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(54) Title: KIT FOR COLORIMETRIC DETERMINATION OF CHLORINE LEVELS IN SAMPLES OF RECREATIONAL WATER

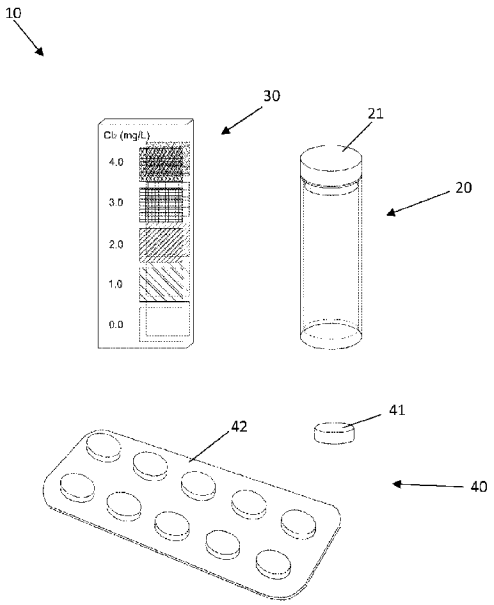

FIG. 3

(57) Abstract: A kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes comprises a liquid container (20) suitable for housing a predetermined amount of liquid of which the concentration of active chlorine is to be measured, a colorimetric reference scale (30), and a chlorine indicator reagent (40) designed to be placed in said container (20) in a specified amount. The reagent contains a weighted amount of TMB so that the whole recommended range of values of chlorine concentration in water is shown by the change in at least three different colours of the solution comparable with corresponding colours of the reference scale (30), so that visual evaluation of the measurement results with the naked eye is simple, accurate and rapid.

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[Continued on next page]

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DISCLOSURE OF THE INVENTION

TITLE

KIT FOR COLORIMETRIC DETERMINATION OF CHLORINE LEVELS IN SAMPLES OF RECREATIONAL WATER

TECHNICAL SECTOR

[0001] The present invention relates to a kit for the colorimetric determination of chlorine levels in recreational waters using the synthetic molecule 3,3',5,5'-tetramethylbenzidine (TMB).

STATE OF THE ART

[0002] Chlorine is widely used for the oxidative inactivation of numerous microorganisms and disease-causing pollutants in water treatment, food preservation and other cleaning procedures. In all cases, the presence of residual chlorine in certain concentrations is necessary to ensure protection from recontamination. Chlorine water treatment is effective and has very low commercial costs. Water treatment with chlorine is effective and has very low commercial costs.

[0003] Colorimetry is one of the most widely used techniques for the quantitative measurement of chlorine in water used for recreational purposes such as swimming pools, water parks or similar.

[0004] The most widely used commercial products used in this respect contain either the yellow dye containing Ortho-Tolidine (OTO), or the pink dye containing Diethyl-Para-Phenylene-Diamine (DPD). The main limitation of commercial tests based on OTO and DPD is that they are monochromatic: the chlorine level in the water is estimated visually with high approximation by comparing the colour intensity obtained from the test with a monochromatic reference scale.

[0005] The synthetic molecule 3,3',5,5'-tetramethylbenzidine (TMB) is also known to be used for the same purposes. This molecule is converted by chlorine into a coloured product through a pH-dependent multi-step oxidation process in which the colour and colour intensity of the TMB solution depend on the total amount of chlorine in the sample. The use of TMB offers several advantages over other commonly used reagents in terms of safety and sensitivity.

[0006] In US 5811254, a mixture, a method and a device for a test intended for determining the concentration of chlorine available in a water sample are described. The patent claims that the indicator capable of detecting the amount of chlorine is contained in a mixture having a pH between 5 and 6. The possibility to use the TMB molecule as an indicator of chlorine concentration is mentioned but no details on how to use it are given as the subject of the patent is the buffer used in the mixture. Therefore, the patent does not suggest any optimised composition for the measurement of residual chlorine in swimming pool water or water used for recreational purposes in general, moreover it has high costs and environmental impact due to the necessary presence of catalysts and surfactants, which are required for the strip formulation of the indicator.

[0007] In US 5783149, colorimetric methods and measurement kits are described for the determination of free and combined residual chlorine, in water, which include the use of TMB. The main limitation of the methods and measurement kits mentioned above is that they are monochromatic as the formulations used are such that an orange colour is obtained, which is visually compared with a stable reference scale. Another major limitation of the technology described is that it operates at very low pH levels, which are potentially irritating. In addition, the measurement kits described involve the use of the indicator in liquid solution with the limitations that this entails.

[0008] US 5972713 also discloses the use of TMB for the determination of total chlorine in a water sample and a measurement kit consisting of the indicator itself and a reference colorimetric scale. The document mentions that the range of detectable chlorine concentration can be modified by changing the concentration of TMB in the indicator. A limitation of the described method is that blue, green, yellow and orange or red are used as reference colours taken from the analysed sample to determine the chlorine concentration in the sample. However, the use of orange and red greatly reduces the reliability of the measurement, as beyond a certain molar ratio between chlorine concentration and TMB concentration there is no longer a direct proportionality between the increase in chlorine concentration and the change in colour shade. Furthermore, the described ratio between the moles of chlorine and TMB and the supposed colour obtained is empirical and in disagreement with the established molecular oxidation equilibria of TMB reported by Josephy DP.

et al., "FREE RADICAL AND CHARGE-TRANSFER COMPLEX INTERMEDIATES", 1982, according to which each mole of molecular chlorine reacts completely with one mole of TMB. Finally, the measurement kits described here also contain the indicator in liquid solution.

[0009] In view of the above-mentioned limitations, a kit for the determination of the concentration of chlorine in water prepared by means of the teachings of the above-mentioned patent can only give a qualitative indication, since no direct and precise link can be established between the absolute amount of chlorine in the water and the colour of the sample analysed.

[0010] A further document from which substantially the same teachings can be derived but which shows the same limitations as the previous document is CN 203 965 329 U. This patent describes an apparatus for measuring the concentration of chlorine in which there is a container of a TMB stock solution, a TMB dispenser, and a chamber containing a sample of water to be analysed wherein a quantity of TMB contained in the container of the stock solution is transferred through said dispenser and suitable valves. The document never defines the composition of the TMB stock solution and, most importantly, it never mentions the criteria on which the amount of TMB to be released into the container of the sample to be analysed is defined. For this reason, as already mentioned, this document does not teach how to make a device for measuring the concentration of chlorine in water that provides not only qualitative but also quantitative information that can be easily detected by naked eye.

SUMMARY OF THE INVENTION

[0011] It is therefore an object of the present invention to provide a kit for the colorimetric determination by the synthetic molecule 3,3',5,5'-tetramethylbenzidine (TMB) of the level of chlorine in water used for recreational purposes which is capable of overcoming the above discussed limitations of the systems of known technique.

[0012] It is a further object of the present invention to provide a kit for the colorimetric determination by TMB of the level of chlorine in water used for recreational purposes with high accuracy.

[0013] Additionally, it is an object of the present invention to provide a kit for the colorimetric determination by TMB of the chlorine level in water used for recreational purposes that has very low cost.

[0014] Another object of the present invention to provide a kit for the colorimetric determination by TMB of the chlorine level in recreational waters having minimal health risks and minimal environmental impact.

[0015] It is a further object of the present invention to provide a kit for the colorimetric determination of chlorine levels in recreational waters by TMB that shows prolonged shelf-life and low packaging costs.

[0016] The above-mentioned purposes and others are achieved by means of a kit for colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to claim 1. The kit of the invention comprises: a container suitable for housing a predetermined amount of liquid of which it is necessary to measure the concentration of active chlorine; a reference colorimetric scale; and a chlorine indicator reagent (40) provided to be inserted into said container (20) in a given amount, wherein said given amount of reagent contains a weighted amount of TMB equimolar to the molecular chlorine contained in the container when the container is filled with water in which a predetermined maximum recommended concentration of chlorine is present.

[0017] The key element of the present invention lies in the appropriate and novel setting of the reaction conditions, which show for the first time that it is possible to precisely adjust the colour change due to the presence of TMB to exactly cover the range of chlorine concentrations recommended by international health agencies for drinking water and swimming pool water treatment.

[0018] The provision of a quantity of TMB equal to the maximum amount of chlorine that must be present in the measuring container allows the chlorine concentration in water to be determined simply and accurately by naked eye, as a yellow colour will indicate the presence of the maximum permissible amount of chlorine, a green colour will indicate the presence of an intermediate and optimal chlorine concentration and a blue colour will indicate a minimum chlorine

concentration sufficient to achieve correct disinfection. In addition, all intermediate shades between blue and yellow, which are immediately recognisable due to the significant differences in colour, will be directly proportional to the increase in chlorine concentration between these minimum and maximum values.

[0019] In contrast to single-colour intensity estimation, which occurs following the known technique based on TMB (yellow or blue) or DPD (magenta), the present invention enables naked-eye quantification of chlorine by means of multi-coloured variations of the TMB solution. The present approach for the first time proposes TMB as a universal indicator for a selected range of typical chlorine concentrations for drinking water or swimming pool. The scale proceeds from a minimum blue level for effective disinfection, through a green colour corresponding to an optimum of concentration, up to the safety level according to specific guidelines corresponding to a yellow colour, which results from chlorine and TMB in an equimolar ratio.

[0020] Further advantageous features of a kit as outlined above are expressed in the dependent claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] Further features and/or advantages of the present invention will become clearer with the following description of an embodiment thereof, made by way of example and not limitation, with reference to the accompanying drawings in which:

- Figure 1 shows the sequence of the TMB oxidation reaction;
- Figure 2 shows the absorbance at three distinctive wavelengths of the TMB solution in water in the presence of chlorine for various chlorine concentrations;
- Figure 3 shows schematically an embodiment of a kit for colorimetric determination by TMB of the concentration of active chlorine in drinking or recreational water according to the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0022] Referring to Fig. 1, the synthetic molecule 3,3',5,5'-tetramethylbenzidine (TMB), denoted (A) produces an uncoloured solution in the reduced state (diamine, λ_{max} at 210 and 285 nm) which turns blue on oxidation (λ_{max} at 370 nm and 652 nm). In particular, the blue-coloured product,

denoted (C), has been characterised as the diamine/diimine charge transfer complex in equilibrium with both the cation radicals, denoted (B), and the mixture of diamine/diimine. Further oxidation leads to a green stage, which is a mixture of the blue and yellow endpoints. The yellow colour is the end-point of the two-electron oxidised state (diimine, λ_{max} at 450 nm), denoted by the letter (D) (Josephy et al., 1982; Bally and Gribnau, 1989).

[0023] With reference to Fig. 2, it is possible to follow all the reaction steps described above exploiting the full potential of the TMB reaction with active chlorine by titration, in plates or cuvettes, without enzymatic catalysts, but by using a fixed amount of TMB in slightly acidic buffer in which chlorine is present mainly as HOCl. In detail, the absorbances at 280 nm, 450 nm (yellow), 655 nm (blue) obtained using a spectrophotometer are shown. The absorbance at 280 nm decreases together with the accumulation of chlorine. The titration curve at 655 nm shows the formation and subsequent disappearance of the blue product with a bell-shaped trend symmetrical to the midpoint corresponding to 0.5 mol HOCl / mol TMB, and the titration curve at 450 nm shows the formation of the yellow product with an almost sigmoidal trend.

[0024] A yellow solution is thus obtained when the concentration of the oxidising agent is equimolar with the TMB. Furthermore, the titration curve of TMB in water in the presence of chlorine shows that the formation of the blue product and its subsequent destruction are almost symmetrical around the mid-point where the concentration of the charge transfer complex is highest. Thus, the blue colour of the solution is obtained when the molar concentration of molecular chlorine is about half that of the TMB. When the concentration of chlorine in relation to TMB is below this threshold, the solution gradually turns from transparent to blue through light blue. When the concentration of chlorine in relation to TMB exceeds this threshold, the green colour begins to appear. The shape and height of the titration curve are determined by the equilibrium constant of the charge transfer complex formation and its molar attenuation coefficient.

[0025] This reaction sequence is sensitive to several factors, such as pH, solvent, temperature, which influence the π-conjugation in each step and the stability of the charge transfer complex. In order to obtain a solution that adheres as closely as possible to the behavioural patterns described above, the pH should be kept as constant as possible within certain values. Although strict

pH control is not necessary in absolute terms, the pH range affects the development of colour in the solution. In detail, a pH from 1 to 2 allows to identify the formation of TMB-diimine (yellow colour) by measuring the absorbance at 450 nm ($\varepsilon_{450nm}^{TMB-D}$= 59 103 M^{-1} cm^{-1}); whereas a pH in the range from 3.8 to 5.8 allows the formation of the TMB charge-transfer complex (blue) to be more clearly identified. The use of a slightly acidic buffer provides the best conditions for measuring the chlorine content in real samples by monitoring all stages of non-catalytic oxidation of the TMB. Indeed, it has been shown that a pH of less than 2 prevents the formation of the blue TMB charge transfer complex in favour of only the formation of yellow TMB-diimine. Similarly, it has been verified that the formation of the charge transfer complex is significantly reduced at a pH above 6. Hence, a buffer capable of stabilising the solution at pH 4.5 is advantageously used.

[0026] The disclosure described above is advantageously exploited in a kit according to the present invention for the colorimetric determination by TMB of the chlorine level in water used for recreational purposes. To achieve effective disinfection, chlorine (in the form of chlorine gas or salt) should be added to drinking water to reach a minimum level of 0.2-0.5 mg L^{-1} (expressed as Cl_2) and the residual concentration should not fall below these values, but should remain below 4-5 mg L^{-1} to avoid eye/nose irritation and stomach upset (WHO, 2011; EPA, 2019). The maximum recommended concentration of residual chlorine in drinking water and swimming pool water is about 4 mg L^{-1}. A kit according to the present invention is therefore designed by adjusting the concentration of TMB so that it is in a stoichiometric ratio of 1/1 for the oxidant/substrate system. Thereby the kit allows both spectroscopic and visual detection of the chlorine concentration by changing the colour of the solution from blue to yellow, passing through green, while remaining within the range of chlorine concentrations recommended for swimming pool water.

[0027] To achieve the above purpose, with reference to Fig. 3, a kit, 10, is hereby provided for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes. The disclosed kit comprises a container, 20, for housing a predetermined amount of liquid, a colorimetric reference scale, 30, and a chlorine indicator reagent, 40, prepared to be placed in said container in a specified amount. Specifically, said given amount of reagent contains a weighted amount of TMB chosen to be equimolar to the molecular chlorine that

should be contained in said container 20, when said container is filled with water in which a certain maximum recommended concentration of residual chlorine, i.e. around 4 mg L^{-1}, is present.

[0028] The container 20 is a transparent container with a capacity of a few millilitres, provided with a cap, 21, and is entirely analogous to the containers used in the kits currently available on the market, which use DPD or OTO solutions as reagents.

[0029] According to a preferred embodiment of the present invention, the indicator reagent 40 is in the solid form of single-dose tablets, 41, which can advantageously be sold in blisters, 42. A single-dose indicator tablet 41 contains a quantity of TMB equivalent to 4.0 mg L^{-1} active chlorine and a suitable buffer to maintain the solution at a pH between 4.0 and 8.0, advantageously from 4.0 to 5.0, and to prevent interference with the correct measurement by Fe (III) that may be present in the solution. In a preferred embodiment, the buffer consists of citric acid / sodium citrate, or a pyrophosphoric salt and more precisely disodium diphosphate ($Na_2H_2P_2O_7$) or tetrasodium diphosphate ($Na_4P_2O_7$) in the presence of acetic acid / sodium acetate.

[0030] By means of a kit as described above the measurement of chlorine concentration in pool water is extremely simple and accurate.

[0031] The container 20 is filled with pool water, a single-dose indicator tablet 41 is added, and is closed with the cap 21 so that the container is substantially full or a liquid level indicated on the container is reached. After briefly shaking the container to dissolve the tablet and promote the oxidation reaction, the results of the measurement can be read within about one minute by comparing the colour of the solution inside the container with the reference colour scale 30.

[0032] The container 20 is filled with pool water, a single-dose indicator tablet 41 is added, and the container is closed with the cap 21 so that it results substantially full or a liquid level indicated on the container is reached. After briefly shaking to dissolve the tablet and promote the oxidation reaction, the results of the measurement can be read within about one minute by comparing the colour of the solution inside the container with the reference colour scale 30.

[0033] The reading is extremely simple and precise. In fact, by virtue of the studied choice of the amount of TMB in the single-dose indicator tablet 41, a yellow colour of the solution corresponds to the maximum recommended concentration of active chlorine, a blue colour corresponds to the

minimum concentration of active chlorine required for efficient water disinfection; whereas a green colour and all shades leading from blue to green and from green to yellow indicate that the concentration of active chlorine in the water is within the optimum range. A transparent or light blue colour indicates too low a concentration of active chlorine, while orange or red colours indicate too high levels of potentially dangerous chlorine. The different colours identifying the limit values of the recommended chlorine concentration range allow immediate and reliable visual identification of the result. In addition, a kit 10 as described above, due to the exact weighting of the indicator quantity is easy to produce in large scale in order to obtain reproducible and therefore reliable measurement results.

[0034] Furthermore, since the concentrations of chlorine to be measured are relatively low, a kit according to the present invention allows the employment of very small quantities of reagent leading to various advantages including lower production costs, lower health risks and reduced risks to the environment.

[0035] The use of a solid-state reagent, particularly in the form of single-dose tablets 41 shows considerable advantages, in relation to not only the possibility of pre-dosing exactly the quantity of indicator according to the capacity of the container 20 and the maximum recommended concentration of chlorine. In fact, the solid-state indicator favours the stability of the solution and allows an optimal logistic management of the kit 10 as it allows savings in packaging and transport.

[0036] Of course, alternative embodiments of a kit according to the invention can be envisaged without losing the main advantages of the invention described above. For instance, a reagent still in a solid state but in powder form may be used. In this case, the kit advantageously comprises a calibrated measuring cup, which allows a dose of powdered indicator corresponding to the amount required for a measurement to be taken from a larger container.

[0037] The above description of certain specific embodiments is capable of showing the invention from a conceptual point of view in such a way that others, using the known technique, will be able to modify and/or adapt in various applications such specific embodiment without further research and without departing from the inventive concept. Therefore, it is understood that such adaptations and modifications will be considered as equivalents of the specific embodiment.

[0038] The means and materials for performing the various functions described may be of various nature without going beyond the scope of the invention.

[0039] It is understood that the expressions or terminology used are purely descriptive and therefore not limiting.

CLAIMS

1. Kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes comprising:
   - a container (20) suitable for housing a predetermined amount of liquid of which the active chlorine concentration is to be measured;
   - a colorimetric reference scale (30), and
   - a chlorine indicator reagent (40) provided to be placed in said container (20) in a predetermined quantity,

   said kit (10) being **characterised in that** said quantity of said reagent contains a quantity of TMB equivalent to the molecular chlorine contained in said container (20) when it is filled with water in which a predefined maximum recommended concentration of chlorine is present.
2. Kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to claim 1, **wherein** said maximum recommended chlorine concentration is in the range from 2 mg L^{-1} to 5 mg L^{-1}.
3. Kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to claim 1, **characterised in that** said chlorine indicator reagent (40) contains a buffer substance suitable for maintaining the pH of the solution within said container (20) between 4 and 8.
4. Kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to the preceding claim, **characterised in that** said buffer substance contains citric acid/sodium citrate.
5. Kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to any one of claims 1 to 3, **characterised in that** said buffer substance contains pyrophosphoric salt.
6. Kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to the preceding claim,

characterised in that said buffer substance contains disodium diphosphate ($Na_2H_2P_2O_7$) or tetrasodium diphosphate ($Na_4P_2O_7$) in the presence of acetic acid / sodium acetate.

7. Kit (10) for colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to any of the preceding claims, **characterised in that** said chlorine indicator reagent (40) is in the form of single-dose tablets (41).

8. Kit (10) for colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to the preceding claim, **characterised in that** said kit (10) comprises a plurality of single-dose tablets (41) contained in at least one blister (42).

9. Kit (10) for the colorimetric determination by TMB of the concentration of active chlorine in drinking water or water used for recreational purposes according to any one of claims 1 to 5, **characterised in that** said chlorine indicator reagent is in powder form and said kit (10) comprises a calibrated measuring cup suitable for containing a single dose of said reagent.

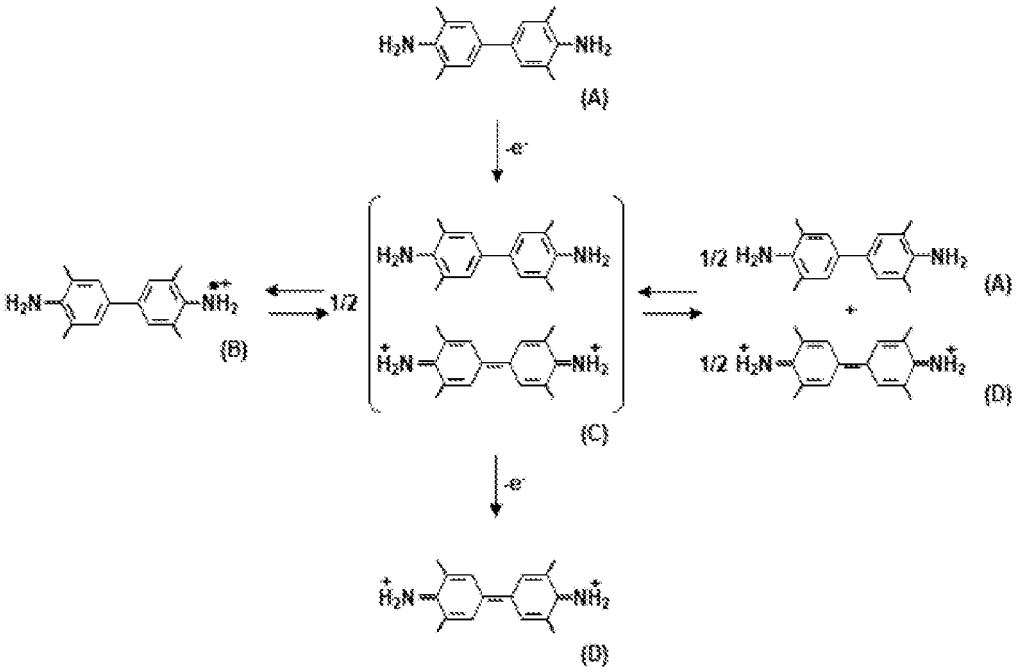
(A)
-e⁻
(B)
1/2
(C)
1/2
(A)
+
1/2
(D)
-e⁻
(D)

FIG. 1

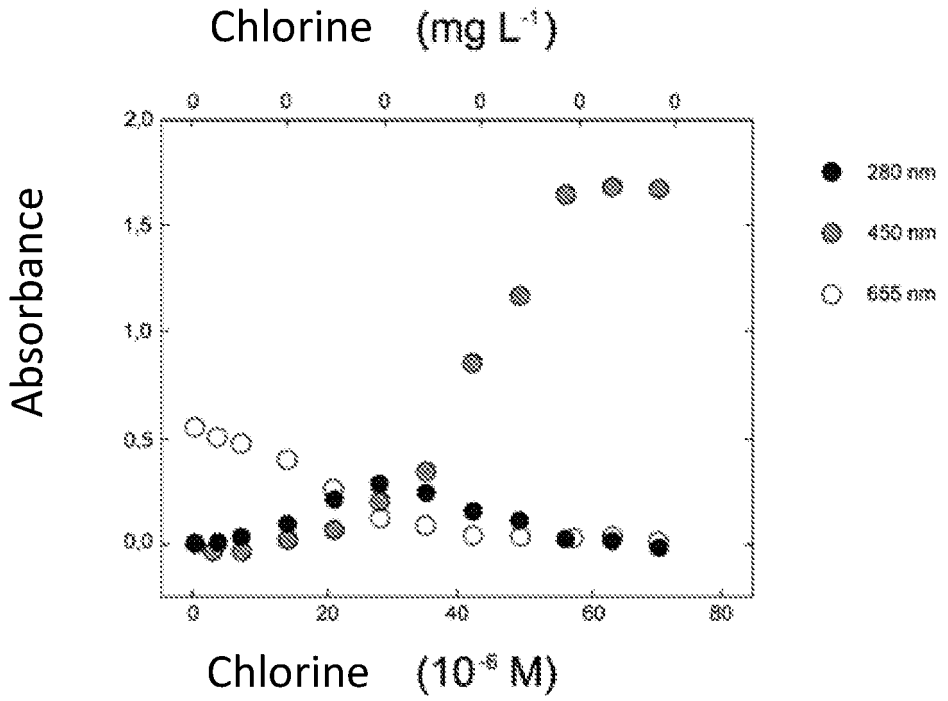
Chlorine (mg L⁻¹)
Absorbance
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
280 nm
450 nm
655 nm
Chlorine (10⁻⁶ M)

FIG. 2

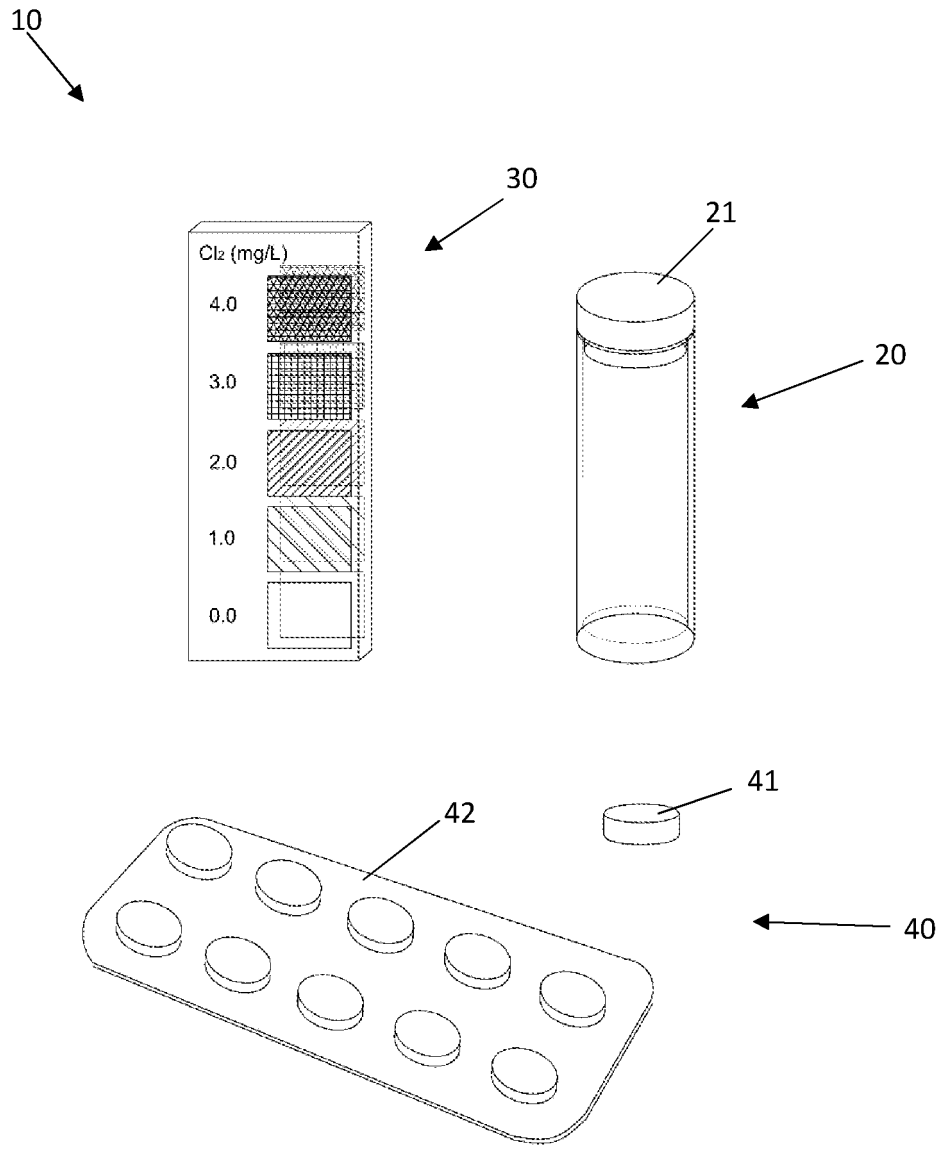

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/062203

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/78 G01N33/18
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 C02F

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 972 713 A (KUZUHARA NORIYASU [JP] ET AL) 26 October 1999 (1999-10-26)	1-6
Y	column 1, lines 7-12 column 7, lines 31-49 column 16, lines 3-10,32-36,51-54 column 19, line 2 tables 1-3,4,6 figure 1	7-9

Y	GB 2 423 358 A (TECHNICAL PRODUCTS & SPECIALIT [GB] ET AL.) 23 August 2006 (2006-08-23) page 4, paragraph 1	7-9

X	CN 2 482 081 Y (GUANGDONG MICROBIOLOGY INST [CN]) 13 March 2002 (2002-03-13)	1-9
Y	the whole document figures 1-3	7-9

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[X] Further documents are listed in the continuation of Box C.

[X] 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

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"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

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Date of the actual completion of the international search	Date of mailing of the international search report
5 March 2021	15/03/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Brauer, Jan

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/062203

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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