



(51) International Patent Classification:

G01N 33/52 (2006.01) G01N 33/14 (2006.01)
C08F 38/02 (2006.01)

(21) International Application Number:

PCT/EP2021/082965

(22) International Filing Date:

25 November 2021 (25.11.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PA 2020 70787 26 November 2020 (26.11.2020) DK

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))
— in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE

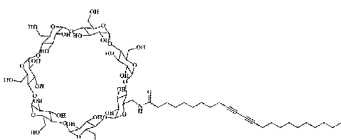
(54) Title: POLY-DIACETYLENE SENSORS AND METHODS FOR CHARACTERIZING SAMPLES

A)

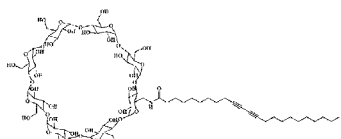


Fig. 1

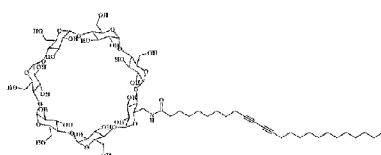
B)



C)



D)



(57) Abstract: A method for characterizing complex samples comprising at least one analyte is provided. The method is based on poly-diacetylene sensors having cyclodextrin moieties and arrays comprising the same. The method is considered particularly relevant for characterization of aqueous samples, such as liquid food products, and with preferred application in characterizing samples comprising esters or other flavouring components usually found in beverages, such as beer. Provision of the poly-diacetylene sensors having cyclodextrin moieties and provision of the arrays comprising the same, is also disclosed.



Poly-diacetylene sensors and methods for characterizing samples

Technical field of the invention

The present invention relates to colorimetric poly-diacetylene (PDA) sensors and arrays thereof for detection of analytes in aqueous solutions. In particular the present

5 invention relates to the use of poly-diacetylene arrays comprising cyclodextrin for detection of analytes such as particularly esters and phenolic flavour compounds and levels thereof, present in for example beverages such as beer and beer precursors, using said sensors.

10 Background of the invention

Methods for fast and reliable characterisation of samples comprising ester and phenolic compounds are of interest within several industries, including the food industry, beverage industry, pharmaceutical industry, environmental industry and others.

Particularly the characterisation of complex mixtures such as for example food/feed
15 samples, biological samples, and environmental samples is of interest. Food samples may particularly include foodstuff, but also dairy products or alcoholic and non-alcoholic beverages where the content of ester or phenolic flavour compounds is of interest for product development, quality and safety. Esters and phenolic flavour compounds are for example important flavour components in beer.

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In relation to products for human consumption, today's dominant approaches remain rather complex and labour intense focusing on gas chromatography and/or sensory panels. Electronic tongue-sensors employing artificial membranes and electrochemical techniques, are an emerging concept but many technical, material and computational
25 challenges need to be tackled before they can become widely applicable. Alternative approaches which would allow for fast on-site screening are therefore in high demand. Especially interesting in this context are colorimetric sensors for instance based on poly-diacetylenes (PDA). Diacetylene (DA) monomers can be polymerized into PDA, typically a blue coloured polymer, within a few minutes without the need of a catalyst
30 or an initiator. In response to various external stimuli including temperature, solvents exposure, or ligand-receptor interactions PDA undergoes a blue-to-red (and non-fluorescent-to-fluorescent) configuration shift which is easy to detect. PDA sensors in the form of vesicles, embedded into electrospun fibres, connected to carbon nanotubes, inorganic porous materials, or paper among others were reported.

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WO 2019/137589 discloses colorimetric PDA sensor arrays for characterizing aqueous solutions, such as the content of esters in beer. The poly-diacetylenes are polymerized from various substituted diacetylene monomers. However, none of the disclosed monomers, or poly-diacetylenes thereof, comprise any cyclodextrin moieties.

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Also, *Qian, X., et al., ACS Applied Nano Materials, 3, 2020, pp. 3439-3448* discloses poly-diacetylenes polymerized from a set of five different linear diacetylene monomers. The PDAs are used to setup sensor arrays for characterizing complex aqueous solutions and with the particular aim of measuring amounts of specific flavoring compounds

10 usually found in beer.

Cho, E., et al. Sci Rep, 6, 31115, 2016 discloses poly-diacetylene vesicles comprising beta-cyclodextrin for use in measuring amounts of amino acids, lysine and arginine. The disclosure does not consider complex samples comprising multiple analytes and is

15 silent in respect of esters and beer compositions.

Thus, development of new methods for quick on-site characterization of complex samples comprising esters is requested by society for use in safe production of high quality products. This is considered particularly relevant for the manufacturing of

20 beverages and similar products wherein esters are important flavouring constituents.

Hence, an improved method for characterizing and/or quantifying samples comprising esters and other analytes would be advantageous, and in particular a more efficient and/or reliable method for characterizing beverages, such as beer would be

25 advantageous.

Summary of the invention

An object of the present invention relates to a method for characterizing samples comprising esters and/or phenolic flavour compounds and/or other analytes. Arrays of

30 poly-diacetylenes are provided wherein each PDA is especially suitable for detecting specific analytes within a sample. An array of the invention contains at least one PDA comprising cyclodextrin that is particularly effective at detecting esters and/or phenolic flavour compounds .

35 In particular, it is an object of the present invention to provide a method that solves the above mentioned problem of fast on-site characterization of complex samples

comprising esters and/or phenolic flavour compounds. The method is considered particularly relevant for use in manufacturing of food products wherein esters and/or phenolic flavour compounds are key components. Fast and reliable characterization of samples obtained after and during production of such products is considered paramount for ensuring a safe and reliable production of high quality products. Particularly, a PDA sensor array capable of providing an improved fingerprint type identification of a beverage or beverage precursor and capable of rapidly distinguishing between e.g. two distinctive beverage batches or brands would be advantageous.

- 10 Hence, a first aspect of the present invention relates to a method for characterizing a sample for at least one analyte comprising the steps of
- a) providing a sensor array comprising at least two different poly-diacetylenes,
 - b) contacting said sensor array with said sample,
 - c) measuring a colorimetric response of said poly-diacetylenes after contact with
- 15 said sample,
- wherein said poly-diacetylenes are polymerised from a composition comprising one or more diacetylene monomers, said diacetylene monomers comprising one or more substituents selected from the group consisting of an optionally substituted C₁-C₃₀ alkyl, an optionally substituted C₂-C₃₀ alkenyl, and an optionally substituted C₂-C₃₀ alkynyl,
- 20 wherein at least one poly-diacetylene is polymerised from a composition comprising a diacetylene monomer comprising a cyclodextrin derivative, and
- wherein at least one analyte is an ester or a phenolic flavour compound.

The diacetylene monomers comprising cyclodextrin derivatives are of particular interest as they are capable of improving the characterising of samples comprising esters or a phenolic flavour compound.

Therefore, yet another aspect of the present invention is to provide a diacetylene monomer comprising one or more substituents selected from the group consisting of an optionally substituted C₁-C₃₀ alkyl, an optionally substituted C₂-C₃₀ alkenyl, and an optionally substituted C₂-C₃₀ alkynyl,

30 wherein said diacetylene monomer comprises an *alpha*-cyclodextrin derivative or *gamma*-cyclodextrin derivative.

35 In summary, the present inventors have surprisingly found that introduction of cyclodextrin derivatives in poly-diacetylene sensors, and arrays thereof, allows for

improved characterisation and differentiation of complex mixtures. In particular it has been found that for mixtures comprising esters or a phenolic flavour compound, for example certain beverages, said cyclodextrin moieties provide for increased sensitivity and specificity, whereby improved characterisation may be achieved.

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Brief description of the figures

Figure 1 shows, chemical structures for a selection of diacetylene monomers. Insert A) depicts the structure of a monomer comprising alpha-cyclodextrin and a tricosadiyne moiety, insert B) depicts the structure of a monomer comprising beta-cyclodextrin and a tricosadiyne moiety, insert C) depicts the structure of a monomer comprising gamma-cyclodextrin and a tricosadiyne moiety, insert D) depicts the structure of a monomer comprising beta-cyclodextrin and a pentacosadiyne moiety.

Figure 2 shows, a schematic illustration of the process in which the diacetylene monomers are reacted to form vesicles of poly-diacetylene. The actual polymerization happens in the last step wherein the aqueous composition comprising the unpolymerized vesicles is exposed to UV-irradiation.

Figure 3 to 5 show, colorimetric responses (CR) measured of PDA sensors comprising alpha-, beta- and gamma-cyclodextrin and a sample comprising an ester from the group of ethyl hexanoate (EH), isoamyl acetate (IA), 2-phenylethyl acetate (PA), ethyl octanoate (EO), and ethyl acetate (EA). The experiments were repeated using four different concentrations of the esters in the sample (i.e. 0.01, 0.1, 0.5, and 1.0 mM). Each individual experiment was repeated at least five time in order to provide the error bars.

Figure 3 shows, the CR measured when the PDA comprises alpha-cyclodextrin,
Figure 4 shows, the CR measured when the PDA comprises beta-cyclodextrin,
Figure 5 shows, the CR measured when the PDA comprises gamma-cyclodextrin.

Figure 6 shows, colorimetric responses measured on samples containing the phenolic flavour compound 4-vinyl guaiacol with three of the PDA sensors comprising cyclodextrins, i.e. T+T-alpha-CD, T+T-beta-CD, and T+T-gamma-CD. The experiments were repeated using four different concentrations of 4-vinyl guaiacol.

Figure 7 shows, colorimetric responses measured on samples containing ethanol with three of the PDA sensors comprising cyclodextrins, i.e. T+T-alpha-CD, T+T-beta-CD,

and T+T-gamma-CD. The experiments were repeated at three different concentration levels of ethanol, i.e. 686 mM (4 vol.%), 1370 mM (8 vol.%), and 2055 mM (12 vol.%).

Figure 8 shows, colorimetric responses measured on samples containing the ester ethyl acetate with three of the PDA sensors comprising cyclodextrins (alpha, beta or gamma) and a number of other PDA sensors not comprising cyclodextrin. The experiments were repeated using four different concentrations of ethyl acetate.

The present invention will now be described in more detail in the following.

Detailed description of the invention

Definitions

Prior to discussing the present invention in further details, the following terms and conventions will first be defined:

Sample

A sample is any solid or liquid sample to be characterised according to the present invention. Herein, the sample comprising at least one analyte may also be called a "complex sample" or just "sample". An analyte as described in the examples is also a sample containing the defined analyte(s). Biological samples and samples in the food and beverage industry are commonly aqueous solutions.

Aqueous solution

In the present context an aqueous solution in the broadest sense is any liquid comprising water in any amount. It includes homogenous solutions or mixtures and inhomogeneous mixtures such as dispersions or emulsions of e.g. fats in water (for example dairy milk). Particularly, the aqueous solutions of the present invention may comprise complex mixtures of many analytes and additional components in water. Aqueous solution may be used interchangeably with aqueous compositions.

Analyte

In the present context an analyte in the broadest sense is any compound or entity capable of interacting with the sensor array of the invention. Analytes may be dissolved, dispersed, or part of an emulsion.

Sensor

In the present context, the term "sensor" refers to a poly-diacetylene as described herein presented on some form of support. It may be positioned on solid support or in a liquid support, including vesicles and micelles. The vesicles or micelles may be formed

5 by the PDAs themselves.

Sensor array

In the present context a sensor array is a collection of individual sensors, wherein each sensor is separated from the other. The collection of sensors may be designed for a

10 specific purpose, such as for characterization of a specific target sample.

Cyclodextrin and derivatives thereof

In the present context, a "cyclodextrin" is an organic compound belonging to the family of cyclic oligosaccharides. The compounds are composed of a number of glucopyranose

15 units joined by alpha-1,4 glycosidic bonds. The number of units may be 5 to 32, preferably a number between 6 to 9, such as 6, 7 or 8 glucopyranose units.

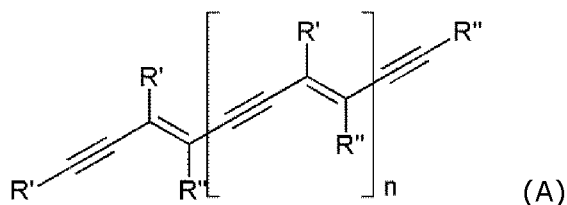
Diacetylene monomer

In the present context "diacetylene monomer" refers to a monomer which may be used

20 in a polymerisation process to produce poly-diacetylenes. A diacetylene group consisting of two acetylene groups separated by a single bond ($R'-\equiv\equiv-R''$) is comprised in such monomers. The monomers may include several diacetylene groups.

Poly-diacetylene

25 In the present context a poly-diacetylene is a polymer obtained from polymerisation of diacetylene monomers. They may be represented by the general formula (A) below, when a single diacetylene monomer with only one diacetylene moiety ($R'-\equiv\equiv-R''$) is used during polymerisation.



30 Such polymerisation results in a linear polymer with R' and R'' groups distributed evenly along the polymer chain. When a mixture of two or more different monomers are used,

the R groups may vary along the polymer chain randomly. Also, if the monomer comprises more than one diacetylene group (e.g. if R' and/or R'' comprises a further diacetylene), cross coupling will occur and non-linear polymers or polymer matrices may be obtained.

5

Characterizing

In the present context characterizing has its usual meaning and involves obtaining a set of data that enables the characterisation of a composition comprising one or more analytes. Characterising may be used interchangeably with identifying. Preferably the characterisation is able to provide a data set which is unique for the specific composition of analytes in the sense, that any changes to analyte amounts or presence of further measurable analytes will provide a measurably different results. In other words the characterisation is ideally able to distinguish between samples having different analyte content and/or different levels of analyte comprised.

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Optionally substituted

In the present context "optionally substituted" means that a chemical moiety or group may or may not be substituted with one or a plurality of compatible substituents known in the field of organic synthesis. In the present context "substituted" means that a chemical moiety or group has one or more substituents (further chemical moiety or group) attached in addition to those implied by the name of the moiety or group.

Alkylene, alkenylene, alkynylene

In the present context alkylene, alkenylene, alkynylene have their usual meaning, i.e. they represent hydrocarbon chains, where alkylenes comprise single bonds only, where alkenylene chains comprise at least one carbon-carbon double bond, and alkynylene comprises at least one carbon-carbon triple bond. The hydrocarbon chains may be straight or branched. The chains are open-ended, i.e. as represented by for example - $(CH_2)_n$, n being an integer.

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Flavour constituent

In the present context a flavour constituent is any molecule or salt capable of contributing to the flavour of e.g. a beverage, i.e. capable of interacting with the human or animal flavour detection system. Particular beverages such as beer, ciders and wine have particular flavour constituents known to the skilled individual. Flavour

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constituents may particularly comprise organic compounds, salts thereof and inorganic salts.

Beverage and precursors thereof

5 In the present context a beverage is an aqueous composition for human consumption comprising analytes, which are typically flavour constituents of the beverage.

Precursors of beverages are aqueous intermediate products at any stage in the production line, prior to arriving at the final product (the beverage). Examples of beverage precursors in beer production is wort, fermentation broth and green beer.

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Amino acid

In the present context amino acids in the broadest sense are any natural or synthetic amino acid that may be present in the analysed sample.

15 *Colorimetric response*

In the present context a colorimetric response is a measurable colour change in one or more of the poly-diacetylenes present on the sensor array induced by one or more analytes in the analysed complex solutions. The colour change may be compared to a reference array (optionally subjected to a reference solution), or compared to the same
20 array prior to subjection to a sample solution. The colour change may be in the visible spectrum, but may also extend into the infrared and ultraviolet spectra. A colorimetric response may also be a colour difference between two or more corresponding poly-diacetylenes on each the array, which have been subjected to different sample solutions.

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Method of characterising samples comprising esters or a phenolic flavour compound

The present invention relates to the characterizing samples comprising at least one ester or a phenolic flavour compound. In this regard, the present inventors have surprisingly found that PDA sensors synthesized by polymerization of different
30 diacetylene monomers and including at least one monomer comprising a cyclodextrin moiety are especially sensitive to esters and phenolic flavour compounds. In addition, said PDA sensors comprising cyclodextrin were also found to be somewhat selective towards compounds of different sizes depending on the type and size of cyclodextrin employed.

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Thus, a first aspect of the present invention relates to a method for characterizing a sample for at least one analyte comprising the steps of

- a) providing a sensor array comprising at least two different poly-diacetylenes,
- b) contacting said sensor array with said sample,

5 c) measuring a colorimetric response of said poly-diacetylenes after contact with said sample,

wherein said poly-diacetylenes are polymerised from a composition comprising one or more diacetylene monomers, said diacetylene monomers comprising one or more substituents selected from the group consisting of an optionally substituted C₁-C₃₀ alkyl, 10 an optionally substituted C₂-C₃₀ alkenyl, and an optionally substituted C₂-C₃₀ alkynyl, wherein at least one poly-diacetylene is polymerised from a composition comprising a diacetylene monomer comprising a cyclodextrin derivative, and wherein at least one analyte is an ester or a phenolic flavour compound.

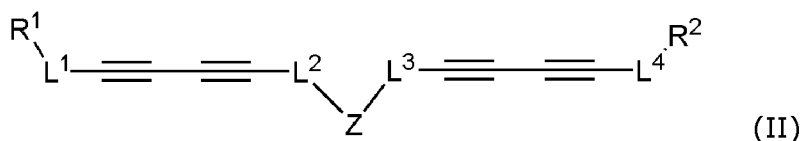
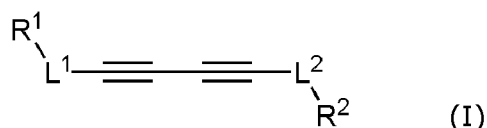
15 The PDA sensor array of the present invention thus comprises at least one PDA polymerized from a DA monomer or a mixture of DA monomers of which at least one monomer comprises a cyclodextrin derivative. The most common types of cyclodextrins are alpha-, beta-, and gamma-cyclodextrin. Hence, the cyclodextrin derivative is preferably a derivative selected from the group consisting of an alpha-cyclodextrin 20 derivative, a beta-cyclodextrin derivative, and a gamma-cyclodextrin derivative. In one embodiment the analyte is an ester.

The diacetylene monomers

The diacetylene monomers used to prepare the poly-diacetylenes are compounds 25 comprising a diacetylene moiety and they are therefore compounds comprising at least two neighbouring triple bonds in their chemical structures. The optional substituent on the diacetylene monomer may preferably be optionally substituted C₂-C₂₀ alkyl, an optionally substituted C₃-C₂₀ alkenyl, and an optionally substituted C₃-C₂₀ alkynyl, such as optionally substituted C₃-C₁₆ alkyl, an optionally substituted C₄-C₁₆ alkenyl, and an 30 optionally substituted C₄-C₁₆ alkynyl.

Hence, one preferred embodiment of the present invention relates to the method, wherein said one or more diacetylene monomers are selected from the group of diacetylenes according to formula (I) or (II)

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or mixtures thereof, wherein

L¹, L², L³ and L⁴ are the same or different and individually selected from the group consisting of an optionally substituted C₁-C₃₀ alkylene, an optionally substituted C₂-C₃₀ alkenylene, and an optionally substituted C₂-C₃₀ alkynylene, R¹ and R² are the same or different and individually selected from the group consisting of -CH₃, -OR³, -SR³, -COOR³, -CONR⁴R⁵, wherein

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R³, R⁴, and R⁵ are individually selected from the group consisting of hydrogen, C₁-C₈ alkyl optionally substituted with a thiol, vinyl, or optionally substituted imidazolium, a polyethylene glycol alkyl ether optionally substituted with a thiol, vinyl, amino acid, or optionally substituted imidazolium, and a cyclodextrin derivative; or are selected so that NR⁴R⁵ constitutes an amino acid,

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Z is selected from the group consisting of optionally substituted alkylene, aryl, -CONH-(CH₂)_x-HNCO- where X is an integer between 1 and 20, and heteroaryl,

with the proviso that at least one poly-diacetylene comprises a diacetylene monomer wherein at least one of R³, R⁴, and R⁵ is a cyclodextrin derivative.

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The diacetylene moiety is an important feature of the monomers because the two neighbouring triple bonds are considered to be key for the polymerization of mixtures of DA monomers to give PDAs. The chromatographic properties and thus, the colorimetric response of a PDA depends on the actual mixture of monomers used. Thus, a PDA sensor prepared from a particular monomer or mixture of monomers may obtain a specific sensitivity to an analyte or group of analytes.

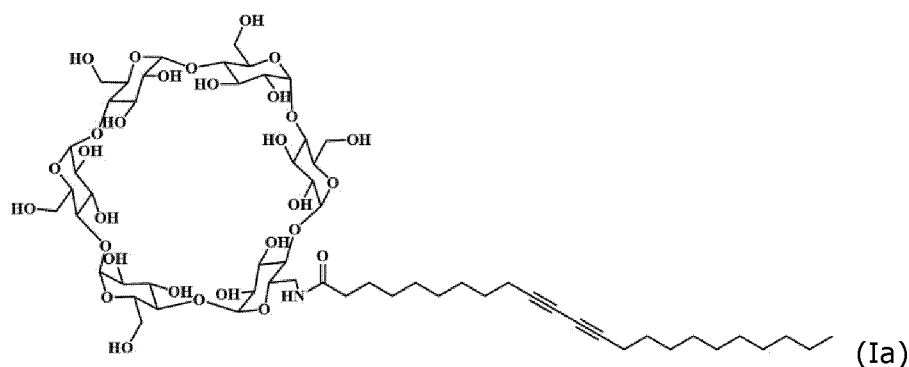
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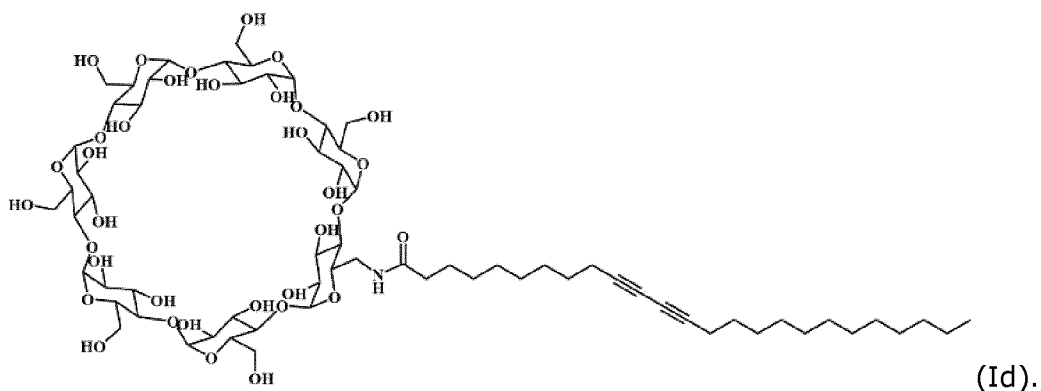
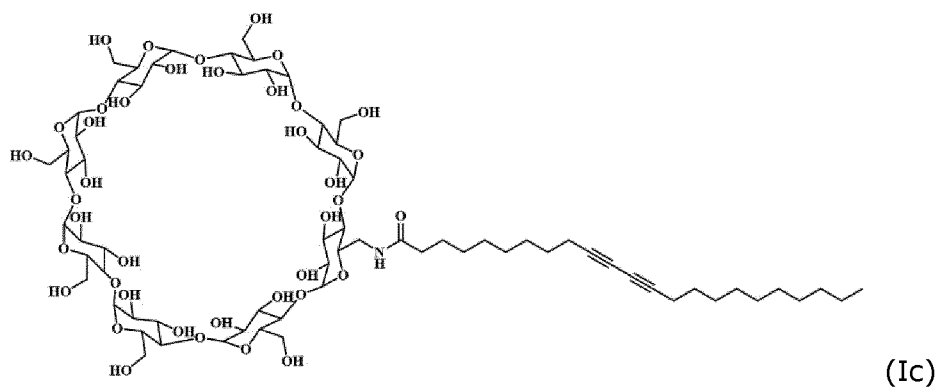
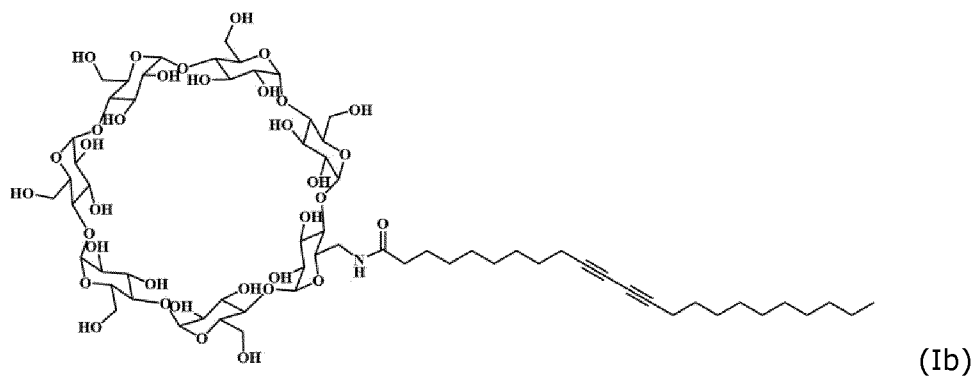
The diacetylene moieties of the DA monomers are attached to a set of side-groups (L and R above) that are important for e.g. solubility properties, colorimetric response, and shape of the synthesized PDA sensors. For instance, in an embodiment wherein one of the side-groups consists of an aliphatic carbon chain and the other one comprises one or more hydrophilic groups, the obtained PDA sensor is typically in the form of a

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vesicle or micelle when prepared in aqueous solution. Thus, a preferred embodiment of the present invention relates to the aliphatic parts of the DA monomers, wherein L^1 , L^2 , L^3 and L^4 are the same or different and individually selected from a $-(CH_2)_n-$ group wherein n is 1-30, such as 1-20, 1-18, 1-15, such as preferably 1-12. The hydrophilic groups are usually installed in the end part of a side-group and a further embodiment of the present invention therefore relates to the method, wherein R^1 and R^2 are the same or different and individually selected from the group consisting of $-CH_3$, $-COOR^3$, and $-CONR^4R^5$.

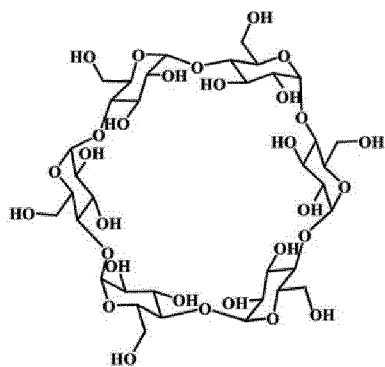
- Another preferred embodiment of the present invention relates to the method, wherein L^1 , L^2 , L^3 and L^4 are the same or different and individually selected from a $-(CH_2)_n-$ group wherein n is 1-20, R^1 and R^2 are the same or different and individually selected from the group consisting of $-CH_3$, $-COOR^3$, $-CONR^4R^5$, wherein R^3 , R^4 , and R^5 are individually selected from the group consisting of hydrogen, and C_1 - C_8 alkyl optionally substituted with a thiol, vinyl, or optionally substituted imidazolium, a polyethylene glycol alkyl ether optionally substituted with a thiol, vinyl, amino acid, or optionally substituted imidazolium, and a cyclodextrin derivative; or are selected so that NR^4R^5 constitutes an amino acid, and Z is selected from the group consisting of optionally substituted alkylene, aryl, $-CONH-(CH_2)_x-HNCO-$ where X is an integer between 1 and 20, and heteroaryl with the proviso that at least one poly-diacetylene comprises a diacetylene monomer wherein at least one of R^3 , R^4 , and R^5 is a cyclodextrin derivative.
- A particular embodiment of the present invention relates to the method, wherein said one or more diacetylene monomers comprising a cyclodextrin derivative are selected from the group of diacetylenes according to formula (Ia), (Ib), (Ic), and (Id):



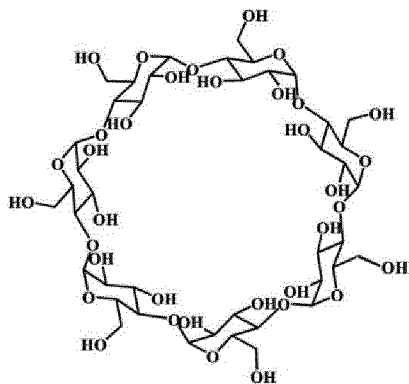


5 The diacetylene monomers comprising cyclodextrin derivatives

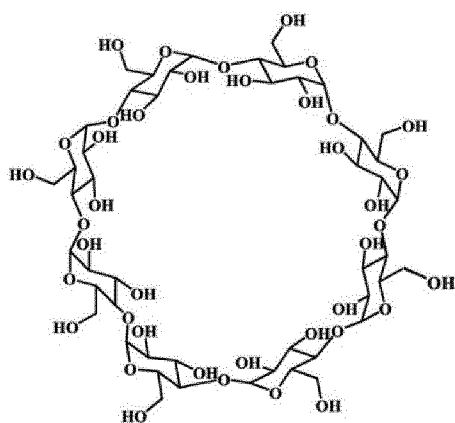
The alpha-, beta-, and gamma-cyclodextrins are molecules composed of 6, 7, and 8 glucopyranose subunits that are joined to form a ring shape with an inner free space of a diameter considered to be in the range of 4.5-5.3 Å, 6.0-7.0 Å, and 7.5-8.5 Å, respectively. Each of the compounds may be illustrated as:



alpha-cyclodextrin,



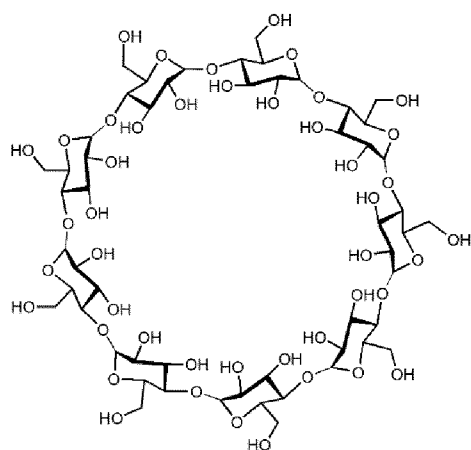
beta-cyclodextrin,



gamma-cyclodextrin.

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However, cyclodextrins composed of more than 8 glucopyranose subunits are also comprised herein. For example, a cyclodextrin comprising 9 subunits illustrated as:

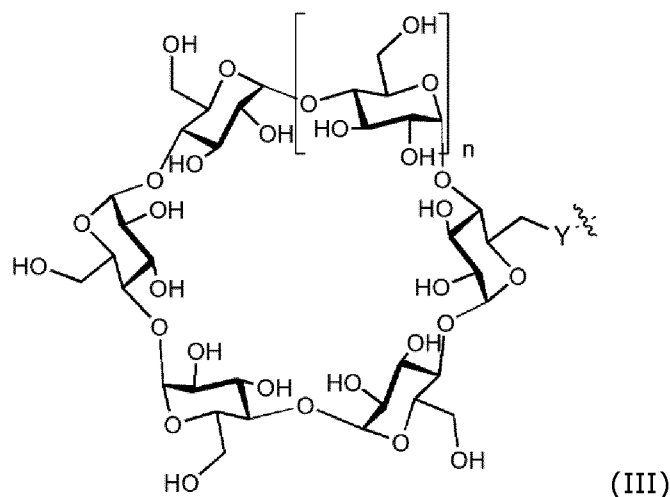


Herein, a cyclodextrin derivative is a radical of an optionally substituted cyclodextrin.

In an embodiment of the present invention the cyclodextrin derivative is an optionally substituted *alpha*-cyclodextrin, *beta*-cyclodextrin, or *gamma*-cyclodextrin. In a more specific embodiment of the present invention the cyclodextrin derivative is an optionally substituted *alpha*-cyclodextrin or *gamma*-cyclodextrin. In a specific embodiment relating to the method as described herein, the optional substituent on the cyclodextrin derivative replaces a glucose hydroxy group. Further thereto, an embodiment of the present invention relates to the method, wherein the optional substituent on the cyclodextrin derivative replaces the glucose hydroxy group selected from hydroxy group in the 2-, 3- or 6-position, preferably the 6-position. The optional substituents are preferably those according to the embodiment of the present invention, wherein the optional substituent on the cyclodextrin derivative is selected from the group consisting of C₁-C₆ alkoxy, acetyloxy, C₁-C₆ alkyl ester (-C(O)O-alkyl), and C₁-C₆ alkyl amide (-C(O)NH-alkyl).

A particular embodiment of the present invention relates to the method, wherein the diacetylene monomer comprising a cyclodextrin derivative is a diacetylene according to Formula (I) or (II),

wherein R¹ and/or R² is selected from -COOR³ and -CONR⁴R⁵, and wherein one of R³, R⁴, and R⁵ is a substituent according to formula (III):



25

wherein

n is an integer selected from 1, 2, and 3,

Y is selected from the group consisting of a bond, a C₁-C₈ alkylene amide (-alkylene-C(O)NH-), a C₁-C₈ alkylene ester (-alkylene-C(O)O-), and -Q-(CH₂-CH₂-O)_m- wherein Q is an ester or an amide, and m is an integer in the range of 1-20. Preferably, n may be 1 or 3.

5

The diacetylene monomer comprising a cyclodextrin derivative may preferably be those of formula (Ia), (Ib), (Ic), or (Id).

The poly-diacetylene sensors and arrays thereof

10 An array is a collection of individual sensors, wherein each sensor is based on a carefully prepared distinct PDA polymer. Thus, in a preferred embodiment of the present invention, said poly-diacetylenes are spatially separated and individually addressable.

An array may be set up using different sensors, however, often at least one of the PDA
15 sensors of the array may be obtained from polymerization of at least two different DA monomers. An embodiment of the present invention therefore relates to the method, wherein at least one of the poly-diacetylenes is a polymer polymerised from a mixture comprising at least two different diacetylene monomers.

20 A PDA sensor used in an array is preferably sensitive to at least one of the analytes in the complex sample to be characterized. One embodiment of the present invention therefore relates to the method, wherein said sensor array for each analyte comprises at least one poly-diacetylene capable of a colorimetric response upon contact with said analyte.

25

A PDA sensor may be created by polymerization of monomers on a solid, such as a paper material, or of monomers in a solution. Thus, one embodiment of the present invention relates to the method, wherein the concentration of diacetylene monomer or mixture thereof during polymerisation on a solid support is in the range of 1-1000 mM,
30 such as 2-500 mM, 5-200 mM, 8-150 mM, 10-100 such as preferably 20-75 mM. In another embodiment the concentration of diacetylene monomer or mixture thereof during polymerisation in liquid phase is in the range of 0.01-2 mM, such as 0.05-1 mM, 0.08-0.8 mM, 0.1-0.5 mM, such as preferably 0.15-0.30 mM.

35 PDA sensors may be prepared in a liquid phase in which they have been found to conform to specific forms. Thus, a preferred embodiment of the present invention

relates to the method, wherein the poly-diacetylene polymers are, of form as part of, vesicles or micelles, preferably vesicles.

A vesicle, also called a liposome, is a spherical structure composed of at least one lipid bilayer whereas a micelle is composed of lipid monolayer. In this context, the lipid bilayer or monolayer may be formed by the aliphatic carbon chains of the side-groups as described herein.

I another embodiment of the present invention relates to the method, wherein the poly-diacetylene polymers are positioned on a solid support, preferably and absorbent solid support, such as preferably paper.

The size of an array may differ depending on different factors, such as the complexity of the sample to be characterized and the desired quality of final characterization. Thus, an embodiment of the present invention relates to the method, wherein the sensor array comprises at least 3 different spatially separated poly-diacetylene polymers, such as at least 4, at least 5, at least 10, such as at least 15 different poly-diacetylene polymers.

In one embodiment of the present invention, at least one of the alpha- and gamma-cyclodextrin PDA sensors described herein are included in an array. In another embodiment the array includes a beta-cyclodextrin PDA sensor in combination with at least one additional PDA sensor.

25 The analytes and samples

The PDA sensors comprising cyclodextrin are sensitive to specific analytes and in particular to analytes where the whole analyte or specific parts of the analyte may fit with the inner free space of the cyclodextrin moiety. Analytes that may fit, are often of a limited size, whereby an embodiment of the present invention relates to the method, wherein the at least one analyte has a molecular weight below 1000 g/mol, such as below 800 g/mol, such as below 700 g/mol, preferably such as below 600 g/mol.

An array comprising sensors of the present invention is considered especially suitable for characterization of aqueous samples. The arrays may therefore be particularly good at characterizing samples like beverages, which usually contains a plurality of different flavouring constituents. Precise fingerprint characterization or efficient mapping of the

concentrations of each flavouring constituent is considered beneficial for the production of uniform products. The arrays may therefore be useful for characterization of such products or precursors thereof. Thus, an embodiment of the present invention relates to the method, wherein the at least one analyte, are flavour constituents of a beverage
5 or a beverage precursor, preferably of a beer or a beer precursor. In another embodiment, the at least one analyte may be multiple analytes.

A flavour constituent according to an embodiment of the present invention may be, selected from the group consisting of ethanol, carbonic acid, hop bitter substances
10 (such as trans-isohumulone), hop oil constituents (such as myrcene, humulene, oxygenated humulenes), maltol, monosaccharides, disaccharides, banana esters (such as 3-methylbutyl acetate, 2-methylpropyl acetate), apple esters (such as ethyl hexanoate and ethyl octanoate), 3-methylbutanol, dimethyl sulfide, C₆-C₁₂ fatty acids (such as octanoic acid), acetic acid, propanoic acid, ethyl acetate, 2,3-butanedione,
15 citric acid, maleic acid, polyphenols (such as leucocyanidin), trisaccharides (such as maltotriose), amino acids (such as proline), diacetyl acetylpropionyl, acetaldehyde, isobutyl acetate, propanol, isobutanol, isoamyl acetate, isoamylalcohol, ethyl caproate, ethyl caprylate, 2-phenylethyl acetate, caprylic acid, caproic acid, capric acid, linalool, limonene, pentanedione, λ -decalactone, 2-phenylethanol, trans-2-nonenal, 4-
20 vinylguaiacol (4-VG), 4-vinyl phenol, 4-ethyl guaiacol, 4-ethyl phenol, 4-Propenyl guaiacol, eugenol, tyrosol, 4-Propyl syringol, hydrogen sulfide, 3-methyl-2-butene-1-thiol, and sodium chloride. In a particular embodiment the at least one analyte is not an amino acid.

25 Apart from the assumed size-exclusion induced by the cyclodextrin moieties the cyclodextrin derived PDA sensors were also found to exhibit good sensitivity towards compounds comprising at least one ester functionality or a phenolic flavour compound. An array of the present invention is therefore considered to be particularly good at characterizing complex samples comprising esters and phenolic flavour compounds.

30

Thus, an embodiment of the present invention relates to the method, wherein the ester is a small molecule ester, such as an ester which has a molecular weight in the range of 50 — 1000 g/mol, such as 70 — 1000 g/mol, such as 80 — 1000 g/mol, such as 80 — 800 g/mol, such as 80 — 600 g/mol, preferably such as 80 — 400 g/mol. Preferably the
35 ester may be a volatile organic compound, such as a flavour component, preferably a flavour component of a beverage, such as a beer.

Efficient and reliable methods for measuring concentrations of esters or a phenolic flavour compound and/or characterize complex samples comprising esters or a phenolic flavour compound is advantageous, especially for application in beer production and other similar beverages wherein esters are important flavour constituents. A selection of some of the most important flavour constituents in beer are listed in Table 1, together with an estimated level of measuring difficulty by existing analytical methods.

Table 1: Beer component concentrations and measuring difficulty

Beer component	Threshold (mg/L)	Concentrations in (mg/L)		Measuring difficulty ^{b)}
		beer	Fermen- tations ^{a)}	
Esters:				
Ethyl acetate	20-40	8–32	Up to 65	Difficult
2-Phenylethyl acetate	0.2–3.8	0.1–1.5	1-5	Difficult
Ethyl hexanoate	0.15–0.25	0.05–0.4	Up to 0.6	Difficult
Isoamyl acetate	0.5–1.7	0.3–3.8	Up to 15	Difficult
Ethyl octanoate	0.9	0.04–0.53	Up to 1.2	Difficult
Alcohols:				
Isoamyl alcohol	50-70	25-150	Up to 250	Medium
Propanol	600-800	4-20	Up to 50	Medium
<i>iso</i> -Butanol	100-200	4-57	Up to 60	Medium
Phenyl ethanol	40-100	5-100	Up to 110	Medium
Carbonyls:				
Acetaldehyde	10-25	2-10	Up to 25	Medium
Diacetyl	0.05–0.15	0.01–0.3	0.01–1.6	Medium
Phenolics:				
4-vinyl guaiacol	0.3	0.2–3.5	0.5-17	Easy

^{a)} high gravity fermentations. ^{b)} difficulty of measuring the concentration of the beer component by known methods.

10 The PDA sensors and arrays of the present invention are thus considered particularly beneficial in regard of characterizing complex samples comprising any of the esters or a phenolic flavour compound as listed in Table 1. An embodiment of the present invention therefore relates to the method, wherein the ester is selected from the group consisting of ethyl acetate, 2-phenylethyl acetate, ethyl hexanoate, isoamyl acetate, and ethyl

octanoate. Another embodiment of the present invention therefore relates to the method, wherein the phenolic flavour compound is selected from the group consisting of 4-vinylguaiacol (4-VG), 4-vinyl phenol, 4-ethyl guaiacol, 4-ethyl phenol, 4-Propenyl guaiacol, eugenol, tyrosol, 4-Propyl syringol.

5

The sample of the present invention may be a solid or liquid sample, however a liquid sample may comprise other non-soluble liquids and/or solids. Thus, one embodiment of the present invention relates to the method, wherein the sample is a composition comprising analytes in solution, emulsion, or suspension. In most applications however, the major part of the complex sample is considered to be water, whereby a preferred embodiment of the present invention relates to the method, wherein the sample is an aqueous solution. A sample may also be predominantly solid, but may comprise liquids. This is particularly relevant for e.g. food samples and biological samples.

10

15 Preferably, the sample is selected from the group consisting of a beverage or a beverage precursor, a food or feed item, aqueous industrial waste, sewage, non-human biological samples, blood plasma, urine, and saliva, preferably a beverage or beverage precursor.

20

The complex sample is preferably a beverage and an embodiment of the present invention therefore relates to the method, wherein the beverage is selected from the group consisting of beer or a beer precursor, cider, white wine, rosé wine, red wine, dairy products, soft-drinks, alcopops and precursors thereof, most preferably beer or a beer precursor.

25

Colorimetric response and methods for performing characterisation

Characterization of the sample of at least one analyte is achieved by measuring the colorimetric response (CR) of the PDA sensors used in the array. Usually, the CR is measured by applying light of a specific wavelength and then determining how much of the light that is transmitted or absorbed. Thus, an embodiment of the present invention relates to the method, wherein the colorimetric response, is the percentage of light transmitted by the poly-diacetylenes in contact with the aqueous solution or the absorbance by which light is attenuated by the poly-diacetylenes in contact with the aqueous solution.

30

35

In one embodiment, the colorimetric response may be determined by determining the red-green-blue (RGB) value or the absorbance of each sensor before and after contact with the sample of at least one analyte. If the sensor is positioned on a solid support, e.g. on paper, the colour may e.g. be determined with the aid of a scanner, whereas a spectrophotometer may be used when the sensor is in solution. The colorimetric response may then be determined as a change in RGB value (ΔRGB) or a change in absorbance. In another embodiment, the colorimetric response is determined by determining the RGB of several sensors before and after contact with the sample of at least one analyte, and analysing the RGB values by standard statistical methods, for example by a principal component analysis (PCA). The PCA may e.g. be used to determine a cluster mean, which can be used as an indication of the colorimetric response. Closeness in space of the cluster mean indicates that two samples of at least one analyte are similar. In yet another embodiment, the colorimetric response may be determined by calculating the change in the percentage of a particular colour (e.g. percentage of red, green or blue) based on the RGB value. The colorimetric response for percentage blue (CR_{blue}) may, for example, be determined by determining the light absorbance at two specific wavelengths (e.g. at 640 nm and 548 nm) of each sensor before and after contact with the sample of at least one analyte and then calculating the percent change in the percentage of a particular absorbance.

20

The instrument used for measuring the colorimetric response may be a colorimeter or a similar device. The process of measuring CR for each of the individual sensors in an array can be done manually or by an automated process. In an embodiment of the present invention, the colorimeter is constructed such that the measuring is an automated process such as continuous flow analysis, flow injection analysis, or preferably as a plate reader.

The wavelength of the light applied is measured in nanometers (nm) and according to an embodiment of the present invention, the light has a wavelength of between 1 — 1000 nm, such as 100 — 1000 nm, preferably such as 370 — 750 nm.

Diacetylene monomers of the invention

Yet another aspect of the present invention is to provide a diacetylene monomer comprising one or more substituents selected from the group consisting of an optionally substituted $\text{C}_1\text{-C}_{30}$ alkyl, an optionally substituted $\text{C}_2\text{-C}_{30}$ alkenyl, and an optionally substituted $\text{C}_2\text{-C}_{30}$ alkynyl,

wherein said diacetylene monomer comprises an alpha-cyclodextrin derivative or gamma-cyclodextrin derivative.

In one embodiment of the present the diacetylene monomer is a compound according
5 to formula (I) or (II) as defined above, wherein at least one of R^3 , R^4 , and R^5 is an alpha-cyclodextrin derivative or gamma-cyclodextrin derivative.

Another aspect of the present invention relates to a sensor array comprising at least two different poly-diacetylenes, wherein at least one poly-diacetylene is polymerised
10 from a diacetylene monomer comprising an alpha-cyclodextrin derivative or gamma-cyclodextrin derivative as described above.

Methods of characterising beverages and flavour components therein

The sample of the present invention may be a beverage and thus the analytes may be
15 any of the flavour constituents in said beverage.

Therefore, another aspect of the present invention is to provide a method for characterizing a beverage for at least one analyte, comprising the steps of:

- a) providing a sensor array comprising at least two different poly-diacetylenes,
- 20 b) contacting said sensor array with a sample of a beverage,
- c) measuring the colorimetric response of said poly-diacetylenes to the beverage, and

wherein said poly-diacetylenes are polymers polymerised from a composition comprising a diacetylene monomer or mixtures thereof, and

25 wherein said sensor array for each analyte comprises at least one poly-diacetylene capable of a colorimetric response upon contact with said analyte, wherein at least one poly-diacetylene is polymerised from a composition comprising a diacetylene monomer comprising a cyclodextrin derivative, and wherein the analytes are flavour constituents of the beverage.

30

A particular embodiment of the present invention relates to the method, wherein the beverage is selected from the group consisting of beer or a beer precursor, cider, white wine, rosé wine, red wine, dairy products, soft-drinks, alcopops and precursors thereof, most preferably beer or a beer precursor. Another embodiment of the present invention
35 relates to the above method, wherein said method is capable of differentiating distinct

beers or beer precursors. Preferably, at least one flavour constituent is an ester or a phenolic flavour compound.

Furthermore, a more specific embodiment of the present invention relates to the method, wherein said diacetylene monomers comprise one or more substituents selected from the group consisting of an optionally substituted C₁-C₃₀ alkyl, an optionally substituted C₂-C₃₀ alkenyl, and an optionally substituted C₂-C₃₀ alkynyl.

Methods of comparing test samples to reference samples

10 The characterization of a complex sample may be achieved by comparing the results obtained by an array of sensors applied to a test sample with the results obtained using an identical array and applying a reference sample. For example, the reference may be a particular batch of a specific beer which subsequent batches of the same beer should resemble as closely as possible, thereby ensuring a consistent and uniform production
15 of the target product.

Hence, a further aspect of the present invention is therefore the provision of a method for comparing a test sample with a reference sample comprising at least one analyte, comprising the steps of

- 20 a) providing at least two identical sensor arrays comprising at least two different poly-diacetylenes,
b) contacting a first sensor array with a sample of the test sample and a second sensor array with a reference sample,
c) comparing the colorimetric response of said poly-diacetylenes of the first
25 sensor array to the colorimetric response of said poly-diacetylenes of the second sensor array,

wherein a similar colorimetric response of the first sensor array and the second sensor array indicates that the test sample is similar to the reference sample; and
wherein said poly-diacetylenes are polymers polymerised from a composition

30 comprising a diacetylene monomer or mixtures thereof, and
wherein at least one poly-diacetylene is polymerised from a composition comprising a diacetylene monomer comprising a cyclodextrin derivative.

The sample may be any liquid sample. Thus, an embodiment of the present invention
35 relates to the method, wherein the test sample is selected from the group consisting of a beverage or a beverage precursor, aqueous industrial waste, sewage, non-human

biological samples, blood plasma, urine, and saliva, preferably a beverage or beverage precursor.

However, a particular embodiment of the present invention relates to the method,
5 wherein the test sample is selected from the group consisting of beer, cider, white wine, rosé wine, red wine, dairy products, soft-drinks, alcopops and precursors thereof, most preferably beer or a beer precursor. Yet, a preferred embodiment of the present invention relates to the method, wherein the test sample is a beer or a beer precursor.

10 The reference sample may be achieved by any means possible, thus including mixing of the components or only some of the components of the targeted product. A specific embodiment of the present invention relates to the method, wherein the reference sample is a sample of a specific composition which is particularly relevant as a beverage and which is selected from the group consisting of beer, cider, white wine,
15 rosé wine, red wine, dairy products, soft-drinks, alcopops, an precursors thereof, most preferably beer or a beer precursor.

However, for most applications the reference sample is considered to be a sample obtained from a specific batch of the target product. Thus, a most preferred
20 embodiment of the present invention relates to the method, wherein the reference sample represents the desired or ideal analyte composition for the test sample.

It should be noted that embodiments and features described in the context of one of the aspects of the present invention also apply to the other aspects of the invention.
25

All patent and non-patent references cited in the present application, are hereby incorporated by reference in their entirety.

The invention will now be described in further details in the following non-limiting
30 examples.

Examples

Materials and methods:

The chemicals were purchased from commercial suppliers (Sigma-Aldrich, Alfa Aesar,
35 Acros Organics, Fluka, and VWR) and used without further purification unless specified. The monoamino-cyclodextrin (CD) derivatives were purchased from Cyclodextrin-Shop

(www.cyclodextrin-shop.com/). Ultrapure water was obtained from an ELGA Purelab Ultra system (ELGA LabWater, Lane End). 4-Vinyl guaiacol (4-VG) was purified with column chromatography before use to remove the impurities. Syringe filters with 0.8 μ M cellulose acetate membrane were purchased from GE Whatman.

5

Thin layer chromatography (TLC) plates were used to monitor the reactions. Visualization of the developed TLC plates was done under UV light (254 nm and 365 nm). Silica gel (250-430 mesh) was used as stuffing in the glass column for column chromatography.

10

^1H -NMR and ^{13}C -NMR spectra were recorded on a Bruker Ascend 400 NMR spectrometer. Chemical shifts are given in parts per million (ppm) with tetramethylsilane (TMS) as the reference.

15 Mass spectra were recorded on a Waters gas chromatography coupled time-of-flight mass spectrometer (GC-TOF-MS) system or liquid chromatography electron-spray ionization coupled quantum time-of-flight mass spectrometer (LC-ESI/Q-TOF-MS) system.

20 Polymerization of diacetylene monomers was conducted with a hand-held UVP UVLS-26 EL Series UV lamp (254 nm, 6 watts). UV-vis absorption spectra were all taken on a PerkinElmer EnSight multimode plate reader with either 48 well plates or 96 well plates. Tip sonication was conducted on a Branson digital sonifier model 450 with a 20% amplitude.

25

GraphPad Prism 8 was used to process the data to remove outliers and to determine statistically significant differences. Principal component analysis and hierarchical clustering analysis were performed using RStudio and the MATLAB PLS Toolbox, respectively.

30

EXAMPLE 1 — Synthesis of monomers

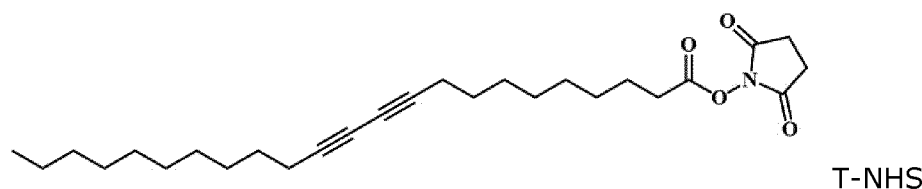
Preparation of 10,12-tricosadiynoic acid:



T

10,12-Tricosadiynoic acid (T) was purchased from commercial vendor and purified by first dissolving in chloroform and then filtered through a filter paper to remove the insoluble polymer parts. Then, the filtrate was dried using a rotary evaporator with the
5 flask protected by aluminium foil. The obtained white powder was used immediately after purification.

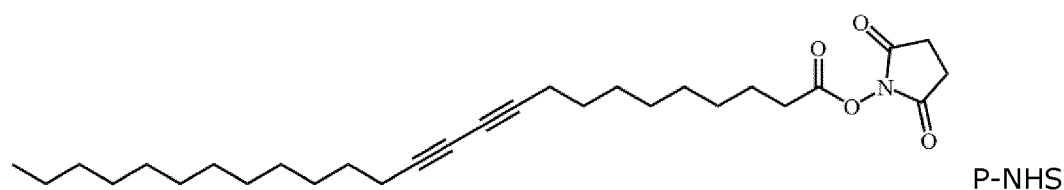
Synthesis of T-NHS:



10

10,12-tricosadiynoic acid (T, 1.12 g, 3 mmol) was dissolved in dichloromethane (30 mL). To this solution, N-hydroxysuccinimide (NHS) (0.448 g, 3.9 mmol) and N,N'-dicyclohexylcarbodiimide (0.805 g, 3.9 mmol) were added and the mixture was kept stirring at room temperature for 4 hours. After thin layer chromatography showed that
15 10,12-tricosadiynoic acid had been fully consumed, the precipitation was filtered and the filtrate was concentrated and purified with column chromatography (eluent: hexane: dichloromethane=1:4, v:v). The product was obtained as a white solid, which was stored in the dark before use. ^1H NMR (400 MHz, CDCl_3) δ 2.84 (s, 4H), 2.60 (t, J = 7.4 Hz, 2H), 2.24 (t, J = 7.0 Hz, 3H), 1.74 (p, J = 7.5 Hz, 2H), 1.52 (dd, J = 14.1, 6.8 Hz, 4H), 1.47 – 1.12 (m, 22H), 0.88 (t, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3)
20 δ 169.21, 168.64, 65.32, 65.23, 31.88, 30.90, 29.55, 29.47, 29.29, 29.08, 28.88, 28.84, 28.77, 28.68, 28.34, 28.27, 25.59, 24.53, 22.67, 19.18, 19.16, 14.10. LC-ESI/Q-TOF-MS m/z = 444.7 $[\text{M}+\text{H}]^+$, calc. for $[\text{M}]$ $\text{C}_{27}\text{H}_{41}\text{NO}_4$ = 443.6.

25 Synthesis of P-NHS:



P-NHS was synthesized in the same manner as T-NHS except that 10,12-pentacosadiynoic acid (P) was used instead of T. ^1H NMR (400 MHz, CDCl_3) δ 2.86 (s, 4H), 2.62 (t, J = 7.5 Hz, 2H), 2.26 (t, J = 7.0 Hz, 4H), 1.76 (p, J = 7.5 Hz, 2H), 1.54
30 (dq, J = 14.7, 7.3 Hz, 4H), 1.47 – 1.20 (m, 26H), 0.90 (t, J = 6.7 Hz, 3H). ^{13}C NMR

(100 MHz, CDCl₃) δ 169.51, 168.04, 66.32, 65.27, 31.88, 30.99, 29.55, 29.43, 29.29, 29.08, 28.88, 28.87, 28.79, 28.69, 28.56, 28.07, 25.69, 24.43, 22.63, 19.33, 19.16, 14.22. LC-ESI/Q-TOF-MS m/z = 472.7 [M+H]⁺, calc. for [M] C₂₉H₄₅NO₄ = 471.6.

5 Synthesis of T-alpha-CD:

Figure 1A, shows a chemical representation of T-alpha-CD. NaHCO₃ (16.8 mg, 0.2 mmol) and 6-monoamino-6-monodeoxy- α -cyclodextrin hydrochloride (86 mg, 0.085 mmol) was dissolved in water (3 mL). To this solution, T-NHS (45 mg, 0.1 mmol) in tetrahydrofuran (3 mL) was added dropwise. The mixture was left to react for 24 hours.

- 10 After that, THF was evaporated and the residue was added to acetone (30 mL). The precipitate was washed twice with small amount of acetone and then dried in oven to obtained T-alpha-CD as a white powder. ¹H NMR (400 MHz, DMSO-d₆) δ 4.85–4.80 (m, 6H), 3.88–3.10 (m, 36H), 2.31 (t, 4H), 2.13 (m, 2H), 1.48 (m, 6H), 1.35–1.28 (br, 22H), 0.90 (t, 3H); ¹³C NMR (100 MHz, DMSO-d₆): δ 173.83, 103.70, 84.58, 82.63, 79.38, 75.99, 71.89, 66.82, 61.51, 36.18, 32.50, 30.30, 29.79, 29.69, 29.00, 27.88, 23.90, 19.71, 15.41. LC-ESI/Q-TOF-MS m/z = 1323.1 [M+Na]⁺, calc. for [M] C₅₉H₉₇NO₃₀ = 1300.4.

Synthesis of T-beta-CD:

- 20 Figure 1B, shows a chemical representation of T-beta-CD. T-beta-CD was synthesized in a similar manner except that 6-monoamino-6-monodeoxy- β -cyclodextrin hydrochloride was used instead of 6-monoamino-6-monodeoxy- α -cyclodextrin hydrochloride. ¹H NMR (400 MHz, DMSO-d₆) δ 4.85–4.80 (m, 7H), 3.88–3.10 (m, 42H), 2.31 (t, 4H), 2.13 (m, 2H), 1.48 (m, 6H), 1.35–1.28 (br, 22H), 0.90 (t, 3H); ¹³C NMR (100 MHz, DMSO-d₆): δ 173.53, 102.70, 88.58, 82.75, 79.68, 75.91, 71.89, 66.82, 61.51, 36.18, 32.50, 30.30, 29.80, 29.69, 29.00, 27.88, 23.90, 19.71, 15.41. LC-ESI/Q-TOF-MS m/z = 1485.0 [M+Na]⁺, calc. for [M] C₆₅H₁₀₇NO₃₅ = 1462.5.

Synthesis of T-gamma-CD:

- 30 Figure 1C, shows a chemical representation of T-gamma-CD. T-gamma-CD was synthesized in a similar manner except that 6-monoamino-6-monodeoxy- γ -cyclodextrin hydrochloride was used instead of 6-monoamino-6-monodeoxy- α -cyclodextrin hydrochloride. ¹H NMR (400 MHz, DMSO-d₆) δ 4.85–4.80 (m, 8H), 3.88–3.10 (m, 48H), 2.31 (t, 4H), 2.13 (m, 2H), 1.48 (m, 6H), 1.35–1.28 (br, 22H), 0.90 (t, 3H); ¹³C NMR (100 MHz, DMSO-d₆): δ 173.93, 103.70, 87.08, 82.28, 79.83, 75.91, 63.82, 61.55,
- 35

36.28, 32.90, 30.10, 29.77, 29.69, 29.00, 27.56, 23.44, 19.09, 15.51. LC-ESI/Q-TOF-MS $m/z = 1647.8$ $[M+Na]^+$, calc. for $[M]$ $C_{71}H_{117}NO_{40} = 1624.7$.

Synthesis of P-alpha-CD:

- 5 P-alpha-CD was synthesized in a similar manner as T-alpha-CD except that P-NHS was used instead of T-NHS. 1H NMR (400 MHz, DMSO- d_6) δ 4.85–4.80 (m, 6H), 3.88–3.10 (m, 36H), 2.31 (t, 4H), 2.13 (m, 2H), 1.48 (m, 6H), 1.35–1.28 (br, 26H), 0.90 (t, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 173.69, 104.70, 84.53, 82.63, 79.38, 75.99, 70.89, 65.82, 62.51, 36.58, 32.50, 30.70, 29.79, 29.59, 29.08, 27.89, 23.00, 19.81, 15.48.
- 10 LC-ESI/Q-TOF-MS $m/z = 1351.7$ $[M+Na]^+$, calc. for $[M]$ $C_{61}H_{101}NO_{30} = 1328.5$.

Synthesis of P-beta-CD:

- Figure 1D, shows a chemical representation of P-beta-CD. P-beta-CD was synthesized in a similar manner as T-beta-CD except that P-NHS was used instead of T-NHS. 1H NMR (400 MHz, DMSO- d_6) δ 4.85–4.80 (m, 7H), 3.88–3.10 (m, 42H), 2.31 (t, 4H), 2.13 (m, 2H), 1.48 (m, 6H), 1.35–1.28 (br, 26H), 0.90 (t, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 174.53, 102.70, 88.58, 82.75, 79.68, 78.66, 71.89, 66.82, 61.51, 36.18, 32.50, 30.60, 29.60, 29.29, 29.00, 28.88, 24.80, 19.59, 15.62. LC-ESI/Q-TOF-MS $m/z = 1513.5$ $[M+Na]^+$, calc. for $[M]$ $C_{67}H_{111}NO_{35} = 1490.6$.

20

Synthesis of P-gamma-CD:

- P-gamma-CD was synthesized in a similar manner as T-gamma-CD except that P-NHS was used instead of T-NHS. 1H NMR (400 MHz, DMSO- d_6) δ 4.86–4.81 (m, 8H), 3.98–3.12 (m, 48H), 2.32 (t, 4H), 2.10 (m, 2H), 1.58 (m, 6H), 1.38–1.28 (br, 26H), 0.90 (t, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 176.98, 103.56, 87.02, 82.89, 79.22, 75.55, 63.82, 61.55, 36.28, 32.90, 30.10, 29.87, 29.63, 29.10, 27.55, 23.22, 19.19, 15.03. LC-ESI/Q-TOF-MS $m/z = 1675.8$ $[M+Na]^+$, calc. for $[M]$ $C_{73}H_{121}NO_{40} = 1652.7$.

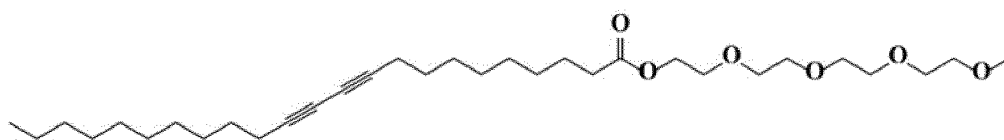
Synthesis of T-PEG1:

- 30 10,12-Tricosadiynoic acid (T, 346 mg, 1 mmol) was dissolved in 20 mL anhydrous dichloromethane (DCM). Oxalyl dichloride (507 mg, 4 mmol) was added dropwise to the solution followed by 2 drops of *N,N*-dimethylformamide (DMF) as catalyst. The mixture was kept stirring for 4 hours at room temperature. After that, the mixture was vacuum dried and the intermediate product, In1, was obtained as a yellowish powder



In1

In1 was immediately used in the following synthetic procedure without further purification and characterization. Methoxytetraethylene glycol (0.25 g, 1.2 mmol) was dissolved in anhydrous DCM (20 mL). Triethylamine (0.3 g, 3 mmol) was added, and the mixture was cooled down in an ice bath. In1 (364 mg, 1 mmol) was dissolved in 10 mL DCM and added dropwise to the solution. The mixture was kept stirring at room temperature overnight. After that, the mixture was washed with water and the organic phase was collected and dried over anhydrous Na_2SO_4 .



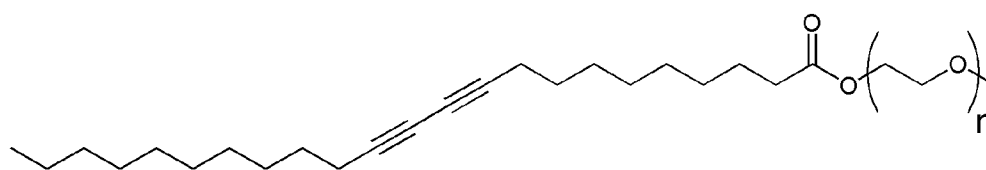
T-PEG1

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T-PEG1 was obtained as a yellowish oil after purification with column chromatography (eluent: DCM: CH_3OH = 100:1). ^1H NMR (400 MHz, CDCl_3) δ 4.22 (t, J = 4.0 Hz, 2H), 3.67 (m, 12H), 3.55 (d, J = 4.2 Hz, 2H), 3.38 (s, 3H), 2.32 (t, J = 7.5 Hz, 2H), 2.23 (t, J = 6.9 Hz, 4H), 1.70 (d, J = 6.1 Hz, 2H), 1.60 (p, J = 7.3 Hz, 2H), 1.50 (d, J = 13.0 Hz, 4H), 1.27 (d, J = 12.9 Hz, 22H), 0.87 (t, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 173.77, 71.90, 70.58, 70.55, 70.52, 70.49, 69.17, 65.29, 65.21, 63.35, 59.01, 34.14, 31.87, 29.54, 29.46, 29.29, 29.08, 29.04, 28.89, 28.83, 28.74, 28.33, 28.28, 24.83, 22.66, 19.17, 19.16, 14.11. GC-TOF-MS 536.4103[M] $^+$, calc. for $\text{C}_{32}\text{H}_{56}\text{O}_6$ = 536.4077.

20

Synthesis of T-PEG2:



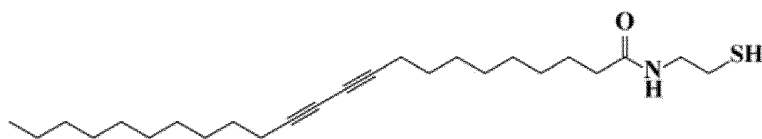
T-PEG2

T-PEG2 was synthesized using a similar procedure as for T-PEG1 except that poly(ethylene glycol) methyl ether ($n \approx 12.5$) was used instead of methoxytetraethylene glycol. ^1H NMR (400 MHz, CDCl_3) δ 4.25 – 4.19 (m, 2H), 3.72 – 3.59 (m, 47H), 3.54 (dd, J = 5.8, 3.5 Hz, 2H), 3.38 (s, 3H), 2.32 (t, J = 7.5 Hz, 2H),

25

2.23 (t, J = 7.0 Hz, 4H), 1.61 (p, J = 7.5 Hz, 2H), 1.56 – 1.45 (m, 4H), 1.42 – 1.20 (m, 22H), 0.87 (t, J = 6.7 Hz, 3H).

Synthesis of T-SH:



5

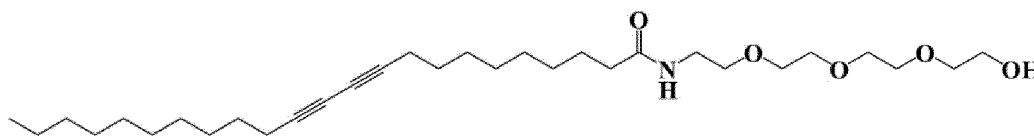
T-SH

The same procedure was used as for the synthesis of T-PEG1 except that cysteamine was used instead of methoxytetraethylene glycol. ¹H NMR (400 MHz, CDCl₃) δ 6.31 (s, 1H), 3.57 (q, J = 6.3 Hz, 2H), 2.83 (t, J = 6.4 Hz, 2H), 2.28 – 2.17 (m, 6H), 1.64 (d, J = 7.0 Hz, 2H), 1.51 (t, J = 7.5 Hz, 4H), 1.28 (m, J = 14.9 Hz, 22H), 0.88 (t, J = 6.6 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 173.68, 65.32, 65.25, 38.39, 37.85, 36.55, 31.89, 29.56, 29.48, 29.30, 29.21, 29.18, 29.10, 28.96, 28.87, 28.79, 28.37, 28.32, 25.66, 22.68, 19.22, 19.20, 14.11. LC-ESI/Q-TOF-MS m/z = 406.3 [M+H]⁺, calc. for [M] C₂₅H₄₃NOS = 405.3.

15

Synthesis of T-PEG-SH:

T-NHS from Example 1 (443 mg, 1 mmol) was dissolved in 20 mL anhydrous DCM. Triethylamine (303 mg, 3 mmol) was added and the solution was cooled in an ice bath. 2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethylamine (232 mg, 1.2 mmol) was dissolved in 10 mL anhydrous DCM and added dropwise to the solution. The mixture was kept stirring overnight at room temperature. After that, it was washed with water and organic phase was collected and dried over anhydrous Na₂SO₄.



In2

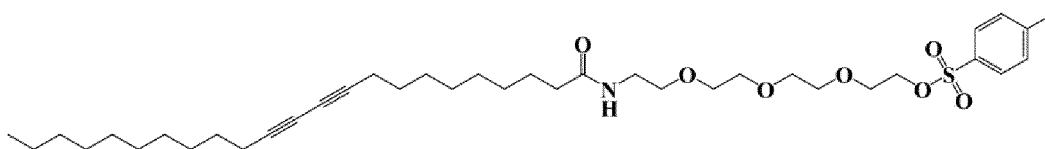
The intermediate product, In2, was obtained as a white solid after purification with column chromatography (eluent: DCM: CH₃OH=30:1), which was stored in the dark. ¹H NMR (400 MHz, CDCl₃) δ 7.00 (s, 1H), 3.73 (q, J = 4.7 Hz, 4H), 3.69 – 3.58 (m, 8H), 3.56 – 3.50 (m, 2H), 3.46 (dq, J = 9.8, 4.9 Hz, 2H), 2.23 (t, J = 7.0 Hz, 4H), 2.20 – 2.12 (m, 2H), 1.67 – 1.59 (m, 2H), 1.50 (ddt, J = 12.0, 7.1, 3.6 Hz, 4H), 1.38 – 1.23 (m, 22H), 0.88 (t, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 173.36, 72.58, 70.67, 70.46, 70.39, 70.04, 70.01, 61.56, 39.06, 36.56, 31.87, 29.54, 29.45, 29.31, 29.28,

30

29.20, 29.07, 28.95, 28.84, 28.79, 28.35, 28.32, 25.75, 22.65, 19.19, 19.18, 14.08.

LC-ESI/Q-TOF-MS $m/z = 522.4$ $[M+H]^+$, calc. for $[M]$ $C_{31}H_{55}NO_5 = 521.4$.

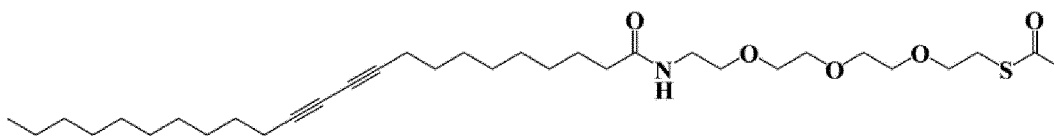
In2 (0.469 g, 0.9 mmol) and 4-toluenesulfonyl chloride (206 mg, 1.08 mmol) were dissolved in 30 mL DCM. The solution was cooled down in an ice bath and grinded KOH powder (202 mg, 3.6 mmol) was slowly added in portions. The mixture was kept stirring at room temperature overnight. After that, the mixture was diluted with 50 mL DCM and washed with water.



In3

The intermediate product, In3, was obtained as a white solid after the solvent was removed. 1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, $J = 8.2$ Hz, 2H), 7.34 (d, $J = 8.0$ Hz, 2H), 5.98 (s, 1H), 4.20 – 4.13 (m, 2H), 3.72 – 3.66 (t, $J = 5.0$ Hz, 2H), 3.61 (m, 8H), 3.54 (t, $J = 5.0$ Hz, 2H), 3.44 (q, $J = 5.2$ Hz, 2H), 2.45 (s, 3H), 2.24 (d, $J = 7.0$ Hz, 4H), 2.19 – 2.11 (m, 2H), 1.59 (q, $J = 7.3$ Hz, 2H), 1.49 (qd, $J = 7.1, 4.4$ Hz, 4H), 1.37 – 1.24 (m, 22H), 0.88 (t, $J = 6.7$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.13, 144.83, 133.04, 129.83, 127.94, 77.42, 70.80, 70.57, 70.45, 70.23, 69.93, 69.20, 68.71, 39.13, 36.64, 31.86, 29.53, 29.44, 29.26, 29.22, 29.16, 29.06, 28.91, 28.83, 28.76, 28.34, 28.30, 25.65, 22.64, 21.61, 19.18, 14.07. LC-ESI/Q-TOF-MS $m/z = 676.5$ $[M+H]^+$, calc. for $[M]$ $C_{38}H_{61}NO_7S = 675.4$.

In3 (543 mg, 0.81 mmol) was dissolved in 30 mL acetone. Potassium thioacetate (103 mg, 0.9 mmol) was added and the mixture was kept stirring at 50 °C for 18 hours. The precipitate was removed by filtration and the filtrate was dissolved in 30 mL DCM and washed with water. The organic phase was collected and dried over anhydrous Na_2SO_4 . After purification with column chromatography (eluent: DCM: $CH_3OH = 40:1$).



In4

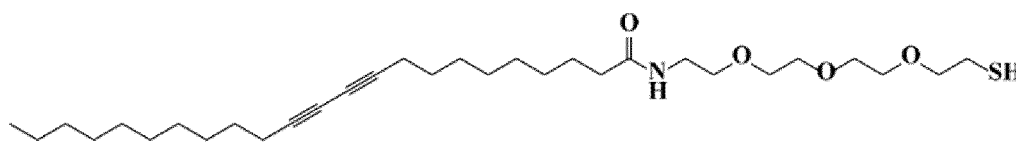
The intermediate product, In4, was obtained as a yellowish powder. 1H NMR (400 MHz, $CDCl_3$) δ 6.00 (s, 1H), 3.67 – 3.54 (m, 12H), 3.45 (q, $J = 5.2$ Hz, 2H), 3.09 (t, $J = 6.5$ Hz, 2H), 2.34 (s, 3H), 2.24 (t, $J = 7.0$ Hz, 4H), 2.17 (t, $J = 7.6$ Hz, 2H), 1.67 – 1.59

(m, 2H), 1.50 (tt, $J = 7.1, 3.6$ Hz, 4H), 1.39 – 1.25 (m, 22H), 0.88 (t, $J = 6.7$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 195.35, 173.07, 70.59, 70.49, 70.32, 70.25, 69.94, 69.79, 65.33, 65.27, 39.15, 36.69, 31.86, 30.52, 29.53, 29.44, 29.27, 29.24, 29.18, 29.06, 28.92, 28.83, 28.78, 28.76, 28.34, 28.31, 25.66, 22.64, 19.19, 19.17, 14.07.

5 LC-ESI/Q-TOF-MS $m/z = 580.4$ $[\text{M}+\text{H}]^+$, calc. for $[\text{M}] \text{C}_{33}\text{H}_{57}\text{NO}_5\text{S} = 579.4$.

In4 (580 mg, 1 mmol) was dissolved in CH_3OH (30 mL). Sodium methoxide (162 mg, 3 mmol) was dissolved in 5 mL CH_3OH and added dropwise under N_2 atmosphere to the solution comprising In4. The mixture was kept stirring at room temperature for 1 hour. After that, 1 M HCl was added to acidify the mixture and CH_3OH was evaporated

10 afterwards. Then the residue was dissolved in DCM and washed three times with water.



T-PEG-SH

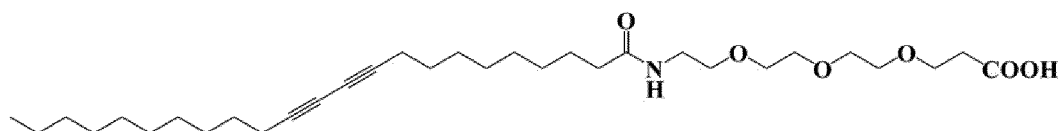
The product, T-PEG-SH, was obtained as yellowish powder after drying the solvent, which was kept in fridge (4 °C) before use. ^1H NMR (400 MHz, CDCl_3) δ 5.99 (s, 1H),

15 3.69 – 3.60 (m, 10H), 3.56 (t, $J = 5.1$ Hz, 2H), 3.45 (q, $J = 5.2$ Hz, 2H), 2.70 (dt, $J = 8.0, 6.4$ Hz, 2H), 2.24 (t, $J = 7.0$ Hz, 4H), 2.20 – 2.11 (m, 2H), 1.62 (t, $J = 8.1$ Hz, 2H), 1.51 (p, $J = 7.8, 7.0$ Hz, 4H), 1.37 – 1.23 (m, 22H), 0.88 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 173.07, 72.87, 70.58, 70.50, 70.26, 70.24, 69.96, 65.33, 65.26, 39.13, 36.71, 31.87, 29.53, 29.45, 29.27, 29.24, 29.18, 29.07, 28.92, 28.83,

20 28.77, 28.35, 28.31, 25.67, 24.27, 22.65, 19.19, 19.17, 14.08. LC-ESI/Q-TOF-MS $m/z = 538.5$ $[\text{M}+\text{H}]^+$, calc. for $[\text{M}] \text{C}_{31}\text{H}_{55}\text{NO}_4\text{S} = 537.3$.

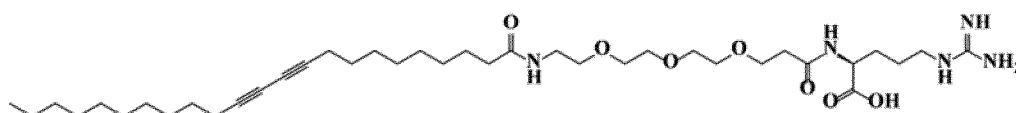
Synthesis of T-PEG-Arg:

T-NHS from Example 1 (0.5 g, 1.13 mmol) was dissolved in 20 mL anhydrous DCM. To
 25 this solution, triethylamine (0.344 g, 3.4 mmol) was added. The solution was cooled down in an ice bath. After that, 3-[2-[2-(2-aminoethoxy)ethoxy]-ethoxy]propanoic acid (0.25 g, 1.13 mmol) was dissolved in 10 mL DCM and added dropwise to the chilled solution. The mixture was left to stir overnight at room temperature. The solution was washed with H_2O and the organic phase was collected and dried over
 30 anhydrous Na_2SO_4 .



In5

The crude product, In5, was purified using column chromatography with DCM:CH₃OH=20:1 (v/v) as the eluent. The product, In5, was obtained as a white powder. ¹H NMR (400 MHz, CDCl₃) δ 6.27 (s, 1H), 3.78 (t, J = 6.0 Hz, 2H), 3.64 (d, J = 4.4 Hz, 8H), 3.59 (t, J = 5.1 Hz, 2H), 3.46 (q, J = 5.4 Hz, 2H), 2.62 (t, J = 6.0 Hz, 2H), 2.22 (dt, J = 16.1, 7.4 Hz, 6H), 1.67 – 1.57 (m, 2H), 1.55 – 1.45 (m, 4H), 1.42 – 1.23 (m, 22H), 0.88 (t, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 175.73, 174.65, 70.47, 70.40, 70.33, 70.11, 66.79, 65.31, 65.26, 39.11, 36.57, 34.90, 31.86, 29.53, 29.45, 29.27, 29.23, 29.20, 29.07, 28.95, 28.84, 28.79, 28.35, 28.33, 25.72, 22.65, 19.18, 14.08. LC-ESI/Q-TOF-MS m/z = 550.4 [M+H]⁺, calc. for [M] C₂₉H₅₀N₄O₃ = 549.4. In5 (110 mg, 0.2 mmol) was dissolved in anhydrous THF (20 mL). To this solution, N-hydroxysuccinimide (30 mg, 0.26 mmol) and N,N'-dicyclohexylcarbodiimide (53.6 mg, 0.26 mmol) were added and the mixture was kept stirring at room temperature. After TLC showed that In5 had been fully consumed, the crude product was used for the next step with further purification. L-arginine (45.3 mg, 0.26 mmol) and NaHCO₃ (21.8 mg, 0.26 mmol) were dissolved in 15 mL H₂O. The crude product in THF from the last step was added dropwise. The mixture was kept stirring overnight at room temperature. THF was then evaporated and 2 M HCl was added to acidify the solution until the pH reached 2. Then water was evaporated and the residue was subjected to column chromatography with DCM:CH₃OH=1:1 (v/v) as the eluent.



T-PEG-Arg

The product, T-PEG-Arg, was obtained as a yellowish solid. ¹H NMR (400 MHz, CD₃OD) δ 4.67 (m, 2H), 4.22 – 4.19 (m, 2H), 3.74 – 3.60 (t, J = 5.0 Hz, 2H), 3.58 (m, 10H), 3.51 (t, J = 5.0 Hz, 2H), 3.40 (q, J = 5.2 Hz, 2H), 2.24 (d, J = 7.0 Hz, 4H), 2.19 – 2.11 (m, 2H), 1.59 (q, J = 7.3 Hz, 2H), 1.59-1.53 (m, 4H), 1.41 (qd, J = 7.1, 4.4 Hz, 4H), 1.31 – 1.20 (m, 22H), 0.98 (t, J = 6.7 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 173.83, 169.06, 155.92, 75.09, 70.89, 70.45, 70.00, 69.23, 68.13, 68.34, 68.13, 68.02, 63.90, 46.98, 32.98, 30.84, 29.30, 29.34, 29.16, 29.06, 29.00, 28.96, 28.91, 28.53, 28.46,

28.34, 28.20, 25. 82, 22.22, 21.01, 19.89, 14.10. LC-ESI/Q-TOF-MS $m/z = 706.5305$
 $[M+H]^+$, calc. for $[M] C_{38}H_{67}N_5O_7 = 705.5040$.

EXAMPLE 2 — Polymerization of monomers to poly-diacetylene

- 5 Poly-diacetylene and poly-diacetylene comprising cyclodextrin was synthesized from the monomers by the procedure illustrated in Figure 2 and as described here:

For poly-diacetylene polymerized of T alone, 0.01 mmol of 10,12-tricosadiynoic acid (T) was weighed into a glass vial and dissolved in 1 mL of chloroform to reach a
 10 concentration of 10 mM. Then, 200 μ L of the solution was transferred into a round-bottomed flask. For poly-diacetylene polymerized of two different monomers, each monomer (0.01 mmol) was first dissolved in 1 mL of chloroform. Then, 100 μ L of each solution was transferred into a round-bottomed flask to mix it well. In each case, the chloroform was subsequently removed by gently blowing dry with a stream of N_2 , and a
 15 thin film was obtained on the wall of the flask. Then, 10 mL of ultrapure H_2O was added into the flask to reach a concentration of 0.2 mM and it was subjected to tip sonication for 30 min. After that, the obtained milky solution was filtered through a 0.8 μ M cellulose acetate membrane to remove large aggregates. The solution was then transferred into a glass vial and kept in the fridge (4 °C) overnight. Polymerization was
 20 carried out by irradiation of the solution sample with a hand-held UV lamp (254 nm) for 2 min. The distance from the lamp to the sample was kept constant at 3 cm throughout the whole preparation process for all the poly-diacetylenes. The obtained composition comprises vesicles of poly-diacetylene and has a blue color.

- 25 Table 2 summarize the different PDA sensors of the present invention and the reference sensors used for comparison.

Table 2: PDA sensors comprising cyclodextrin and reference PDA sensors.

PDA sensors of the invention			
Name	Polymerized of		
	First monomer	Second monomer	Molar ratio ^{a)}
T+T-alpha-CD	T	T-alpha-CD	1:1
T+T-beta-CD	T	T-beta-CD	1:1
	T	T-beta-CD	8:2
T+T-gamma-CD	T	T-gamma-CD	1:1
P+P-beta-CD	P	P-beta-CD	1:1

Reference PDA sensors			
T	T	-	-
T+T-PEG1	T	T-PEG1	1:1
T+T-PEG2	T	T-PEG2	1:1
T+T-SH	T	T-SH	1:1
T+T-PEG-SH	T	T-PEG-SH	1:1
T+T-PEG-Arg	T	T-PEG-Arg	1:1

a) Molar ratio of first monomer/second monomer.

EXAMPLE 3 — Measurement of colorimetric response

A composition comprising vesicles of poly-diacetylene according to Example 2 (300 μ L) was transferred into four wells (48-well plate). From left to right, 297, 270, 150, and 0 μ L of water were added into the four wells. Then, 3, 30, 150, and 300 μ L of analyte solution (2 mM in water) were added to reach a total volume of 600 μ L in each well. The mixtures were shaken and then subjected to a multiplate reader to measure the absorbance. These experiments were performed with at least five independent repeats, whereby error bars could be obtained to each experiment.

The concentrations may be indicated in parts per million (ppm, 10^6) instead of molar concentrations. Herein, parts per million is the concentration of analyte in mg/L which may be converted to mmol/L (mM) by dividing the number of ppm with the molar weight, M_w (g/mol) of the analyte: (mM = ppm / M_w).

A composition comprising poly-diacetylene is blue as long as it is not responding to any analytes. However, a change from blue to red color happens when such a composition is brought into contact with an analyte to which the utilized poly-diacetylene is responsive. This was used to evaluate the sensitivity of the poly-diacetylenes towards a range of different analytes.

Sensitivity of the poly-diacetylenes toward certain analytes was calculated based on absorbance and quantitatively evaluated by the colorimetric response (CR), which is defined as the relative change from blue to red. CR is calculated as:

$$CR = \frac{(PB_b - PB_a)}{PB_b} \times 100\%$$

where PB_b and PB_a are the before and after percentages of blue (PB) of the system. Meanwhile, PB is defined as:

$$PB = \frac{A_{\text{blue}}}{A_{\text{blue}} + A_{\text{red}}} \times 100\%$$

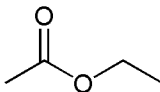
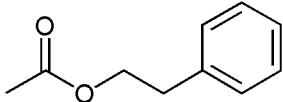
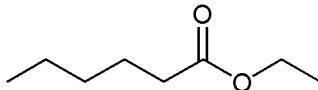
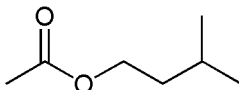
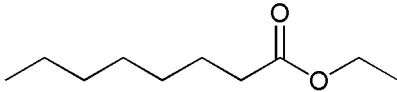
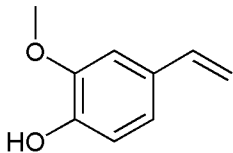
where A_{blue} and A_{red} are the absorbance of the blue form ($\lambda = \sim 650 \text{ nm}$) and the red form ($\lambda = \sim 550 \text{ nm}$) of the system, respectively. Therefore, a higher CR indicates a larger color change from blue to red of the system, which means the sensor is more sensitive to that analyte.

EXAMPLE 4 – colorimetric response of poly-diacetylenes comprising

10 cyclodextrin

The analytes used for the experiments related to Figures 3 to 7 are listed in Table 3. These were dissolved in water to give 2 mM solutions of the respective esters.

Table 3: Esters and other analytes of the analyte solution.

Abbreviation	Name of analyte	Chemical structure of analyte
EA	Ethyl acetate	
PA	2-Phenylethyl acetate	
EH	Ethyl hexanoate	
IA	Isoamyl acetate	
EO	Ethyl octanoate	
4-VG	4-vinyl guaiacol	
EtOH	Ethanol	CH ₃ CH ₂ OH

Figures 3, 4 and 5 show the colorimetric responses of each of the PDA sensors comprising cyclodextrin towards samples comprising the esters: EH, IA, PA, EO and EA in concentrations of 0.01 mM, 0.1 mM, 0.5 mM, and 1 mM (e.g. 3, 30, 150, and 300 μ L of 2 mM aqueous analyte solution). As mentioned, these experiments were performed with at least five independent repeats whereby error bars could be obtained to each experiment. Figure 3, shows the colorimetric response of T+T-alpha-CD, Figure 4 of T+T-beta-CD, and Figure 5 of T+T-gamma-CD.

Notably, the colorimetric response for T+T-beta-CD in Figure 4 increased proportionally with increasing ester concentration and the trend was common for all of the tested esters. Compared to the result shown in Figures 3 and 5, this common trend of proportionality between concentration and colorimetric response seems to be an effect which was particularly pronounced for T+T-beta-CD. The poly-diacetylene comprising beta-cyclodextrin was therefore considered a particularly good sensor for these esters. In addition, the colorimetric responses obtained in the experiments using T+T-beta-CD were generally higher than for the alpha-CD and gamma-CD PDAs. Without being bound by theory, we believe this was due to the cavity in beta-CD which may match the molecular size-ranges of the tested analytes better than the smaller cavity of alpha-CD and the larger cavity of gamma-CD. This theory is further supported by the results obtained for the phenolic flavour compound, 4-VG. The colorimetric responses for the PDA sensors comprising cyclodextrin were tested towards different concentrations of 4-VG: 1.5 ppm (0.01 mM), 15 ppm (0.1 mM), 75 ppm (0.5 mM), and 150 ppm (1 mM) and the results are shown in Figure 6. The molecular structure of 4-VG is bulkier than the tested esters in figure 3-5, which are mostly linear molecules. The increased CR responses for the larger gamma-CD and the decreased responses for the smaller alpha-CD, when compared to utilization of beta-CD, was considered to be in line with the proposed connection between molecular size of the analytes and the inner free space of the cyclodextrin. The poly-diacetylene comprising gamma-cyclodextrin was therefore considered a particularly good sensor for bulkier analytes, such as phenolic flavour compounds, and in particular 4-VG.

The colorimetric responses to ethanol of the PDA sensors comprising cyclodextrin were measured and the results are shown in Figure 7. Three different concentrations were investigated, and thus, only 3 wells were used for the sample preparation. The concentrations were selected to mimic normal beer of 4 vol%, 8 vol% and 12 vol% which corresponds to molar concentrations of 685 mM, 1370 mM, and 2055 mM,

ethanol respectively. These concentrations are several orders of magnitude higher than the concentrations used for the other analytes of the Example. The colorimetric responses for ethanol were therefore considered to be fairly low in light of the high analyte concentrations. The PDA sensors comprising cyclodextrin may therefore be able to characterise samples comprising esters or phenolic flavour compounds, even in a sample which also comprises ethanol. It is therefore envisaged that the PDA sensors comprising cyclodextrins, and maybe particularly the PDA sensor comprising beta-cyclodextrin, are especially useful for measuring amounts of esters in complex samples, beverages, or beer. Likewise, it is envisaged that the PDA sensors comprising cyclodextrins, and maybe particularly the PDA sensor comprising gamma-cyclodextrin, are especially useful for measuring amounts of phenolic flavour compounds in complex samples, beverages, or beer.

EXAMPLE 5 — Comparison with reference poly-diacetylene sensors

The present example compares the colorimetric response of PDA sensors with or without incorporated cyclodextrins to the ester ethyl acetate (EA).

Four samples of ethyl acetate in concentrations of 0.01 mM, 0.1 mM, 0.5 mM, and 1 mM were prepared and colorimetric responses to these samples using either the PDA sensors comprising cyclodextrin, or any of the previously reported reference poly-diacetylenes, were obtained according to the procedure described in Example 3.

The results are shown in Figure 8. The PDA sensors comprising cyclodextrin mostly gave colorimetric responses greater than the responses obtained for any of the reference poly-diacetylenes. Especially, T+T-alpha-CD and T+T-beta-CD yielded very high CR values to the ester analyte at all concentration levels, whereas the responses of the T+T-gamma-CD and the reference PDA sensors were much weaker. The very strong signals obtained when using the PDA sensors comprising alpha-CD and beta-CD were considered to be linked to the small inner free spaces of these cyclodextrins, which seemingly fit well with the molecular size of ethyl acetate.

Based on this it can be concluded that PDA sensors comprising cyclodextrins are better at measuring concentrations of esters, such as ethyl acetate when compared to the reference PDA sensors. In addition, the correlation between the inner free space of the utilized cyclodextrin with the size of the analyte seems to be important for the strength of the colorimetric response. It is therefore proposed that the PDA sensor comprising

cyclodextrin to be used in a specific experiment is selected based on the molecular size of the target analyte.

EXAMPLE 6 — Improved characterisation of beer using CD-PDA array

- 5 When using the a PDA array comprising PDAs formed from the polymerisation of monomers where cyclodextrins are comprised an improved characterisation of samples comprising analytes including esters and phenolic is achieved.

Thus, when comparing two PDA arrays where one comprises cyclodextrin monomers
10 and one does not, better separation and specificity is achieved for the cyclodextrin array, when subjecting the arrays to commercial beers.

Figure 9 A) and B) shows Principal Component Analysis (PCA) plots of 4 different commercial beers (Carlsberg 1664, Carlsberg alcohol free Pilsner, Non-alcoholic GO
15 pilsner, OKOCIM 0,0%), where Figure 9A) is the result with a reference PCA array (1) and Figure 9B) is the result with a PCA array (2) comprising cyclodextrin PDAs. Without CD sensors (Fig 9A), OKOCIM severely overlapped with Carlsberg AF pilsner. With the CD sensors (Fig 9B), OKOCIM no longer overlaps with Carlsberg AF pilsner, and Carlsberg AF pilsner only slightly overlaps with GO. This shows that the CD sensors
20 helps the separation of these beers during colorimetric characterisation.

The PCA arrays were manufactured according to the method in example 2. The two arrays were:

- 25 **PCA array (1) (Figure 9A):** 5 non-CD sensors were used: T, T+T-SH (1:1), T+T-PEG1 (1:1), T+T-PEG-SH (1:1), T+T-PEG-Arg (1:1).

PCA array (2) (Figure 9B): 5 non-CD combined with 5 CD sensors were used: The 5 non-CD were the same as in PCA array (1) and the CD sensors were: T+T-alfa-CD
30 (1:1), T+T-beta-CD (1:1), T+T-gamma-CD (1:1), T+T-beta-CD (8:2), and P+P-beta-CD (1:1).

Each sensor had 8 valid repeats, that's the 8 small dots you see in the PCA for each beer. In both figures, principle component 1 (Dim1) and component 2 (Dim2) are used.

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- 5 • Xiaomin Qian, Carina L. Gargalo, Krist V. Gernaey, and Brigitte Städler
"Distinguishing Commercial Beers Using a Solution-Based Sensor Array Derived from Nanoscale Polydiacetylene Vesicles", *ACS Applied Nano Materials*, 3, 2020, pp. 3439-3448.

Claims

1. A method for characterizing a sample for at least one analyte comprising the steps of

- 5 a) providing a sensor array comprising at least two different poly-diacetylenes,
- b) contacting said sensor array with said sample,
- 10 c) measuring a colorimetric response of said poly-diacetylenes after contact with said sample,

wherein said poly-diacetylenes are polymerised from a composition comprising one or more diacetylene monomers, said diacetylene monomers comprising one

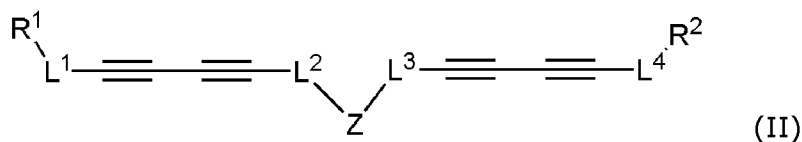
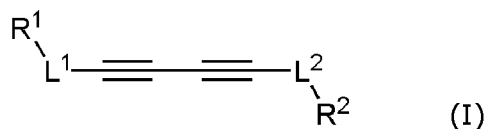
- 15 or more substituents selected from the group consisting of an optionally substituted C₁-C₃₀ alkyl, an optionally substituted C₂-C₃₀ alkenyl, and an optionally substituted C₂-C₃₀ alkynyl,

wherein at least one poly-diacetylene is polymerised from a composition comprising a diacetylene monomer comprising a cyclodextrin derivative, and

- 20 wherein at least one analyte is an ester or phenolic flavour compounds.

2. The method according claim 1, wherein said one or more diacetylene monomers are selected from the group of diacetylenes according to formula (I) or (II)

25



30

or mixtures thereof, wherein

L¹, L², L³ and L⁴ are the same or different and individually selected from the group consisting of an optionally substituted C₁-C₃₀ alkylene, an optionally substituted C₂-C₃₀ alkenylene, and an optionally substituted C₂-C₃₀ alkynylene,

5 R¹ and R² are the same or different and individually selected from the group consisting of -CH₃, -OR³, -SR³, -COOR³, -CONR⁴R⁵, wherein R³, R⁴, and R⁵ are individually selected from the group consisting of hydrogen, C₁-C₈ alkyl optionally substituted with a thiol, vinyl, or optionally substituted imidazolium, a polyethylene glycol alkyl ether optionally substituted with a thiol, vinyl, amino acid, or optionally substituted imidazolium, and a cyclodextrin derivative; or are selected so that NR⁴R⁵ constitutes an amino acid,

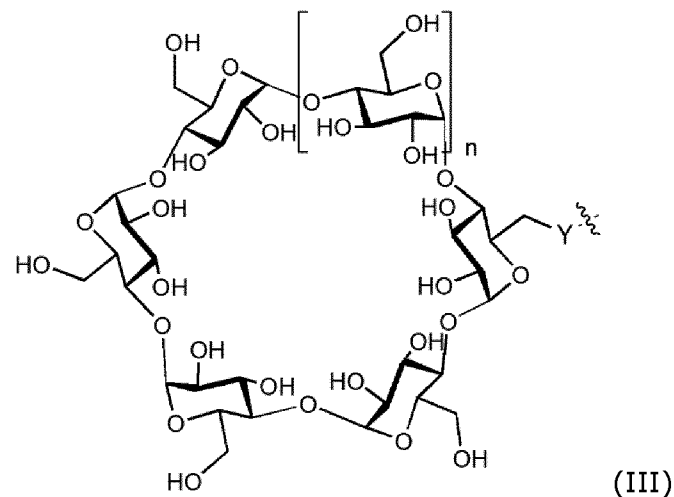
10 Z is selected from the group consisting of optionally substituted alkylene, aryl, -CONH-(CH₂)_x-HNCO- where X is an integer between 1 and 20, and heteroaryl,

15 with the proviso that at least one poly-diacetylene comprises a diacetylene monomer wherein at least one of R³, R⁴, and R⁵ is a cyclodextrin derivative.

3. The method according to claim 2, wherein L¹, L², L³ and L⁴ are the same or different and individually selected from a -(CH₂)_n- group wherein n is 1-30, such as 1-20, 1-18, 1-15, such as preferably 1-12.

4. The method according to any one of claims 1-3, wherein the cyclodextrin derivative is an optionally substituted *alpha*-cyclodextrin, *beta*-cyclodextrin, or 25 *gamma*-cyclodextrin.

5. The method according to any one of claims 2-4, wherein the diacetylene monomer comprising a cyclodextrin derivative is a diacetylene according to Formula (I) or (II),
30 wherein R¹ and/or R² is selected from -COOR³ and -CONR⁴R⁵, and wherein one of R³, R⁴, and R⁵ is a substituent according to formula (III):



wherein

5 n is an integer selected from 1, 2, and 3,

Y is selected from the group consisting of a bond, a C_1 - C_8 alkylene amide (-alkylene- $C(O)NH$ -), a C_1 - C_8 alkylene ester (-alkylene- $C(O)O$ -), and $-Q-(CH_2-CH_2-O)_m-$ wherein Q is an ester or an amide, and m is an integer in the range of 1-20.

10

6. The method according to any one of claims 1-5, wherein said poly-diacetylenes are spatially separated and individually addressable.

15 **7.** The method according to any one of claims 1-6, wherein the poly-diacetylene polymers are vesicles or micelles, preferably vesicles.

8. The method according to any one of claims 1-6, wherein the poly-diacetylene polymers are positioned on a solid support.

20 **9.** The method according to any one of claims 1-8, wherein the at least one analyte, are flavour constituents of a beverage or a beverage precursor, preferably of a beer or a beer precursor.

10. The method according to claim 9, wherein the flavour constituent is selected
25 from the group consisting of ethanol, carbonic acid, hop bitter substances (such as trans-isohumulone), hop oil constituents (such as myrcene, humulene,

oxygenated humulenes), maltol, monosaccharides, disaccharides, banana esters (such as 3-methylbutyl acetate, 2-methylpropyl acetate), apple esters (such as ethyl hexanoate and ethyl octanoate), 3-methylbutanol, dimethyl sulfide, C₆-C₁₂ fatty acids (such as octanoic acid), acetic acid, propanoic acid, ethyl acetate, 2,3-
5 butanedione, citric acid, maleic acid, polyphenols (such as leucocyanidin), trisaccharides (such as maltotriose), amino acids (such as proline), diacetyl acetylpropionyl, acetaldehyde, isobutyl acetate, propanol, isobutanol, isoamyl acetate, isoamylalcohol, ethyl caproate, ethyl caprylate, 2-phenylethyl acetate, caprylic acid, caproic acid, capric acid, linalool, limonene, pentanedione, λ-
10 decalactone, 2-phenylethanol, trans-2-nonenal, 4-vinylguaiacol (4-VG), 4-vinyl phenol, 4-ethyl guaiacol, 4-ethyl phenol, 4-Propenyl guaiacol, eugenol, tyrosol, 4-Propyl syringol, hydrogen sulfide, 3-methyl-2-butene-1-thiol, and sodium chloride.

11. The method according to any one of claims 1-10, wherein the ester is selected
15 from the group consisting of ethyl acetate, 2-phenylethyl acetate, ethyl hexanoate, isoamyl acetate, and ethyl octanoate.

12. The method according to any one of claims 1-10, wherein the phenolic flavour compound is selected from the group consisting of 4-vinylguaiacol (4-VG), 4-vinyl
20 phenol, 4-ethyl guaiacol, 4-ethyl phenol, 4-Propenyl guaiacol, eugenol, tyrosol, 4-Propyl syringol.

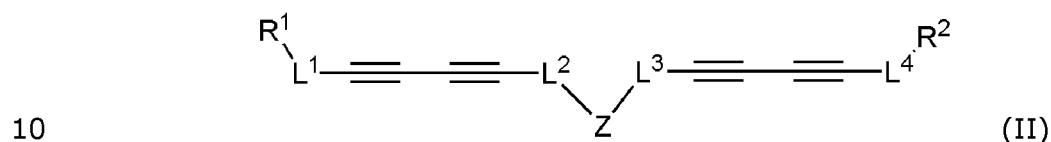
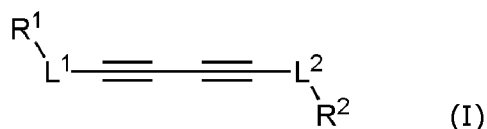
13. The method according to any one of claims 1-12, wherein the sample is selected from the group consisting of a beverage or a beverage precursor, a food
25 or feed item, aqueous industrial waste, sewage, non-human biological samples, blood plasma, urine, and saliva, preferably a beverage or beverage precursor.

14. The method according to claim 13, wherein the beverage is selected from the group consisting of beer or a beer precursor, cider, white wine, rosé wine, red
30 wine, dairy products, soft-drinks, alcopops and precursors thereof, most preferably beer or a beer precursor.

15. A diacetylene monomer comprising one or more substituents selected from the group consisting of an optionally substituted C₁-C₃₀ alkyl, an optionally
35 substituted C₂-C₃₀ alkenyl, and an optionally substituted C₂-C₃₀ alkynyl,

wherein said diacetylene monomer comprises an *alpha*-cyclodextrin derivative or *gamma*-cyclodextrin derivative.

- 16.** The diacetylene monomer according to claim 15, wherein the monomer is a
5 compound according to formula (I) or (II)



wherein

- L^1 , L^2 , L^3 and L^4 are the same or different and individually selected from the
group consisting of an optionally substituted C_1 - C_{30} alkylene, an optionally
15 substituted C_2 - C_{30} alkenylene, and an optionally substituted C_2 - C_{30}
alkynylene,

- R^1 and R^2 are the same or different and individually selected from the
group consisting of $-\text{CH}_3$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{COOR}^3$, $-\text{CONR}^4\text{R}^5$, wherein
 R^3 , R^4 , and R^5 are individually selected from the group consisting of
20 hydrogen, C_1 - C_8 alkyl optionally substituted with a thiol, vinyl, or optionally
substituted imidazolium, a polyethylene glycol alkyl ether optionally
substituted with a thiol, vinyl, amino acid, or optionally substituted
imidazolium, and a cyclodextrin derivative; or are selected so that NR^4R^5
constitutes an amino acid,

- Z is selected from the group consisting of optionally substituted alkylene,
25 aryl, $-\text{CONH}-(\text{CH}_2)_x-\text{HNCO}-$ where X is an integer between 1 and 20, and
heteroaryl,

- with the proviso that at least one R^1 and R^2 is selected from the group consisting
of $-\text{OR}^3$, $-\text{SR}^3$, $-\text{COOR}^3$, $-\text{CONR}^4\text{R}^5$, and at least one of R^3 , R^4 , and R^5 is an α -
30 cyclodextrin derivative or γ -cyclodextrin derivative.

17. A sensor array comprising at least two different poly-diacetylenes, wherein at least one poly-diacetylene is polymerised from a diacetylene monomer according to any one of claims 15-16.

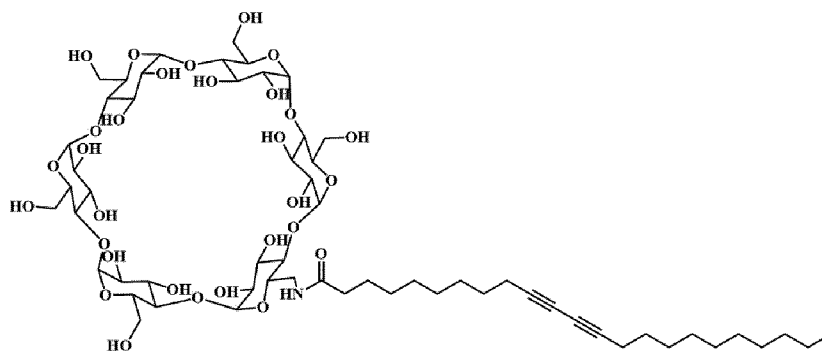
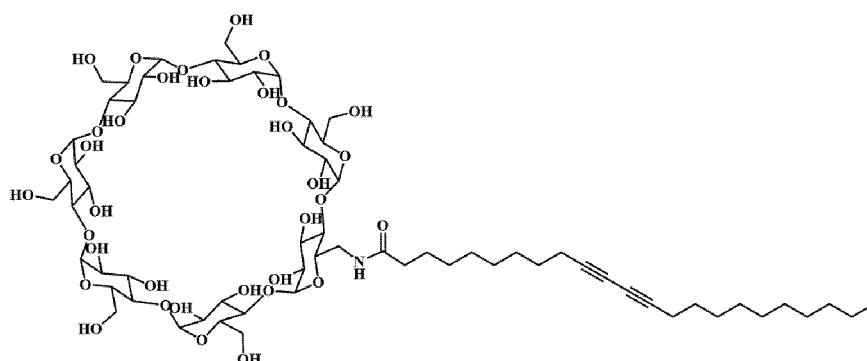
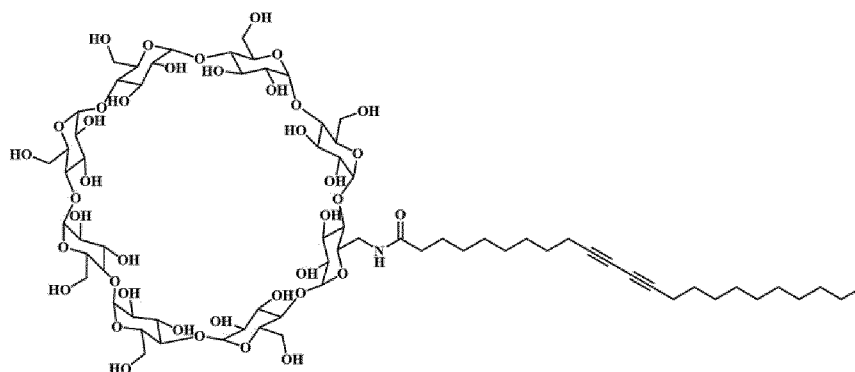
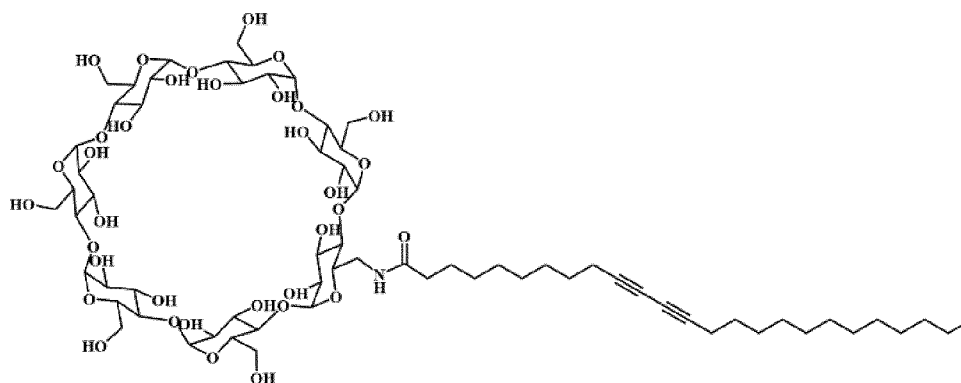
A)**B)****C)****D)**

Fig. 1

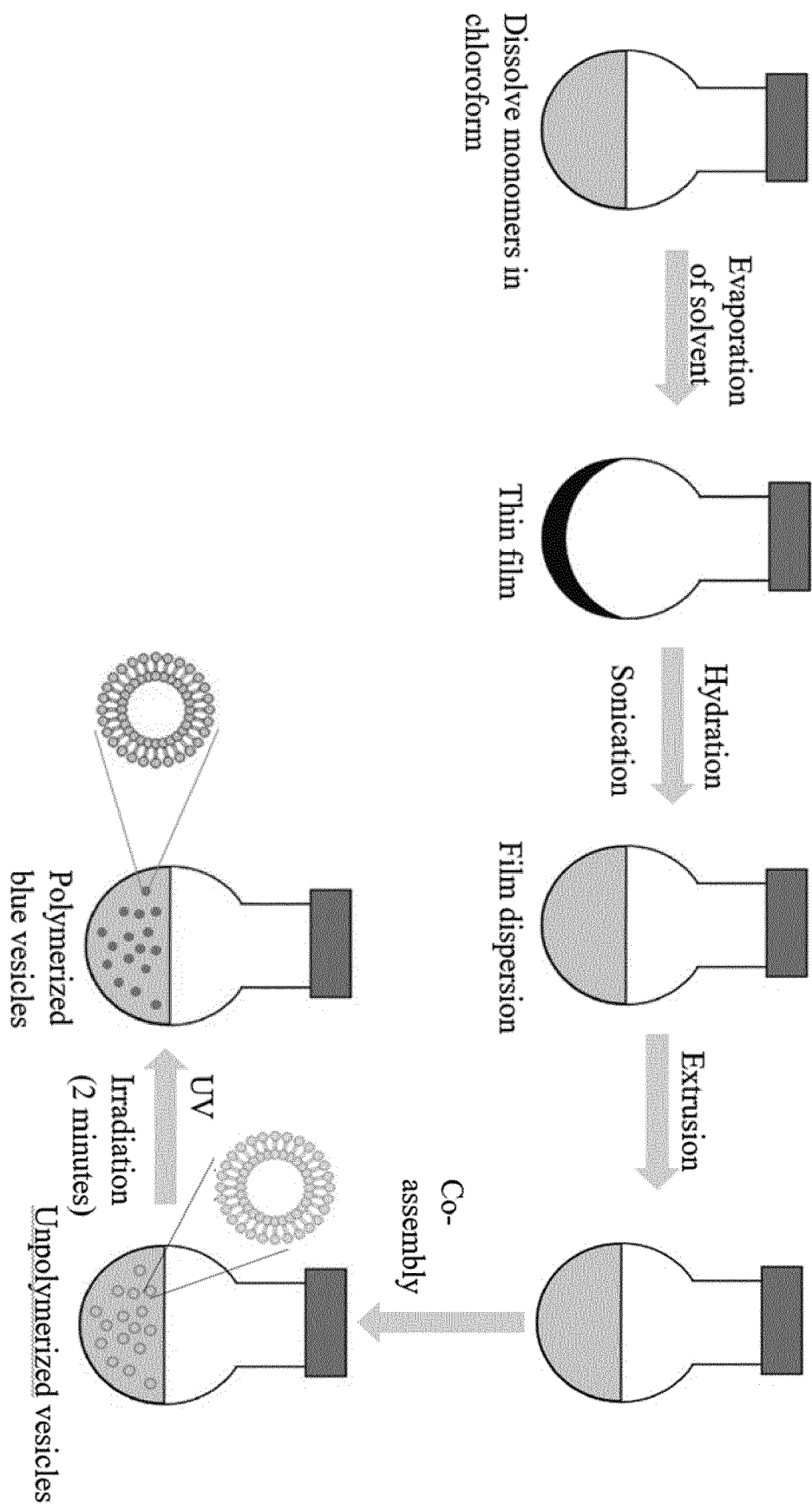


Fig. 2

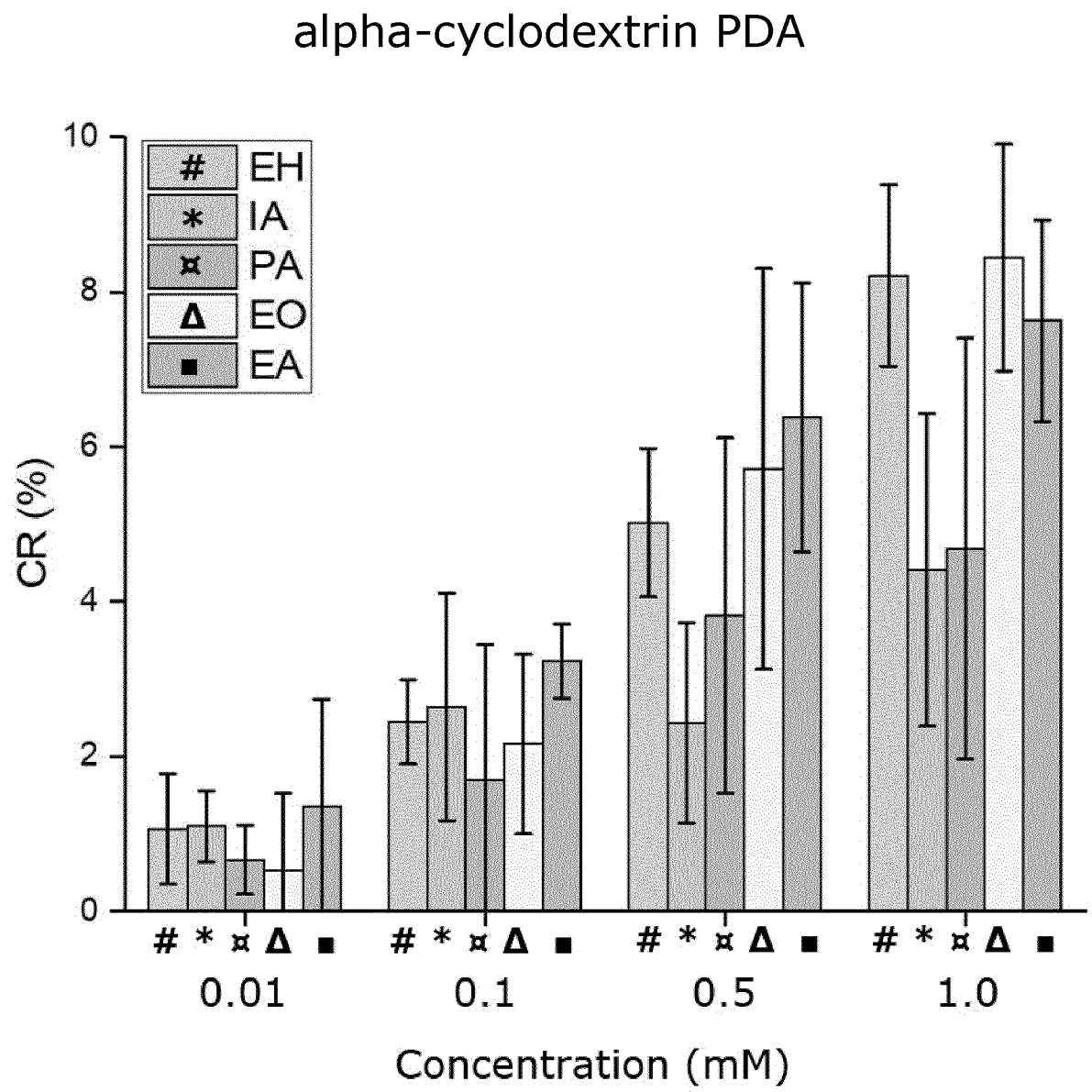


Fig. 3

beta-cyclodextrin PDA

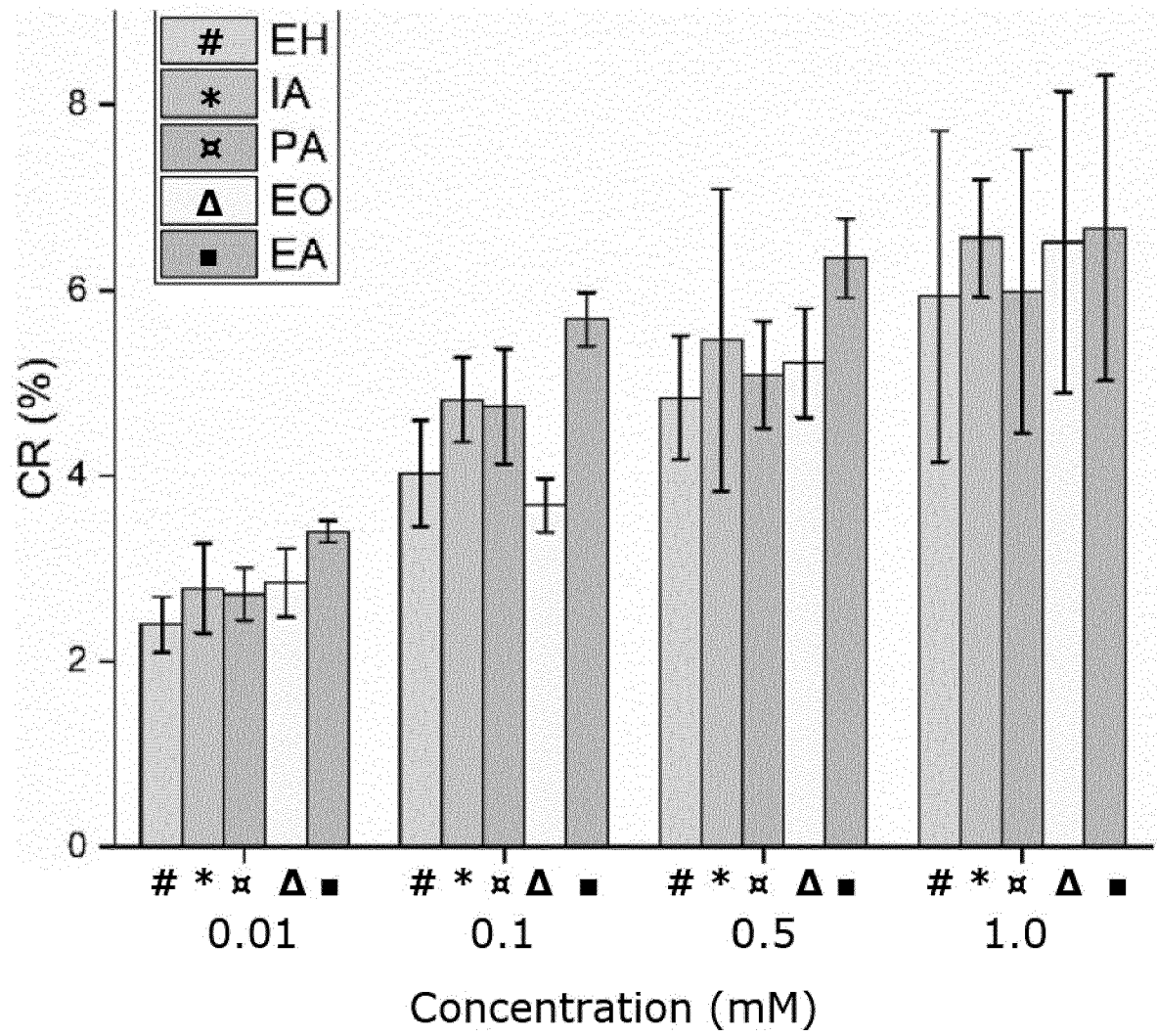


Fig. 4

gamma-cyclodextrin PDA

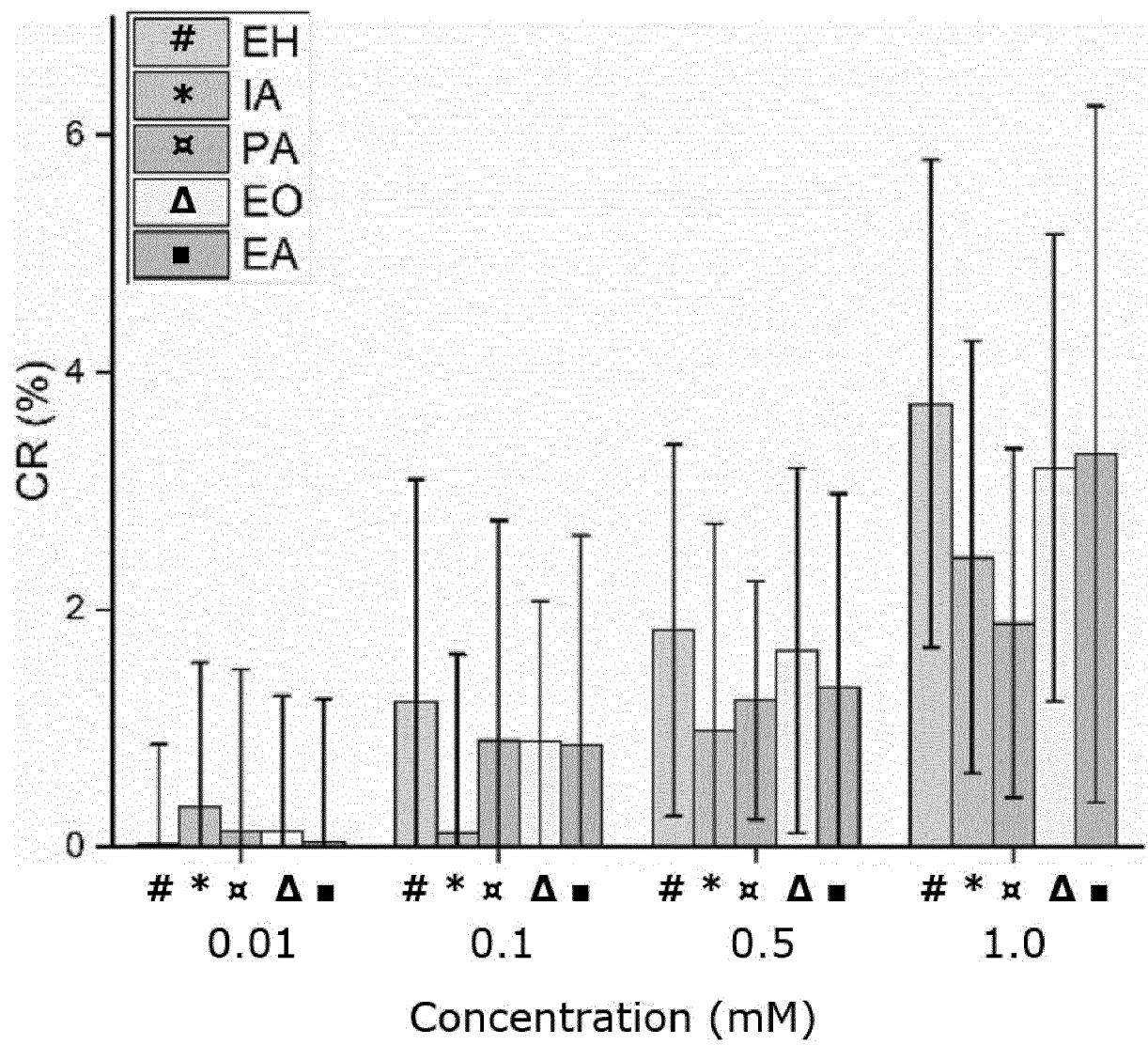


Fig. 5

CR against 4-vinyl guaiacol (4-VG)

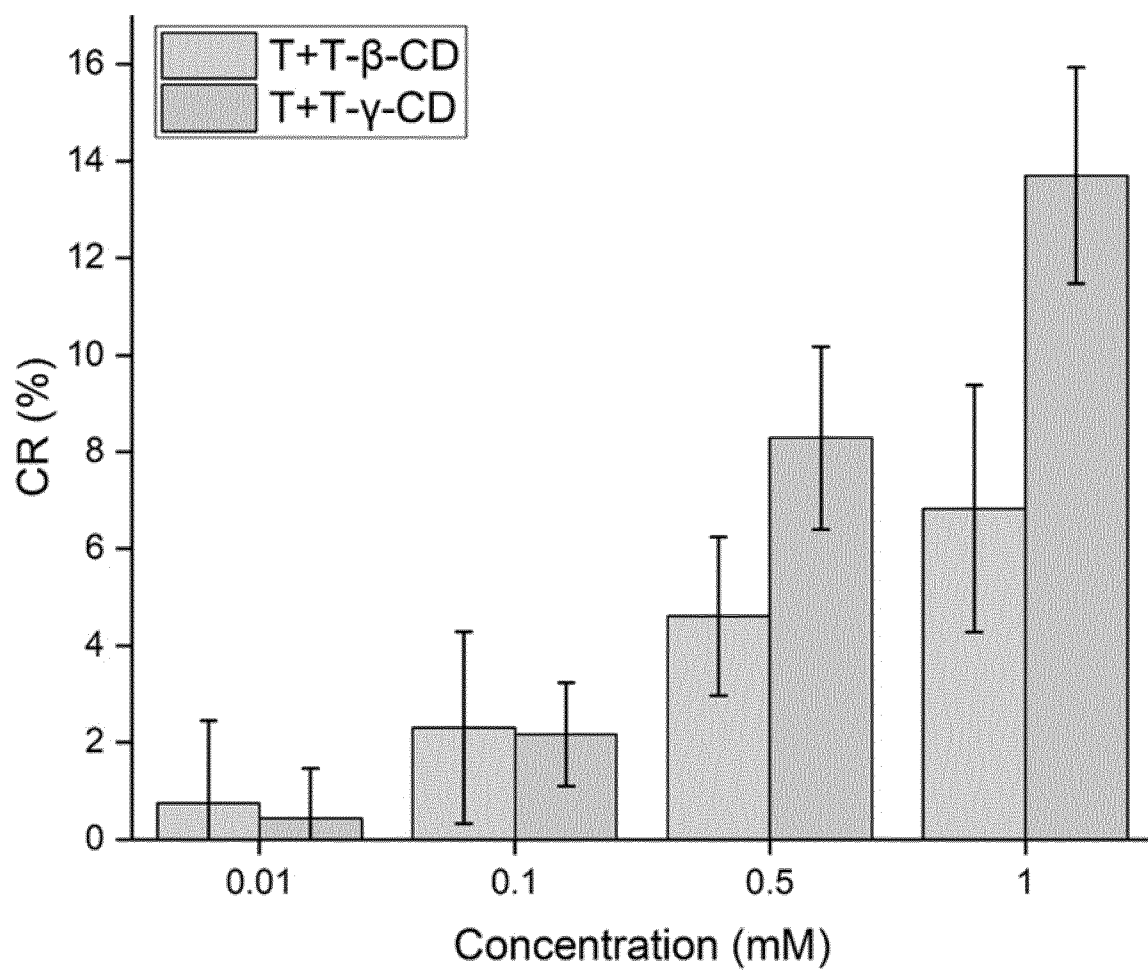


Fig. 6

CR against Ethanol

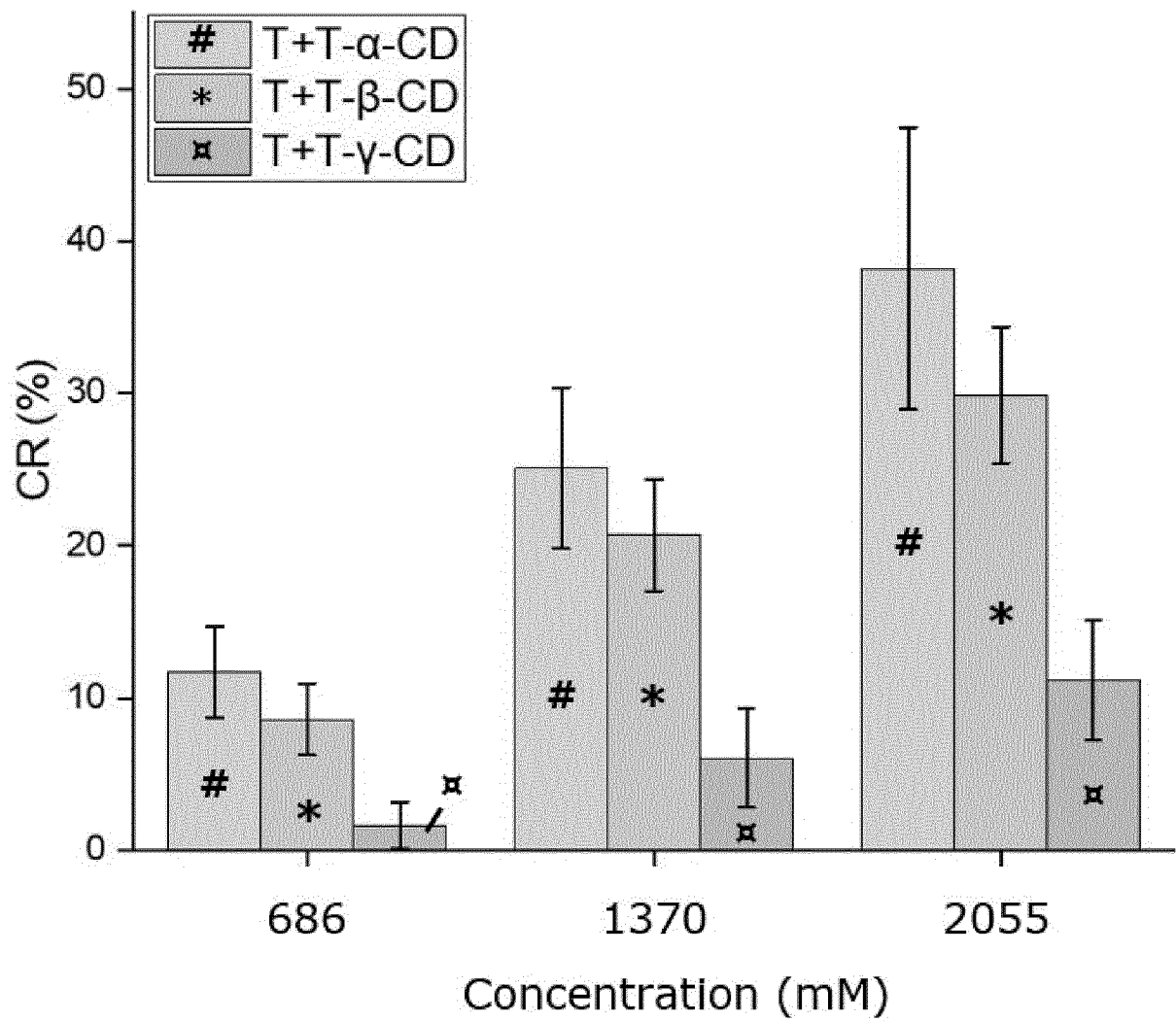


Fig. 7

CR against Ethyl Acetate (EA)

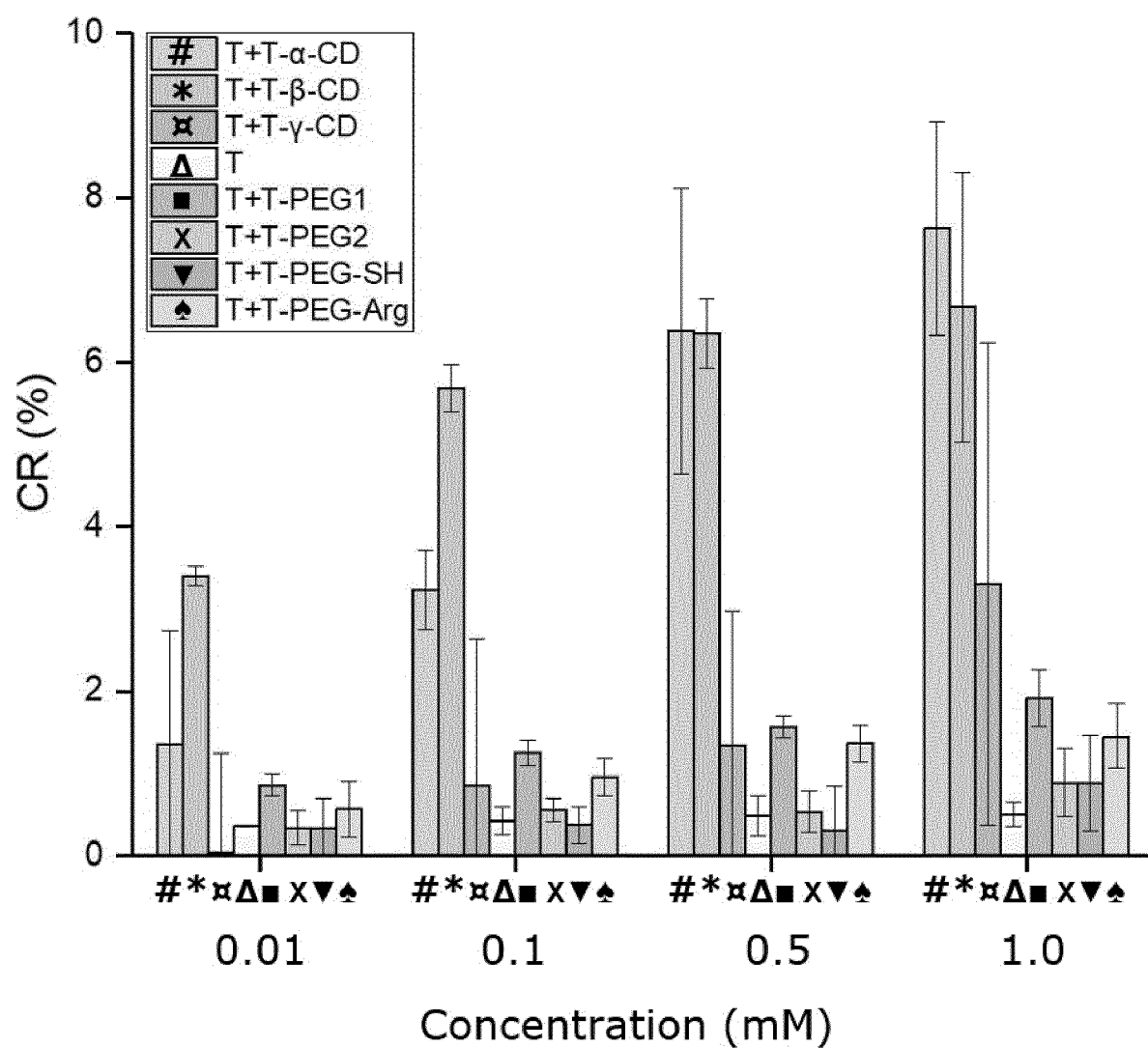


Fig. 8

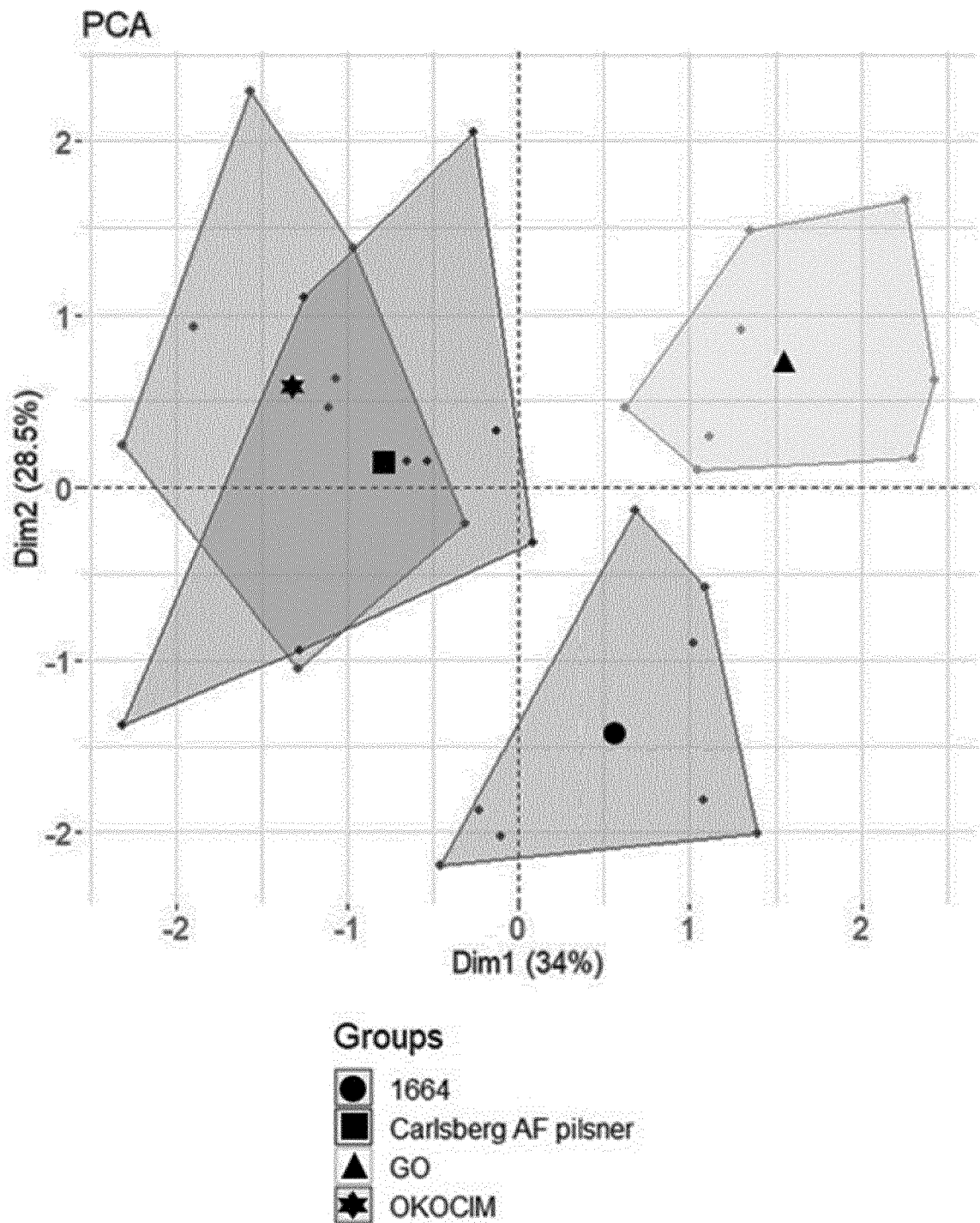


Fig. 9A

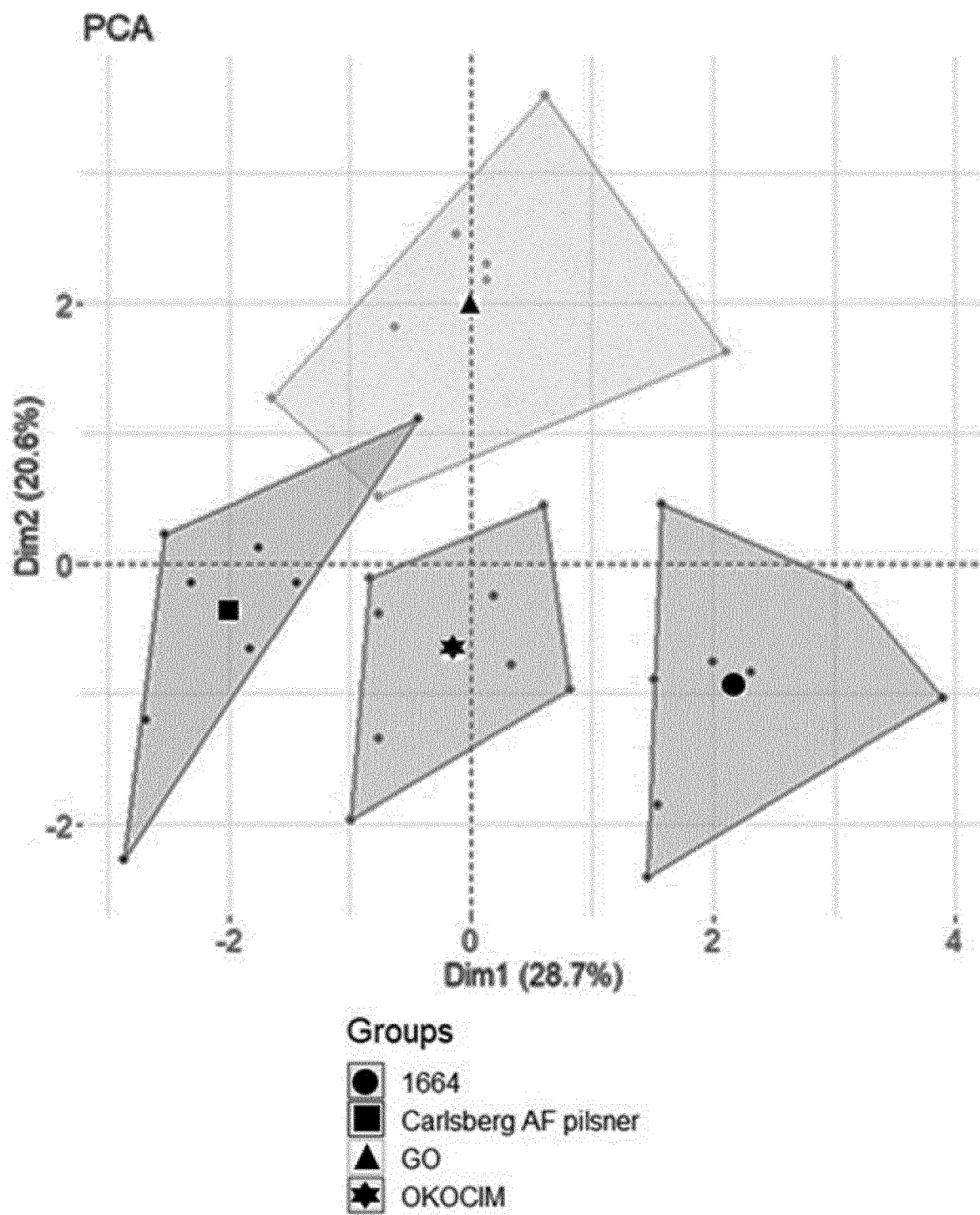


Fig. 9B

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2021/082965

A. CLASSIFICATION OF SUBJECT MATTER INV. G01N33/52 C08F38/02 G01N33/14 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) G01N C08F		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHO EUNAE ET AL: "Supramolecular self-assembled aggregates formed by pentacosa-10,12-diynyl amidomethyl-[beta]-cyclodex", CARBOHYDRATE RESEARCH, PERGAMON, GB, vol. 391, 8 April 2014 (2014-04-08), pages 37-42, XP028650640, ISSN: 0008-6215, DOI: 10.1016/J.CARRES.2014.03.022	15,16
Y	p. 38, col. 1, par.1; Scheme 1 ----- --/--	1-14,17
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 18 February 2022		Date of mailing of the international search report 01/03/2022
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Hohwy, Morten

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2021/082965

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHO EUNAE ET AL: "Polydiacetylenyl [beta]-cyclodextrin based smart vesicles for colorimetric assay of arginine and lysine", SCIENTIFIC REPORTS, vol. 6, no. 1, 1 August 2016 (2016-08-01), XP55891338, DOI: 10.1038/srep31115 Retrieved from the Internet: URL: http://www.nature.com/articles/srep31115	1-9, 13, 15-17
Y	abstract; Fig. 5; "Methods" -----	10-12, 14
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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