Universität für Bodenkultur Wien

Department of Food Sciences and Technology

Institute of Food Technology
Process Technology

Advisor/Advisors:
Ao.Univ.Prof. Dipl.-Ing. Dr.techn. Senad Novalin

# DESCRIPTION OF A SYNTHESIS PATHWAY FOR THE PRODUCTION OF CARBOHYDRATES FROM $CO_2$ AND $H_2O$ AS A THEORETICAL ALTERNATIVE TO PHOTOSYNTHESIS

Dissertation
for obtaining a doctorate degree
at the University of Natural Resources and
Life Sciences Vienna

Submitted by
Dipl.-Ing. Thomas Zweckmair

Vienna, May 2011

## Acknowledgements

The present dissertation has been carried out at the Department of Food Sciences and Technology, Institute of Food Technology, Working Group Process Technology, University of Natural Resources and Life Sciences (BOKU Vienna), Austria from October 2008 to May 2011. The work has been supervised by Prof. Dr. Senad Novalin. The financial support for this work has partly been provided by Gradient Process Technology GmbH, Pyrkergasse 8/1, 1190 Vienna.

First of all, I would like to dedicate my greatest thank to my supervisor, Prof. Dr. Senad Novalin. He has been helping me a lot and encouraged me to overcome any difficulty I have faced during the research work. My sincere gratitude goes to Prof. Dr. Dietmar Haltrich for taking part in the supervision-team. Without his enthusiastic support, I would have been not able to initiate the investigation of new issues during the progress of the dissertation.

With regard to the development of analytical methods I want to dedicate my sincere gratitude to Fritz, Stefan and Manuel and to the Christian Doppler Research Society (Christian Doppler lab ‘‘Advanced cellulose chemistry and analytics’’) for the financial support. I wish to thank very much my colleagues Thomas, Moritz and Bernhard for their inspiring help in technical assistance during conducting trials and performing subsequent analysis. I also want to express my sincere gratitude to the trainees Vera, Lina and Maria for their encouragement and their enthusiastic technical assistance. My appreciation goes to the staffs and students of the Division of Food Biotechnology, Department of Food Science and Technology (BOKU Vienna). I will always remember their help in my work and their friendlyness they spread all over the laboratories. In particular, I acknowledge Cindy Lorenz for her help in providing resources whenever I asked for.

I want to thank my parents for the education and guidance they gave me in my way to adulthood; especially my mother for her patience and her understanding. Sincerest thanks to my girlfriend Claudia for all her love I have been privileged from her. Her understanding and patience encourages me very much to complete this dissertation. Looking at you is sufficient to overcome any difficulty.

Vienna, May 2011

## Abstract

The present dissertation deals with a multi-step process for the synthesis of carbohydrates from $CO_2$ and $H_2O$. As a preliminary step water electrolysis is applied in order to generate oxygen and hydrogen. Subsequently hydrogenation of $CO_2$ to methanol is carried out. Once having methanol, conversion to formaldehyde, the key component, is conducted using conventional catalysis systems. By carrying out a controlled aldol-condensation using formaldehyde, metabolizable carbohydrates can be generated. Isolating the later one and providing oxygen from the preliminary electrolysis, the metabolizable hexoses can be consumed in cell respiration. Recycling and reusing of the remaining reaction components (e.g. toxic formaldehyde) plays a decisive role in such a closed cycle. Thus, the entire system could be considered e.g. as an artificial photosynthesis process.

With regard to the controlled aldol-condensation $C_3$-carbohydrates e.g. could be suggested as intermediate products for the manufacture of synthetic hexoses. Based on this, in a simple bottom-up approach, formaldehyde can be converted with glycolaldehyde in a controlled one-step aldol-condensation to produce $C_3$-carbohydrates (DL-glyceraldehyde and dihydroxyacetone). This reaction represents a so-called formose reaction, the aldol-condensation of formaldehyde and carbohydrate-fragments, in its initial phase yielding racemic carbohydrates. Subsequently, in an anion-exchange resin catalyzed aldol-condensation, $C_3$-carbohydrates are condensed to e.g. DL-fructose and DL-sorbose with a considerable yield enabling partial diastereoselective control. This cascade provides a system which can be used for the synthesis of racemic hexoses from $CO_2$ and $H_2O$.

On the basis of this process chain many extensive research and problem areas can be identified. Some of them were investigated in the present work: a) examining the formose reaction with regard to delivering appreciable product distributions; this includes the development of a simple and versatile method for the analysis of complex carbohydrate mixtures, b) examining separation technologies for the isolation of desired carbohydrates, c) deducing a possible pathway for the entire process and d) discussing the individual research areas with regard to the identification of principal problems.

The analysis of complex carbohydrate mixtures represents a major challenge, which needs the combination of LC, CE and GC. For LC-UV analysis per-*O*-benzoylation of alditols (PBAs) and reversed phase chromatography was introduced. Validation was carried out based on LC-ESI-MS (liquid chromatography-electrospray ionization-mass spectrometry)-data.

It is recommended to operate the formose reaction e.g. at moderate temperatures (room temperature) using homogeneous catalysis, which delivers appreciable product distributions.

The separation of $C_3$-carbohydrates after aldol-condensation of formaldehyde and glycolaldehyde was examined using the membrane processes nanofiltration (NF) and reverse osmosis (RO). Two fractions were obtained, namely a $C_3$-enriched and a fraction containing mainly formaldehyde, glycolaldehyde, methanol and formic acid. The later fraction has to be used for a subsequently repeated aldol-condensation. In a first test series of experiments, approximately 80% of formaldehyde, methanol and formic acid were removed in case of a RO-process. About 40% of $C_3$-carbohydrates were found in the permeate. Also NF may fulfill this separation task.

The assessment of different problem areas did not uncover principal problems with regard to deducing an artificial photosynthesis process, at least theoretically.

**Keywords:** artificial photosynthesis, $CO_2$-reduction, DL-glyceraldehyde and dihydroxyacetone, synthetic hexoses, nanofiltration, reverse osmosis, aldol-condensation; per-*O*-benzoylation of alditols

## Abstract

Die vorliegende Dissertation beschäftigt sich mit einem Mehrschritt-Prozess zur Synthese von Kohlenhydraten aus $CO_2$ und $H_2O$. In einem vorgelagerten Schritt wird die Elektrolyse von Wasser eingesetzt, wobei Sauerstoff und Wasserstoff gewonnen werden. Anschließend wird $CO_2$ zu Methanol hydriert und weiter zu Formaldehyd umgesetzt, wobei konventionelle Katalysesysteme verwendet werden. Auf der Basis von Formaldehyd wird nunmehr eine kontrollierte Aldolkondensation vorgeschlagen um metabolisierbare Kohlenhydrate zu generieren. Isoliert man letztere und stellt Sauerstoff durch die vorgelagerte Elektrolyse zur Verfügung, so können die metabolisierbaren Kohlenhydrate durch Zellatmung verbraucht werden. Dem Recycling und der Wiederverwendung von den verbleibenden Reaktionskomponenten (z.B. giftiges Formaldehyd) kommt in solch einem geschlossenen Kreislauf eine entscheidende Rolle zu. Dadurch kann das Gesamtsystem beispielsweise als ein künstlicher Photosyntheseprozess betrachtet werden.

In Bezug auf die kontrollierte Aldolkondensation werden beispielsweise $C_3$-Kohlenhydrate (DL-Glyceraldehyd und Dihydroxyaceton) als Zwischenprodukte zur Darstellung von synthetischen Hexosen vorgeschlagen. Diese können in einem einfachen Bottom-Up-Ansatz durch die Umsetzung von Formaldehyd mit Glycolaldehyd in einer kontrollierten Aldolkondensation erzeugt werden. Diese Reaktion stellt eine sogenannte Formosereaktion in der Anfangsphase dar, welche racemische Kohlenhydrate liefert. Im Anschluss können $C_3$-Kohlenhydrate miteinander kondensiert werden um beispielsweise DL-Fruktose und DL-Sorbose mit einer signifikanten Ausbeute zu erhalten. Dabei ist eine teilweise Kontrolle der entstehenden Diastereomere möglich. Diese Prozesskette stellt ein System dar, welches zur Produktion von racemischen Hexosen aus $CO_2$ und $H_2O$ verwendet werden könnte.

Im Zusammenhang mit dieser Kaskade können viele umfassende Forschungs- und Problemfelder identifiziert werden. Ein Teil dieses Mehrschritt-Prozesses wurde in der vorliegenden Arbeit erforscht: a) Untersuchung der Formosereaktion in Bezug auf Generierung einer erwünschten Produktverteilung. Das beinhaltet die vordergründige Entwicklung einer einfachen und vielseitigen Methode zur Analyse von komplexen Kohlenhydratmischungen, b) Erforschung von Trenntechnologien zur Isolierung von gewünschten Kohlenhydraten, c) Ableiten eines möglichen Pfades, welcher die Realisierung des vorgeschlagenen Prozesses unter spezifischen Bedingungen zulässt und d) Diskussion der einzelnen Forschungsfelder in Bezug auf die Identifizierung prinzipieller Probleme.

Die Analytik von komplexen Kohlenhydratmischungen stellt eine große Herausforderung dar, welche die Kombination von LC, CE und GC benötigt. Zur LC-UV-Analyse wurden die per-O-Benzoylierung von Alditolen (PBAs) und die Umkehrphasenchromatographie eingeführt. Die Validierung wurde anhand von LC-ESI-MS (liquid chromatography-electrospray ionization-mass spectrometry)-Daten durchgeführt.

Es wird empfohlen, die Formosereaktion beispielsweise bei moderaten Temperaturen (Raumtemperatur) unter homogener Katalyse ablaufen zu lassen um wünschenswerte Produktverteilungen zu erzielen.

Zur Untersuchung der Fraktionierung von $C_3$-Kohlenhydraten, nach erfolgter Aldolkondensation von Formaldehyd und Glycolaldehyd, wurden die Membranprozesse Nanofiltration (NF) und Reverse Osmose (RO) eingesetzt. Dabei ergaben sich zwei Fraktionen: die eine enthält angereicherte $C_3$-Kohlenhydrate, die andere hauptsächlich Formaldehyd, Glycolaldehyd, Methanol und Ameisensäure. Letztere muss notwendigerweise zur wiederholten Aldolkondensation von Formaldehyd und Glycolaldehyd rezykliert werden. Im Falle eines RO-Prozesses wurden in einer ersten Versuchsreihe ungefähr 80% an Formaldehyd, Methanol und Ameisensäure abgetrennt. Zirka 40% an $C_3$-Kohlenhydraten wurden im Permeat detektiert. Auch die NF scheint für diese Trennaufgabe geeignet zu sein.

Die Bewertung unterschiedlicher Problemfelder ergab zumindest theoretisch keine prinzipiellen Ausschlussgründe um einen künstlichen Photosynthese-Prozess darstellen zu können.

**Schlagwörter:** künstliche Photosynthese, $CO_2$-Reduktion, DL-Glyceraldehyd und Dihydroxyaceton, synthetische Hexosen, Nanofiltration, Reverse Osmose, Aldolkondensation, per-*O*-Benzoylierung von Alditolen

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# 1 Introduction

## 1.1 Initial situation - $CO_2$ as a $C_1$-source

Considering the system “earth” as a thermodynamically closed system and neglecting e.g. meteorites and nuclear chemical processes, the carbon-content of the system can be supposed to be constant. Within the carbon cycle, $CO_2$ is appearing as the highest oxidized $C_1$-source [1].

$CO_2$ is thermodynamically stable and kinetically inert [2]. Enabling selective reactions for converting $CO_2$ at moderate conditions represents a challenge for a scientist. Numerous pathways for $CO_2$ activation have been investigated [2-4]. These include bioconversion [5-9], photochemical reduction [10-21], electrochemical reduction [3, 22-38], thermal heterogeneous and homogeneous reductions [3, 39-48] as well as coordination to transition metals [2, 3, 39, 49]. In Table 1 the individual standard potentials for the reduction of $CO_2$ are listed.

Table 1: Half-cell reactions for the electroreduction of $CO_2$ [23].

| Reaction | | E° [V][a] |
|---|---|---|
| a | $2\ CO_2 + 2\ H^+ + 2e^- \rightleftharpoons H_2C_2O_4$[b] | -0.475 |
| b | $CO_2 + 2\ H^+ + 2e^- \rightleftharpoons HCOOH$ | -0.199 |
| c | $CO_2 + 2\ H^+ + 2e^- \rightleftharpoons CO + H_2O$ | -0.109 |
| d | $CO_2 + 4\ H^+ + 4e^- \rightleftharpoons HCHO + H_2O$ | -0.071 |
| e | $CO_2 + 6\ H^+ + 6e^- \rightleftharpoons CH_3OH + H_2O$ | 0.030 |
| f | $CO_2 + 8\ H^+ + 8e^- \rightleftharpoons CH_4 + 2\ H_2O$ | 0.169 |

[a]: E° versus normal hydrogen electrode at 298 K.
[b]: oxalic acid.

There are many catalysis-systems which convert carbon dioxide to a mixture of the products as shown in Table 1, and surprisingly ethylene or ethane [12, 50, 51]. It should be mentioned,

that $CO_2$ can be converted to formic acid catalyzed by transition-metal complexes in homogeneous phase or in polymer matrix [3, 37, 39], by a $FeS_2$ electrode [25] or by a Pb-granule-electrode [26, 52] to name just a few.

### 1.1.1 Products obtained by industrial processing of $CO_2$

Synthesis strategies using $CO_2$ as a building block mainly rely on the formation of C-O bonds. The formation of C-C bonds is much more difficult and only a few realized examples are known [53]. Interesting target compounds using $CO_2$ as raw material are carbon acids, esters, lactones, polyesters, polylactones, polycarbonates, cyclic carbonates, urea derivates and ketals under maintenance of the C=O or the COO-functional groups (see Table 2).

Table 2: Products manufactured by the conversion of $CO_2$.

| Product | Usage | Worldwide production [t * $a^{-1}$] |
|---|---|---|
| Urea | fertilizer, urea-melamine-resin, feed additive | 100 * $10^6$ [54] |
| Alkylen carbonates | solvent | [a] |
| β-oxynaphtoic acid | raw material for dyes | [a] |
| Salicylic acid | pharmaceuticals | 40 * $10^3$ [55] |
| Methanol | fuel component, solvent, raw material for formaldehyde and acetic acid synthesis, energy storage | 52.7 * $10^6$ [56]; 2 * $10^6$ via the syngas route [55] |
| Cyclic carbonates | dimethylcarbonate used as reagents for methylation and for manufacturing of polycarbonates | 40 * $10^3$ [55] |
| Formic acid | feed additive, raw material for the synthesis of formamids and formic acid esters | 3 * $10^5$ [57] |

[a]: only produced in small amounts [39].

Further pathways in which $CO_2$ is taking part, would be e.g. the direct hydrogenation in order to yield higher alcohols or oxalic acid, the copolymerization with olefins, the hydrocarbonylation of alkenes, ketons and imins, the conversion to isocyanats, carbamins or the carboxylation of C-H-bonds [55].

## 1.2 $CO_2$ and photosynthesis

Photosynthesis is a well known and highly complex biological process. Although not every detail is understood so far, some of the key elements of natural photosynthesis should be summarized at this point: a) light absorption, b) water oxidation, c) products of light-induced reactions (e.g. ATP and NADPH) enable the formation of C-C covalent bonds utilizing $CO_2$ in order to obtain carbohydrates [58]. As shown in literature, only some key steps of biological photosynthesis have already been mimicked artificially [59-68]. Only some attempts were undertaken to produce metabolizable carbohydrates from $CO_2$ and $H_2O$, but no one shows a technically acceptable solution [69-73]. Currently, there is no artificial solution available, which addresses the comprehensive conversion of $CO_2$ and $H_2O$ to metabolizable carbohydrates taking the recycling of the byproducts accumulated into account.

## 1.3 Objective and definition of the topic

The aim of this dissertation is to investigate a process for the synthesis of carbohydrates from $CO_2$ and $H_2O$, which could be considered as a possible artificial photosynthesis. To the best knowledge of the author there is only a very limited number of approaches, which probably could reach this goal as shown in this work. Two novel pathways are proposed:

a) conversion of $CO_2$ to formaldehyde and performing a controlled aldol-condensation to synthetic hexoses using $C_3$-carbohydrates as intermediate products; separation and entirely recycling of every reaction compound.
b) conversion of $CO_2$ to formaldehyde; suggesting hydroformylation of formaldehyde to desired carbohydrates using synthesis gas [74-76] and again separation and recycling of every reaction compound.

Pathway a) is studied in the present work to a certain extend. The later one represents an alternative route which remains to be investigated.

### 1.3.1 Artificial production of carbohydrates from $CO_2$ and $H_2O$ - proposed conversion pathway in detail

Despite the toxicity of formaldehyde, its production from $CO_2$ is selected to act as the preliminary process step. Doing so, in a first step, $CO_2$ is reduced to methanol by hydrogenation (see Table 3). The hydrogen necessary is produced by electrolysis of water generating oxygen at the same time. In a second step, methanol is partly oxidized/dehydrogenized to yield formaldehyde (see Table 4). Simultaneously in hydroformylation, formaldehyde can react to yield glycolaldehyde [77, 78] (without assessment). These preliminary steps represent the basis for the following cascade:

In a simple bottom-up synthesis approach, formaldehyde and glycolaldehyde are condensed in a controlled one-step aldol-condensation to produce $C_3$-carbohydrates (DL-glyceraldehyde and dihydroxyacetone). Subsequently, in an anion-exchange resin catalyzed aldol-condensation, $C_3$-carbohydrates are converted e.g. to DL-fructose and DL-sorbose with a considerable yield [79, 80] enabling partial diastereoselective control [81, 82]. This cascade provides a system, which can be used for the production of racemic hexoses from $CO_2$ and $H_2O$. By isolating metabolizable hexoses, simultaneously recycling of the byproducts and providing oxygen from the preliminary electrolysis, metabolizable hexoses can be consumed in cell respiration. Thus, a closed cycle is achieved.

#### 1.3.1.1 Conversion step one – $CO_2$ → methanol → formaldehyde

With regard to the preliminary processing steps first of all the conversion of $CO_2$ to methanol should be considered closer. In general, methanol can be produced from $CO_2$ by e.g. photochemical, electrochemical and thermal conversion processes [14, 41, 83]. With regard to photochemical reduction of $CO_2$ to methanol, a conceptual approach was proposed using a multifunctional photocatalyst based on semiconductor material [84]. Although the model system is kept simple, there are significant scientific difficulties to overcome for making the system work. First reported in 1979, the photocatalytic reduction of $CO_2$ in aqueous solution to produce formaldehyde, formic acid, methanol and trace amounts of methane has been carried out using various semiconductors, such as tungsten trioxide ($WO_3$), titanium dioxide ($TiO_2$) or zinc oxide (ZnO) to name just a few [14]. In the following years, many research groups have studied the photocatalytic reduction of $CO_2$ using a variety of semiconductors.

There are severe limitations to improve the productivity of photocatalytic reduction. E.g. it was shown, that the net yield of fuel product in photochemical energy storage reaction is

unlikely to be greater than 12 to 13 percent [85]. The physical separation of the water photodissociation from the $CO_2$ reduction stage could overcome some of the limitations [68, 86].

Concerning the electrochemical reduction of $CO_2$ to methanol the reader is referred to appropriate literature e.g. [83]. With regard to thermal conversion of $CO_2$ to methanol, e.g. the following catalysis systems can be listed (see Table 3).

Table 3: Conversion of $CO_2$ to methanol (selection of examples).

| Catalysis | Reductant | Comments | Ref. |
|---|---|---|---|
| $Cu/SiO_2$ and $Cu/ZrO_2/SiO_2$ | $H_2$ | stepwise hydrogenation of $CO_2$ to methanol takes place on Cu-surface; a bi-functional mechanism for methanol synthesis from $CO_2/H_2$ is proposed | [41] |
| p-type semiconductor electrode, photochemical cell | pyridine | - | [42] |
| optical fiber photoreactor, $Ag/TiO_2$-catalyst | $H_2O$ | - | [13] |
| Ru-Ti-oxide-electrodes | - | - | [43] |
| Cu/Zn/Al/Zr-fibrous catalyst | $H_2$ | it is suggested that the high dispersion and stability of the Cu/Zn crystallites due to the fibrous structure enhanced $CO_2$ hydrogenation and the added Zr component further improved the catalyst | [44] |
| Cu/ZnO-catalyst | $H_2$ | the role of ZnO in Cu/ZnO catalysts can be ascribed to both increases in the Cu dispersion and the specific activity | [45-47] |
| $Cu/TiO_2$ | NaOH, $H_2O$ | photocatalytically catalyzed reduction of $CO_2$ | [21] |

| Catalysis | Reductant | Comments | Ref. |
|---|---|---|---|
| Cu, Ni/Cu-catalyst | $H_2$ | submolar quantities of Ni lead to a strong increase in the rate of methanol formation from CO, $CO_2$ and $H_2$-mixtures | [87] |
| $Ga_2O_3$-Pd/$SiO_2$-catalyst | $H_2$ | the closeness between the Pd crystallites and the $Ga_2O_3$ surface patches enhance the activity | [88] |
| $ZrO_2$ doped CuZnO-catalyst | $H_2$ | the presence of $ZrO_2$ leads to a high copper dispersion | [89] |
| Cu/ZnO-catalyst | $H_2$ | ZnO has no promotional effect on the methanol synthesis activity except for the role of ZnO to create the active site | [90-93] |
| Cu/ZnO-catalyst on a $Al_2O_3$-support | $H_2$ | - | [94, 95] |
| Ru-catalysts supported by micro- and mesoporous oxides | $H_2$ | addition of Co promotes methanol formation | [48] |
| transparent Ti-containing mesoporous silica thin film materials | $H_2O$ | films having hexagonal pore structure exhibited higher photocatalytic activity than the powdered catalyst even with the same pore structure | [20] |

After having methanol, the subsequent conversion to formaldehyde should be highlighted as depicted in Table 4.

Table 4: Conversion of methanol to formaldehyde (selection of examples).

| Catalyst | Comment | Ref. |
|---|---|---|
| Ag-catalyst | conversion of methanol and the selectivity to formaldehyde appeared to increase with respect to the methanol ballast process with no added water | [96, 97] |

| Catalyst | Comment | Ref. |
|---|---|---|
| Ag-catalyst using $CH_3I$ as additive | while the conversion of methanol and oxygen decreased, the selectivity to formaldehyde increased after treating the Ag catalyst with $CH_3I$ | [98] |
| $Na_2CO_3$ in a circulating fluidized bed | compared to the fixed bed reactor higher productivities could be obtained | [99] |
| Nanostructured vanadium containing composite membranes | oxidative dehydrogenation; catalytic structures showed different behavior depending on the mode of supply of methanol and oxygen to the catalytic layer | [100] |
| Cu/Nb-silicate, niobosilicate and alumnosilicate mesoporous sieves | depending on the chemical composition of the support, various copper species were found to be formed and a range of catalytic activities could be achieved; the highest catalytic activity towards methanol could be obtained by applying Si/Nb = 57 in bulk and 43 on the surface, Nb/Cu = 0.8 on the surface | [101] |
| $MoO_3$ and $Fe_2(MoO_4)_3$ | the addition of excess crystalline $MoO_3$ to the crystalline $Fe_2(MoO_4)_3$ phase significantly increases the overall steady-state catalytic performance towards formaldehyde | [102] |
| Vanadium, molybdenum and chromium oxide clusters supported on rutile $TiO_2$ | - | [103, 104] |

Based on the catalysis systems shown in Table 3 and 4, the subsequent process can be proposed as shown in section 1.3.1.2.

#### 1.3.1.2 Conversion step two – formaldehyde → aldol-condensation → separation/recycling → metabolizable carbohydrates

Once formaldehyde is provided, the following route for generating metabolizable carbohydrates could be proposed as shown in Figure 1.

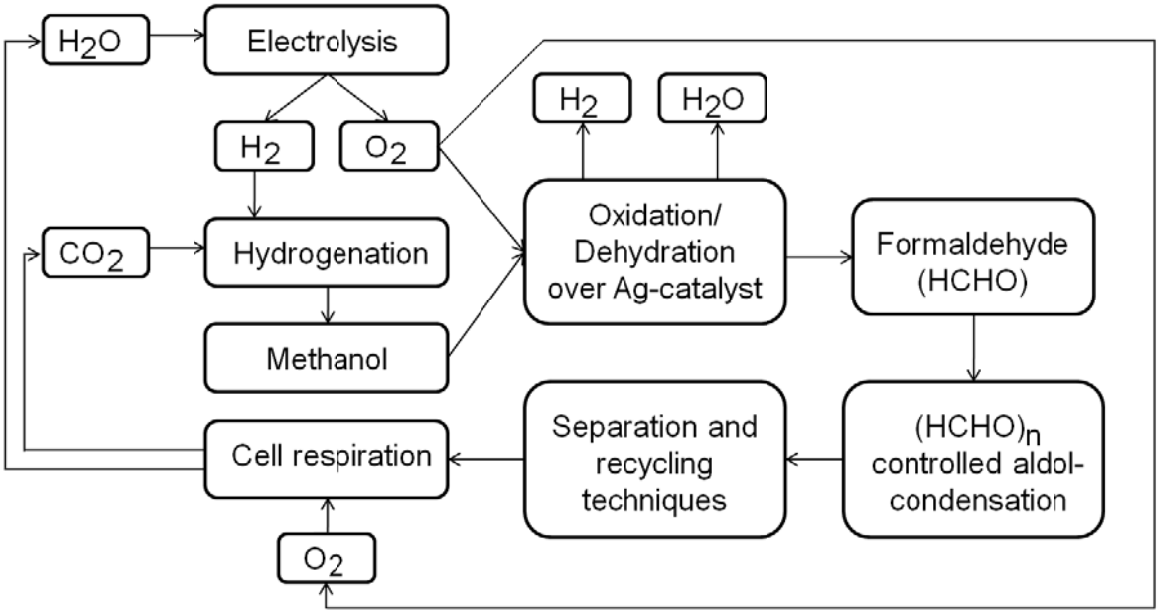



Figure 1: Possible synthesis route for generating metabolizable carbohydrates based on a preliminary water-electrolysis, conversion of $CO_2$ to formaldehyde via methanol and subsequently a controlled aldol-condensation of formaldehyde to metabolizable carbohydrates.

The scheme depicted in Figure 1 represents a selected synthesis pathway for the production of metabolizable carbohydrates from $CO_2$ and $H_2O$ as a theoretical alternative to photosynthesis. As the conversion step one (see section 1.3.1.1) is known in principle, the following main research and problem areas can be identified:

a) investigation of the aldol-condensation of formaldehyde and carbohydrate fragments with regard to delivering appreciable product distributions. The aldol-condensation of formaldehyde and carbohydrate fragments plays a major role within the so-called formose reaction. The investigations are summarized in section 3.2. In order to allow complex carbohydrate mixtures to be investigated, an appropriate method necessarily has to be developed. A simple and versatile one is presented in section 3.1.

b) examination of separation technologies with regard to isolating desired carbohydrates. Some investigations about membrane fractionation, which depicts a part of this research area, has been summarized in section 3.3.

c) deduction of a possible pathway which could enable the realization of the process proposed under specific conditions. The investigations are summarized in section 4.

d) discussion the individual problem areas with regard to uncovering principal problems to be solved.

At this point, the formose reaction, which mainly includes the aldol-condensation of formaldehyde and carbohydrate fragments, should be considered closer.

# 2 The formose reaction system – data basis

## 2.1 Introduction

In 1861 Butlerow [105] discovered the formose reaction, the alkaline polymerization of formaldehyde using e.g. calcium hydroxide, which represents a system for the non-enzymatic total synthesis of carbohydrates. It plays an important role in relation to the prebiotic and interstellar synthesis of carbohydrates [106, 107].

The majority of microorganisms cannot metabolize formose carbohydrates [108]. Among the carbohydrates produced, branched-chain carbohydrates are formed, which are considered to be toxic due to potentially blocking glucose-oxidase [109]. Residual formaldehyde and L-carbohydrate enantiomers are expected to account for the toxicity of formose carbohydrates. In addition, rare hexoses such as D-tagatose only adsorbed to a small degree probably could only be less tolerated by the by the digestive tract [110].

In classical polymer-synthesis formose carbohydrates can be used e.g. for the production of polyurethane-resins or of reactive isocyanate mixtures [111, 112]. Beside the production of carbohydrates, the formose reaction can be applied to eliminate formaldehyde in industrial effluents [113] and plays an inferior role in technical invert-sugar degradation taking place in saccharose production from sugar-beet [114].

In order to better understand the nature of formaldehyde, a primary educt used in the formose reaction, some properties should be considered.

### 2.1.1 Physicochemical properties of formaldehyde

At this point, physical constants (e.g. vapor pressure, molecular mass) are not displayed; the reader is referred to appropriate literature. At ordinary temperatures, formaldehyde gas is readily soluble in water, alcohols, and other polar solvents with a pungent, suffocating odor [115]. An overview about the relation of the different types of formaldehyde-species commercially available is given in Figure 2.

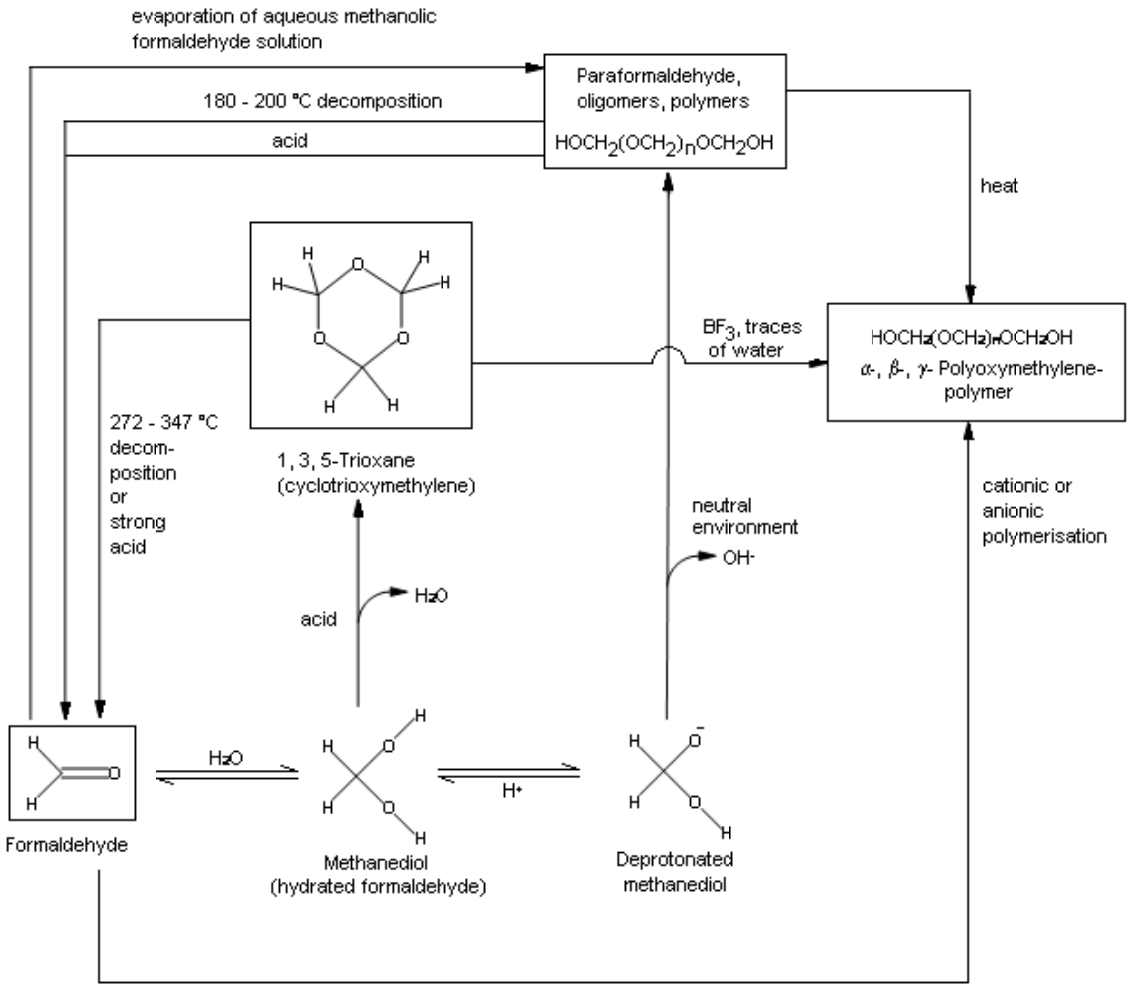



Figure 2: Interconversion of formaldehyde species and polymerization products; interaction of formaldehyde with methanol not shown; scheme adapted from [116].

Especially (a) 30 - 40% (w/v) aqueous formaldehyde solution (containing approximately 0.5 to 15% (w/v) methanol), (b) 1,3,5-trioxane, a formaldehyde-trimer, and (c) paraformaldehyde powder, formaldehyde oligomers [116, 117], play an important role. In aqueous methanolic solution formaldehyde is present in various species, which should be explained shortly.

#### 2.1.1.1 Aqueous methanolic formaldehyde solution

Formaldehyde and water react to form (oxymethylene)glycol (Eq. (1)) and poly(oxymethylene)glycol (Eq. (2)).

$$HCHO + H_2O \underset{k^*_{MG}}{\overset{k_{MG}}{\rightleftharpoons}} HOCH_2OH \qquad (1)$$

$$HO(CH_2O)_{n-1}H + HOCH_2OH \underset{k^*_{MGn}}{\overset{k_{MGn}}{\rightleftharpoons}} HO(CH_2O)_nH + H_2O \quad n \geqslant 2 \qquad (2)$$

With methanol, formaldehyde polymerizes to (oxymethylene)hemiformal (Eq. (3)) and poly(oxymethylene)hemiformal (Eq. (4)) [118].

$$HCHO + CH_3OH \underset{k^*_{HF}}{\overset{k_{HF}}{\rightleftharpoons}} CH_3OCH_2OH \qquad (3)$$

$$CH_3O(CH_2O)_{n-1}H + CH_3OCH_2OH \underset{k^*_{HFn}}{\overset{k_{HFn}}{\rightleftharpoons}} CH_3O(CH_2O)_nH + CH_3OH \qquad (4)$$

Methanol is used as a stabilizing agent for formaldehyde in aqueous solution. Formaldehyde, initially bound in long-chain, poorly soluble poly(oxymethylene)glycols, subsequently is transferred to short-chain and therefore better soluble poly(oxymethylene)-hemiformals [119]. The fact that methanol might be a more favorable solvent for the formaldehyde oligomers than water should also be considered [119].

Formaldehyde oligomers containing up to five carbon units have been detected by quantitative NMR spectroscopy [120]. Hydrated formaldehyde can be deprotonated to form an anionic formaldehyde species featuring a small $K_s$-value of approximately $1 \times 10^{-14}$ [121]. Despite this physical constant and the fact that a rather small amount of hydrated formaldehyde (< 1%) is present in aqueous formaldehyde solution [122], a pH-value of approximately 3 is measured.

#### 2.1.1.2 Paraformaldehyde powder

As Figure 2 shows, paraformaldehyde is easily produced in neutral environment by means of polymerization of formaldehyde monomers [116, 117]. In addition, paraformaldehyde can be generated by evaporation of aqueous methanolic formaldehyde solution [117]. Acid-treatment or exposure to elevated temperatures (180 - 200 °C) releases formaldehyde-monomers from paraformaldehyde [116].

#### 2.1.1.3 1-,3-,5-trioxane

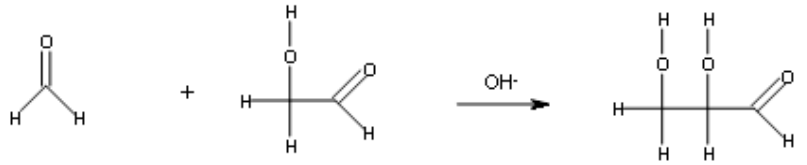
1-,3-,5-trioxane (trioxane), the industrial source of formaldehyde, is produced by means of acid-catalyzed conversion of hydrated formaldehyde [117, 123]. Trioxane is a stable crystalline solid compound at ambient conditions. Applying elevated temperature (272 – 347 °C) or treating with a strong acid releases formaldehyde-monomers. Besides the formation of trioxane, higher cyclic oligomers such as tetrosane and pentoxane are known to occur [124].

#### 2.1.1.4 Polyoxymethylene- and further plastics manufactured from formaldehyde

Poly(oxymethylene)-polymer (POM-plastic) can be produced from formaldehyde-monomer or trioxane by anionic or cationic polymerization [123, 125]. Treatment of paraformaldehyde by heat also yields poly(oxymethylene)-polymer as shown in Figure 2. In addition to the industrial production of polyoxymethylene-plastics, formaldehyde is used for the production of a number of further polymers e.g. urea- and melamine formaldehyde resins or for Resol- and Novolak-plastics [123, 125].

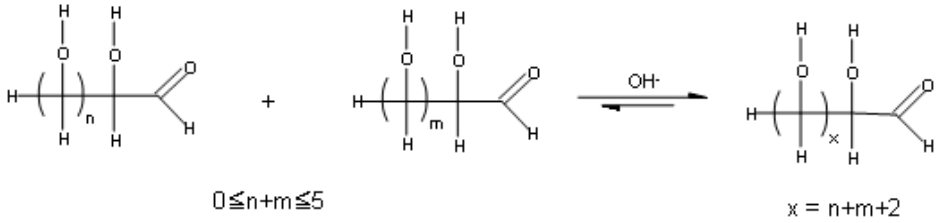
## 2.2 The formose reaction

The formose reaction addresses a catalytic system consisting of a series of interfering reactions, which have not been entirely discovered up to the present. A major reaction type has been identified as the aldol-condensation between formaldehyde and carbohydrate fragments (e.g. glycolaldehyde to mention the simplest, Eq. (5)).




(5)

The initial step of the formose reaction is closely accompanied by subsequent addition-steps of carbohydrate fragments and retro-aldol-splitting of polymerization products (Eq. (6)).




(6)

### 2.2.1 Side reactions

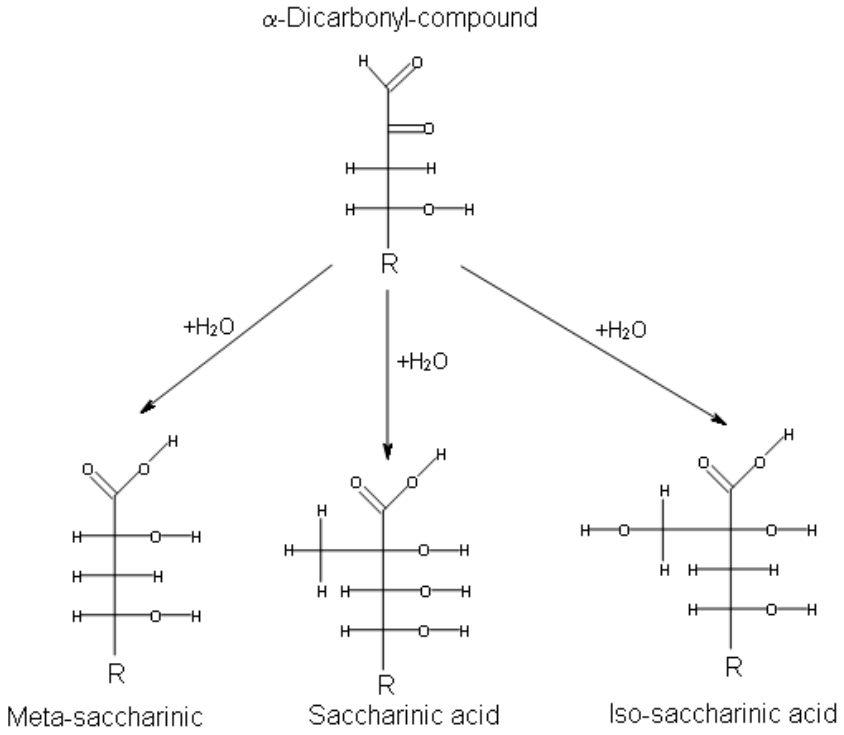
The cannizzaro-reaction, a disproportionation-reaction of formaldehyde yielding methanol and formic acid, competes with the formose reaction [126]. In addition to the formation of straight chain also branched chain carbohydrates can be formed. In a so-called cross-cannizzaro-reaction, a disproportionation-reaction, branched chain carbohydrates not any more carrying α-hydrogen, can only react with formaldehyde to produce a branched chain sugar alcohol and formic acid [127].

Apart from cannizzaro and cross-cannizzaro reaction the formation of volatile compounds (see Table 5) as well as of saccharinic acids (see Eq. (7)) could take place in the formose reaction-system.

Table 5: Formation of volatile compounds by means of alkaline degradation (pH 8-10) of fructose [128].

| |
|---|
| Acetic acid |
| Hydroxyacetone |
| 1-Hydroxy-2-butanone |
| 3-Hydroxy-2-butanone |
| 4-Hydroxy-2-butanone |
| Furfuryl alcohol |
| 5-Methyl-2-furfuryl alcohol |
| 2,5-Dimethyl-4-hydroxy-3-(2H)-furanone |
| 2-Hydroxy-3-methyl-2-cyclopenten-1-one |
| 3,4-Dimethyl-2-hydroxy-2-cyclopenten-1-one |
| 3,5-Dimethyl-2-hydroxy-2-cyclopenten-1-one |
| 3-Ethyl-2-hydroxy-2-cyclopenten-1-one |
| γ-Butyrolactone |

Saccharinic acid formation is explained in terms of a two-step reaction: in a first step, α-dicarbonyl-formation from ene-diol-anions and subsequent β-elimination [114, 129] takes place. In a second step, α-dicarbonyls undergo a benzilic-acid-rearrangement yielding saccharinic, meta-saccharinic and iso-saccharinic acids (see Eq. (7)). Saccharinic acid formation preferably takes place at high carbohydrate and low formaldehyde concentrations in the formose reaction-system [127, 130].




(7)

2,4-dihydroxybutyric and other acids are major products of the alkaline degradation of glucose [131]. In addition, partially unknown reactions seem to be involved in the formose reaction-system if almost quantitative conversion of formaldehyde is considered. Up to the present only 30 different carbohydrate species have been identified by gas chromatography-mass spectrometry as their trifluoroacetylated-*O*-butyloxime derivates [132].

## 2.3 Formose reaction catalysis

Considering the time dependent progress of the batch-formose reaction a typical development is observed as shown in Figure 3.

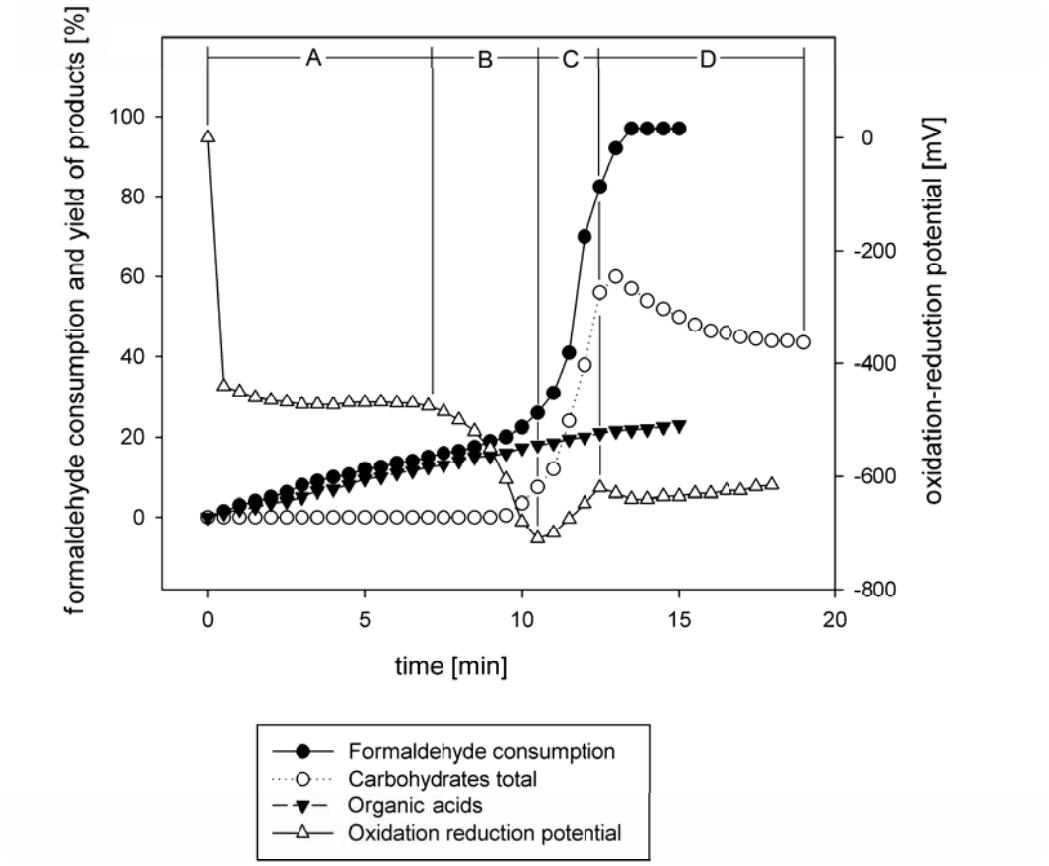



Figure 3: Batch-formose reaction catalyzed by calcium hydroxide; starting conditions: 1.4 M formaldehyde, 0.13 M calcium hydroxide, no initiator, 60 °C; modified from ref. [133, 134].

Figure 3 shows a representative oxidation-reduction potential (ORP) curve of the formose reaction, which features four characteristic sections:

Section (A): Almost no change in ORP is detected and no formation of carbohydrates can be observed with a small conversion of formaldehyde. This section can be considered as an induction period, after which only a small amount of formic acid is produced by cannizzaro reaction. The duration of the induction period is strongly depending on the type and quantity of initiator used [135-137].

Section (B): Subsequent to the induction period the ORP value begins to decrease and reaches a minimum point at the end of section (B). At this ORP-minimum point the yield of carbohydrates accounts only for approximately 3% (quantified as glucose based on formaldehyde). The condensation of formaldehyde to carbohydrates is initiated slowly (see the slope of the formaldehyde consumption curve in section (B)). The rate of organic acid

formation is comparable to that in the previous step indicating that cannizzaro reaction is taking place simultaneously independent from the condensation of formaldehyde to carbohydrates.

Section (C): The ORP curve turns upwards from the minimum point and reaches a small maximum, where a little decrease in ORP is observed. Simultaneously the color of the solution turns to pale yellow; the so-called yellowing point is reached. The formation of α-dicarbonyl-compounds is considered to be responsible for the appearance of colored compounds [138]. As the ORP of the solution increases the reaction proceeds rapidly (see consumption of formaldehyde at the end of section (C)). During this phase a significant rise in temperature is known to occur [133]. At the end of section (C) the formaldehyde consumption accounts for 95% and the yield of the carbohydrates amounts to 50-70%. An increase in reaction volume is observed until reaching the yellowing point at the end of section (C). Afterwards the reaction volume starts to decrease again [139, 140]. Based on the ORP-curve observed, section (C) could be termed as the period of carbohydrate formation [133, 134].

Section (D): Concerning ORP a steady state appears repeatedly, where the carbohydrates begin to degrade. The total amount of carbohydrates decreases whereas the residual amount of formaldehyde remains constant as time passes [133, 134]. The temperature of the reaction mixture is lowered slowly while at the same time the color changes from light yellow to brown [116].

Oxygen has been identified to have a quenching effect on the formose reaction [140]. Following sugar decomposition a final product distribution is obtained similar to the products obtained from alkaline degradation of monosaccharides [138]. This hint partially answers the question where the “equilibrium” of a formose reaction could lie.

### 2.3.1 Attempts to increase the selectivity of the formose reaction

Up to the present the following products could be preferably produced from the formose reaction as summarized in Table 6.

Table 6: Preferred production of carbohydrates using the formose reaction-system.

| Product | ref. |
|---|---|
| 2,4-di-C-(hydroxymethyl)-3-pentulose | [141] |
| 2-hydroxymethylglycerol | [142] |
| 3,3-di-C-hydroxymethyl-3-deoxyfuranorono-1,4-lactone | [143] |
| 3-C-hydroxymethyl-pentofuranose | [144] |
| trioses, especially dihydroxyacetone | [145-147] |
| DL-2-C-hydroxymethyl-3-pentulose | [148, 149] |
| DL-dendroketose | [150] |
| ethyleneglycol | [151] |
| glycolaldehyde | [152] |
| L-dendroketose | [153] |
| pentaerythritol | [142, 152, 154] |
| threo-3-pentulose | [155] |

#### 2.3.1.1 Formose reaction-catalysts

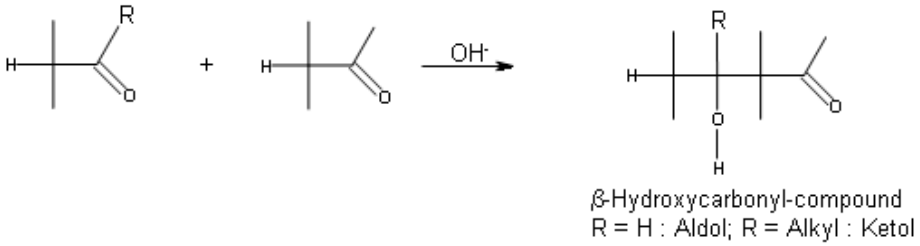
A broad spectrum of catalysts presented in Table 20 and 21 (see appendices) can be used to successfully catalyze the formose reaction if a certain concentration of catalyst is exceeded [156]. Among inorganic catalysts alkaline earth hydroxides and oxides, thallium hydroxide and lead oxide show the highest catalytic activity [157]. In addition, tertiary amines, lanthanide hydroxides and aluminosilicates have already been applied in formose reaction-catalysis [157]. The type of catalyst influences the ratio of formose/cannizzaro reaction. E.g. thallium hydroxide is known to be an exclusive catalyst for the formose reaction whereas calcium or sodium hydroxide is not [116]. Tertiary amines only seem to catalyze the formose reaction without catalyzing the cannizzaro reaction at all [158]. As shown in Table 22 and 23 (see appendices) a number of heterogeneous catalysts as well as physical influences have been applied in the formose reaction-catalysis.

## 2.4 General aspects of the formose reaction

### 2.4.1 Reaction mechanism

The reaction-mechanism explaining the (retro)-aldolization of carbohydrates in aqueous alkaline solution can be attributed to an anionic chain-growing reaction, a reaction type well

known in polymer-synthesis [125]. The addition of formaldehyde to e.g. glycolaldehyde, the simplest initial step of the formose reaction, can be classified as an aldol-reaction: in alkaline environment aldehydes and ketones react to β-hydroxycarbonyl-compounds (aldols, ketols) if the α-carbon atom is essentially linked to hydrogen (Eq. (8); adapted from ref. [159]).




(8)

At first a Lewis-base (here $OH^-$) abstracts a proton at the α-carbon atom. This equilibrium reaction produces a small amount of a mesomerie-stabilized carbanion. The nucleophilic attack of this carbanion at the electrophilic neighbour carbonyl-c-atom leads to an alkoxid-anion; the acid-base reaction thereof with water yields an aldol or ketol [159]. The role of the cation introduced by applying a certain amount of catalyst (e.g. calcium hydroxide) is not completely clarified. Nevertheless, a certain influence of divalent cations onto the diastereomeric selectivity of the aldol-condensation between e.g. $C_3$-carbohydrate fragments could be observed [81]. Based on UV-spectroscopic data there is evidence for the formation of an ene-diol-complex from calcium and carbohydrates [116]. As soon as α-hydrogen is present in the formose reaction system, the subsequent unidirectional addition of formaldehyde takes place in alkaline media and competes with the cannizzaro reaction [126].

For the sake of completeness it must be mentioned that aldoses can be reacted e.g. in a so-called Kiliani-Fischer-Synthesis in order to extend the carbohydrate backbone by one carbon unit [160]. For a controlled degradation of the carbohydrate chain organic synthesis methods according to e.g. Wohl and Ruff are well known [160]. Despite these examples a number of further ascending and descending synthesis methods for monosaccharides are available [161].

### 2.4.2 Influence of reaction parameters

#### 2.4.2.1 pH-value

A pH-value >10 seems to be required to start the formose reaction [126]. In case of a calcium hydroxide-catalyzed formose reaction, the pH-value over reaction time shows a typically

shaped curve as indicated in Figure 4. This behavior might be explained in terms of calcium hydroxide interacting with formaldehyde in the initial reaction phase, the subsequent autocatalytic condensation-phase and finally in terms of significant amounts of formic acid being present [126].

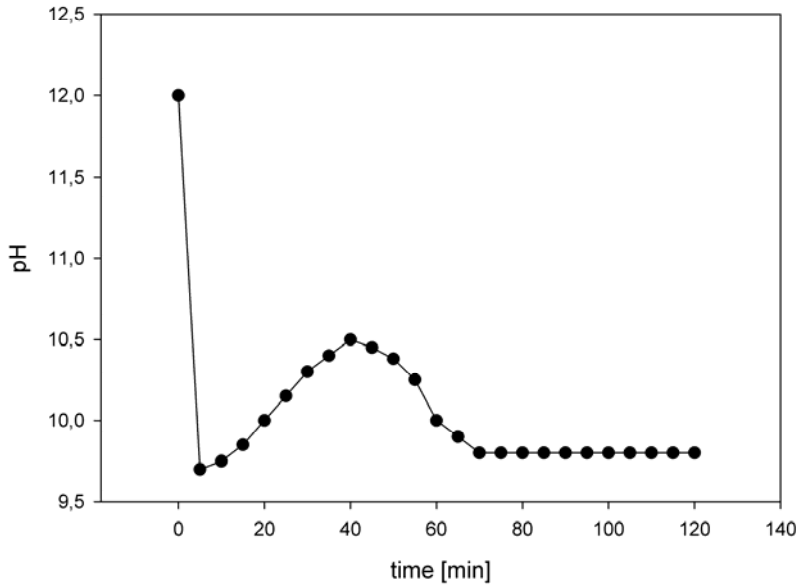



Figure 4: pH-value vs. time typically acquired during formose reaction-catalysis; batch-setup, 50°C, starting conditions: 1.67 M formaldehyde, 0.135 M calcium hydroxide; no initiator; modified from ref. [126].

#### 2.4.2.2 Temperature

The extent of the formose reaction is strongly dependent on the temperature applied. In case of using sodium hydroxide, a particularly high temperature leads to a significant amount of formose carbohydrates. E.g. conducting the reaction using 1.6 M formaldehyde, 2.5 M sodium hydroxide at 100°C using no initiator yields 20% formose carbohydrates within 5 min reaction time [156]. Significantly lower reaction temperature only yields cannizzaro products [156]. In case of using e.g. calcium hydroxide, moderate reaction temperatures e.g. 50°C are sufficient for successfully catalyzing the formose reaction. Working at ambient conditions in case of calcium hydroxide catalysis yields less complicated carbohydrate mixtures compared to elevated temperatures [162].

### 2.4.2.3 Concentration of base

The higher the concentration of base the more the formose reaction is favored over the cannizzaro reaction [156]. In general, every base can be used to catalyze the formose reaction, if it is applied in a sufficiently high amount [156]. When the concentration of base is increased continuously, the induction time period is no longer being shortened and apparently becomes constant. Simultaneously, the time-period of carbohydrate synthesis is continuously shortened [163].

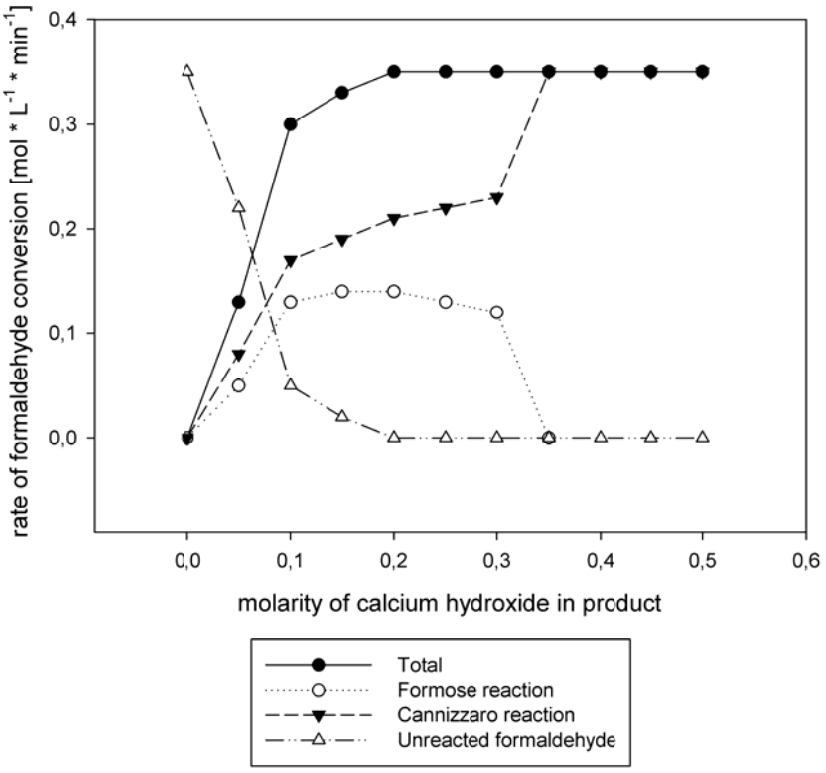



Figure 5: Dependence of the formose reaction and cannizzaro reaction on the concentration of base determined in a continuous-stirred-tank-reactor ; 60 °C; formaldehyde-feed rate: 0.35 [mol * $L^{-1}$ * $min^{-1}$]; modified from ref. [164, 165].

Operating in a continuous-stirred-tank-reactor indicates that there is only a small ratio of base/formaldehyde where the formose reaction is successfully catalyzed [164, 165]. If a ratio of calcium hydroxide/formaldehyde = 0.35 is exceeded (see Figure 5), formose reaction is not catalyzed any more. Instead, formaldehyde is only converted by the cannizzaro reaction.

#### 2.4.2.4 Concentration of formaldehyde

The higher the concentration of formaldehyde the smaller the relative extent of formose reaction with respect to total converted formaldehyde. In general, every base, which is applied in a certain concentration, has a typical upper limit of formaldehyde-concentration where conversion within formose reaction takes place. Above this limit only the cannizzaro reaction is proceeding [156].

In order to successfully catalyze the formose reaction with a particular chosen catalyst one can decrease the concentration of formaldehyde or increase the concentration of base. In case of only low soluble bases, the base/aldehyde-ratio, which is necessary for the successful formose catalysis, can be used for calculating the highest formaldehyde concentration, which can barely be converted to carbohydrates using the formose reaction [156].

#### 2.4.2.5 Methanol

A rather low concentration (< 25% (v/v)) of methanol represses the final formaldehyde-conversion without affecting the initial rate. Despite this fact, a rather high concentration (> 75% (v/v)) of methanol significantly decreases the initial rate without affecting the final formaldehyde-conversion [166]. At high methanol concentrations the retarded delivery of formaldehyde affecting the initial rate might at least be explained by significant poly(oxymethylene)hemiformal-formation (Eq. (2) - Eq. (4)).

#### 2.4.2.6 Initiator

Several initiators have been found to start the formose reaction and in general show first-order dependency with regard to formaldehyde conversion. The usage of a high excess of initiator eliminates the autocatalytic character of the formose reaction and decreases the induction period to a great extend. The capability of reducing the duration of the induction period is increasing e.g. in the following order: glucose < ribose < fructose < sorbose < dihydroxyacetone < glycolaldehyde [135].

Table 24 (see appendices) summarizes different initiator/catalyst-combinations, which have already been applied according to data reported in literature. As indicated in Eq. (6), during the progress of the reaction the initiator is consumed. By means of dealdolization species capable of continuously initiating the formose reaction are regenerated, which explains the

autocatalytic nature of the formose reaction (Eq. (6)). Glycolaldehyde can be regenerated easily by retro-aldol splitting of e.g. tetroses [167]. If partial formaldehyde conversion is considered, a transient influence on the product distribution is evident [136]. Based on the autocatalytic nature of the formose reaction specific phenomena e.g. hysteresis and bistablility are known to occur [168, 169].

In addition, selected reactions between e.g. glyceraldehyde and dihydroxyacetone [80], glycolaldehyde and dihydroxyacetone [170], glycero-tetrulose and glycolaldehyde [171], 2-pentulose and formaldehyde [171] as they appear in the formose reaction have been investigated in detail. The formation of e.g. dendroketose [80] or 3-hexuloses [171] has been shown. Considering reactions between lower carbohydrates, special interest has focused on the diastereoselectivity of their calcium hydroxide catalyzed aldol-reaction [162]. In case of using only a low base concentration and moderate temperatures the reverse-aldol-reaction might be quenched [162].

## 2.5 Conclusion

Full conversion of formaldehyde within the formose reaction leads to a very complex mixture of carbohydrates. Defining a formose reaction system working with aqueous formaldehyde solution, calcium hydroxide catalyst and glycolaldehyde acting as the initiator, it is concluded that the controllability of the formose reaction requires moderate reaction conditions.

Considering the stereoselective production of hexoses e.g. in a first aldol-reaction, enantioselective dimerization of α-oxyaldehydes carrying different protecting groups catalyzed by L-proline is carried out [172]. In a second, a so-called Lewis acid-mediated Mukaiyama aldol-carbohydrate cyclization, diastereoisomeric control is accomplished by the choice of the Lewis acid and by the reaction solvent [173]. The synthesis methodology does need solvent exchange as well as the introduction of protecting groups. Using e.g. this synthesis approach, such manipulations represent severe or even unsolvable difficulties with regard to recycling of byproducts, which would accumulate in a technical scale.

$C_3$-carbohydrates, intermediate products which could be suggested for the manufacture of synthetic hexoses for instance, can be produced by various synthesis-routes [174]. In a top-down approach D-glyceraldehyde can be obtained from D-fructose while L-glyceraldehyde is derived from L-sorbose [175]. Glyceraldehyde derivates e.g. their *O*-isopropylidene-, 2-*O*-methyl- or 2-*O*-benzyl-derivates are simply generated from D-mannitol or ascorbic acid

[176, 177]. In a simple bottom-up approach for the synthesis of $C_3$-carbohydrates (DL-glyceraldehyde and dihydroxyacetone) not enabling stereoselective control e.g. a controlled one-step aldol-condensation could be applied, in which formaldehyde is added onto glycolaldehyde acceptor. At the same time ending up in an uncontrollable autocatalytic formose-cycle must be avoided by critically controlling the one step aldol-condensation of formaldehyde and glycolaldehyde. Doing so, the formose reaction could be limited to its initial phase. Glycolaldehyde, which is necessary for the one step aldol-condensation, can be generated by the hydroformylation of formaldehyde [77, 78] (without assessment).

# 3 Results and Discussion

In order to provide valid analytical results from the one-step aldol-condensation of formaldehyde and glycolaldehyde, an analytical method has been developed and a great number of standard analytical methods has been validated.

## 3.1 Analysis of complex monosaccharide mixtures by LC-UV and LC-ESI-MS

### 3.1.1 Introduction

It is the aim of this chapter to present a simple and versatile method for LC-UV analysis of complex carbohydrate mixtures using inexpensive standard analytical equipment. Especially higher molecular weight as well as temperature sensitive compounds should be made easily amenable to analysis. Thus, the limitations of conventional gas chromatographic analysis of carbohydrates could be overcome. The resolution of enantiomers is beyond the scope of this work.

The formose reaction, the autocatalytic anionic polymerization of formaldehyde to carbohydrates initiated by the presence of a carbohydrate containing α-hydrogen in alkaline media, is used as a model reaction delivering representative carbohydrate mixtures. This reaction is of great importance to the question of the origin of life because it is considered as a potential synthesis route for the generation of complex monosaccharides, a non-enzymatic source of sugars [131, 178-181]. Due to its autocatalytic kinetics a formose reaction could develop into a self-organizing non-enzymatic system continuously producing sugars including branched-chain sugars as precursors of amino acids, of the isoprene moiety and of the branched chains of valine, leucine and isoleucine in a prebiotic scenario [132]. The formose reaction, as well as transition-metal-catalyzed reactions of carbohydrates [182, 183] provide

tremendously complex carbohydrate mixtures. Besides these examples of chemical origin carbohydrate degradation products formed during saccharose production [114] and in pulp production processes [184], as well as carbohydrates in biological fluids [185, 186] deliver similar complex carbohydrate spectra. Developing analytical methods for such complex monosaccharide mixtures represents a major challenge, which needs the combination of LC, CE and GC.

Direct analysis of formose carbohydrates using weak anion-exchange Carbopak- or cation-conditioned polymer based-columns is not practicable since a considerable amount of residual formaldehyde as well as salts are anticipating the analysis. LC-UV analysis of formose carbohydrates has been conducted by 2,4-dinitrophenylhydrazine (DNPH) labeling [187], but shows only limited applicability due to peak multiplicity (syn- and anti-isomer formation) complicating the analysis. In addition, a huge number of carbohydrate species remains to be separated independent form the analytical system chosen.

In light of this difficulty, reduction of monosaccharides to their corresponding alditols was conducted to eliminate peak multiplicity since the resulting alditols cannot anomerize. Hence, much simpler chromatograms could be obtained [188-190]. In addition, formaldehyde is converted to methanol by reduction, which is considered as less bothering the analysis. The introduction of a reduction step is accompanied by a loss of information since different monosaccharides yield the same alditol [191] as well as elaborate removal of residual borate species before analysis has to be taken into account. Contrary to aldoses and ketoses, which are converted to their corresponding alditols, carboxylic acids such as formic acid are not affected by $NaBH_4$-reduction due to its limited reduction strength [192]. Since the borate species present do not affect DNPH-labeling, formic acid is easily quantified in LC-UV (see supplementary material). Nevertheless, the elimination of borate species as trimethylborate is simply carried out by applying appropriate aliquots of methanol followed by treating under a stream of nitrogen. During the progress of the formose reaction branched-chain alditols are formed by cross-cannizzaro reaction. In order to estimate the extent of cross-cannizzaro reaction representative branched-chain alditols, namely 2-hydroxymethylglycerol (2-HMG; IUPAC: 2-(hydroxymethyl)propane-1,2,3-triol) and 2-hydroxymethyltetritol (2-HMT) were synthesized (see supplementary material).

After an appropriate ion-exchange sample preparation procedure direct analysis of alditols using weak anion-exchange Carbopak- or e.g. Pb-conditioned polymer based-columns could be carried out. However, this approach is not practicable since the majority of carbohydrates are adsorbed onto the resin in a non-reproducible manner.

Introducing per-*O*-benzoylation of alditols enables UV-detection as well as provides favorable capacity factors on reversed-phase columns, which has already been described by several authors in literature [189, 193-197]. Since derivatization not only yields per-*O*- but also partially-*O*-benzoylated alditols, particular attention is focusing on this issue. Linking the method to ESI-MS technique necessarily using internal standard calibration yields valuable information about a) the validity of UV-data analyzed, b) the identification of probably co-eluting compounds and c) the probable improvement of limit-of-detection.

In addition, benzoylation provides a significant increase in ESI-response as a key advance, which facilitates the detection of otherwise hardly amenable compounds. Coupling the analysis of benzoylated compounds to an ESI-MS has been investigated focusing on the analysis of some sugars, polyols and amino acids in biological fluids [194] as well as on the analysis of low molecular weight organic acids derived from root exudation [198]. For the sake of completeness it should be mentioned that benzoylation is used for the LC-MS analysis of bases, ribosides, intact nucleotides, diethylene glycol in sea water as well as of 1-monomycoloyl glycerol [199-202]. For the analysis of sialooligosaccharides, benzoylation provides a valuable tool for generating intensive signals during matrix-assisted laser desorption-ionization (MALDI) mass spectrometric analysis. In addition, some terminal units of oligosaccharides undergo characteristic structural changes during benzoylation providing easily recognizable mass spectral patterns [203].

Among formose reaction products more than thirty carbohydrates have been identified as their trifluoroacetylated-*O*-butyloximes [132] in gas chromatography-mass spectrometry. To the best knowledge of the author, no work about CE-analysis of formose carbohydrates is evident in literature. Apart from LC, GC and CE the application of 2-hydroxymethylboronate reagent in cost-intensive Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) allows a deep insight in the autocatalytic formose reaction [204].

Several coupled analytical techniques based on LC and GC deliver detailed analytical information about complex formose carbohydrates formed as shown in this chapter.

### 3.1.2 Materials and methods

#### 3.1.2.1 Formose reaction setups

Experiments were conducted using a batch reactor-system. 6.75 mL of aqueous formaldehyde solution was diluted to a final volume of 25 mL containing 5 mg initiator (glycolaldehyde, D-glyceraldehyde, D-erythrose (Ery), D-xylose (Xyl) or D-glucose (Glc)).

After suspending 0.56 g calcium oxide in 25 mL of water in another vessel, the reactants were pre-heated to the reaction temperature 60 ± 2°C using an oil-bath. The formose reaction was started by transferring the formaldehyde solution containing the dissolved initiator into the vessel containing base. This reaction setup provides 1.66 M formaldehyde, 0.2 M calcium hydroxide and 100 ppm initiator as starting conditions.

The formose reaction was stopped after 20 min by adding 5.6 mL of 10% (w/v) hydrochloric acid to the batch reactor. After cooling down to room temperature pH=7 was adjusted by adding 5% (w/v) sodium hydroxide.

#### 3.1.2.2 Analytical equipment

LC-UV of per-*O*-benzoylated alditols (PBA) was performed on a Dionex Ultimate 3000 HPLC (Dionex, Vienna, Austria) equipped with a diode-array-detector operated by Chromeleon software version 6.80 SR9 applying the following chromatographic conditions: Zorbax Eclipse XDB C18-column 5.0 µm material (Agilent Technologies, Waldbronn, Germany), 150 x 4.6 mm, precolumn Zorbax Eclipse XDB-C18 (Agilent) 12.5 x 4.6 mm operated at 25°C; gradient: 0-40 min: 20-95% $CH_3CN$ applying 1 mL*min$^{-1}$ flow rate, 41-50 min: 100% $CH_3CN$ applying 1.1 mL*min$^{-1}$ flow rate, 51-55 min: 20% $CH_3CN$ applying 1 mL*min$^{-1}$ flow rate; the eluents used contain 0.01% (v/v) formic acid maintaining pH~4; detection wavelength: 240 nm; injection volume 10 µL. The resulting dwell volume was determined to be 400 µL.

LC-ESI-MS-analysis was performed using the same HPLC which was used for LC-UV of PBAs. An Agilent MSD 6320 IonTrap MS (Agilent Technologies, Palo Alto, CA, United States) controlled by Bruker LC/MSD Trap software version 5.3 together with an ESI source from Agilent Technologies was used as a mass-selective detector in positive ion mode. Source parameters were set as follows: drying gas temperature 350°C, drying gas flow 8 L*min$^{-1}$, nebulizer pressure 20 psi and capillary voltage 2000 V. The Agilent MSD 6320

IonTrap MS was connected to the LC with a fixed flow splitter (split ratio 1:4). In addition, 20 µL*min$^{-1}$ ionization buffer consisting of 0.1 mM formic acid, 0.1 mM sodium formate and 10% (v/v) methanol were supplied postcolumn to the flow splitter. Scanning was carried out from 50 to 2200 m/z in Ultra Scan mode at 26.000 m * z$^{-1}$ * sec$^{-1}$ using 500 m/z as target mass. Data interpretation and peak integration was based on extracted ion chromatograms ± 0.5 m/z peak width.

For investigations on analyte recovery a Thermo Savant SPD SpeedVac SPD131DDA operated at 65°C and maximum vacuum combined with a Thermo Savant Refrigerated Vapor Trap RVT405DDA and a Thermo Savant OFP-400 pump were used.

#### 3.1.2.3 Reduction as a pretreatment step

A 500 µL formose sample aliquot was diluted with 2.5 mL of HQ-water. After adding 30 mg of $NaBH_4$ reduction was carried out for two hours at room temperature. 400 µL of 10% (w/v) hydrochloric acid was added in order to stop the reaction. The completeness of reduction was confirmed by reducing carbohydrate model solutions and checking subsequently for the absence of the characteristic UV-signal after DNPH-derivatization [187].

In order to remove borates as their volatile trimethylborate-esters the following optimized evaporation procedure (see supplementary material) was carried out: 80 µL of a reduced formose sample, which did not contain more than 600 µg of carbohydrates, were transferred to a 1.5 mL glass-vial. After adding 0.5 mL of methanol, evaporation was carried out in a stream of $N_2$ at 45°C. After carrying out the addition of methanol and subsequent evaporation fifteen times for up to 40 samples in parallel the samples were allowed to dry. The entire evaporation procedure did not take more than two hours.

#### 3.1.2.4 Benzoylation

For the analysis of formose reaction-samples the following optimized reaction conditions were applied (optimization see supplementary material): the reduced dry samples were dissolved in 600 µL of pyridine/dimethylformamid 2:1 at 62°C for 20 min. After cooling to room temperature 50 µL of benzoylchloride was added and subsequent derivatization was carried out at 80°C for 90 min. After quenching the excess of reagent by adding 300 µL of methanol the samples were ready for analysis.

### 3.1.3 Results and discussion

#### 3.1.3.1 Qualitative characterization of PBAs by means of LC-UV and LC-ESI-MS

As summarized in Table 7, the qualitative characterization of PBAs has been carried out on the basis of extracted ion chromatograms of the mother ions, which are explained in terms of stoichiometric calculations [194, 205]. As an example the structure of D-Glc as well as 2-hydroxymethylglyceraldehyde converted to their corresponding PBAs amenable to analysis is shown in Figure 6.

Table 7: Retention time, nominal masses of PBAs and characteristic ions observed in LC-ESI-MS

| | retention time [min] | M [Da] of PBA | mother ion (MI) [Da] | explanation |
|---|---|---|---|---|
| Glycolaldehyde | 24.4 | 270 | 293 | $[M+Na]^+$ |
| 2-HMG | 26.3 | 418 | 457 | $[M+K]^+$ |
| D-Glyceraldehyde | 30.9 | 404 | 427 | $[M+Na]^+$ |
| 2-HMT | 31.0 | 552 | 591 | $[M+K]^+$ |
| D-Threose | 34.8 | 538 | 561 | $[M+Na]^+$ |
| D-Ery | 35.2 | 538 | 561 | $[M+Na]^+$ |
| D-Xyl/D-Ara/D-Lyx | 37.6 | 672 | 695 | $[M+Na]^+$ |
| D-Rib | 38.2 | 672 | 695 | $[M+Na]^+$ |
| L-iditol/D-Gal/D-Glc/D-Man | 39.3 | 806 | 829 | $[M+Na]^+$ |
| D-Alt | 39.8 | 806 | 829 | $[M+Na]^+$ |
| D-All | 40.3 | 806 | 829 | $[M+Na]^+$ |
| Heptoses | 40.3 - 41.5 | 941 | 964 | $[M+Na]^+$ |
| Octoses | 41.5 - 42.7 | 1075 | 1098 | $[M+Na]^+$ |

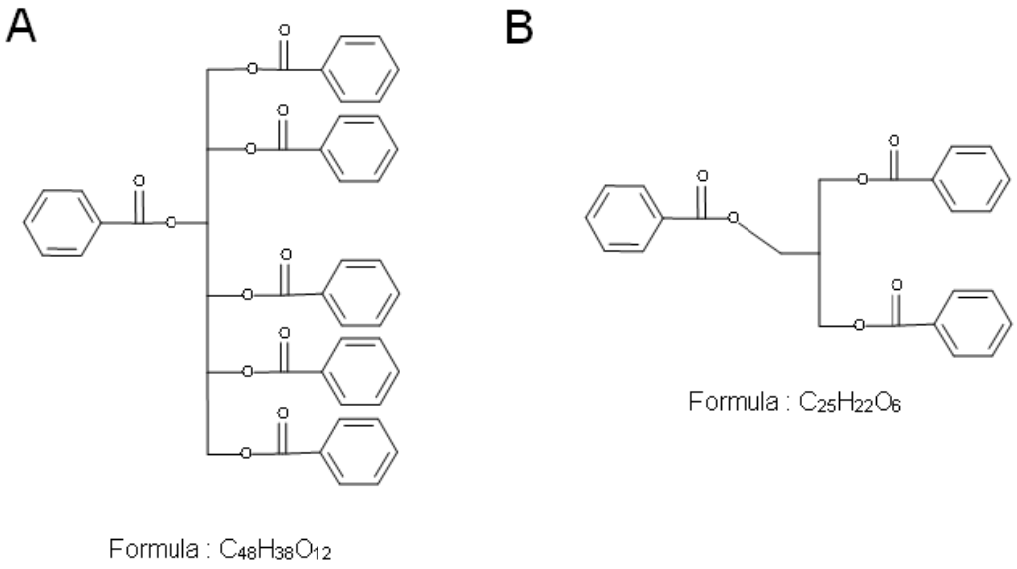



Figure 6: Structure of $NaBH_4$-reduced and per-*O*-benzoylated carbohydrates detected in LC-UV and LC-ESI-MS: (A) D-glucose and (B) 2-hydroxymethylglyceraldehyde.

Considering individual standards injected, the overlapping of the characteristic ions detected with the UV-signal was found to be in good agreement. Based on this fact, the following peaks could be assigned considering a real formose sample as depicted in Figure 7 (characteristic ion chromatograms ± 0.5 m/z extraction width see Figure 12, supplementary material). In case of glycolaldehyde detected at 293 m/z in a real formose sample the characteristic retention time could be doubtlessly determined taking LC-UV data into account. However, at 293 m/z a rather big peak is detected around 10 min retention time, which represents an unknown byproduct (see Figure 12, supplementary material).

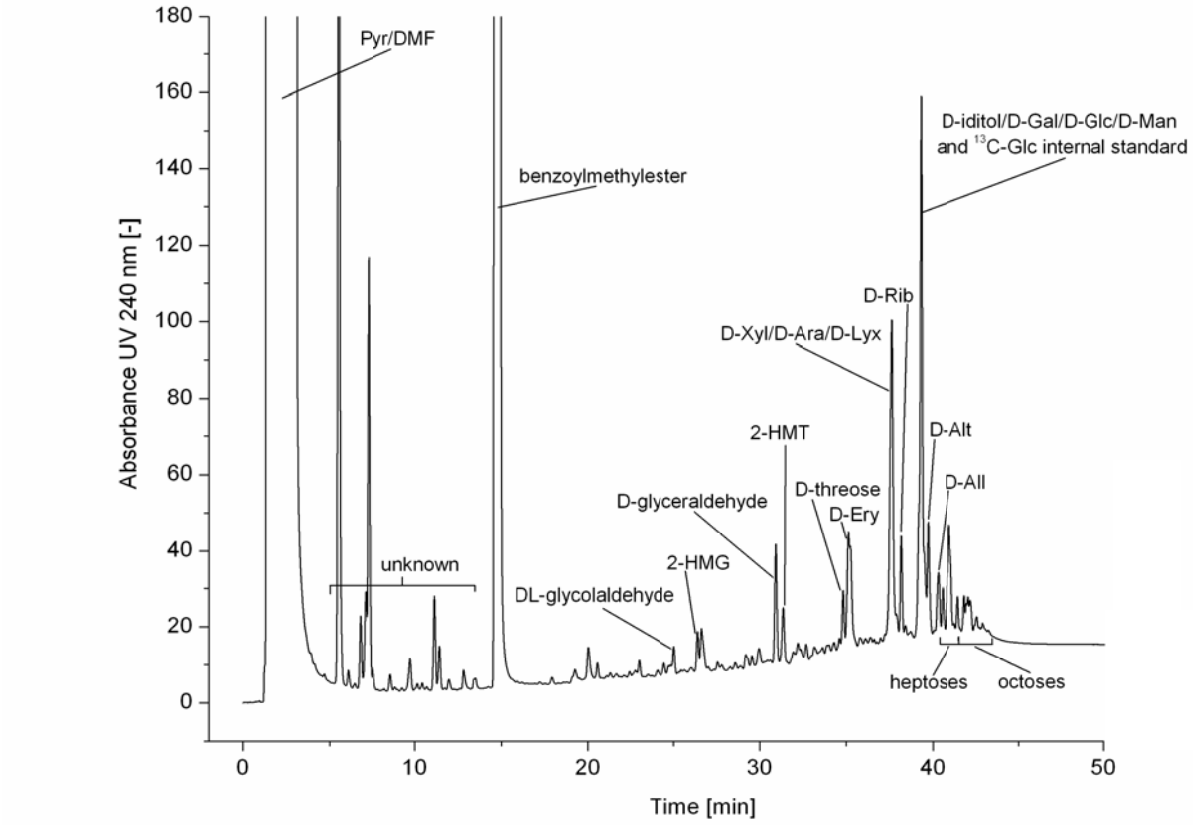



Figure 7: LC-UV chromatogram obtained from a real formose sample using glycolaldehyde initiator; carbohydrates were analyzed as their per-*O*-benzoylated alditols.

Fragmentation of PBAs does not yield further specific information for compound identification. Nevertheless, MS/MS-spectra of the PBAs showed typical mass shifts of 144 Da and 122 Da, which can be explained in terms of elimination of a sodium benzoate or a benzoic acid unit, respectively [202, 205].

As indicated in Figure 7 by the UV-signal, benzoylmethylester generated by the addition of methanol to the excess of benzoylchloride, the solvent applied in derivatization and unknown products most probably arising from derivatization are easily separated from the PBAs quantified. Except for D-talose, which is expected to elute in the range of 39.3 to 40.3 min, the complete row of $C_3$- to $C_6$-aldoses and D-idose in form of its alditol is subject to LC analysis as presented in Table 7. In the real formose samples analyzed, the presence of heptoses and octoses could be indicated by matching the calculated mass of the PBA-

sodium adducts at the expected retention time (~ 40 min). Due to the limited availability of heptose as well as octose carbohydrate standards these analytes have only been determined qualitatively.

As the groups of D-Xyl, D-Ara, D-Lyx and D-iditol, D-Gal, D-Man, D-Glc are coeluting it appeared to be necessary to enhance separation efficiency. However, separation of all the pentoses and hexoses present as their PBA seems to be very difficult even when 3.0 µm particles are used [193]. Apart from reversed phase (RP)-C18, RP-C8 (data not shown), and a phenyl-phase operated in normal phase elution mode were used for separating PBAs but no significant improvement in separation is evident [197]. As the group of D-Xyl/D-Ara/D-Lyx and L-iditol/D-Gal/D-Glc/D-Man show comparable slopes in UV-calibration (data not shown) quantification was carried out on the basis of D-Xyl and D-Man calibration data in UV- as well as in MS-quantification.

#### 3.1.3.2 Evaluation of partial benzoylation of alditols

Apart from the PBAs identified (see Table 7 and Figure 7) a number of unknown peaks appear in LC-UV, which could correspond to partially benzoylated as well as unknown compounds. In order to estimate the presence of partially-*O*-benzoylated alditols interfering in LC-UV, the following nominal masses of characteristic ions were calculated originating from partial benzoylation as shown in Table 8.

Table 8: Nominal masses of partially benzoylated alditols probably amenable to detection in LC-ESI-MS; the formation of the corresponding cation-adduct is assumed.

| | fully benzoy-lated | Number of benzoyl-units missing | | | | | | | | explana-tion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| Glycolaldehyde | 293 | 190 | 86 | - | - | - | - | - | - | $[M+Na]^+$ |
| Trioses | 427 | 324 | 220 | 117 | - | - | - | - | - | $[M+Na]^+$ |
| 2-HMG | 457 | 354 | 250 | 147 | - | - | - | - | - | $[M+K]^+$ |
| Tetroses | 561 | 458 | 354 | 251 | 147 | - | - | - | - | $[M+Na]^+$ |
| 2-HMT | 591 | 488 | 384 | 281 | 177 | - | - | - | - | $[M+K]^+$ |
| Pentoses | 695 | 592 | 488 | 385 | 281 | 178 | - | - | - | $[M+Na]^+$ |
| Hexoses | 829 | 726 | 622 | 519 | 415 | 312 | 208 | - | - | $[M+Na]^+$ |
| Heptoses | 964 | 861 | 757 | 654 | 550 | 447 | 343 | 240 | - | $[M+Na]^+$ |
| Octoses | 1098 | 995 | 891 | 788 | 684 | 581 | 477 | 374 | 271 | $[M+Na]^+$ |

By scanning for the masses of partially *O*-benzoylated alditols calculated in Table 8 the presence of the ions probably corresponding to alditols mainly missing one but rarely two benzoyl-units could be confirmed (see Figure 13, supplementary material; probably interfering partially *O*-benzoylated alditols were only assigned to the peaks doubtlessly identified, small peaks detected around 35-40 min retention time are considered as unspecific). The interference of partially-*O*-benzoylated compounds with the targets amenable to UV-quantification was found to be probable in several cases as indicated in Figure 8. Potential interference occurs in case of 2-HMG by tetrose (-1; indicates the number of benzoyl-units missing) and octose (-2); 2-HMT by pentoses (-1); D-Ery by hexoses (-1) as well as D-Xyl/D-Ara/D-Lyx by heptoses (-1). In addition, the potential presence of 2-HMT (-1), hexoses (-2) as well as heptoses (-2) not coeluting with the target compounds amenable to UV-quantification has been confirmed since their corresponding mother-ions could be detected (see Figure 8 and Figure 13).

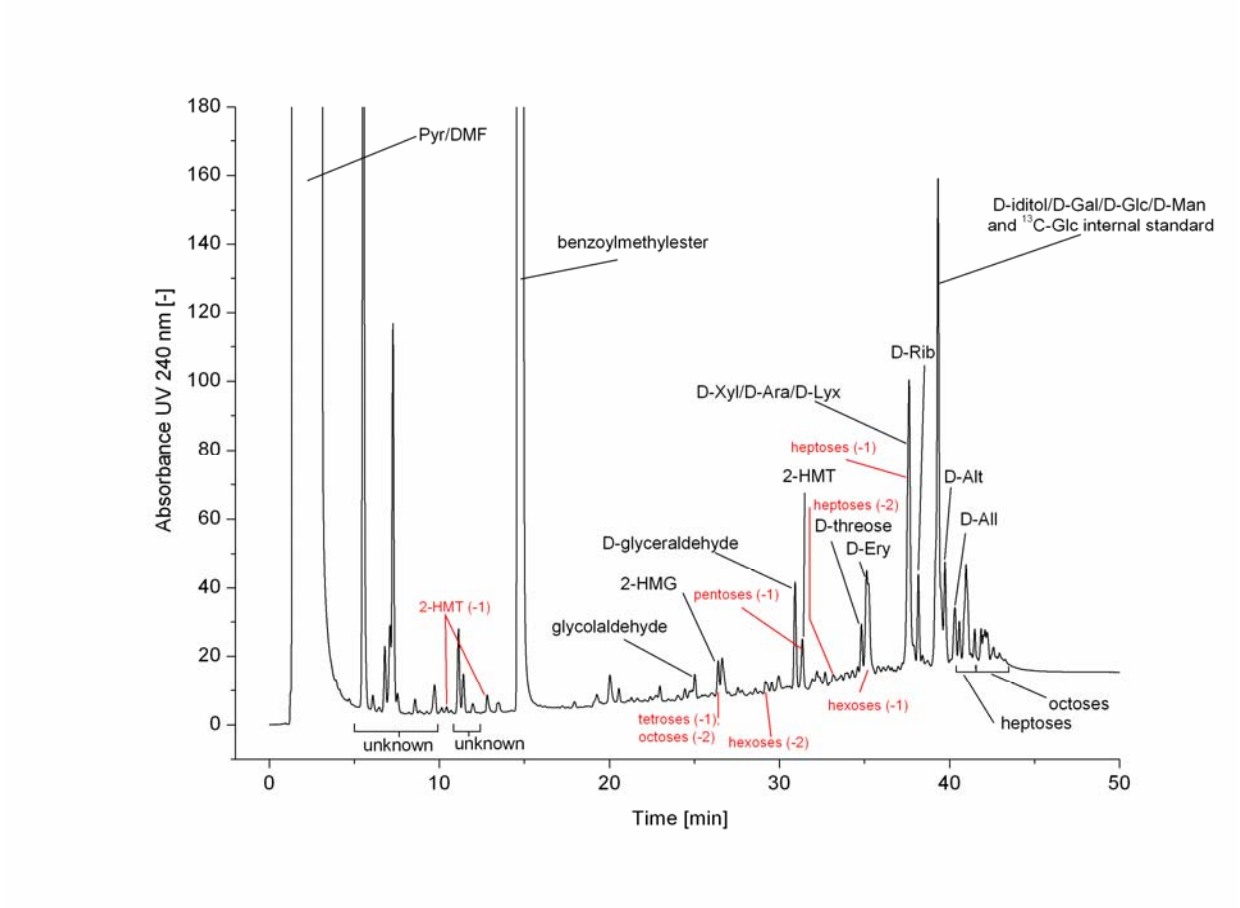



Figure 8: LC-UV chromatogram obtained from a real formose sample using glycolaldehyde initiator; carbohydrates were analyzed as their per-*O*-benzoylated alditols; potentially partially-*O*-benzoylated alditols are marked in red; negative value indicates the number of benzoyl-units missing.

Although the reaction conditions applied in benzoylation have been carefully selected (see supplementary material) considerable amounts of probably partially-*O*-benzoylated alditols could be detected (extracted ion chromatograms see Figure 13 in supplementary material, note the y-axis scaling). As the yield of fully benzoylated alditols is depending on several parameters, calibration was carried out on the basis of aldoses, which were converted to their corresponding PBAs by applying the whole derivatization procedure. Thus, the need to determine the derivatization yield, as well as the analyte recovery for calibration, has been eliminated. Only in case of 2-HMG the alditol was used for calibration since the corresponding aldose could not be easily provided by synthesis (see supplementary material). Hence, the content of 2-HMG and 2-hydroxymethylglyceraldehyde, quantified as 2-HMG in the real formose sample, can only be estimated by the calibration procedure applied since 2-HMG detected in its per-*O*-benzoylated form is not exposed to the whole sample preparation procedure.

Moreover, moderate concentrations of $K_2HPO_4$ are known to favor the formation of partially benzoylated derivates whereas particularly high concentrations of sodium hydroxide enhance the formation of fully benzoylated derivates [194].

### 3.1.3.3 Quantification of carbohydrates as their PBAs

#### 3.1.3.3.1 UV-validation data

Linear regression modeling using five-point external standard calibration demonstrated a good linearity over 3 orders of magnitude for every aldose as well as for 2-HMG (see Table 9). The resulting regression coefficients varied from 0.951 to 0.996. The LOD and LOQ were calculated according to the 3σ- and 10σ-criterion, i.e. the three- and tenfold standard deviation of the noise quantified via single point calibration (after DIN 32465:2008-11) [206].

Table 9: Linearity, detection limits, working range and repeatability precision (n … number of replicates) of carbohydrates quantified as their PBAs in LC-UV; chromatographic conditions see section 3.1.2.2.

| | regression coefficient ($R^2$) | LOD $_{on\ column}$ [nmol] | LOQ $_{on\ column}$ [nmol] | method working range[b] [ng*µL$^{-1}$] | RSD [%; n=3] |
|---|---|---|---|---|---|
| Glycolaldehyde | - | - | - | - | - |
| 2-HMG | 0.98 | 5.5 | 18.3 | 0.2 – 27.0 | 6.0 |
| D-Glyceraldehyde | 0.98 | 115.5 | 385.1 | 3.5 – 90.6 | 5.3 |
| 2-HMT | 0.98 | 7.7 | 35.7 | 0.3 – 69.1 | 2.1 |
| D-Threose | 0.987 | 6.9 | 22.9 | 0.3 – 117.2 | 3.5 |
| D-Ery | 0.989 | 12.6 | 42.1 | 0.5 – 105.0 | 3.6 |
| D-Xyl/D-Ara/D-Lyx | | | | | 4.8 |
| D-Xyl | 0.989 | 7.2 | 23.9 | 0.4 – 120.0 | |
| D-Ara[a] | 0.996 | 7.5 | 25.0 | 0.4 – 117.6 | |
| D-Lyx[a] | 0.986 | 6.9 | 22.9 | 0.4 – 97.6 | |
| Rib | 0.993 | 7.5 | 24.9 | 0.4 – 122.3 | 4.2 |
| L-iditol/D-Gal/D-Glc/D-Man | | | | | 2.6 |

| | regression coefficient ($R^2$) | LOD $_{on\ column}$ [nmol] | LOQ $_{on\ column}$ [nmol] | method working range[b] [ng*µL$^{-1}$] | RSD [%; n=3] |
|---|---|---|---|---|---|
| L-iditol | - | - | - | - | - |
| D-Gal | 0.951 | 339.2 | 1130.5 | 20.4 – 110.6 | |
| D-Glc | 0.975 | 75.1 | 250.4 | 4.5 – 108.2 | |
| D-Man | 0.976 | 239.9 | 799.6 | 14.4 – 120.0 | |
| D-Alt | 0.973 | 5.8 | 19.3 | 0.3 – 98.8 | 5.7 |
| D-All | 0.995 | 5.7 | 19.2 | 0.3 – 104.7 | 6.3 |

[a]: yield identical alditols; [b]: LOQ and highest standard for calibration considered.

The lowest absolute LOD and LOQ (on column) were achieved for 2-HMG (5.5 and 18.3 nmol), while D-Gal featured the highest LOD and LOQ (0.3 µmol and 1.1 µmol). Satisfying repeatability precision ranging from 2.1 (2-HMT) to 6.3 (D-All) %RSD was determined by injection of independent standards in triplets (see Table 9).

Accuracy was determined by performing standard addition to a real formose reaction sample. Quantitative recovery (99.9 to 100.2%) of carbohydrates was observed except for glycolaldehyde. Thus, analysis of glycolaldehyde using $NaBH_4$-reduction and benzoylation as done in this work only yielded qualitative data. The concentration of glycolaldehyde in formose reaction-samples was quantified by DNPH-derivatization of a 1:500 diluted raw formose reaction-sample (see supplementary material). However, due to a moderate LOD of 46.3 ng*µL$^{-1}$ an alternative way for the determination of glycolaldehyde in formose reaction-samples remains to be developed.

#### 3.1.3.3.2 MS-validation data

Except for early eluting peaks (2-HMG, D-glyceraldehyde and 2-HMT) internal standard calibration using $^{13}$C-glucose was carried out as indicated in Table 10. The regression models were chosen according to the best quality of fit using a five-point calibration. The resulting regression coefficients for the particular regression model selected varied from 0.966 to 0.999. The LOD and LOQ were calculated according to the 3σ- and 10σ-criterion, i.e. the three- and tenfold standard deviation of the noise quantified via single point calibration (after DIN 32465:2008-11) [206].

Table 10: Regression model, quality of fit, detection limits, working range and repeatability precision of carbohydrates (n … number of replicates) quantified as their PBAs in LC-ESI-MS; improved LOD compared to LC-UV written in bold style; chromatographic conditions see section 3.1.2.2.

| | Calibration type[a] | regression coefficient ($R^2$) | LOD on column [nmol] | LOQ on column [nmol] | method working range[c] [ng*µL$^{-1}$] | RSD [%; n=3] |
|---|---|---|---|---|---|---|
| Glycolaldehyde | - | - | - | - | - | - |
| 2-HMG | Q, ES | 0.999 | 10.3 | 34.2 | 0.6 – 54.0 | 12.1 |
| D-Glyceraldehyde | Q, ES | 0.977 | 190.8 | 636.0 | 4.6 – 90.6 | 20.3 |
| 2-HMT | Q, ES | 0.999 | **6.3** | 21.2 | 0.2 – 46.1 | 5.9 |
| D-Threose | Q, IS | 0.961 | **2.4** | 8.2 | 0.1 – 117.2 | 7.9 |
| D-Ery | L, IS | 0.981 | 8.3 | 27.8 | 0.3 - 105 | 3.1 |
| D-Xyl/D-Ara/D-Lyx | L, IS | | | | | 17.8 |
| D-Xyl | | 0.991 | 6.0 | 20.1 | 0.3 - 120 | |
| D-Ara[b] | | 0.992 | 9.8 | 32.8 | 0.5 – 117.6 | |
| D-Lyx[b] | | 0.997 | 18.4 | 61.5 | 1.1 – 97.6 | |
| Rib | L, IS | 0.994 | 16.6 | 55.5 | 0.8 – 122.3 | 8.6 |
| L-iditol/D-Gal/D-Glc/D-Man | L, IS | | | | | 7.8 |
| L-iditol | | - | - | - | - | - |
| D-Gal | | 0.979 | **30.9** | 103.9 | 2.5 – 110.6 | |
| D-Glc | | 0.997 | **15.8** | 52.8 | 1.3 – 108.2 | |
| D-Man | | 0.999 | **57.0** | 190.2 | 4.6 – 118.8 | |
| D-Alt | Q, IS | 0.966 | 22.0 | 73.5 | 1.2 – 65.9 | 8.3 |
| D-All | Q, IS | 0.984 | 16.1 | 53.8 | 0.9 – 69.8 | 6.3 |

[a]:L ... linear, Q ... quadratic, IS ... internal standard calibration, ES ... external standard calibration;
[b]: yield identical alditols; [c]: LOQ and highest standard for calibration considered.

The lowest absolute LOD and LOQ (on column) were achieved for D-threose (2.4 and 8.2 nmol) while D-glyceraldehyde featured the highest LOD and LOQ (190.8 and 636.0 nmol; detailed data see Table 10). This rather weak LOD could be explained in terms of obtaining a

very small signal for D-glyceraldehyde in the blanks. Compared to LC-UV, a significant improvement in LOD (on column) could be obtained in case of 2-HMT: 6.3 nmol (7.7 nmol in UV), D-Gal 30.9 nmol (339.2 nmol in UV), D-Glc 15.8 nmol (75.1 in UV) and D-Man 57.0 (239.9 nmol in UV). However, by using e.g. highly purified solvents further improvement in LOD seems to be realizable.

As sodium formate was used in the ionization buffer inter-day repeatability precision has been evaluated. LC-ESI-MS was found to work stable without any indication of drift (data not shown). Satisfying intra-day repeatability precision ranging from 3.1 (D-Ery) to 20.3 (D-glyceraldehyde) %RSD has been determined by injection of independent standards in triplets (detailed data see Table 10). Acceptable RSD-values were achieved in case of early eluting peaks (2-HMG, D-glyceraldehyde and 2-HMT), even though external standard calibration was used. As a matter of fact, for the precise quantification of formose carbohydrates using LC-ESI-MS the application of at least one multiple $^{13}$C-labeled internal standard turned out to be essential. The implementation of multiple $^{13}$C-labeled internal standards might be beneficial in terms of improving repeatability precision. However, for routine analysis the rise in costs caused by usage of multiple $^{13}$C-labeled internal standards is considerable.

Accuracy has been determined by performing standard addition to a real formose reaction-sample. Almost quantitative recovery (81.1 to 113.3%) of carbohydrates except for glycolaldehyde has been observed.

#### 3.1.3.3.3 Evaluation of UV-quantification using MS-data

After quantifying real formose samples in LC-UV the samples were re-quantified using LC-ESI-MS-calibration. As $^{13}$C-glucose was used as an internal standard in MS-quantification, the samples were analyzed twice, once in LC-UV and once in LC-ESI-MS, since the internal standard yields a UV-signal for per-*O*-benzoylated sorbitol. As the repeatability precision accounts for ≤ 6.3% RSD (n=3) in LC-UV as well as ≤ 20.3% RSD (n=3) in LC-ESI-MS, cross-validation of UV-quantification relative to MS-data has been carried out. The results are shown in Table 11.

Table 11: Evaluation of the quantification in LC-UV on the basis of MS-data; figures in [%] relative to UV.

| | Initiator used for formose reaction | | | | | Quality of match |
|---|---|---|---|---|---|---|
| | Glycolaldehyde | D-Glyceraldehyde | D-Ery | D-Xyl | D-Glc | |
| D-Glyceraldehyde | 99.1 | 121.1 | 104.3 | 124.7 | 119.7 | good |
| D-Threose | 47.8 | 43.8 | 76.5 | 88.1 | 66.5 | moderate |
| D-Ery[d] | 44.6 | 26.7 | 48.4 | 68.0 | 70.5 | moderate |
| D-Xyl[a,e] | 76.5 | 49.0 | 98.5 | 128.6 | 120.3 | good |
| D-Rib | 43.5 | 29.2 | 101.3 | 143.9 | 139.1 | moderate |
| D-Man[b] | 31.3 | 27.8 | 35.8 | 50.1 | 42.8 | bad |
| D-Alt[f] | 79.8 | 78.1 | 104.4 | 133.9 | 110.0 | good |
| D-All[g] | 29.6 | 29.1 | 43.9 | 61.6 | 63.0 | bad |
| 2-HMG[h] | 124.0 | 130.2 | 94.2 | 120.3 | 120.8 | good |
| 2-HMT[c] | 19.3 | 36.0 | 20.7 | 29.6 | 32.5 | bad |

[a]: representing D-Xyl/D-Ara/D-Lyx; [b]: representing L-iditol/D-Gal/D-Glc/D-Man; [c]: interference probably by pentoses (-1); [d]: interference probably by hexoses (-1); [e]: interference probably by heptoses (-1); [f]: interference probably by D-iditol/D-Gal/D-Glc/D-Man (see Table 7); [g]: interference probably by heptoses (see Table 7); [h]: quantification probably distorted by the calibration approach applied, interference probably by tetroses (-1) and octoses (-2).

Independent from the initiator used for conducting the formose reaction, LC-UV-quantification was found to be in good agreement with LC-ESI-MS in case of D-glyceraldehyde, D-Xyl, D-Alt as well as 2-HMG. This agreement could be achieved although D-Xyl is used for the quantification of the group of D-Xyl/D-Ara/D-Lyx and probable interference with partially-*O*-benzoylated compounds was found to take place in case of D-Xyl, D-Alt and 2-HMG. In case of 2-HMG the quantification in the real formose samples only represents an estimation since the alditol instead of the corresponding aldose has been applied in calibration.

Considering D-threose, D-Ery as well as D-Rib, LC-UV-quantification was found to be in moderate agreement with LC-ESI-MS. Although no interference of partially benzoylated

alditols was found in case of D-threose and D-Rib only a moderate agreement could be achieved.

Considering D-Man, D-All as well as 2-HMT to be analyzed in LC-UV, significant deviation based on the LC-ESI-MS quantification was found. As D-Man is used for the quantification of the group of L-iditol/D-Gal/D-Glc/D-Man and the quantification of D-All and 2-HMT is probably interfered by the presence of heptoses and partially-*O*-benzoylated alditols, the significant discrepancy could be explained. It is supposed that a significant amount of unknown UV-absorbing compounds is probably eluting at the retention times corresponding to D-threose, D-Rib, D-Man, D-All as well as 2-HMT. This reasonable suspicion could be confirmed by observing the MS-spectra obtained (data not shown).

#### 3.1.3.3.4 Quantification of formose carbohydrates as their PBAs

Formose carbohydrates were analyzed as their PBAs in LC-ESI-MS and standard analytical tools were used for the analysis of key compounds (e.g. residual formaldehyde; see supplementary material). Quantitative conversion of formaldehyde (98.5 to 98.8%) was obtained independent from the type of initiator used. As a rather large amount of formaldehyde is converted within the formose reaction (89.3 to 94.2%) the extent of cannizzaro reaction accounts only for 3.4 to 10.3% (detailed data not shown). Based on stoichiometric calculations of the cross-cannizzaro reaction the total conversion of branched chain-sugars to branched-chain alditols is estimated to account for 0.8 to 2.4% of total converted formaldehyde.

For the quantification of formose carbohydrates as their PBAs commercially available standards as well as 2-HMG and 2-HMT synthesized were used (see Table 7). The fraction of carbohydrates quantified as their PBAs in LC-ESI-MS has been calculated as a benchmark in order to assess the strength of the method presented. 19.3 to 37.3% (w/w) of formose carbohydrates detected as their PBAs (without consideration of glycolaldehyde) is acceptable with regard to the exclusive use of commercially available as well as two synthesized standards (detailed data not shown).

As depicted in Figure 9, the ratio of the individual carbohydrate-classes with regard to chain-length as well as 2-HMG and 2-HMT remains almost constant irrespectively of the type of initiator used.

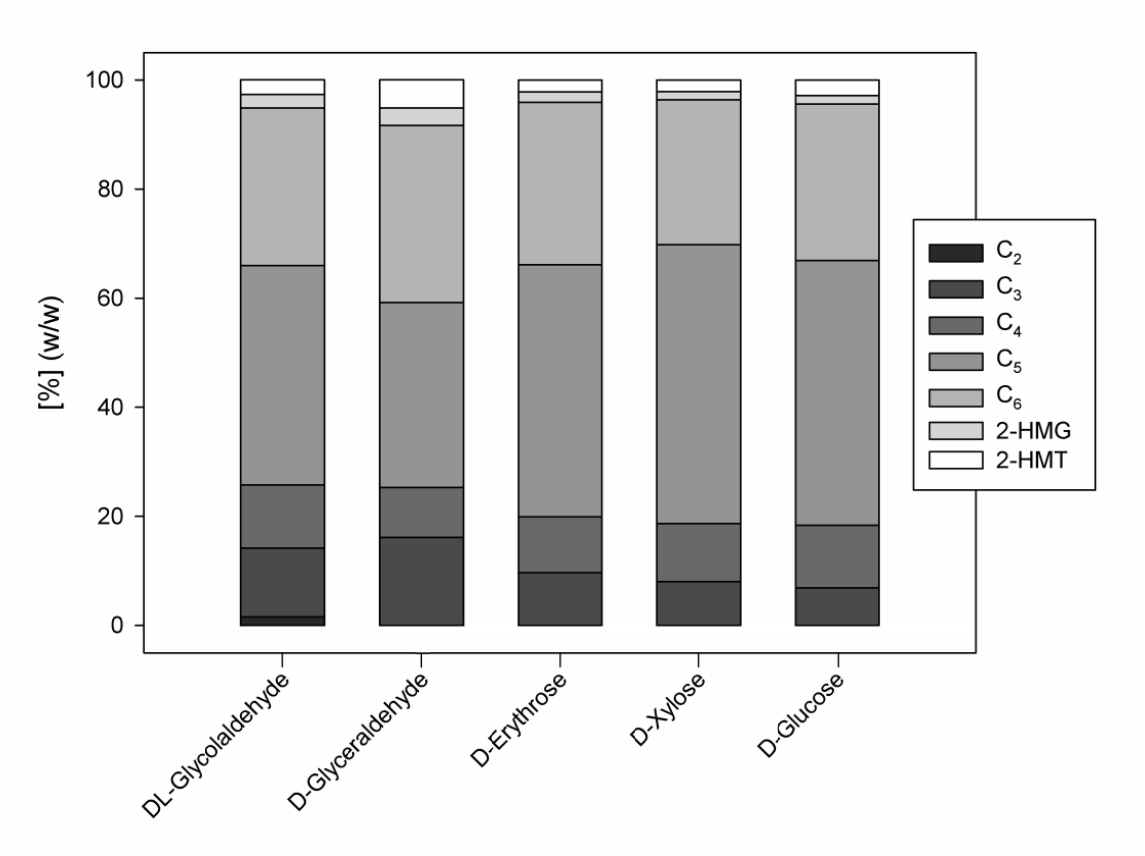



Figure 9: Product distribution (w/w) of formose carbohydrates quantified as their PBAs in LC-ESI-MS expressed as reducing carbohydrates with respect to the type of initiator used.

As indicated in section 3.1.3.1, the probable presence of heptoses and octoses has been confirmed qualitatively in all formose samples investigated (see Figure 12, supplementary material).

The formose product distribution is particularly sensitive to reaction conditions e.g. reaction temperature as shown in ref. [207]. The distributions shown in Figure 9 were obtained by selecting arbitrary reaction conditions and do not allow any conclusion about the particular dependence on specific reaction parameters.

### 3.1.4 Conclusions

1. The analytical method presented, in which carbohydrates are analyzed as their per-*O*-benzoylated alditols, depicts a simple and versatile procedure for LC-UV analysis of complex carbohydrate mixtures using inexpensive standard analytical equipment. Especially higher molecular weight and temperature sensitive compounds are easier

amenable to analysis by the method applied, which overcomes the limitations of conventional gas chromatographic analysis of carbohydrates.

2. Quantifying carbohydrates as their per-*O*-benzoylated alditols in LC-UV provides valid data in case of D-glyceraldehyde, 2-hydroxymethylglycerol, which depicts a branched-chain alditol occurring in the formose reaction, D-Xyl as well as D-Alt although a significant amount of carbohydrate degradation products is present. With regard to the determination of these analytes, this method delivers valid results at minimum effort. Considering samples with a similar complex carbohydrate spectrum, the methodology applied in this work can be extended just straight forward.

3. As a key advance benzoylation provides a significant increase in ESI-response, which facilitates the detection of otherwise hardly amenable compounds. In case of formose reaction samples generated for providing representative carbohydrate mixtures, limit-of-detection could be improved in case of 2-hydroxymethyltetritol, which represents another branched-chain alditol, D-threose, D-Gal, D-Glc and D-Man. The amount of formose carbohydrates quantified as their PBAs in LC-ESI-MS ranges from 19.3 to 37.3% demonstrating the strength of the method presented.

Apart from the analytes quantified as their PBAs in this work the formose reaction delivers a broad spectrum of byproducts formed such as sugar acids as well as α-dicarbonyl-compounds, as some preliminary investigations showed. Investigating different GC/MS-methods for the analysis of formose reaction-samples for discovering the broad variety of carbohydrates is under way (manuscript in preparation). Due to an unsatisfactory LOD of 46.3 ng*μL$^{-1}$ for glycolaldehyde after DNPH-labeling and LC-UV-analysis an alternative way for the determination of glycolaldehyde in formose reaction-samples remains to be developed.

### 3.1.5 Supplementary material

#### 3.1.5.1 Analysis of formose reaction key compounds and semi-preparative chromatography

Analysis of formaldehyde, glycolaldehyde and formic acid as their corresponding DNPH-derivate as well as semi-preparative chromatography for the production of 2-hydroxymethylglycerol, a representative branched-chain alditol, was conducted on a VWR-Hitachi EzChromElite HPLC (VWR, Vienna, Austria) consisting of a L 2130 pump, L 2200

autosampler, L 2350 column oven, L 2450 diode array detector- and a L 2490 RI-detector operated by EzChromElite Client/Server software version 3.1.7. The chromatographic conditions for the analysis of DNPH-derivatives were identical with those described in [187] except for applying a truncated gradient and injecting 10 µL: 0-33 min: 5-57.2% $CH_3CN$ applying 1 mL*min$^{-1}$ flow rate, 33.1-43 min: 100% $CH_3CN$ applying 1.2 mL*min$^{-1}$ flow rate, 43.1-48 min: 5% $CH_3CN$ applying 1 mL*min$^{-1}$ flow rate; the eluents used contain 0.025% (v/v) formic acid maintaining pH~3. Formaldehyde was determined by derivatization of a 1:500 diluted raw formose sample with an acceptable precision (0.9% RSD (n=3); 6.2 ng*µL$^{-1}$ LOD; 20.8 ng*µL$^{-1}$ LOQ). Glycolaldehyde was quantified by derivatization of a 1:500 diluted raw formose sample with an acceptable precision (1% RSD (n=3)) but shows only a moderate LOD of 46.3 ng*µL$^{-1}$ and 154.5 ng*µL$^{-1}$ LOQ. Formic acid was measured by performing DNPH-labeling of an undiluted $NaBH_4$-reduced formose sample, reversed phase (RP)-chromatography as described before and UV-detection at 360 nm providing 1% RSD (n=3), 2.7 ng*µL$^{-1}$ LOD, 9.1 ng*µL$^{-1}$ LOQ, and accuracy of 95%.

In case of semi-preparative chromatography a Biorad Aminex HPX 87C 7.8 x 300 mm HPLC column was operated at a flow rate of 0.7 mL*min$^{-1}$ HQ-water, oven temperature 80°C and RI-detection. A Besta multipositon-valve was used for collecting fractions.

Methanol was analyzed using a Shimadzu GC17A (Shimadzu, Korneuburg, Austria) equipped with a HP-PLOT U column, 320 µm x 30 m, 10 µm film thickness (Agilent Technologies) and a flame ionization detector (FID). The column was operated using He carrier gas at 120 kPa constant head-pressure, splitless injection, 3 min sampling time, and the following temperature program: 125°C, 0.2 min equilibration time; 10°C*min$^{-1}$ gradient; 180°C for 10 min finally. The temperature of the injector has been set to 150°C, whereas the temperature of the FID has been maintained at 280°C. GC-solution software version 2.30.00 was used for controlling the gas-chromatograph and for data processing. Headspace samples were injected using a HP 7694E Headspace sampler (Agilent Technologies) equipped with a 3 mL sample loop.

Residual formaldehyde is almost quantitatively converted to methanol by reduction, which was checked with model solutions. The concentration of methanol was determined by headspace GC-analysis. By subtracting the formaldehyde-value, which has been determined by DNPH-derivatization, expressed as methanol from the methanol-value determined after reduction, the content of methanol in the raw formose sample was calculated easily. Methanol was determined using standard addition and isopropanol as an internal standard featuring 7.1% RSD (n=3), 0.5 ng*µL$^{-1}$ LOD and 1.6 ng*µL$^{-1}$ LOQ.

#### 3.1.5.2 Synthesis of representative branched-chain alditols

It should be taken into account that the amount of 2-HMG and 2-HMT quantified is corresponding to the sum of aldose or ketose, which did not yet react within a cross-cannizzaro reaction and 2-HMG as well as 2-HMT, the final product of the cross-cannizzaro reaction, since reduction is carried out as a sample preparation step. Two representative branched-chain alditols namely 2-HMG (IUPAC: 2-(hydroxymethyl)propane-1,2,3-triol) and 2-HMT were synthesized.

NMR-spectroscopy was carried out by Deutero GmbH, Kastellaun, Germany by using a Varian VXR-300S instrument. NMR spectra were recorded at 398.908 MHz for $^{1}H$ and 100.567 MHz for $^{13}C$, respectively, using DMSO-$d_6$-solvent.

##### 3.1.5.2.1 Synthesis of 2-hydroxymethylglycerol

2-HMG was produced from tris by diazotation and subsequent decomposition of the corresponding alkyldiazonium-salt [208]. Conditions similar to those reported in literature were chosen [190]: 17.1 ml of concentrated acetic acid was added to 100 mL of water; 12.1 g of tris was dissolved and the solution was kept at 4°C. 6.9 g of sodium nitrite was dissolved in 25 mL of water and also kept at 4°C. After adding the sodium nitrite solution to the dissolved tris, the solution was vigorously mixed for 12 hours at 4 °C. Finally the solution was warmed to room temperature. Dowex 50WX2-400 cation exchange resin was added to an aliquot of the reaction solution. After centrifugation at 3000 rpm for 5 min the supernatant was subjected to semipreparative-chromatography in order to isolate 2-HMG (see section 4.5.2). Sufficient substance was obtained to be useful as a standard for quantification and for NMR-spectroscopy for structure confirmation of 2-hydroxymethylglycerol (2-HMG; IUPAC: 2-(hydroxymethyl)propane-1,2,3-triol):

$^{1}H$-NMR (DMSO-$d_6$): δ = 3.3 (d, J = 6.6, $CH_2$-groups), 4.0 (s, tertiary OH-group), 4.3 (t, J = 6.7, primary OH-group) ppm.

$^{13}C$-NMR (DMSO-$d_6$): δ = 62.84 ($CH_2$-groups), 74.29 (quaternary carbon) ppm.

The NMR-data obtained ($^{1}H$-peak area ratio of 6:1:3 and a matching coupling pattern) indicates the formation of 2-hydroxymethylglycerol (2-HMG; IUPAC: 2-(hydroxymethyl) propane-1,2,3-triol).

#### 3.1.5.2.2 Synthesis of 2-hydroxymethyltetritol

2-HMT was produced by $NaBH_4$-reduction of apiose, a naturally occurring branched chain-sugar. LC-ESI-IonTrap-MS only provides nominal mass resolution. Nevertheless, identification of 2-HMT has been carried out by comparison of the mass of the mother ion observed at the expected retention time based on LC-UV with the one calculated, which corresponds to the sodium-adduct of the PBA (see Table 7).

### 3.1.5.3 Analyte recovery with respect to the removal of borate species

Critical evaluation of the analyte recovery observed during removal of borate species as their methylborate ester has been carried out by exposing model solutions to the derivatization procedure. If methanol was evaporated in a stream of nitrogen at 45°C, glycolaldehyde could only be recovered to a very low extend (~2%). Carrying out the evaporation in a Speedvac at lowered pressure, only $C_5$-$C_6$ alditols could be recovered almost quantitatively. Apart from the loss of approximately 40% of $C_4$- and 95% of $C_3$-alditols ethyleneglycol was lost almost quantitatively.

### 3.1.5.4 Evaluation of the benzoylation of alditols

In order to ensure a quantitative labeling of hydroxyl containing compounds and due to little information available in literature [189, 193-197] a critical evaluation of the benzoylation of alditols was performed. For the optimization of derivatization quantification has been carried out at 254 nm, which allows to quantify rather high alditol concentrations (c = 1 $\mu g*\mu L^{-1}$) due to moderate detector response. 240 nm has been selected as the standard wavelength for analysis due to an appreciable signal-to-noise-ratio as well as acceptable sensitivity.

To check the influence of the chain length of alditols on the success of labeling the following trials were carried out: 5 mg of D-sorbitol, D-xylitol and D-threitol were dissolved individually in 1 mL pyridine/dimethylformamid 2:1 at 62°C for 20 min, 600 µL of 1:5 diluted aliquots were transferred into other 1.5 mL glass-vials. After cooling to room temperature different amounts of benzoylchloride-reagent (5 to 120 µL; if less than 120 µL was used solvent was used in order to fill up to 120 µL) were added, which corresponds to a ratio from 2 to 45 of derivatization reagent with respect to OH-functions. Reacting 2.5 h at 80°C was considered sufficient for quantitative derivatization. After cooling down to room temperature the excess of reagent was quenched by adding 300 µL of methanol. As shown in Figure 10 almost

quantitative derivatization was obtained if at least a 10-fold excess of reagent with respect to OH-functions was applied.

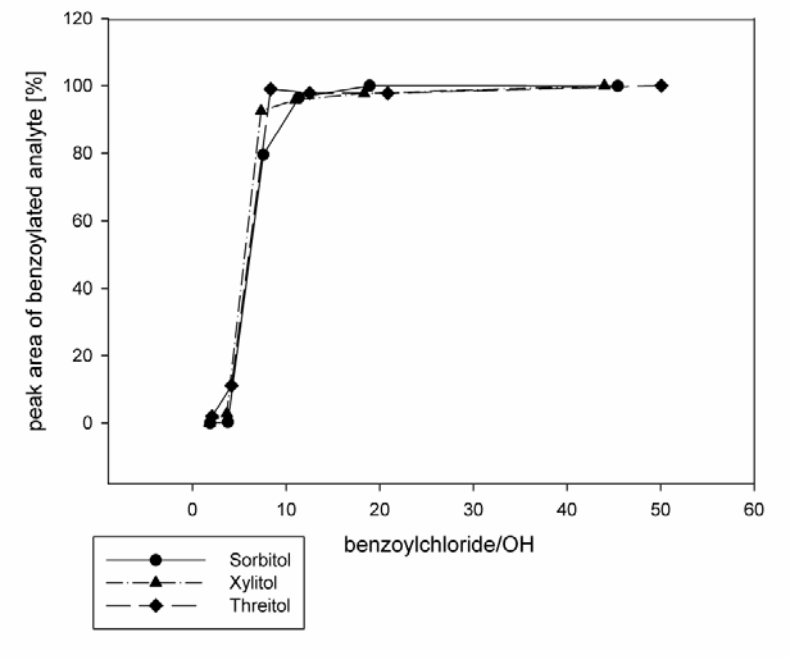



Figure 10: Relation between the relative peak area observed and the molar ratio of benzoylchloride/OH in case of D-sorbitol, D-xylitol and D-threitol; derivatization at 80°C for 2.5 hours.

In order to ensure sufficient reagent to be present with respect to OH-functions a 20-fold excess of benzoylchloride was selected as the optimal ratio for derivatizing alditols. Based on this, further investigations were carried out:

To determine the optimal reaction time, a 1 µg*µL$^{-1}$ D-sorbitol-standard was selected for derivatization starting from 15 to 150 min. As shown in Figure 11, optimal reaction time was determined as 90 min to obtain maximum peak area.

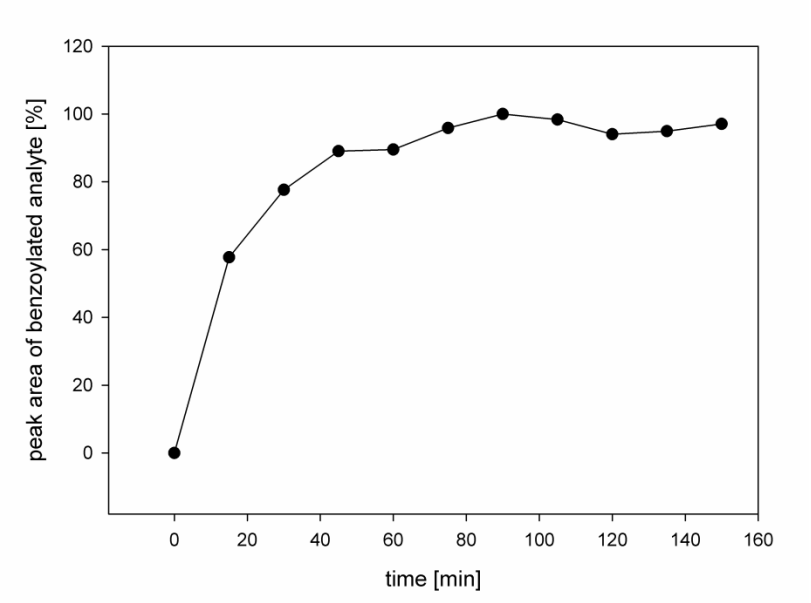



Figure 11: Relation between the relative peak areas of a 1 µg*µL$^{-1}$ D-sorbitol standard according to reaction time using a 20-fold excess of benzoylchloride with respect to OH-functions and 80°C reaction temperature.

### 3.1.5.5 Detection of extracted ions in LC-ESI-MS for compound identification

#### 3.1.5.5.1 per-*O*-benzoylated alditols

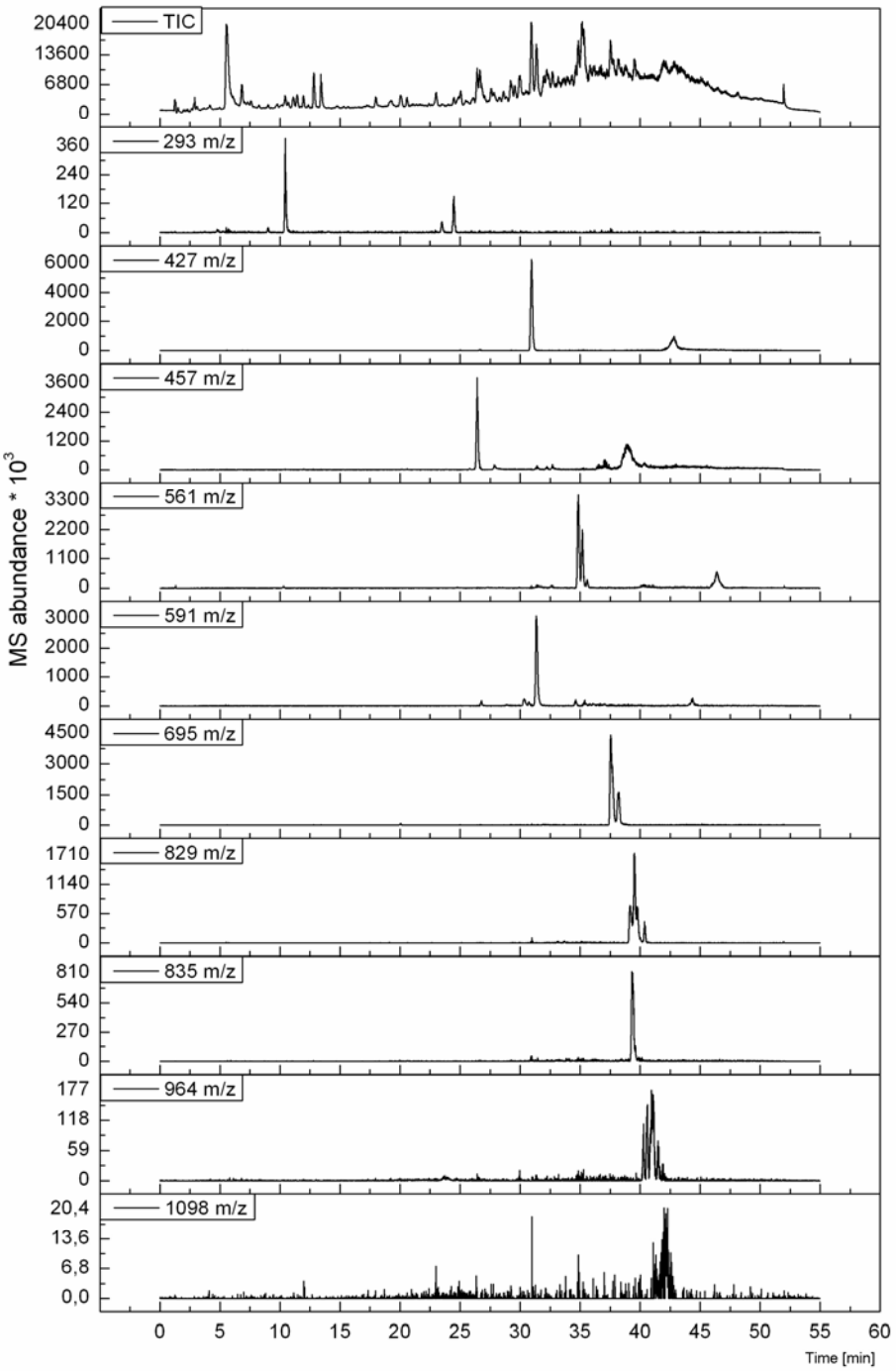



Figure 12: Detection of per-*O*-benzoylated alditols in LC-ESI-MS for assigning the retention time to the corresponding peaks in LC-UV.

### 3.1.5.5.2 partially-*O*-benzoylated alditols

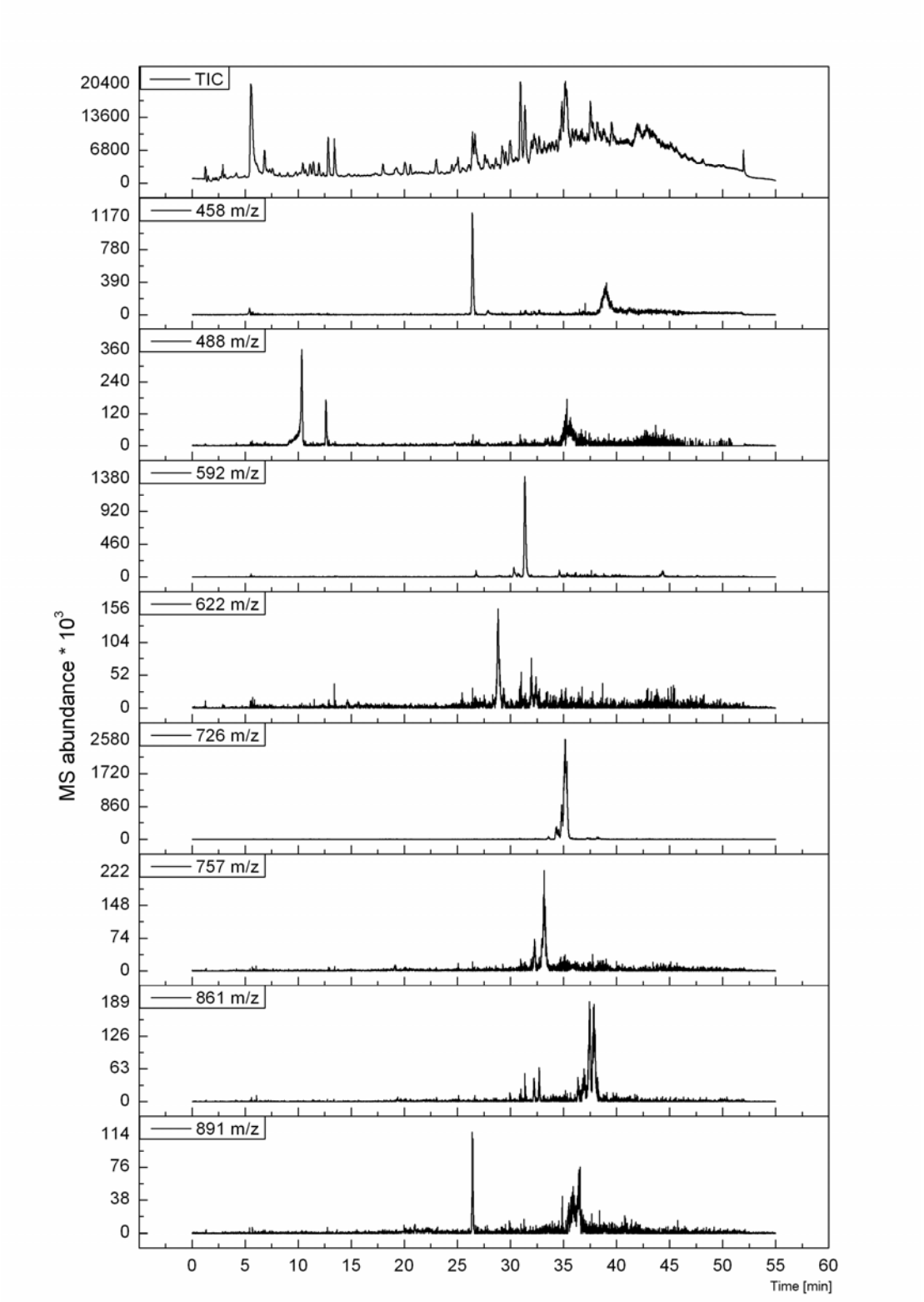



Figure 13: Detection of partially-*O*-benzoylated alditols in LC-ESI-MS for assigning the retention time to the corresponding peaks in LC-UV.

## 3.2 Investigation of the aldol-reaction of formaldehyde and glycolaldehyde

In the present chapter the selection of appropriate reaction conditions, in order to realize the aldol-condensation step yielding $C_3$-carbohydrates, was investigated systematically. Experiments were conducted using a batch reactor-system. Conducting the aldol-reaction at room temperature (25°C) yields viable product distributions as some preliminary trials showed (data not shown). The detailed parameters applied are listed in Table 12.

Table 12: Standard aldol-condensation setup, data in g * $L^{-1}$ unless otherwise stated.

| Catalysis-system | Heterogeneous | Homogeneous |
|---|---|---|
| Formaldehyde | 50 | 50 |
| Methanol | 13.5 | 13.5 |
| Calcium hydroxide | 14.8 | 0.58 |
| Glycolaldehyde | 1.25 | 1.25 |
| Temperature [°C] | 25 | 25 |
| Reaction volume [mL] | 50 | 50 |
| 10% (w/v) Hydro-chloric acid used as stopping solution [mL] | 6 | 2.5 |

In case of heterogeneous catalysis the reactions were conducted using a Millipore stirred ultrafiltration cell (type XFUF07601) equipped with a 0.3 µm membrane (type JX MFPVDF Osmonics), which was used repeatedly without any indication of deterioration of the membrane. In case of homogeneous catalysis the reaction setups were carried out in a stirred 100 mL Schott Duran™ glass flask.

108 mL of aqueous 37% (w/v) formaldehyde solution were diluted to a final volume of 0.5 L containing 1.25 g glycolaldehyde, which represents solution A. Calcium hydroxide was suspended in 25 mL of water in the filtration cell (heterogeneously catalyzed) or in another vessel (homogeneously catalyzed) representing solution B. The reactants (25 mL of solution A and solution B) were pre-heated to the reaction temperature ± 2°C using an oil-bath. The aldol-reaction was started by transferring the formaldehyde-solution containing dissolved

glycolaldehyde into the vessel containing base. This reaction setup provides 1.33 M formaldehyde and 20.8 mM glycolaldehyde as starting conditions.

After carrying out the reaction in case of heterogeneous catalysis filtration has been carried out by applying 5 bar nitrogen pressure. The reaction time is defined as the time between adding the formaldehyde solution containing dissolved glycolaldehyde and starting filtration. Dependent on the properties of the cleaned membrane filtration time accounted for approximately 2 min. The permeate obtained was collected by usage of a measuring flask containing the stopping solution being continuously stirred at ambient conditions.

In case of homogeneous catalysis the reaction was quenched by adding stopping solution to the continuously stirred batch reactor. After cooling down to room temperature pH=7 was adjusted by adding 5% (w/v) sodium hydroxide independent from the catalysis system chosen.

Quantification of the carbohydrates as their PBAs in LC-UV provides valid data for D-glyceraldehyde, 2-HMG, D-Xyl and D-Alt as shown in Table 7. Since partial formaldehyde conversion is considered, which is expected to yield just traces of carbohydrates degradation products, only minor interference of the remaining analytes is expected. However, in order to confirm the identity of the target-compounds such as $C_3$-carbohydrates particular attracting attention in this work, e.g. mature GC/MS-technique including confirmation of the spectra observed with those available in libraries is considered to be essential.

### 3.2.1 Heterogeneous catalysis

With respect to the data reported in literature (see section 3.2) the formation of $C_3$-carbohydrates has been investigated applying a rather big amount of glycolaldehyde acting as formaldehyde acceptor. As shown in Figure 14 the preferable formation of $C_3$-carbohydrates was proven to be feasible.

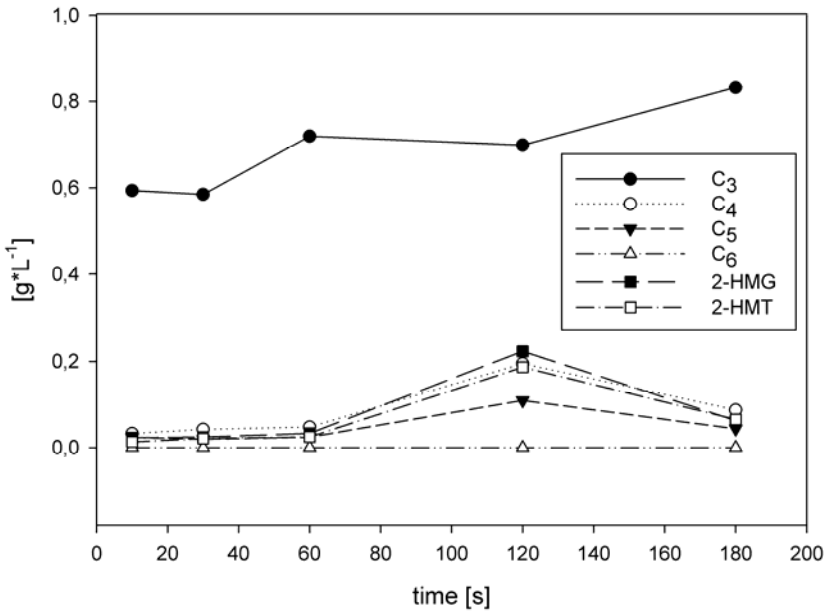



Figure 14: Time dependency of the $C_3$-carbohydrate formation using heterogeneous calcium hydroxide catalysis; total formaldehyde conversion ≤ 13.5% within 180 s; formic acid ≤ 0.45 g*$L^{-1}$.

Considering elevated reaction time (> 100 s) the formation of higher carbohydrates as well as cross-cannizzaro products e.g. 2-HMG and 2-HMT is evident. With regard to minimizing the formation of byproducts, 45 s reaction time was selected to be appropriate. As indicated in Figure 14 the amount of byproducts formed is decreasing from 120 to 180 sec. This phenomenon could be explained in terms of partially unknown side reactions taking place, e.g. the formation of saccharinic acid, which is not covered by the analytical methods applied. The permeate obtained in the heterogeneously catalyzed aldol-reaction was investigated with regard to time-dependent composition. As shown in Figure 15 the permeate collected was divided into five different fractions and analytical results are shown in Figure 16.

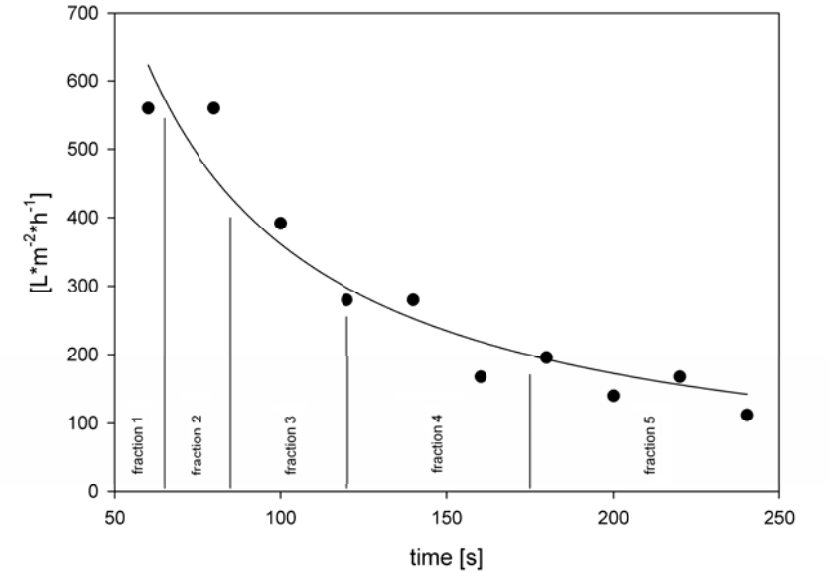



Figure 15: Flux behavior and fractions obtained from heterogeneously catalyzed aldol-condensation.

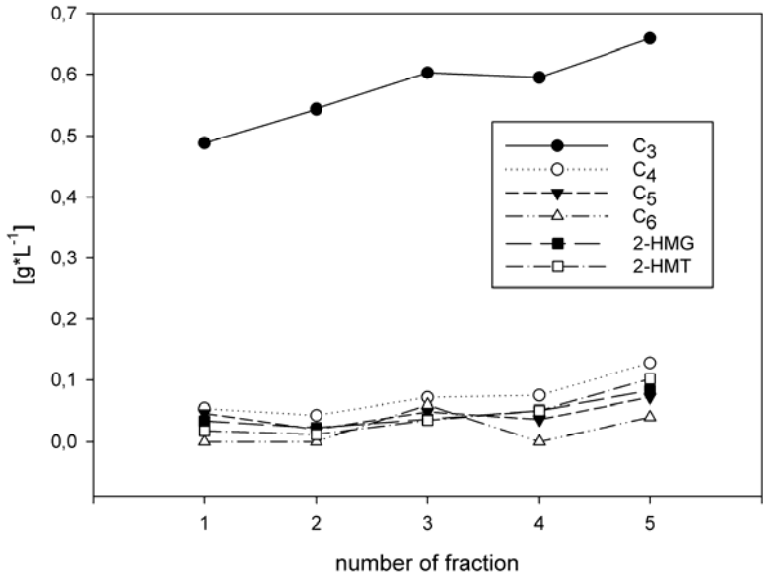



Figure 16: Time dependency of the carbohydrate-distribution obtained in the permeate-fractions using heterogeneous catalysis.

Within 45 s reaction time and subsequent filtration total formaldehyde conversion accounted for ≤ 2% whereas ≤ 0.23 g*$L^{-1}$ formic acid was generated. As shown in Figure 16, the yield of $C_3$-carbohydrates increases as filtration time passes. Decreasing the content of formaldehyde trough filtration might result in a more favorable ratio of formaldehyde/base, which could explain the increasing amount of $C_3$-carbohydrates formed.

Heterogeneous catalysis of the aldol-reaction is considered as disfavorable with regard recycling a rather high amount of salts in a process technological scale. Thus, trials were conducted applying homogeneous catalysis.

### 3.2.2 Homogeneous catalysis

#### 3.2.2.1 Time dependency

As shown in Figure 17 in a homogeneously catalyzed aldol-reaction the time scale is shifted towards longer intervals at a first glance (compared with Figure 14). Applying moderate reaction conditions the controllability of the reaction seems to be feasible.

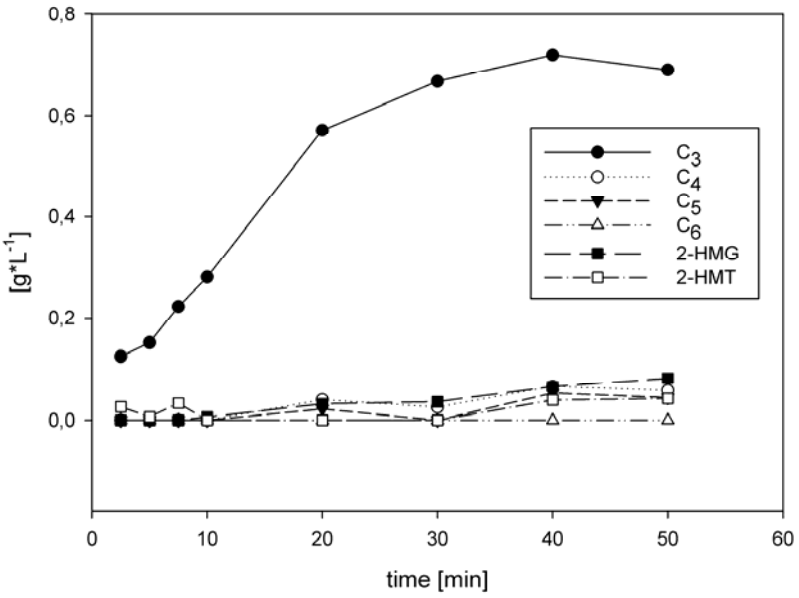



Figure 17: Time dependency of the carbohydrate-distribution obtained in the permeate using homogeneous calcium hydroxide catalysis; formaldehyde conversion ≤ 5% within 50 min; formic acid ≤ 0.03 $g*L^{-1}$.

As indicated in Figure 17 the accumulation of significant amounts of byproducts can be prohibited to a certain extend by selecting an appropriate reaction time. In addition, taking the ratio of $C_1/C_3$ as well as $C_3/C_4$ into account, 30 min of reaction time was selected to be appropriate (see Table 13; line marked in bold style).

Table 13: Homogeneously catalyzed formation of $C_3$-carbohydrates with respect to reaction time.

| [min] | $C_1/C_3$ | $C_3/C_4$ |
|---|---|---|
| 2.5 | 944.8 | - |
| 5 | 789.1 | - |
| 7.5 | 540.2 | - |
| 10 | 399.8 | - |
| 20 | 204.5 | 18.9 |
| **30** | **193.3** | **34.6** |
| 40 | 176.2 | 14.4 |
| 50 | 167.1 | 15.7 |

### 3.2.2.2 Concentration dependency

#### 3.2.2.2.1 Glycolaldehyde

The dependency of the $C_3$-carbohydrates formed with respect to the amount of glycolaldehyde applied is summarized in Table 14.

Table 14: Dependency of the homogeneously catalyzed formation of $C_3$-carbohydrates on the concentration of glycolaldehyde ($C_2$) applied using 30 min reaction time; formaldehyde conversion ≤ 9%; formic acid ≤ 0.03 g*L-1; data in g * $L^{-1}$.

| $C_2$ applied | 2.5 | 3.75 |
|---|---|---|
| $C_3$ | 1.2 | 1.7 |
| $C_4$ | 0.1 | 0.1 |
| $C_5$ | 0.1 | ≤ LOD |
| $C_6$ | ≤ LOD | ≤ LOD |
| 2-HMG | 0.1 | 0.1 |
| 2-HMT | ≤ LOD | ≤ LOD |
| $C_3/C_4$ | 15.5 | 15.8 |
| $C_1/C_3$ | 96.5 | 64.3 |

As shown in Table 14 the yield of $C_3$-carbohydrates obtained is directly proportional to the amount of glycolaldehyde applied. In order to obtain a significant amount of $C_3$-carbohydrates, 3.75 g*$L^{-1}$ glycolaldehyde was selected for performing the aldol-condensation setups. As the aldol-reaction velocity is inverse proportional to the concentration of formaldehyde applied (see section 2.4.2.4) the influence on formaldehyde concentration was investigated.

#### 3.2.2.2.2 Formaldehyde

As indicated in Table 14 and 15 decreasing formaldehyde concentration with respect to glycolaldehyde preferably yields higher condensation-products e.g. $C_4$- and $C_5$-carbohydrates.

Table 15: Dependency of the homogeneously catalyzed formation of $C_3$-carbohydrates on the concentration of formaldehyde; 30 min reaction time; formaldehyde conversion ≤ 12%; data in g* $L^{-1}$; formic acid ≤ LOD.

| formal-dehyde applied | 40 | 30 | 20 | 10 |
|---|---|---|---|---|
| methanol applied | 10.8 | 8.1 | 5.4 | 2.7 |
| $C_3$ | 1.8 | 1.8 | 1.8 | 1.5 |
| $C_4$ | 0.2 | 0.2 | 0.3 | 0.4 |
| $C_5$ | 0.1 | 0.1 | 0.1 | 0.2 |
| $C_6$ | ≤ LOD | ≤ LOD | ≤ LOD | ≤ LOD |
| 2-HMG | 0.1 | 0.1 | 0.1 | 0.1 |
| 2-HMT | ≤ LOD | ≤ LOD | ≤ LOD | 0.1 |
| $C_3/C_4$ | 14.6 | 10.5 | 8.7 | 4.6 |
| $C_1/C_3$ | 53.5 | 38.5 | 26.3 | 15.7 |

With regard to obtaining a significant amount of $C_3$-carbohydrates 40 g*$L^{-1}$ formaldehyde was selected based on the $C_1/C_3$- and $C_3/C_4$- ratios observed (grey column in Table 15).

### 3.2.2.3 Dependency on the type of base

Recycling of the byproducts accumulated during aldol-condensation of formaldehyde and glycolaldehyde also includes inorganic compounds. As hydrochloric acid is used as stopping solution for the calcium hydroxide catalyzed aldol-condensation, the calcium chloride formed has to be recycled. Converting calcium salts to their corresponding acids and bases is particularly difficult in electrodialysis using bipolar membranes due to the limited solubility of calcium hydroxide in aqueous solution [209]. Just the removal of calcium chloride can be realized by e.g. usage of a very cumbersome ion-exchange process. Thus, trials have been carried out in order to replace calcium with sodium. Based on the data reported in literature (see section 2.3.1.1) the following trials were carried out using the reaction conditions shown in Table 16.

Table 16: Aldol-condensation setup using sodium hydroxide catalysis, data in g * $L^{-1}$ unless otherwise stated.

| | | | | |
|---|---|---|---|---|
| Formaldehyde | 50 | 75 | 150 | 50 |
| Methanol | 1.4 | 2.0 | 4.0 | 1.4 |
| Sodium hydroxide | 50 | 50 | 100 | 200 |
| Glycolaldehyde [mg * $L^{-1}$] | 2.5 | 2.5 | 2.5 | 2.5 |
| Temperature [°C] | 80 | 80 | 80 | 90 |

However, as the results showed within a short reaction period (2 min) the majority of formaldehyde (approx. 80%) is being spent. Apart from detecting significant amounts of cannizzaro products e.g. methanol and formic acid negligible amounts of carbohydrates were quantified. Thus, sodium hydroxide has been excluded to be used for the aldol-condensation of formaldehyde and glycolaldehyde under the conditions specified in Table 16.

### 3.2.3 Summary

Based on the data obtained, the following reaction conditions can be summarized in order to obtain a significant amount of $C_3$-carbohydrates:

Table 17: Aldol-condensation setup for maximizing the yield of $C_3$-carbohydrates, data in g * $L^{-1}$ unless otherwise stated.

| Catalysis-system | Homogeneous |
|---|---|
| Formaldehyde | 40 |
| Methanol | 10.8 |
| Calcium hydroxide | 0.58 |
| Glycolaldehyde | 3.75 |
| Temperature [°C] | 25 |
| Reaction time [min] | 30 |

The reaction conditions summarized in Table 17 were applied for producing the starting material used in nanofiltration (NF) and reverse osmosis (RO) experiments as shown in the subsequent chapter.

## 3.3 Fractionation of reaction components derived from aldol-condensation by reverse osmosis and nanofiltration

The selection of an appropriate separation technology for the fractionation of the $C_3$-carbohydrates after aldol-condensation of formaldehyde and glycolaldehyde seems to be difficult since e.g. in distillation the vapor pressures of the individual substances are of the same order of magnitude [210]. Considering chromatography as a further separation technique the isolation of $C_3$-carbohydrates seems to be problematic as well since the significant amount of residual formaldehyde is disturbing during chromatographic separation (data not shown).

It is the aim of this chapter to investigate the separation of $C_3$-carbohydrates after an aldol-condensation of formaldehyde and glycolaldehyde using the membrane processes NF and RO. In such a separation process formaldehyde and glycolaldehyde are being recycled for a repeated aldol-condensation. In addition, pervaporation and membrane distillation might be applicable for fulfilling this separation task but is beyond the scope of this investigation. NF and RO have been selected due to known selectivity properties with regard to the molecular mass of $<C_3$- and $\geq C_3$-carbohydrates (the fractions obtained are termed as fraction $<C_3$-carbohydrates and fraction $\geq C_3$-carbohydrates in the text).

Only a few studies have investigated the retention of small uncharged or charged organic compounds by RO- and NF-membranes, which represents a crucial membrane feature especially in the light of water-purification [211, 212]. In the present case $C_3$-carbohydrates are accompanied by considerable amounts of formaldehyde, methanol, formic acid, carbohydrates in small amounts facilitating different carbohydrate chain length and divalent cations.

To the best of the authors' knowledge it appears that only one work deals with the membrane separation of formaldehyde from aqueous solution [213]. In ref. [213] the nature of formaldehyde oligomerization in aqueous methanolic solution is not taken into account, which seems to play an important role (see section 2.1.1.1). For the sake of completeness it should be mentioned that the removal of formaldehyde from air was studied using zeolite-, dense polymeric and hydrophobic hollow fiber membranes [214-216].

### 3.3.1 Materials and methods

#### 3.3.1.1 Membranes

The organic RO-membranes UTC70B (Toray, Switzerland) and Desalogics AK (GE Osmonics, USA) were applied in the membrane process experiments. In addition, one organic NF-membrane, namely SelRO MP34 (Koch, Germany), was tested. The detailed properties of all membranes are shown in Table 18.

Table 18: RO- and NF-membranes used in separation experiments.

| | RO-UTC70B | RO-Desalogics AK | NF-SelRO MP34 |
|---|---|---|---|
| Manufacturer | Toray | GE Osmonics | Koch |
| Retention NaCl [%] | 99.4 | 99.0 | 35 |
| Pure water permeability at 20°C [L * $h^{-1}$ * $m^{-2}$ * $MPa^{-1}$] | 7.2 | 17.62 | 12.0 |
| pH operating range | 2-11 | 4-11 | 0-14 |
| Maximum temperature [°C] | 40 | 50 | 70 |

#### 3.3.1.2 Membrane process setups

The membrane separation setup consisted of a HydraCell 231317 pump (HydraCell, Minneapolis, USA) and two DMS membrane separation test cells series 03 (Dauborn MembranSysteme, Ratzeburg, Germany). Each was equipped with a 23 mils spacer and total membrane area was 0.016 $m^2$. Figure 18 shows the experimental setup used for operation.

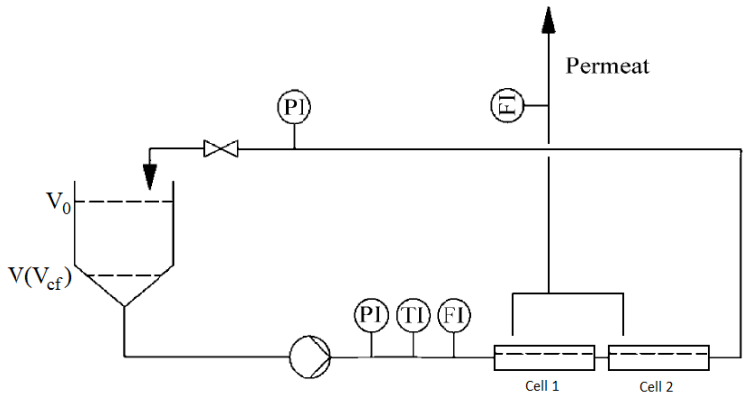



Figure 18: Experimental membrane process setup; PI: pressure indication, FI: flow indication, TI: temperature indication.

In case of the membranes AK and UTC the investigations were carried out at 3 MPa whereas in case of MP34 2.5 MPa was applied. Only during fractionating the aqueous methanolic formaldehyde model-solution (see section 3.3.2.2.1) using a MP34-membrane 2.0 MPa pressure was applied. By adjusting the valve displayed in Figure 18 the transmembrane pressure has been controlled. The pump was operated at a volumetric flow of 2.2 x $10^{-5}$ $m^3$ $s^{-1}$. The permeates of the two membrane cells were collected together. Two membrane cells were used in order to increase the ratio of membrane area with respect of the void volume of the system, which accounted for 150 mL in the present case. The feed temperature was continuously monitored and adjusted utilizing an ice bath as a cooling source.

The permeate flux $J_p$ was measured in volume per time and results were plotted as flux versus volumetric concentration factor ($V_{cf}$). $V_{cf}$ is defined as:

$$V_{cf} = \frac{V_0}{V_0 - V_p(t)} \qquad (9)$$

where $V_0$ is the initial volume of the solution and $V_p(t)$ is the volume of permeate at time $t$. Diafiltration ratio $D_f$ is defined as:

$$D_f = \frac{V_{DF}}{V_C} \qquad (10)$$

where $V_{DF}$ is the total volume of permeate obtained after a batchwise addition of solvent with the same volume as the concentrate $V_c$ for diafiltration. The permeate flux $J_p$ was measured in volume per time and results were plotted as flux versus diafiltration ratio ($D_f$). Samples were taken after reaching the desired $V_{cf}$ or $D_f$ from the concentrate and permeate accumulated. For the compounds investigated the observed retentions $R$ were calculated as:

$$R = \left(1 - \frac{C_p}{C_f}\right) * 100 \qquad (11)$$

where $C_p$ is the end concentration, which was established in the permeate collected until a final $V_{cf}$ of 4 was reached. Only in the case of UTC70B, a $V_{cf}$ of 6 was realized. $C_f$ is the initial concentration of the feed.

### 3.3.2 Results and discussion

#### 3.3.2.1 Flux behavior

Batch experiments with AK- and MP34-membranes fractionating the feed obtained after aldol-condensation of formaldehyde and glycolaldehyde showed that the mean permeate fluxes $J_p$ were comparable with many other applications. Figure 19 shows that in case of AK and MP34 the flux decreased relatively slowly. In addition to those two membranes a further membrane, namely UTC70B, was tested. In this case a $V_{cf}$ = 6 was realized.

After the initial membrane separation two, and in the case of UTC70B four diafiltration steps were carried out and the results are shown in Figure 19. The behavior of Koch MP34-NF membrane in diafiltration is contrary to the RO-membranes. The difference in concentration polarization between NF- and RO-membranes might be responsible for this mannerism. The two RO-membranes showed a typical flux behavior during diafiltration, which primarily is attributed to a reduction in concentration polarization. At this point it has to be remarked that in case of Toray UTC70B membrane a significant difference between the first and the second two diafiltration steps is evident.

Contrary to a conventional membrane separation step, in which a substance is retained to a high degree, the interesting behavior of Toray UTC70B membrane in diafiltration with respect to flux is explained by the significantly lower retention degrees of the compounds investigated. The concentrations of the substances considered are being continuously reduced during the diafiltration steps. Consequently, the number of diafiltration steps is limited.

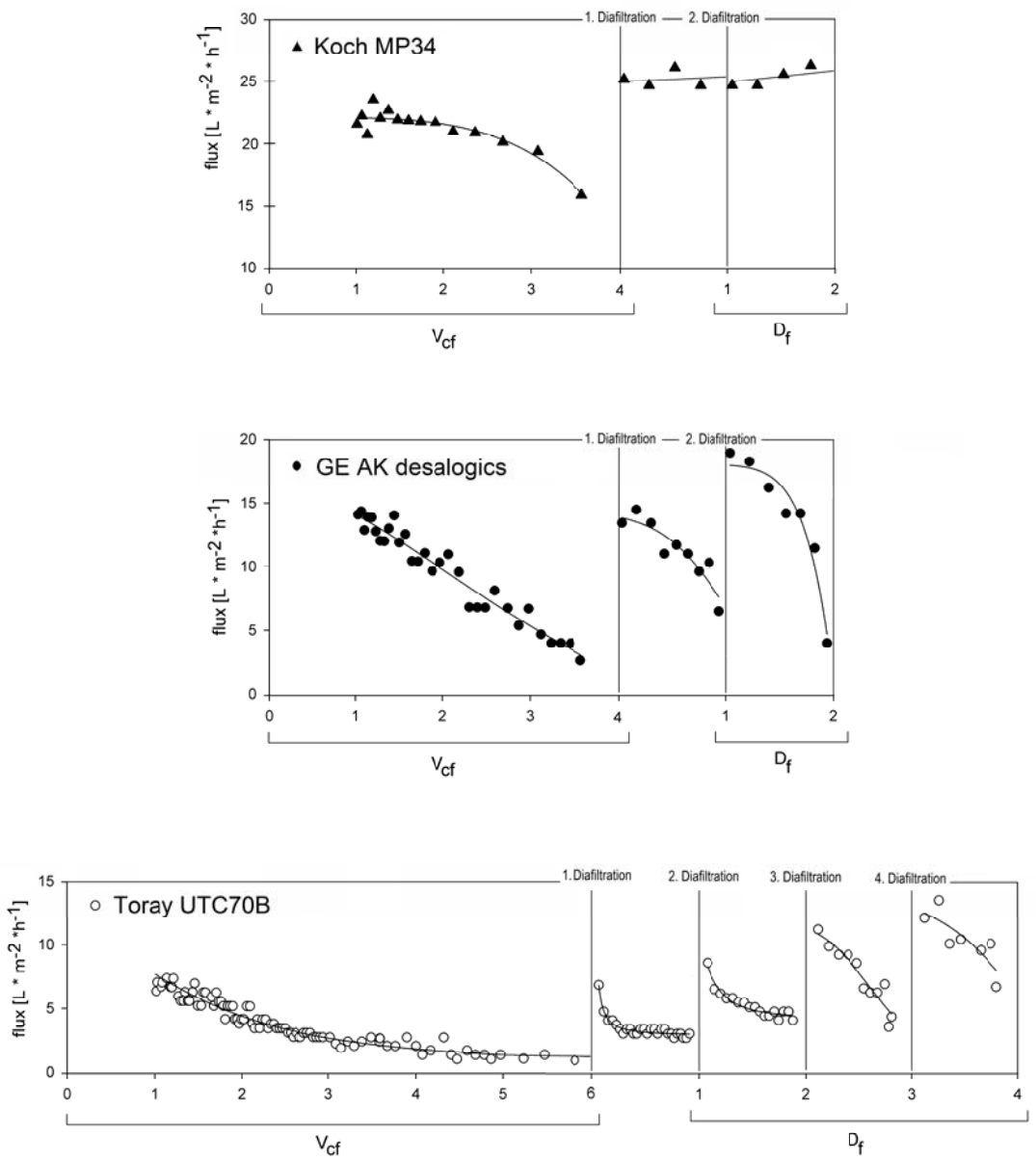



Figure 19: Flux behavior of selected RO- and NF-membranes; process parameters: *p* (AK- and UTC-membrane) = 3MPa, *p* (MP34-membrane) = 2.5 MPa, *T* = 20 °C, 2.2 x $10^{-5}$ $m^3$ $s^{-1}$ feed flow rate; each data point represents a flux measured at a particular $V_{cf}$; each membrane was tested once.

When reaching an integral $D_f$, the intended $V_{cf}$ in preliminary filtration is reached repeatedly. At the same $V_{cf}$, the flux after diafiltration is higher than at the end of the preliminary filtration. Comparing the diafiltration results shown in Figure 19 with each other the primarily influence of formaldehyde on the membrane flux is evident.

With regard to fouling in case of AK-membrane did water permeabilities before and after the membrane separation steps show a significant decrease, about 40%. The other membranes showed a decrease in water permeability of ≤15%.

### 3.3.2.2 Retention

After investigating the permeate fluxes, shown in Figure 19, the retention of the individual compounds should be considered and the results are summarized in Figure 20.

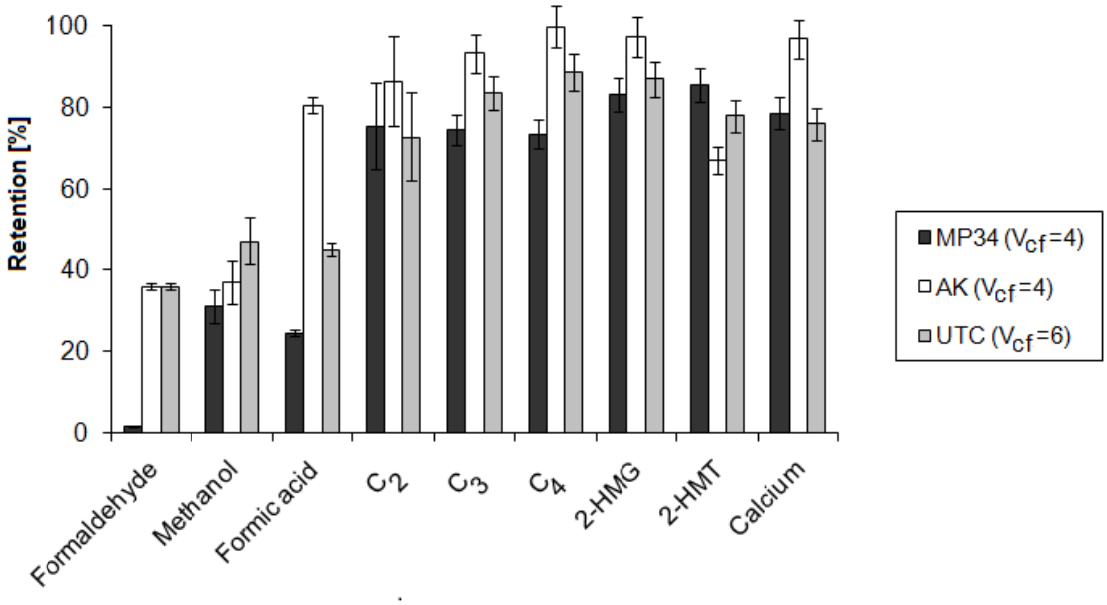



Figure 20: Retention of individual compounds obtained from aldol-condensation of formaldehyde and glycolaldehyde; 2-HMG: 2-hydroxymethylglycerol, a branched-chain alditol occurring in the formose reaction; 2-HMT: 2-hydroxymethyltetritol, another branched-chain alditol; carbohydrates of different chain length termed as $C_2$, $C_3$ and $C_4$; error bars indicate standard deviation arising from analysis (n=3).

Although formaldehyde is present mainly in oligomers in aqueous methanolic solution as shown in Eq. (1)-(4), only a relatively small retention of formaldehyde is observed. One explanation could be that formaldehyde monomers are continuously delivered by the chemical equilibrium, which may not be retained due to the very low molecular weight. During the separation experiments the pH-value was found to fulfill the following relation 4.63 ≤ $pH_{(membrane\ fractionation)}$ ≤ 6.44. As the pH observed during the experiments satisfies the following inequation $pK_{s\ (formic\ acid)}$ = 3.77 < $pH_{(membrane\ fractionation)}$ = 6.5 < $pK_{s\ (hydrated\ formaldehyde)}$ =

14 [121, 209], the majority of the hydrated formaldehyde monomer is present in its protonated form whereas the majority of the formic acid is present in its anionic form. In this regard, the difference in retention of formaldehyde and formic acid might be attributed to their difference in charge. Retention of formic acid significantly depends on the concentration and can be negatively affected by the presence of salts [217]. In case of the AK-membrane, approximately 80% retention of formic acid was observed, which can be explained by electrostatic interactions between the formate anion and the membrane surface.

With regard to methanol in case of MP34-membrane surprisingly the retention is rather high. This phenomenon could be explained by the interaction with formaldehyde (Eq. (3)-(4)) forming oligomers being partially retained. In this regard the concentrations of formaldehyde and methanol have to be noticed. In general, methanol is expected to influence the retention of solutes due to solute-solvent affinity causing solvation (of the solute) and interaction between the membrane and the solvent, which may lead to solvation of the pore wall (swelling) [218].

To a certain extent all carbohydrates are retained for the membranes investigated. Glycolaldehyde, as shown in Figure 20 as $C_2$, is surprisingly retained in the case of MP34, which might be explained in terms of dimerization [219]. As expected, divalent cations are significantly retained by all the membranes investigated. The AK-membrane shows the highest retention.

#### 3.3.2.2.1 Influence of methanol on the retention of formaldehyde in diafiltration

As shown in Eq. (3)-(4), formaldehyde strongly interacts with methanol. According to this consideration an increase of methanol concentration during separation might result in an increased retention of formaldehyde. In order to get a first insight into the dependence of methanol on formaldehyde retention, trials were carried out using an aqueous methanolic formaldehyde model-solution containing 1.4% (w/v) methanol and 5% (w/v) formaldehyde. The influence of methanol on formaldehyde retention was considered only during the diafiltration steps.

After a preliminary membrane separation of aqueous methanolic formaldehyde model solution until reaching a $V_{cf}$ of 4 (see Figure 22) two different diafiltration trials were realized using the following solvents: a) 1% (v/v) methanol and b) RO-water.

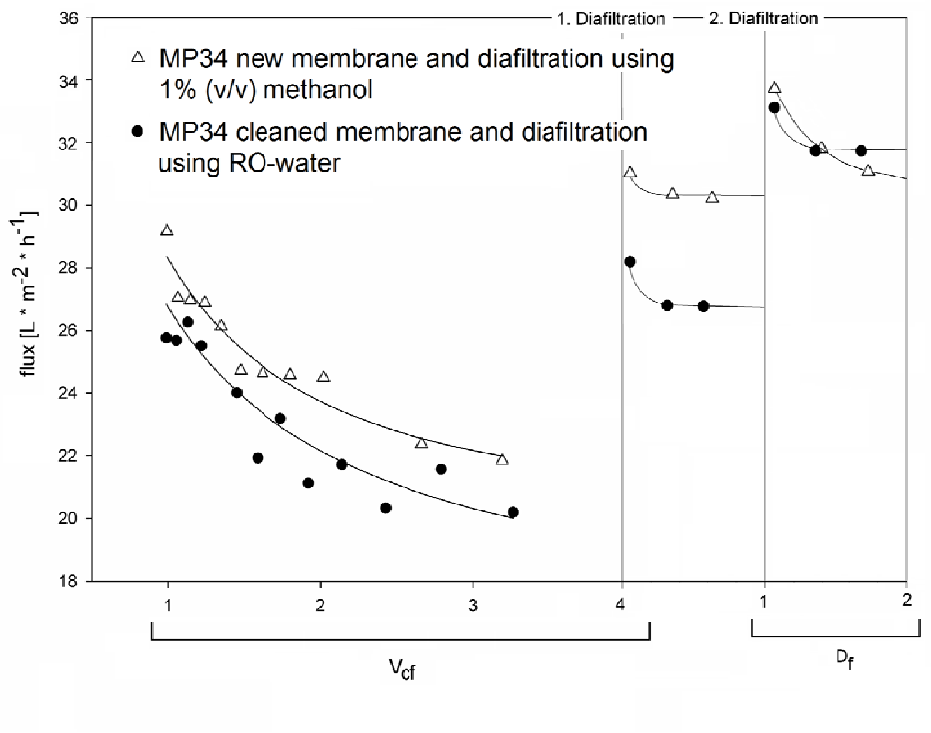



Figure 21: Nanofiltration and diafiltration of an aqueous methanolic formaldehyde model solution containing 1.4% (w/v) methanol and 5% (w/v) formaldehyde using MP34-membrane; process parameters: $p$ = 2.0 MPa, $T$ = 20 °C, 2.2 x $10^{-5}$ $m^3$ $s^{-1}$ feed flow rate; each data point represents a flux measured at a particular $V_{cf}$; each membrane was tested once.

Compared to the membrane separation of the feed obtained from aldol-condensation higher permeate fluxes were observed (compare Figure 19 and 21). Retention of formaldehyde accounted for 36.8% (new membrane in Figure 21) whereas after diafiltration and membrane cleaning using 1% (w/v) sodium hydroxide in a repeated filtration (cleaned membrane in Figure 21) 47.3% was observed. After cleaning the water flux was 90% compared to the new membrane.

Diafiltration using 1% (v/v) methanol or RO-water did not lead to significantly different fluxes (note the y-axis scaling). Using 1% (v/v) methanol 45.2% of formaldehyde can be removed in two diafiltration steps compared to 43.4% which can be realized when RO-water is used. Contrary to the hypothesis tested methanol does not seem to enhance the retention of formaldehyde in diafiltration.

### 3.3.2.3 Mass balance for the individual compounds separated

As Figure 22 shows formaldehyde, methanol and formic acid representing the fraction <$C_3$-carbohydrates can be removed using a MP34-membrane up to approximately 80% (w/w) from the feed obtained from aldol-condensation of formaldehyde and glycolaldehyde by applying two diafiltration steps. Depending on the membrane type, the fraction ≥$C_3$-carbohydrates suffers from the loss of approximately 40% of $C_3$-carbohydrates (w/w).

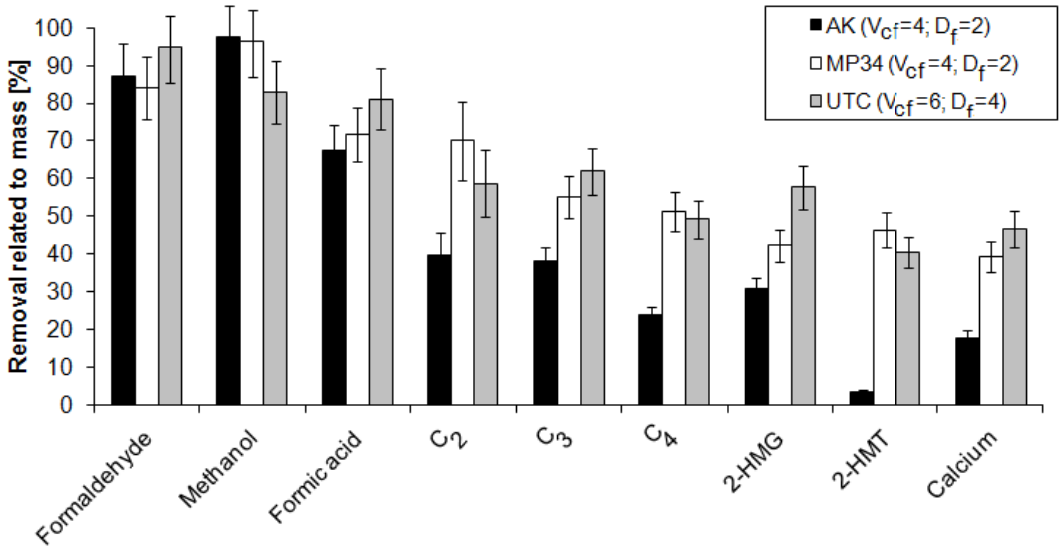



Figure 22: Removal of compounds in mass [%]; 2-HMG: 2-hydroxymethylglycerol; 2-HMT: 2-hydroxymethyltetritol; carbohydrates of different chain length termed as $C_2$, $C_3$ and $C_4$; error bars indicate standard deviation arising from analysis (n=3).

The results indicate that a second or a third separation step of the permeate may lead to satisfying results regarding the fractionation of $C_3$-carbohydrates. However, a broad spectrum of membranes has to be investigated in detail in order to determine which particular membrane is the most suitable for the intended application.

As the fractions obtained are subject to subsequent repeated aldol-condensation, the concentrations generated are of special interest. The following table should give an overview about the concentration ranges obtained after fractionation and subsequent diafiltration steps.

Table 19: Concentration of the individual compounds obtained after membrane separation and subsequent diafiltration experiments; 2-HMG: 2-hydroxymethylglycerol; 2-HMT: 2-hydroxymethyltetritol; data in [g*L$^{-1}$].

| | | GE Osmonics AK desalogics | | Koch SelRO MP34 | | Toray UTC70B | |
|---|---|---|---|---|---|---|---|
| | feed | concentrate after filtration | concentrate after diafiltration 2 | concentrate after filtration | concentrate after diafiltration 2 | concentrate after filtration | concentrate after diafiltration 4 |
| Formaldehyde | 32.6 | 35.7 | 16.9 | 40.6 | 20.7 | 54.9 | 10.2 |
| Methanol | 23.9 | 37.6 | 2.2 | 22.5 | 3.3 | 129.1 | 24.4 |
| Formic acid | 2.3 | 3.0 | 3.0 | 3.2 | 2.6 | 3.4 | 2.6 |
| | | | | | | | |
| $C_2$ | 1.6 | 5.0 | 3.1 | 3.1 | 1.9 | 2.2 | 4.0 |
| $C_3$ | 1.6 | 4.3 | 4.2 | 3.7 | 2.9 | 3.9 | 3.7 |
| $C_4$ | 0.2 | 0.7 | 0.6 | 0.5 | 0.4 | 0.7 | 0.6 |
| 2-HMG | 0.1 | 0.4 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 |
| 2-HMT | 0.1 | 0.3 | 0.3 | 0.2 | 0.2 | 0.3 | 0.2 |
| $Ca^{2+}$ | 0.2 | 0.0 | 0.8 | 0.6 | 0.6 | 2.6 | 0.8 |

As Table 19 shows membrane fractionation using NF or RO allows to separate $C_3$-carbohydrates from a mixture having a particularly high formaldehyde concentration. After appropriate pretreatment of the permeate (e.g. adjusting concentrations) subsequent aldol-condensation of formaldehyde and glycolaldehyde should be enabled.

### 3.3.3 Conclusion

$C_3$-carbohydrates are obtained from a controlled one step aldol-condensation of formaldehyde and glycolaldehyde. In a single membrane separation step $C_3$- and higher carbohydrates can be separated from the synthesis product as shown in this work. By utilizing two or three membrane separation steps in series the removal of formaldehyde, methanol and formic acid is possible to a high extend while concentrating a fraction ≥$C_3$-carbohydrates.

As the aldol-condensation is carried out using formaldehyde and glycolaldehyde both educts should be accumulated in the permeate in order to prepare for the subsequent aldol-condensation. RO- and interestingly also NF-membranes may fulfill this task. Using a RO-process operated until a $V_{cf}$ of 4 and two sequenced diafiltration steps approximately 80% of formaldehyde, methanol and formic acid were removed. Meanwhile, only 40% of $C_3$-carbohydrates were found in the permeate. 1% (v/v) methanol does not seem to enhance the retention of formaldehyde in diafiltration.

In order to isolate pure $C_3$-carbohydrates after aldol-condensation of formaldehyde and glycolaldehyde, chromatography necessarily has to be applied but is beyond the scope of this work. However, the significant concentration of formaldehyde in the feed would prevent chromatographic selectivity from being maintained. As this chapter shows this problem can be solved by applying membrane processes NF or RO.

Moreover it has to be pointed out, that a great number of different separation methods are necessary to realize the isolation of metabolizable carbohydrates based on the pathway investigated in this work. In particular, separation techniques enabling the recycling of reaction compounds in a closed system must be taken into account.

# 4 Summary and conclusions

In this work theoretical pathways for the conversion of $CO_2$ and $H_2O$ to metabolizable carbohydrates were considered and discussed. A selected one, namely the conversion of $CO_2$ to formaldehyde and conducting a controlled aldol-condensation was illuminated and some aspects were investigated. There is no doubt that many remaining research and problem areas still need to be investigated. Some of them should be mentioned in general: toxic substances, stereoselective catalysis, provision of glycolaldehyde, product degradation in alkaline media, energetic efficiency, etc. Nevertheless, the assessment of the entire system did not uncover principal problems within a synthesis pathway for the production of metabolizable carbohydrates form $CO_2$ and $H_2O$. Thus, this system could also be considered as an artificial photosynthesis process.

Based on the results and particularly of ref. [220] including the conversion of $CO_2$ to formaldehyde and the provision of glycolaldehyde the following process chain could be proposed:

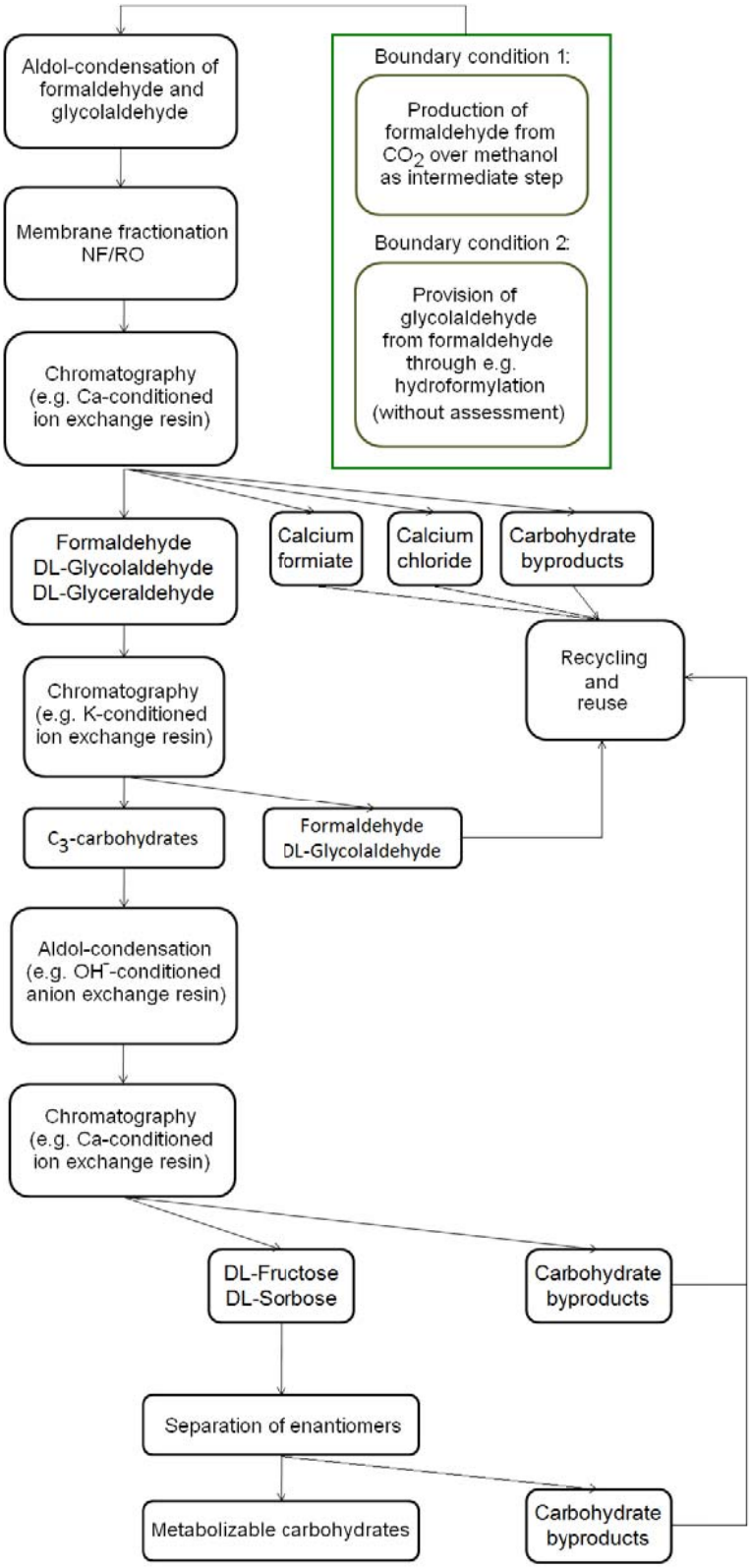



Figure 23: Process scheme, which could represent a possible artificial photosynthesis [220].

As the investigation of every process step is necessary in detail, many issues remain to be illuminated.

It should be pointed out, that $C_3$-carbohydrates were synthesized in laboratory scale and subsequently used in anion-exchange resin catalyzed aldol-condensation in order to produce metabolizable carbohydrates. However, surprisingly the product distribution entering the enantiomer separation step was shown to be very narrow as indicated in Figure 23 [220]. The product distribution was just determined by matching the retention time of authentic standards in LC-RI-detection [220]. The results are consistent with the data reported in literature where mainly DL-fructose and DL-sorbose were detected after such a reaction step using $C_3$-carbohydrate model solutions [79-81].

## 4.1 Bottle necks of the process proposed

The process shows serious limitations with regard to the following issues (this list doesn't claim to be complete):

a) The introduction of calcium-salts and the yield of only racemic glyceraldehyde represent major drawbacks of the first aldol-reaction. It remains to be shown whether an alternative pathway may lead to higher yields of $C_3$-carbohydrates starting from $CO_2$ and $H_2O$.
b) Only restricted diastereoselective control is enabled during conducting aldol-condensation of $C_3$-carbohydrates with each other using anion-exchange resins.
c) The simultaneous presence of carbohydrates as well as base causes e.g. the transformation of carbohydrates (anomerization, aldose-ketose isomerization (Lobry de Bruyn-Alberda van Ekenstein reaction), reversible aldol reaction, and β-elimination or benzilic acid rearrangement after the aldol reaction [161]). Thus, product degradation is difficult or perhaps impossible to be avoided.
d) With regard to recycling of cations in such a closed system, the introduction of electrodialysis using bipolar membranes seems to be essential.
e) Carbohydrate byproducts accumulated by the process must be subject to degradation e.g. by the application of stoichiometric oxidation processes using ozone.

In the light of the bottle necks listed in a) and b) it must be mentioned, that induced stereoselectivity in aldol-reactions is easily generated by appropriate combinations of reactants [221]. However, the introduction of stereoselective aldol-reactions should not increase the complexity of the entire system in the end.

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# 6 Appendices

## 6.1 Chemicals and reagents

Calcium oxide, calcium hydroxide, carbohydrate standards, abs. ethanol, benzoylchloride, pyridine, dimethylformamid, tris(hydroxymethyl)aminomethane (tris), acetic acid, sodium nitrite, Dowex 50WX2-400, 37% formaldehyde aqueous solution (containing approximately 10% methanol as stabilizer), methanesulfonic acid, formic acid used for the preparation of ionization buffer and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Sigma Aldrich, Vienna, Austria. D-glucose-$^{13}C_{6,}$ 99 atom% $^{13}C_6$ (termed $^{13}C$-glucose in the text) was supplied by Isotec, Miamisburg, OH, USA. Methanol, sulfuric acid and hydrochloric acid were obtained from Carl Roth, Graz, Austria. Sodium formate, formic acid used for the preparation of eluents, sodium borohydride, isopropanol, calcium nitrate standard for ion chromatography and sodium hydroxide were obtained from Merck, Vienna, Austria. Acetonitrile was purchased from YMC Europe, Dinslaken, Germany. High quality (HQ)-water was supplied by a SG-water Ultra Clear Basic UV water supply system (SG-water, Barsbüttel, Germany). All standards, chemicals and reagents meet the required purity for analysis and were used without further purification.

## 6.2 Formose reaction catalysis

### 6.2.1 Inorganic catalysts

Table 20: Inorganic catalysts applied in the formose reaction; water is used as solvent unless otherwise noted.

| Catalyst | ref. |
|---|---|
| adding oxalic acid or phosphoric acid at the begin of the carbohydrate formation period to initiate precipitation (or adding EDTA or nitrilotriacetic acid for chelating); subsequent using of KOH for adjusting pH and adding $Mg(OH)_2$, $Fe(OH)_3$, FeO or $Al_2O_3$, $Ba(OH)_2$, $Pb_2O(OH)_2$ | [222] |
| $Al(OH)_3$ | [156, 157] |
| $Al_2O_3$ | [106, 157] |
| aluminosilicate | [157] |
| $Ba(OH)_2$ | [79, 136, 145, 156-158, 223] |
| and KCl | [141] |
| and vitamine $B_1$ in dimethyl-formamide (DMF) | [145] |
| in DMF | [145] |
| $BaCl_2$ | |
| and KOH | [141] |
| and NaOH | [141] |
| $BaCO_3$ | [156, 158] |
| $Bi_2O_3$ | [157] |
| $Ca(OH)_2$ | a |
| and vitamine $B_1$ in DMF | [145] |
| in DMF | [145] |
| in methanol | [166] |
| in presence of borates | [107] |
| $CaCl_2$, KOH and | |
| $Ba(OH)_2$, benzoin, $Cu(OH)_2$, $Fe(OH)_3$, L-ascorbic acid, LiOH, $Mg(OH)_2$, $Pb_2O(OH)_2$, phenacyl alcohol and $Sr(OH)_2$ | [224] |
| $CaCO_3$ | [156-158] |
| in neutral aqueous media | [225] |
| CaO | [157] |
| in $CH_3OH$ | [143, 166] |

| Catalyst | ref. |
|---|---|
| $CaO\text{-}Al_2O_3$; $PbO\text{-}Al_2O_3$ | [226] |
| carbonate containing apatite | [157] |
| $Cd(OCOCH_3)_2$) | [156] |
| CdO | [156, 157] |
| $Ce(OH)_4$ | [164] |
| $CrO_3$ | [116] |
| $Dy(OH)_3$ | [164] |
| $Er(OH)_3$ | [164] |
| $Eu(OH)_3$ | [164] |
| $Fe(OH)_3$ | [116, 157] |
| Fe(OH)O | [227] |
| FeO | [116] |
| HgO | [157] |
| Hydroxides of<br>Dy, Gd, La, Tb, Tm, Yt | [116] |
| illit | [106] |
| $K_2CO_3$ | [157] |
| kaolinite | [106] |
| KOH | [79, 145, 156, 158, 228, 229] |
| KOH and | |
| $Ca(C_2H_5COO)_2$, $Ca(CH_3COO)_2$, $Ca(HCOO)_2$, $CaBr_2$ | [230] |
| KOH in DMF | [145] |
| KOH and vitamine $B_1$ in DMF | [145] |
| Lanthanide hydroxides of | |
| La, Tb, Ho, Tm, Gd, Dy, Er, Sm and Yb | [157] |
| LiOH | [79, 116, 145, 223] |
| in DMF | [145] |
| and vitamine $B_1$ in DMF | [145] |
| Manganese oxide | [157] |
| $Mg(OH)_2$ | [116] |
| in DMF | [145] |
| and vitamine $B_1$ in DMF | [145] |
| $MgCl_2$ | [231] |
| $MgCO_3$ | [116] |
| MgO | [116, 157, 231] |

| Catalyst | ref. |
|---|---|
| $MgSO_4$ | [116, 157] |
| MnO | [157] |
| $MoO_3$ | [116] |
| $Na_2CO_3$ | [157] |
| NaOH | [79, 145, 156-158, 171, 223, 232] |
| and Ca-acetate | [169] |
| and $CaCl_2$ | [207] |
| and $SrCl_2$ | [171] |
| and zeoliths (NaX, 5A or Na mordenite) | [151] |
| in DMF | [145] |
| and vitamine $B_1$ in DMF | [145] |
| $Pb(CH3COO)_2.Pb(OH)_2$ | |
| in DMF | [145] |
| and vitamine $B_1$ in DMF | [145] |
| $Pb(OH)_2$ | [156, 158] |
| $Pb_2O(OH)_2$ and thiamine.HCl in dimethylsulfoxide (DMSO) | [155] |
| $PbCO_3$ | [156] |
| PbO | [116, 156, 157, 233] |
| $PbO(OH)_2$ | |
| in DMF | [145] |
| and vitamine $B_1$ in DMF | [145] |
| phosphate-buffer | [79] |
| phosphates (homogeneous and heterogeneous) in neutral aqueous media | [225, 234] |
| removing of Ca ions with the addition of oxalic acid at the end of the induction period, adding of $Pb_2O(OH)_2$ and adjusting the pH to 10.0 with aqueous KOH | [144] |
| $Sb_2O_3$ | [157] |
| $Sm(OH)_3$ | [164] |
| $Sn(OH)_2$ | [156] |
| SnO | [157] |
| $Sr_2CO_3$ | [156] |
| $Sr(OH)_2$ | [79, 136, 145, 156] |
| $Sr(OH)_2$ in DMF | [145] |
| $Sr(OH)_2$, vitamine $B_1$ in DMF | [145] |

| Catalyst | ref. |
|---|---|
| $Th(OH)_2$ | [164] |
| $ThO_2$ | [116] |
| $TiO_2$ | [116] |
| TlOH | [156-158, 223] |
| in dioxane | [223] |
| in tetrahydrofuran | [223] |
| $V_2O_5$ | [116] |
| $WO_3$ | [116] |
| $Zn(OH)_2$ | [156] |
| $ZnCl_2$ | [231] |
| $ZnCO_3$ | [229] |
| ZnO | [116, 156, 157, 231, 235] |

[a]: no references listed since it is the standard catalyst.

### 6.2.2 Organic catalysts

Table 21: Organic catalysts applied in the formose reaction.

| Catalyst | ref. |
|---|---|
| 1,4-diazabicyclo[2.2.2]octane | [236] |
| 2-(dimethylamino)ethanol, vitamine $B_1$ in DMF | [145] |
| 2-methyl-piperidine | [158] |
| 3-quinuclidinol | [236] |
| aminoethanol | [236] |
| collidine | [156, 158] |
| dimethylamine, vitamine $B_1$ in DMF | [145] |
| dimethylaminoethanol<br>in water | [154, 156, 158, 236] |
| and thiamine HCl in DMF | [145, 148, 149] |
| and vitamine $B_1$, $Ca(OH)_2$ in DMF | [237] |
| ethylaminoethanol | [236] |
| glycine | [79] |
| imidazole | [79, 236] |

| Catalyst | ref. |
|---|---|
| morpholine, vitamine $B_1$ in DMF | [145] |
| N,N-dimethylaniline, vitamine $B_1$ in DMF | [145] |
| N-methyl-imidazole | [79] |
| N-methyl-morpholine<br>in water | [79, 156, 158] |
| and vitamine $B_1$ in DMF | [145] |
| N-methyl-piperidine | |
| in water | [156] |
| and vitamine $B_1$ in DMF | [145] |
| potassium lactylhydroxamate | [236] |
| | |
| *Pyridine-catalysts:* | |
| 2-(β-methylamino-ethyl)pyridine,<br>2,3-dimethylpyridine,<br>2,4,6-trimethylpyridine,<br>2,4-dimethylpyridine,<br>2,5-dimethylpyridine,<br>2,6-dimethylpyridine,<br>2-aminopyridine,<br>2-ethylpyridine,<br>2-methylpyridine,<br>3,4-dimethylpyridine,<br>3,5-dimethylpyridine,<br>3-methylpyridine,<br>4-(β-methylamino-ethyl)pyridine,<br>4-methylpyridine | [79] |
| pyridine | [79, 156, 158, 236] |
| technical pyridine base | [158] |
| | |
| pyrrolidine | [79] |
| quinuclidine | [236] |
| tetramethylammonium hydroxide | [158] |
| thiamine HCl, triethylamine in DMF or ethanol | [146] [147] |
| | |
| *Thiazolium-catalysts:* | |
| 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride and triethylamine | [238] |

| Catalyst | ref. |
|---|---|
| in DMF | |
| 3-benzylthiazolium chloride, triethylamine | |
| in dioxane; adding NaOH, $Na_2CO_3$, quinuclidine, anion-exchange resin in a cascade | [150] |
| 3-ethylbenzothiazolium bromide in dioxane or ethanol-solvent adding | |
| imidazole, pyridine, quinuclidine, sodium ethoxide, NaOH, tetraethylammonium hydroxide, triethylamine, trioctylamine | [147] |
| 3-ethylbenzothiazolium bromide and triethylamine in | |
| butanol, diglyme, DMSO, ethyl propionate, heptane, N,N-DMF, water | [147] |
| 3-ethylbenzothiazolium iodide, triethylamine in ethanol | [147] |
| 3-ethylthiazolium bromide, triethylamine in ethanol | [147] |
| 3-isopropylbenzothiazolium bromide, triethylamine in ethanol | [147] |
| 3-methyl-4-phenylthiazolium iodide, triethylamine in DMF | [146] |
| 3-methylbenzothiazolium iodide, triethylamine in | |
| DMF | [146] |
| ethanol | [147] |
| triethanolamine | |
| in water | [156, 158] |
| and vitamine $B_1$ in DMF | [145] |
| triethylamine | |
| in water | [156, 158] |
| and thiamine.HCl in DMF | [148, 149, 239] |
| triethylammonium hydroxide | [156] |
| trimethylamine, vitamine $B_1$ in DMF | [145] |
| $Zn(Pro)_2$ | [240] |
| α-picolin | [156, 158] |
| β.γ-picolin mixture | [156, 158] |
| β-picolin, vitamine $B_1$ in DMF | [145] |

### 6.2.3 Heterogeneous catalysts

Table 22: Heterogeneous catalysts for the formose reaction.

| Catalyst | ref. |
|---|---|
| acylaminocompound produced from amberlite XE 64 and p-aminobenzoylcarbinol-acetat in presence of CaO or PbO | [233] |
| Amberlite IRA-400 $OH^-$ resin | [80, 171] |
| anion exchange resin | [79] |
| Dowex-1 $OH^-$ resin | [80, 171] |
| polyethyleneimine (containing primary, secondary, and tertiary amino-groups (mol ratio: 1 : 2 : 1)) | [236] |
| thiazolium catalyst immobilized on polymer using triethylamine in DMF; effect of different counter ions on formose reaction-catalysis using thiazolium groups immobilized on resin and dimethylaminoethanol in 1,4-dioxane; effect of different solvents using thiazolium groups immobilized on resin and dimethylaminoethanol | [146, 241] |
| zeoliths coated with thiazolium catalyst using dimethylaminoethanol in different solvents; reused zeoliths | [242, 243] |

### 6.2.4 Physical influences applied in formose reaction-catalysis

Table 23: Physical influences applied in formose reaction-catalysis.

| Physical influence | ref. |
|---|---|
| photochemical catalysis (mercury lamp) in presence of $Na_2CO_3$ | [142] |
| photochemical catalysis (xenon lamp) in presence of NaOH | [152] |
| tesla currents in presence of CaO and $Ca(OH)_2$ | [244] |
| UV-irradiation in acidic media in the absence of catalyst and initiators | [245] |
| UV-irradiation in presence of potassium nitrate or nitrite | [246] |
| zeolith catalysis | [247] |
| γ-irradiation using Co60 γ-rays in presence of | |
| $Ca(OH)_2$, $CaCO_3$, KOH, $Mg(OH)_2$, $Na_2CO_3$, sodium phosphate tribasic | [248] |
| NaOH | [152, 248] |
| molecular sieves | [152] |

### 6.2.5 Initiators

Table 24: Combination initiator/catalyst applied in the formose reaction-catalysis.

| Catalyst | ref. |
|---|---|
| 2-deoxy-D-ribose and dimethylaminoethanol (DMAE) | [236] |
| acetonaphtoylcarbinol and $Ca(OH)_2$ | [249] |
| acetoin | |
| and $CaCl_2$, KOH | [224] |
| and DMAE | [236] |
| acetylacetone and DMAE | [236] |
| arabinose and DMAE | [236] |
| benzoylcarbinol | |
| and $BaCO_3$ | [156, 158] |
| and $Ca(OH)_2$ | [249] |
| and CaO | [233] |
| and DMAE | [158] |
| and PbO | [233] |
| dihydroxyacetone | |
| and $CaCl_2$, NaOH | [135] |
| and DMAE | [236] |
| dihydroxyacetone dimer, $CaCl_2$ and KOH | [224] |
| dioxyacetone and $Ca(OH)_2$ | [249] |
| dioxyacetone and TlOH | [223] |
| DL-glyceraldehyde, $CaCl_2$ and KOH | [224] |
| ethyleneglycol and DMAE | [236] |
| fructose | |
| and 1,4-diazabicyclo-[2.2.2]octane | [236] |
| and 3-quinuclidinol | [236] |
| and aminoethanol | [236] |
| and $Ca(OH)_2$ | [137, 138, 156, 249] |
| and $CaCl_2$, KOH | [224] |
| and $CaCl_2$, NaOH | [135] |
| and DMAE | [154, 236] |
| and ethylaminoethanol | [236] |
| and imidazole | [236] |

Table 24: Combination initiator/catalyst applied in the formose reaction-catalysis.

| Catalyst | ref. |
|---|---|
| and NaOH in $CH_3OH$ | [143] |
| and polyethyleneimine* | [236] |
| and potassium lactylhydroxamate | [236] |
| and pyridine | [236] |
| and quinuclidine | [236] |
| galactose | |
| and $CaCl_2$ and NaOH | [135] |
| and DMAE | [236] |
| glucose | |
| and $Ca(OH)_2$ | [127, 137, 249, 250] |
| and $Ca(OH)_2$, NaOH | [139, 140] |
| and $CaCl_2$, KOH | [224] |
| and $CaCl_2$, NaOH | [135] |
| and DMAE | [154] |
| and TlOH | [223] |
| and ZnO | [235] |
| glyceraldehyde | |
| and $Ca(OH)_2$ | [137, 156] |
| and $CaCl_2$ and NaOH | [135] |
| glycerol and DMAE | [236] |
| glycolaldehyde | |
| and $Ba(OH)_2$ | [136] |
| and $BaCO_3$ | [156] |
| and $Ca(OH)_2$ | [127, 136, 137, 249] |
| and $CaCl_2$, NaOH | [135] |
| and $Sr(OH)_2$ | [136] |
| lactose and DMAE | [236] |
| L-rhamnose and DMAE | [236] |
| lyxose, $CaCl_2$ and NaOH | [135] |
| maltose and DMAE | [236] |
| mannose and DMAE | [236] |
| methyl α-D-glucoside and DMAE | [236] |

Table 24: Combination initiator/catalyst applied in the formose reaction-catalysis.

| Catalyst | ref. |
|---|---|
| monooxyaceton and $Ca(OH)_2$ | [249] |
| naphtoylcarbinol and $Ca(OH)_2$ | [249] |
| p-acetaminobenzoylcarbinol and $Ca(OH)_2$ | [249] |
| p-acetamino-benzoylcarbinol and CaO or PbO | [233] |
| phenylacyl | |
| -polyaminostryrol,<br>-wolfatit N, -wolfatit MD,<br>-acetamid, -anilin,<br>-benzamid, -morpholin, -p-phenetidin in presence of CaO or PbO | [233] |
| p-methoxy-benzoylcarbinol and $Ca(OH)_2$ | [249] |
| ribose and | |
| $Ca(OH)_2$ | [137] |
| $CaCl_2$, NaOH | [135] |
| DMAE | [154, 236] |
| sorbose | |
| and $Ca(OH)_2$ | [137] |
| and DMAE | [236] |
| $CaCl_2$ and NaOH | [135] |
| sucrose and DMAE | [236] |
| triose reductone, $CaCl_2$ and KOH | [224] |
| xylose | |
| and $CaCl_2$, KOH | [224] |
| and DMAE | [154, 236] |
| α-cyclodextrin and DMAE | [236] |

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**Personal data**

| | |
|---|---|
| Name | Thomas Zweckmair |
| Date of Birth | 10th January 1982 |
| Place of Birth | Steyr |
| Country | Austria |
| Nationality | Austria |
| Marital status | single |
| University | University of Natural Resources and Life Sciences (BOKU Vienna) |

**Education**

1997-2002

Agricultural college St. Florian/Linz

**Professional experience**

2003-2006

Bachelor in Food Science and Biotechnology

2006-2008

Master Biotechnology

2008-2011

Doctorate

Vienna May 2011

Dissertations and dissertation print of the University of Natural Resources and Applied Life Sciences Vienna

Module 1: Dissertations

Imprint of this document:
© 2004 Guthmann-Peterson Publisher
Elßlergasse 17, A-1130 Vienna
Phone. +43 (0)1 877 04 26, Fax: +43 (0)1 876 40 04
Dr.-Simoneit-Straße 36, D-45473 Mülheim a. d. Ruhr
E-Mail: verlag@guthmann-peterson.de
http://www.guthmann-peterson.de