of the amylopectin molecule containing a branching point was incorporated into a cyclic structure, one or two glucosyl or maltosyl side chains were attached by α-(1→6) linkages to the ring formed (Frömming and Szejtli 1994). During the production of native cyclodextrins, these branched cyclodextrins were also produced. It was only during the mid-1990s that the existence of the large cyclodextrins has been fully proven (Miyazawa et al. 1995; Endo et al. 1997, 1999; Larsen 2002; Qi et al. 2004; Taira et al. 2006; Crini 2014).

1.3.3 Cyclodextrin Chemistry

For over 20 years, Pringsheim and his various collaborators penned an abundant literature on dextrins. Indeed, Pringsheim is considered to be the first researcher to have published prolifically on their preparation and chemistry (Pringsheim and Langhans 1912; Pringsheim and Eissler 1913, 1914; Pringsheim 1915, 1919, 1922, 1924, 1925, 1926, 1927, 1928a, b, 1931a, b, 1932; Pringsheim and Lichtenstein 1916; Pringsheim and Persch 1921, 1922; Pringsheim and Dernikos 1922; Pringsheim and Goldstein 1922, 1923; Pringsheim and Beiser 1924, 1932; Irvine et al. 1924; Pringsheim and Leibowitz 1924, 1925a, b, 1926a, b; Pringsheim and Steingroever 1924, 1926; Pringsheim and Wolfsohn 1924; Pringsheim and Schapiro 1926; Pringsheim and Meyersohn 1927; Irvine et al. 1929; Pringsheim et al. 1930, 1931a, b). However, these studies suffered from numerous errors due to the use of dextrins that were not pure and to problems arising from separation of the fractions and from the use of unsuitable analytical methods, e.g., determination of the masses by cryoscopy (Freudenberg and Jacobi 1935; Samec and Blinc 1941; French 1957a; Thoma and Stewart 1965; Caesar 1968). In 1935, Freudenberg dismissed the work of Pringsheim as practically valueless, since "most of it was based upon work with dextrin mixtures and upon serious misconceptions relating to the structural principles of high polymers" (Freudenberg and Jacobi 1935). French (1957a) also wrote: "Pringsheim's literature was voluminous but much of it was repetitive, controversial, or based on erroneous concepts."

From 1910, Pringsheim repeated Schardinger's experiments. He reported higher yields of β-dextrin from glycogen crude preparations of amylopectin, and this is the reason why he postulated that amylose was polymerized α-diamylose and amylopectin and glycogen were polymerized β-triamylose. Like Schardinger, Pringsheim observed that the relative proportions of α- and β-dextrins depended on the different substrates used (Pringsheim and Langhans 1912). Pringsheim described the

chemical behavior of dextrins and their properties, in agreement with the previous results published by Schardinger. The dextrins were soluble in water but insoluble in alcohol, ether, and chloroform. They do not reduce Fehling's solution. To precipitate the dextrins, different solvents including benzene, toluene, xylene, bromobenzene, nitrobenzene, and petroleum ether were proposed (Pringsheim and Eissler 1913, 1914; Pringsheim 1915; Pringsheim and Lichtenstein 1916). Pringsheim confirmed that the simplest means to distinguish between the α- and β-dextrins was the iodine reaction (Pringsheim 1922; Pringsheim and Dernikos 1922). Pringsheim was the first to study the halogen complexes of dextrins (Pringsheim and Wolfsohn 1924; Pringsheim and Schapiro 1926). The first methylated β-dextrin was also obtained by his group: 43.6% of degree of methylation as against 45.6% required by theory. The compound was crystallized from ether (Pringsheim and Goldstein 1923). Several data can also be found referring to the preparation of dextrin derivatives including acetates, nitrates, and ethers (Pringsheim 1927, 1928a, b, 1931b; Pringsheim and Meyersohn 1927; Irvine et al. 1929; Pringsheim et al. 1930, 1931a, b; Pringsheim and Beiser 1932). However, all Pringsheim's data are essentially of historic interest (Samec and Blinc 1941; French 1957a; Thoma and Stewart 1965; Caesar 1968; Szejtli 1982a; Crini 2014). From 1920 to 1925, Karrer also contributed greatly to the knowledge of the chemistry of the Schardinger dextrins (Thoma and Stewart 1965; Caesar 1968; Szejtli 1998; Crini 2014). Karrer published several important works on dextrins (Karrer 1920, 1921, 1922, 1923, 1925; Karrer and Nägeli 1921a, b; Karrer et al. 1921, 1922; Karrer and Bürkin 1922). Like Schardinger and Pringsheim, Karrer studied the crystallized dextrins with the expectation that they would shed some light on the features of starch. In 1921, Karrer published the first conclusions on the acetolysis of α-dextrin and β-dextrins. He demonstrated that this reaction gave essentially the same excellent yield of maltose as starch or maltose itself gives, when treated similarly (Karrer 1921; Karrer and Nägeli 1921a, b; Karrer et al. 1921). Karrer also investigated the interactions between dextrins and ions such as barium, sodium, and potassium (Karrer 1922; Karrer and Bürkin 1922; Karrer et al. 1922). In 1925, Karrer summarized the whole of his works and conclusions on dextrins in a famous comprehensive book (Karrer 1925).

Between 1911 and 1935, epoch called by Crini (2014) the "period of doubt," other researchers have also published interesting works on the chemistry of the Schardinger dextrins (Biltz 1913; Biltz and Truthe 1913; Freudenberg and Ivers 1922; Miekeley 1930, 1932; Ulmann 1932, Ulmann et al. 1932; Hess et al. 1933). Miekeley (1930, 1932) published experimental data on the chemical composition of dextrins, which complemented those of Pringsheim. In 1933, Ulmann's group observed that the α-dextrin-ethanol complex had two different crystal modifications which could be interconverted. This was the first observation that a same guest may form different crystal structures with the same dextrin (Hess et al. 1933). However, this period did nothing to stimulate the development of Schardinger dextrins, considered as by-products of starch degradation. So, the work on cyclodextrins reported before 1935 was of little consequence (Samec and Blinc 1941; Thoma and Stewart 1965; Caesar 1968; Szejtli 1998; Crini 2014). This can be explained by the fact that researchers used

incompletely separated fractions and based too much reliance on cryoscopic measurements of molecular weights, which led to many anomalous results.

From 1935 to 1950, epoch called by French (1957a) the "maturation period," the works of Freudenberg on the chemistry of the Schardinger dextrins were acknowledged to have made an important contribution to the cyclodextrin science (Freudenberg and Jacobi 1935; Freudenberg and Rapp 1936; Freudenberg et al. 1936, 1938, 1939, 1947a, b; Freudenberg and Meyer-Delius 1938, 1939; Freudenberg 1939, 1943; Freudenberg and Cramer 1948, 1950). Indeed, Freudenberg is recognized as a pioneer in this domain (Thoma and Stewart 1965; Caesar 1968). As far back as 1922, Freudenberg was the first researcher to focus on the chemical modification of dextrins, in particular of tosylated residues (Freudenberg and Ivers 1922). Later, the Schardinger dextrins were oxidized by iodite, "probably by a glycol-cleavage reaction" (Freudenberg 1934). Enzymatic hydrolysis gave no trace of a sugar unit other than *D*-glucose (Freudenberg and Jacobi 1935). During the hydrolysis of dextrins, Freudenberg also observed an increase in rotation due to hydrolysis of the β-linkage. During acetolysis, the dextrins were shown to be more nearly similar to starch than to compounds of the levoglucosan type. Using a cryoscopic method for the determination of molecular weights, Freudenberg reported (erroneously) the number of glucose units that the Schardinger dextrins contained: five for A-dextrin and six for β-dextrin (Freudenberg and Jacobi 1935). In 1936, Freudenberg confirmed that enzymatic hydrolysis gave no trace of a sugar unit other than D-glucose. He also reported that methylation studies failed to reveal the presence of any *D*-glucose units, concluding that glucose was the only product of acid hydrolysis of dextrins (Freudenberg and Rapp 1936). The following pieces of experimental evidence were also published: (i) the rate of hydrolysis of dextrins in 51% sulfuric acid was too low for there to be any labile β-linkages present; (ii) the Schardinger dextrins were non-reducing, that is, they did not have a reducing chain termination; and (iii) methylation studies on dextrins gave no products than 2,3,6-*O*-methyl-*D*-glucose (Freudenberg and Rapp 1936). The same year, Freudenberg prepared fully methylated α- and β-dextrins and finally demonstrated that 2,3,6-tri-methylglucose was the only product of methylation of dextrins followed by hydrolysis (Freudenberg et al. 1936). Later, acetate derivatives of the dextrins were proposed and characterized for the first time (Freudenberg et al. 1947a, b). In 1955, Freudenberg published a detailed description of the chemistry of the three main cyclodextrins (Freudenberg 1955), and in 1962, he summarized all his results (Freudenberg 1962).

Between 1942 and 1950, French published numerous important contributions on the chemistry of the Schardinger dextrins (French and Rundle 1942; Rundle and French 1943; Bates et al. 1943; French et al. 1948, 1949a, b, 1950a, b, 1954; French and McIntire 1950; Norberg and French 1950). Very quickly, like Freudenberg, French became a pioneer in the understanding of their chemistry (Thoma and Stewart 1965; Caesar 1968; Szejtli 1998; Crini 2014). French showed that the Schardinger dextrins, being cyclic, had no non-reducing end group and they were extremely resistant to alpha-type amylases. Using data from periodate oxidation and methylation reactions, he demonstrated that Schardinger dextrins could not be open-chain compounds. Periodate oxidation was slow with Schardinger dextrins in

comparison with that of straight-chain amylodextrin. French published protocols for the methylation of Schardinger dextrins and showed that 2,3,6-tri-methylglucose was the only product of methylation of cycloamyloses followed by hydrolysis (French et al. 1950b), in agreement with the previous results published by Pringsheim (Pringsheim 1924, 1925, 1926; Pringsheim and Beiser 1924) and Freudenberg (Freudenberg and Meyer-Delius 1938; Freudenberg et al. 1938). French also published solubility data on cycloamyloses, especially in presence of organic liquids. Solubility data of dextrins in water at room temperature were as follows: α-dextrin 14.5 g/100 mL, β-dextrin 1.8 g/100 mL, and γ-dextrin 23.2 g/100 mL (French et al. 1949a). Using data from periodate oxidation and methylation reactions, French definitively demonstrated that Schardinger dextrins could not be open-chain compounds and they were regarded as conical cylinders (French and McIntire 1950; Norberg and French 1950; French et al. 1950a, b). At that time, Schardinger dextrins were also found to be rather anomalous structures with interesting complexing properties when compared with the linear oligosaccharides. French indeed suggested for the first time the fact that cycloamyloses were capable of forming particular complexes. The nature of the complexes between halogen and Schardinger dextrins, particularly the iodine complexes, depended much on the amount of the halides added. However, the cavity of dextrins was referred to as hydrocarbon in nature by French. This result has been definitively abandoned in 1965 with the advent of the modern conformational theory.

1.3.4 Molecular Structure of Schardinger Dextrins

Schardinger was the first to hypothesize that the crystalline substances were "cyclic polysaccharides" (Schardinger 1907, 1909, 1911). However, he never managed to elucidate their structure.

In 1920, Karrer was the first to suggest that the dextrins were made up of several components (Karrer 1920), and 1 year later, he proved it using detailed acetolysis data (Karrer 1921; Karrer and Nägeli 1921a, b; Karrer et al. 1921). In 1923, Karrer was also the first to propose that dextrins are composed of maltose units only joined by α-(1→4) glucosidic linkages (Karrer 1923, 1925), although Pringsheim (1922, 1924) remained unconvinced by Karrer's conclusions. Figure 1.6 is a schematic illustration of two glucopyranose units of a dextrin molecule showing details of the α-(1→4) glucosidic/glycosidic linkage and the numbering systems employed to describe the glucopyranose rings. Later, Miekeley (1930, 1932) also came to the same conclusions as Karrer. In 1926, Pringsheim is finally convinced by Karrer's conclusions (Pringsheim 1926) although he continued to regard the polyamyloses as the basic units of the starch "molecule" (Pringsheim 1928a, 1931a). However, just like Schardinger, Karrer, and Miekeley, Pringsheim failed to elucidate the cyclic structure of the dextrins.

From 1934 for a period of approximately 25 years, the main contributions toward the molecular structure and size of the Schardinger dextrins were developed by

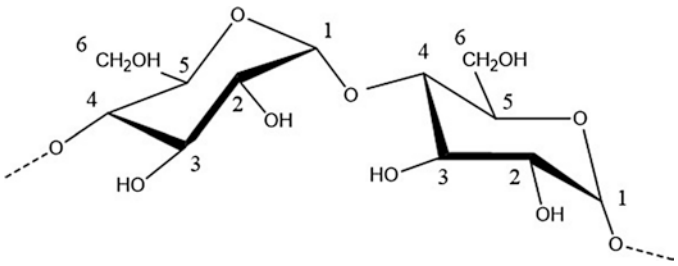


Fig. 1.6 Schematic illustration of two glucopyranose units of a dextrin molecule showing details of the α-(1→4) glycosidic linkage and the numbering systems employed to describe the glucopyranose rings

Freudenberg (Freudenberg 1934, 1939, 1943, 1955, 1957a, b; Freudenberg and Jacobi 1935; Freudenberg and Rapp 1936; Freudenberg et al. 1936, 1938, 1939, 1947a, b, 1953; Freudenberg and Meyer-Delius 1938, 1939; Freudenberg and Cramer 1948, 1950). From 1922, Freudenberg was attracted by Schardinger dextrins since he wanted to obtain information on the degradation products of starch to be able to elucidate its structure (Freudenberg and Ivers 1922). For Freudenberg, Schardinger dextrins were first laboratory curiosities and/or unwanted by-products of starch degradation (Freudenberg 1934), and their chain molecules were intermediate between maltose and starch with non-reducing end groups. Indeed, it is only at the end of the 1930s that Freudenberg concluded that the dextrin-α and dextrin-β molecules were cyclic. In 1935, α-dextrin was considered as a mixture of chain molecules containing 4–5 *D*-glucose units (Freudenberg and Jacobi 1935). Using results of constructing molecular models with the monomer units in a boat rather than a chain conformation, the dextrins were lined with a hydrocarbon interior. One year later, studying the nature of the glycosidic bonds, Freudenberg showed that the dextrins gave rotation-time curves closely parallel to those given by starch and the rigid models such as Kekulé model did not allow free rotation about the individual bonds (Freudenberg and Rapp 1936). The presence of a *Konstellation*, i.e., a ring conformation, is suggested, and in 1936, Freudenberg hypothesized that A-dextrin and β-dextrin have a cyclic structure (Freudenberg et al. 1936). During 2 years, he tried to prove it. On the basis of results obtained from methylation reactions and enzymatic hydrolysis of the dextrins, Freudenberg came, in 1938, to the "same conclusion" as Schardinger, Karrer, Pringsheim, and Miekeley, concerning the cyclic chemical structure of A-dextrin and β-dextrin (Freudenberg and Meyer-Delius 1938; Freudenberg et al. 1938). Ten years later, Freudenberg and his doctoral student Cramer finally demonstrated his conclusion using optical activity data (Freudenberg and Cramer 1948). Schardinger dextrins had a cyclic structure composed of maltose units bound together by α-(1→4) glycosidic linkages. At that time, both French and Borchert also confirmed the cyclic structure of dextrins by X-ray crystallography (French et al. 1948; Borchert 1948). However, although Freudenberg had determined for the first time the correct chemical structure for the Schardinger dextrins, the number of D-glucosyl residues that he gave for the α- and β-dextrin

rings, i.e., five and six, respectively, using a cryoscopic method were incorrect. The correct values were determined by French using both X-ray diffraction and crystal density measurements.

Between 1942 and 1965, French also contributed greatly to the molecular structural knowledge of the Schardinger dextrins, or, as he preferred to call them, cycloamyloses (Caesar 1968; Szejtli 1998; Crini 2014). Very quickly, French became a pioneer in the understanding of their structure, publishing an impressive number of results on cycloamyloses which are still used as references today (French and Rundle 1942; Rundle and French 1943; Bates et al. 1943; French et al. 1948, 1949a, b, 1950a, b, 1954; French and McIntire 1950; Norberg and French 1950; French 1957a, b, 1960, 1962; Bailey and French 1957; Thoma and French 1958, 1959, 1960, 1961; James et al. 1959; Thoma et al. 1959; Whelan et al. 1960; Pulley and French 1961; Robyt and French 1964; French and Abdullah 1965; French et al. 1963, 1965). French's first work concerned the molecular weights of the Schardinger dextrins, considered as cyclic molecules in agreement with the previous results published by Freudenberg (Freudenberg and Meyer-Delius 1938; Freudenberg et al. 1938). French and Rundle (1942), using the X-ray diffraction technique and crystal density measurements, determined the molecular weights of α-dextrin and β-dextrins and discovered the exact number of glucose units per dextrin, i.e., six and seven, respectively, in disagreement with the results published by Freudenberg and Jacobi (1935). French and Rundle demonstrated that molecular weights were integral multiples of the value 162.1 for a glucose residue. They concluded that the X-ray diffraction technique was better suited to the determination of the molecular weights of high molecular weight crystalline substances since impurities, such as solvent of crystallization and inorganic ash, were of minor importance (French and Rundle 1942). In this paper, French also suggested that Schardinger dextrins were cyclic "macromolecules," formed from starch polysaccharide (French and Rundle 1942). They were non-reducing "D-glucopyranosyl polymers" containing 6, 7, or 8 units linked by α-*D*-(1→4) bonds, in agreement with the results published by Karrer (1923) and Miekeley (1932). In each cycloamylose "macromolecule," the *D*-glucose units were in the C1 conformation. Schardinger dextrins were then regarded as cylinders (French and Rundle 1942). However, Freudenberg did not agree with this point of view (Freudenberg 1943).

French pointed out three interesting features: (1) as a consequence of the C-1 conformation of the glucopyranose units, all the secondary hydroxyl groups were located on one side of the cylinder, whereas all the primary hydroxyl groups were located on the opposite side of the cylinder; (2) the interior of the cylinder consisted only of a ring of C-H groups, a ring of glucosidic oxygens, and another ring of C-H groups; and (3) the interior of the cavity was relatively apolar compared to water (French and Rundle 1942; Rundle and French 1943; Bates et al. 1943; French et al. 1948, 1949a, b). Freudenberg claimed again that all the structural and conformational conclusions of French were ambiguous due to "the use of products that were not pure" (Freudenberg et al. 1947a, b). One year later, Freudenberg and Cramer concurred with French's results, after studying the X-ray measurements of Borchert (1948) and also his optical rotation data, publishing similar interpretations

(Freudenberg and Cramer 1948; Cramer 1949). In 1950, French, studying the periodate oxidation of the three cycloamyloses, finally concluded that all three molecules had a cyclic structure in which each *D*-glucose unit was linked to the next by an α-*D*-(1→4)-glucosidic bond, and the interior of the cavity was apolar (French and McIntire 1950; Norberg and French 1950; French et al. 1950b). Schardinger dextrins were then regarded rather as conical cylinders than cylinders, in agreement with Cramer's suggestion. Another interesting feature is made by French: γ-dextrin was "a noncoplanar, more flexible structure," and therefore, it was the "most soluble of the three dextrins." Later, cycloamyloses were finally regarded as truncated cones or "capsules" by French (French 1957a), in agreement with the results published by Cramer (Cramer 1952, 1953, 1956; Dietrich and Cramer 1954).

Cramer also contributed greatly to the molecular structural knowledge of the Schardinger dextrins. In 1948, the young student Cramer published his first result on Schardinger dextrins (Freudenberg and Cramer 1948). Using optical activity, Cramer demonstrated the cyclic nature of α- and β-dextrins. The same year, he discovered γ-dextrin and suggested that the three dextrins possessed an apolar cavity. One year later, Cramer received his PhD at Heidelberg University, under the supervision of Freudenberg (Cramer 1949). He introduced the cyclodextrin-based nomenclature, demonstrated the cyclic nature of cyclodextrins using optical activity data, and showed that the three cyclodextrins had different internal diameters and each cavity was filled with water molecules (Cramer 1949). His doctoral work was then published between 1951 and 1952 (Cramer 1951a, b, c, 1952), adding to the previous results of Freudenberg but mostly "confirming those of French" on the physical (cavity size) and chemical (reactivity) properties, the structure, and chemistry of cyclodextrins. For instance, investigating the configuration at the anomeric centers by hydrolytic methods, Cramer came to the same conclusions as Karrer (1923), Miekeley (1932), and French (French and Rundle 1942) as to the existence of α-(1→4) glucosidic/glycosidic linkages. Cramer also published for the first time a variety of other interesting features. Studying the molecular size of the three dextrins, he showed that a same dextrin could exist in different crystal forms. Cramer then discovered the toroidal form of the cyclodextrin molecules, considering cyclodextrins as truncated cones or "capsules" rather than cylinders, like previously reported by French (French et al. 1948, 1949a, b). The numbering system employed to describe the glucopyranose rings, reported in Fig. 1.5, was then accepted, and Cramer schematized his conclusions on the chemical structure of α-, β-, and γ-cyclodextrins by the two schemes reported in Fig. 1.7. Cramer finally concluded that cyclodextrins were non-reducing oligosaccharides containing 6, 7, or 8 units linked by α-*D*-(1→4) bonds, having both hydrophobic and hydrophilic regions. On the side where the secondary hydroxyl groups were situated, the diameter of the cavity was larger than on the side with the primary hydroxyls, since free rotation of the latter reduced the effective diameter of the cavity. Figure 1.8 illustrates the hydrophobic and hydrophilic regions of an α-dextrin "capsule" (Cramer 1953, 1956; Dietrich and Cramer 1954).

In 1965, both Casu et al. (1965) and Hybl et al. (1965) confirmed the conclusions published by French and Cramer on the cyclic structure of cyclodextrin and its

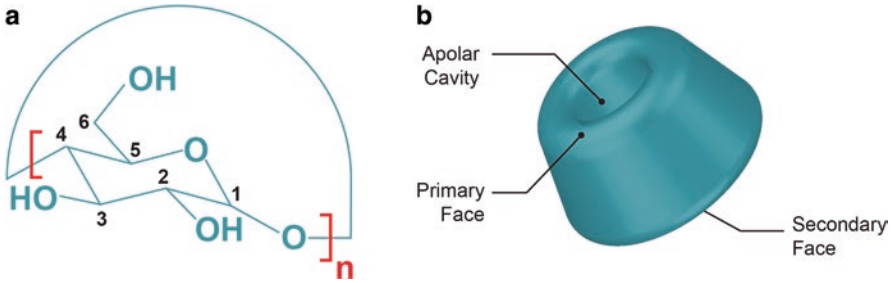


Fig. 1.7 Schematic representations of the (**a**) general chemical structure for cyclodextrins (n = number of glucose units; n = 6, 7, and 8 for α-, β-, and γ-cyclodextrin, respectively) and (**b**) their particular structure showing the apolar cavity of a cyclodextrin "capsule" or torus

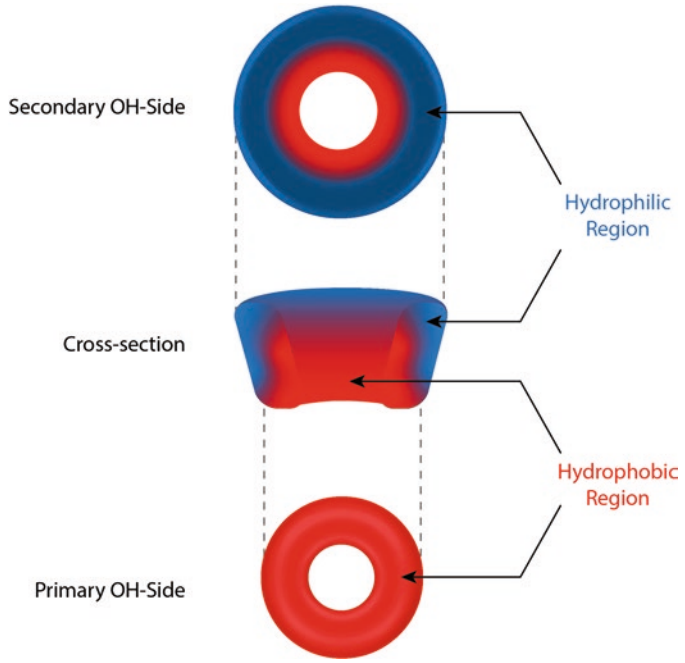


Fig. 1.8 Schematic representation of a dextrin "capsule" showing the hydrophobic and hydrophilic regions

features, using NMR spectra in dimethylsulfoxide solution and using X-ray crystallography of the α-cyclodextrin-potassium acetate complex, respectively. Their results clearly demonstrated that (i) all the glucose residues of cyclodextrins were in the ^{4}C1 chair conformation; (ii) the cavity was lined by the hydrogen atoms and the glycosidic oxygen bridges, respectively; and (iii) the nonbonding electron pairs of the glycosidic oxygen bridges were directed toward the inside of the cavity,

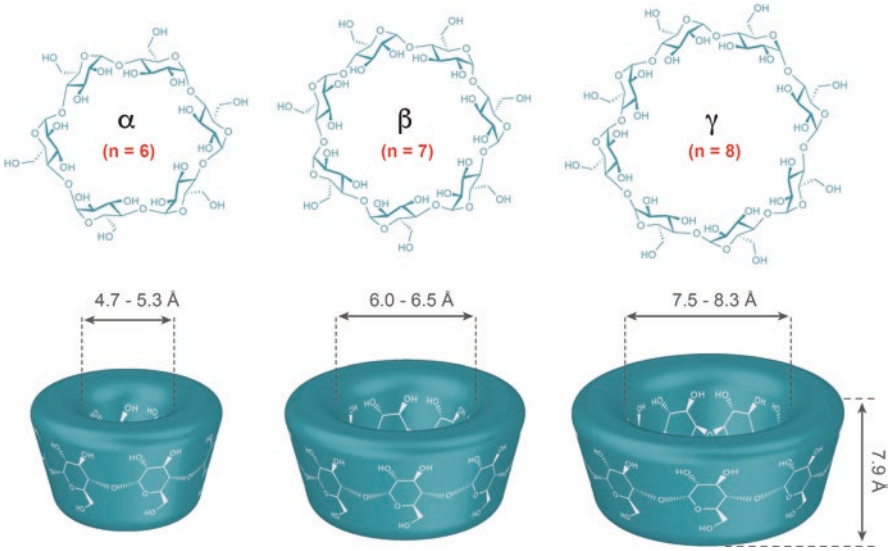


Fig. 1.9 Schematic representations of the chemical tridimensional structure and dimensions for α-, β-, and γ-cyclodextrins (*n* = 6, 7, and 8, respectively) accepted in the 1960s

producing a high electron density. The schematic diagram of two glucopyranose units of a cyclodextrin molecule showing details of the α-(1→4) glycosidic linkage reported in Fig. 1.6 and the schematic representations of the chemical tridimensional structure and dimensions (Fig. 1.9) for α-, β-, and γ-cyclodextrin are finally accepted at the mid-1960s. Later, a more precise study of the conformation of α-cyclodextrin in solution was made by Saenger's group using NMR spectroscopy (Wood et al. 1977). All six glucose units had identical conformations and the molecule had hexagonal symmetry. The secondary hydroxyl groups, which were located in one side of the torus of cyclodextrins, formed hydrogen bond with the secondary hydroxyl groups of contiguous glucose units, in agreement with the previous conclusions published by Casu et al. (1965) and by Hybl et al. (1965). In the cyclodextrin molecule, a complete secondary belt was formed by hydrogen bonds, making it a rigid structure. This was proposed to explain the fact that, among the three native cyclodextrins, β-cyclodextrin had the lowest solubility (Wood et al. 1977). The hydrogen belt was incomplete in the α-cyclodextrin molecule, and γ-cyclodextrin was a noncoplanar, more flexible structure, confirming the results published by French and McIntire (1950). At the beginning of the 1960s, French indicated the possible existence of "a Schardinger dextrin family," describing the structure of δ-dextrin, ε-dextrin, ξ-dextrin, and η-dextrin containing 9, 10, 11, and 12 glucose units, called larger homologues of cycloamyloses (Pulley and French 1961; French et al. 1965). These larger dextrins were not regular cylinder-shaped structures. Indeed, they were collapsed and their real cavity was even smaller than the γ-dextrin (Fig. 1.10).

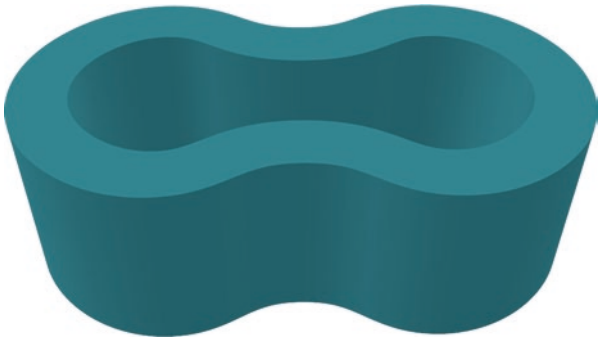


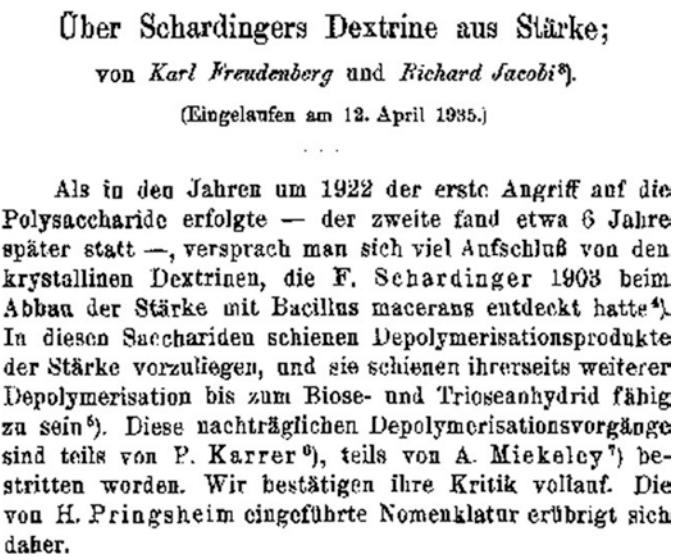
Fig. 1.10 Collapsed cylinder structure of δ-dextrin

1.3.5 Preparation and Separation of Schardinger Dextrins

Between 1905 and 1911, Schardinger studied the first preparation, fractionation/separation, and purification of the two *cellulosines* (Schardinger 1905, 1907, 1909, 1911). In 1911, he published the first fractionation and purification scheme of the dextrins. Later, both Freudenberg, French, and Cramer published other important schemes: see the references French (1957a) and Thoma and Stewart (1965).

The dextrins were synthesized from several sources of starch, e.g., potatoes, rice, and wheat, and bacteria, e.g., the formation of dextrins depended on the type of bacteria digesting starch. About 25–30% of the starch was converted to crystalline dextrins depending on these parameters. The yield was tenfold those reported by Villiers (Schardinger 1907). Schardinger also based his method of separation on the ease of crystallization of the β-dextrin from water and its low solubility, about 1.5% at room temperature, followed by precipitation of the α-dextrin from the mother liquor by the addition of alcohol. Schardinger' protocol was modified by Lange in 1925 who introduced trichloroethylene as a precipitating agent for the crystalline dextrins (Lange 1925). This protocol is described in detail in Pringsheim's book (Pringsheim 1932).

In 1935, Freudenberg and his student Jacobi described a method for the synthesis of Schardinger dextrins with high purity (Freudenberg and Jacobi 1935) (Fig. 1.11). Freudenberg is indeed recognized as the first to prepare almost pure dextrins with high yields (Thoma and Stewart 1965; Caesar 1968; Clarke et al. 1988; Szejtli 1998; Crini 2014). Freudenberg improved the separation of dextrins and produced a scheme based not only on solubility differences of the dextrins themselves, as initially proposed by Schardinger, but also on the differences in solubilities and rates of crystallization of their acetates (Freudenberg and Jacobi 1935). However, the protocol was difficult since it involved many acetylation and saponification reactions. During more than 10 years, this protocol was studied and modified, and in 1947, Freudenberg's group described the first scheme for the isolation of pure fractions of dextrins using bromobenzene as precipitant: α-dextrin did not precipitate, while β-dextrin and γ-dextrin were readily precipitated (Freudenberg et al. 1947a,



Über Schardingers Dextrine aus Stärke;

von *Karl Freudenberg* und *Richard Jacobi*[3].

(Eingelaufen am 12. April 1935.)

. . .

Als in den Jahren um 1922 der erste Angriff auf die Polysaccharide erfolgte — der zweite fand etwa 6 Jahre später statt —, versprach man sich viel Aufschluß von den krystallinen Dextrinen, die F. Schardinger 1903 beim Abbau der Stärke mit Bacillus macerans entdeckt hatte[4]. In diesen Sacchariden schienen Depolymerisationsprodukte der Stärke vorzuliegen, und sie schienen ihrerseits weiterer Depolymerisation bis zum Biose- und Triosean hydrid fähig zu sein[5]. Diese nachträglichen Depolymerisationsvorgänge sind teils von P. Karrer[6], teils von A. Miekeley[7] bestritten worden. Wir bestätigen ihre Kritik vollauf. Die von H. Pringsheim eingeführte Nomenklatur erübrigt sich daher.

Fig. 1.11 First page of the article of Professor Freudenberg published in 1935 where he described a method for the synthesis of Schardinger dextrins with high purity

b). This scheme was comprehensively discussed by French (1957a). In 1950, Freudenberg and Cramer also confirmed the possible existence of dextrins with 9 or 10 glucose units, identified during the preparation of α-, β-, and γ-dextrins (Freudenberg and Cramer 1950). However, these findings were only substantiated a decade later by French (Pulley and French 1961).

French was also among the early researchers, along with Freudenberg, to focus on improving the production of dextrins. French became a pioneer in the preparation of the compounds in a very pure state. Knowing the works of the group of Hudson on the enzymolysis conditions which affected the yield and proportion of the dextrins (Tilden and Hudson 1939, 1942; Tilden et al. 1942; McClenahan et al. 1942; Wilson et al. 1943) and using his own results on the solubilities of Schardinger dextrins (French et al. 1949a), French proposed in 1949 a new protocol for the separation and purification of dextrins (French et al. 1949b), which did not require the acetylation and saponification steps used by Freudenberg. Treatment of starch with the amylase of *Bacillus macerans* gave crude starch digests containing the three cycloamyloses, i.e., ~60% α-dextrin, ~20% β-dextrin, and ~ 20% γ-dextrin, together with small amounts of higher cycloamyloses. Moreover, the protocol permitted the facile separation of pure dextrins by differential precipitation using specific precipitants such as bromobenzene and propan-1-ol (French et al. 1949b). Later, French showed that high temperature cellulose column chromatography was one of the most effective methods for the quantitative analysis of mixtures of cycloamyloses (Pulley and French 1961; French et al. 1965). This method was required in connection with the production of cycloamyloses since these products were simultaneously produced from starch together with the higher series of cycloamyloses. In 1961,

French also reported the preparation, isolation, and partial characterization of large cyclodextrins with 9, 10, 11, and 12 glycosyl units in the macrocycle (Pulley and French 1961), identified during the preparation of α-, β-, and γ-dextrins like Freudenberg.

In the mid-1950s, Cramer also investigated the enzymatic production of cyclodextrins, their separation and purification, and characterization (Cramer 1955, 1956; Cramer and Steinle 1955; Cramer and Henglein 1957a, b). Cramer described an easy protocol to separate α-, β-, and γ-cyclodextrins from the digest by selective precipitation using appropriate organic compounds and optimize parameters, e.g., pH = 6 and temperature = 40 °C (Cramer 1956). The three cyclodextrins are precipitated by addition of a tetrachloroethylene-tetrachloroethane mixture, followed by the addition of *p*-cumene. α-Cyclodextrin was isolated by selective precipitation with cyclohexane, β-cyclodextrin with fluorobenzene, and γ-cyclodextrin with anthracene. Cramer explained his results by the difference in the sizes of cavities of the three cyclodextrins and concluded that the superiority of his method over previous procedures, particularly those of French, resided in the technical ease and the completeness of precipitation.

To summarize, during the periods of reaching maturity from 1935 to 1950 and of exploration from 1950 to 1970, the separation and the purification of the mixture were difficult (Crini 2014; Crini et al. 2018). The period of reaching maturity was also marked by several contradictory results, due, at least in part, to differences in the protocols used for the preparation of Schardinger dextrins and dubious purity of the samples (Thoma and Stewart 1965; Caesar 1968; Szejtli 1998). The work during these periods was even marred by hot debate between the different laboratories, especially those of Freudenberg and Cramer and of French. In addition, in the early 1950s, researchers had not fully realized the potential of cycloamyloses and had little faith in their complexation properties (Szejtli 1998). The three main cyclodextrins were considered just laboratory curiosities difficult to produce. In 1963, French was the first to propose the preparation of cycloamyloses on a larger-than-laboratory scale (French et al. 1963). However, at the end of the 1960s, French concluded that "cycloamyloses were very promising molecules although they remained very expensive products, available only in small amounts as fine chemicals, and also toxic."

1.3.6 The Action of Amylases on Cycloamyloses

Up to 1939, the Schardinger dextrins were known only as products of the bacterial breakdown of starch. For Freudenberg's opinion, *Bacillus macerans* was able to transform starch structure into cyclic and linear breakdown products, and starch was based upon a cyclic Schardinger nucleus with side branches which would be broken in the bacterial breakdown (Fig. 1.11). During the same period, Tilden and Hudson (1942), studying the bacteria that produced the dextrins, also concluded that the resulting Schardinger dextrins were derived from some basic configuration

pre-existing in the starch "molecule." Similar conclusions were published by Kerr (1942, 1943), Myrbäck (1942), and Samec (1942).

Using this concept, Freudenberg was the first scientist to investigate the enzymatic production of dextrins and to propose a first mechanism of action for *Bacillus macerans* (Freudenberg et al. 1938, 1939; Freudenberg and Meyer-Delius 1938, 1939; Freudenberg 1939). This mechanism indicated that the dextrins were preformed within the starch macromolecules (Freudenberg 1939), in agreement with the results of Hudson' group (Tilden and Hudson 1939). However, Freudenberg did not agree with the generally accepted point of view at that time concerning the nature of the bonding of the *D*-glucose units, and he rapidly abandoned this mechanism. Using the helical model of the structure of starch proposed by Hanes (1937) and the α-*D* nature of the glucose units, Freudenberg proposed a second mechanism based on a transglucosylation reaction (Fig. 1.12). He suggested that the enzyme involved was able to degrade the helical structure of the starch, i.e., the amylose fraction, and that there ensued a rearrangement of the glucose units which were then able to form a ring structure (Freudenberg et al. 1939; Freudenberg and Meyer-Delius 1939). Because of the helical arrangement, the first and fifth or sixth

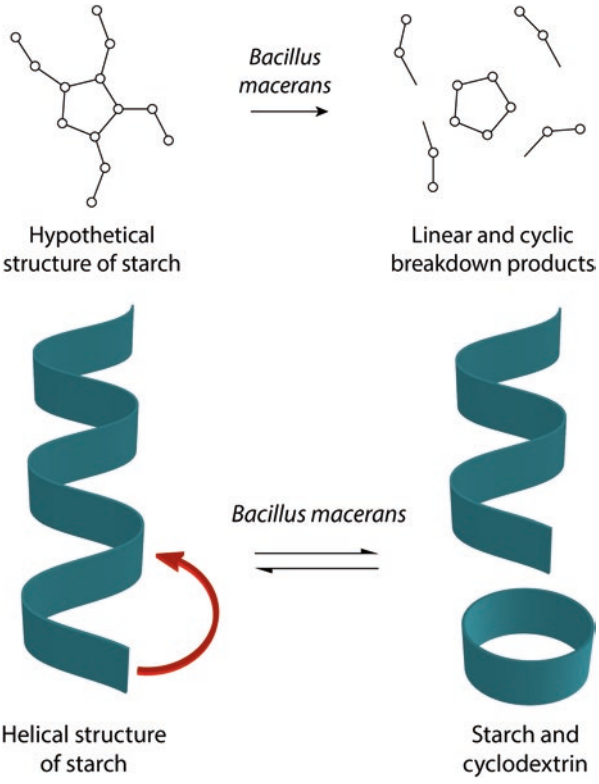
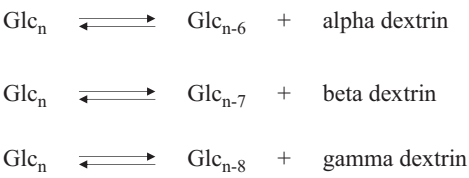


Fig. 1.12 Freudenberg's initial (above) and final (below) model of formation of Schardinger dextrins/cyclodextrin; adapted from Freudenberg (1939)



Scheme 1.1 Reactions proposed by Freudenberg (1939) to explain the formation of dextrins (Glc = a *D*-glucose or a *D*-glucosyl residue)

D-glucosyl residues were situated close to one another and were able to unite to form rings of five or six D-glucose units. The reactions proposed by Freudenberg to explain the formation of dextrins are given in Scheme 1.1 (Freudenberg 1939). Freudenberg concluded that the cyclodextrins were not preformed in starch "molecule" but that formation was made possible by the helicity of the starch chain. However, he was unable to prove this mechanism. It would be confirmed a few years later by French using chromatography (French et al. 1954) and later by Takeo and Kuge (1969) using X-ray crystallography.

From the 1940s, numerous research groups worked on the bacteria that produced the dextrins (Myrbäck 1938, 1942, 1949a, b; Samec and Blinc 1939, 1941; Myrbäck and Ahlborg 1940; Blinc 1941, 1942; Samec 1942; Tilden and Hudson 1939, 1942; Tilden et al. 1942; Kerr 1942, 1943, 1949; Kerr and Severson 1943; Wilson et al. 1943; Myrbäck and Gjorling 1945; Cori and Cori 1946; French et al. 1948; Kerr and Cleveland 1949; Hale and Rawlins 1951). However, the discovery of the enzyme in *Bacillus macerans*, responsible for the conversion of starch into dextrin, is attributed to Tilden and Hudson. In 1939, Tilden and Hudson isolated a cell-free enzyme preparation from *Bacillus macerans*, i.e., *Acrobacillus macerans*, that had the ability to convert starch into crystalline dextrins with interesting yields, ~55%, (Tilden and Hudson 1939). They introduced the name of cycloamylose glucanotransferase, i.e., CGTase or cyclodextrin glucanotransferase. Prior to this discovery, dextrins were made using live cultures of *Bacillus macerans*. In 1942, the authors proposed the following protocol (Tilden and Hudson 1942; Tilden et al. 1942): they cultivated *Bacillus macerans* on sterilized potato slices or on a medium containing 5% oatmeal, in presence of 2% calcium carbonate; after 2–3 weeks of cultivation at 37–40 °C, the cell mass was recovered by filtering or centrifuging; the filtrate contained the enzyme in an activity of 0.7 units/mL, which was separated either by freeze-drying or, after concentration, by precipitation with acetone. Their results mainly showed that it was essential to determine the optimal culturing conditions for the production of the enzyme and the optimal pH, temperature, and fermentation time for enzyme activity for effective use of the enzyme. Later, Hale and Rawlins (1951) also attained similar yields of the enzyme on a scale of 20 L in 10–12 days in an aerated culture. Tilden and Hudson were also the first to propose a simple protocol for purifying the amylase of *Bacillus macerans* using both precipitation by acetone, adsorption, and dialysis steps (Tilden and Hudson 1942; Tilden et al. 1942). The enzyme purified had an activity 140 times that of the initial enzyme solution and was able to convert 1000 times its weight of starch in 30 min at 40 °C.

Since Tilden and Hudson's discovery of *Bacillus macerans* cycloamylose glucanotransferase, effort was devoted to working out methods for cyclodextrin