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(54) LIQUID COMPOSITION FOR INHALATION FOR ELECTRONIC CIGARETTES WITH REDUCED CYTOTOXICITY

(57) The present invention relates to a liquid inhalation composition for electronic cigarettes with reduced cytotoxicity.

In particular, the present invention relates to a composition for inhalation by means of electronic cigarettes comprising:

- a glycerol/propylene glycol mixture in a percentage by volume between 80% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%; 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- optionally, flavorings in a percentage by volume less than or equal to 15%, preferably less than or equal to 10%;
- a pH adjuster,

wherein the composition has a pH between 7.0 and 7.6.

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Description

Field of application of the invention

[0001] The present invention relates to a liquid inhalation composition for electronic cigarettes with reduced cytotoxicity.

Background art

[0002] Recognizing that cigarette smoking causes serious damage to health has long prompted the industry to develop alternative forms of inhalation which can give smokers adequate satisfaction and effectively replace traditional cigarettes in daily use.

[0003] Starting from the evidence that the intake of nicotine is a key factor which causes the continuous desire to smoke in smokers, but that the greatest damage comes from the substances of the high-temperature combustion of tobacco, such as polycyclic aromatic hydrocarbons, tar and carbon monoxide, the art has followed two parallel paths. On the one hand, systems have been developed which include heating tobacco at a controlled temperature (generally no higher than 350°C), so as to avoid the harmful effects of high-temperature combustion. On the other hand, so-called "electronic cigarettes" have been created which vaporize a liquid comprising, in addition to a base capable of providing a sufficiently dense body to the vapor released, also a controlled amount of nicotine and various types of flavorings.

[0004] The heated-tobacco inhalation systems are those which are most similar to traditional smoking for smokers, both in terms of the flavoring of the tobacco and of consumption methods and times, similar to those of a cigarette. However, it should be remembered that some toxic substances contained in tobacco are formed even before the combustion thereof, such as, in addition to nicotine, nitrosamines. These substances are therefore not eliminated by reducing the combustion temperature to no more than 350°C.

[0005] The compositions for electronic cigarettes, on the other hand, do not contain such toxic or carcinogenic substances, apart from a controlled amount of nicotine, which is essential to make the inhalation of the composition pleasant by the traditional smoker. Furthermore, the possibility of flavoring the composition in various manners forms a further attraction.

[0006] Therefore, electronic cigarettes largely solve the toxicity problems associated with tobacco use, even if they are not completely safe. In fact, the base composition, which consists for the most part of a mixture of propylene glycol and glycerol in a volume ratio of 50:50, or for a denser vapor of 30:70, has an inherent toxicity, albeit much more reduced than that of a traditional cigarette and the heated-tobacco system described above.

[0007] Therefore, the problem of being able to further lower the toxicity of a composition for inhalation by means of electronic cigarettes so as to make the prolonged consumption thereof much safer is still felt.

Summary of the invention

[0008] The aforesaid technical problem is substantially solved by a composition comprising the technical features set out in one or more of the appended claims, the definitions of which form an integral part of the present description for the purpose of sufficiency of description.

[0009] Therefore, the present invention relates to a composition for inhalation by means of an electronic cigarette comprising:

- a glycerol/propylene glycol mixture in a percentage by volume between 80% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- optionally, flavorings in a percentage by volume less than or equal to 15%, preferably less than or equal to 10%;
- a pH adjuster,

  in which the composition has a pH between 7.0 and 7.6.

[0010] The invention also relates to a base composition comprising:

- a glycerol/propylene glycol mixture in a percentage by volume between 93% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- a pH adjuster, in which the base composition has a pH between 7.0 and 7.6.

[0011] The invention still further relates to a kit comprising:

a) a container comprising a base composition comprising

- a glycerol/propylene glycol mixture in a percentage by volume between 93% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- a pH adjuster, in which the base composition has a pH between 7.0 and 7.6;

b) one or more containers comprising flavorings in a percentage by volume less than or equal to 10%.

[0012] Further features and advantages of the present invention will become more apparent from the indicative and thus non-limiting description of a preferred, but not exclusive embodiment of the invention.

Brief description of the drawings

[0013]

Figure 1 depicts a graph of cell viability vs composition concentration with a control compound;
Figure 2 depicts a graph of cell viability vs composition concentration with a composition according to the invention;
Figure 3 depicts a graph of cell viability vs composition concentration with a composition for electronic cigarettes according to the prior art;
Figure 4 depicts a graph of cell viability vs composition concentration with a heated-tobacco system according to the prior art.

Detailed description of the invention

[0014] The present invention is directed to a composition for inhalation by means of electronic cigarettes comprising:

- a glycerol/propylene glycol mixture in a percentage by volume between 80% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- optionally, flavorings in a percentage by volume less than or equal to 15%, preferably less than or equal to 10%;
- a pH adjuster,

in which the composition has a pH between 7.0 and 7.6.

[0015] The glycerol/propylene glycol mixture is at a volume ratio between 50:50 and 70:30.
[0016] If the composition contains flavorings, the percentage by volume of the glycerol/propylene glycol mixture will not be greater than 90%.
[0017] The glycerol is preferably vegetable glycerol.
[0018] The percentage of nicotine optionally present in the composition should not be greater than the amount allowed by current legislation, in particular it should be less than or equal to 20 mg/ml.
[0019] The water is preferably demineralized water.
[0020] The flavorings can be of any type normally used for electronic cigarettes, such as fruit flavorings, tobacco flavorings, mentholates or mixtures thereof. The flavorings can be added to a base composition which does not contain them directly by the smoker. In this case, glycol-based flavorings are available to add to the composition as desired.
[0021] Therefore, the invention further relates to a base composition comprising

- a glycerol/propylene glycol mixture in a percentage by volume between 93% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- a pH adjuster, in which the base composition has a pH between 7.0 and 7.6.

[0022] The pH adjuster is preferably an organic or inorganic acid, more preferably selected from orthophosphoric acid, lactic acid and citric acid.
[0023] The weight percentage of the pH adjuster in the composition depends on both the nature of the pH adjuster and the percentage of the various components used in the composition. For example, in a composition containing about 90% by volume of 50:50 glycerol/propylene glycol mixture, about 5% water, about 10% flavorings (apple/kiwi), nicotine 8 mg/ml, orthophosphoric acid in a weight percentage of 0.1% adjusted the pH of the composition to 7.5.
[0024] The pH is preferably between 7.2 and 7.6, more preferably between 7.3 and 7.6 or between 7.4 and 7.6 or is

of about 7.5.

[0025] The invention still further relates to a kit comprising:

a) a container comprising a base composition comprising

- a glycerol/propylene glycol mixture in a percentage by volume between 93% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- a pH adjuster, in which the base composition has a pH between 7.0 and 7.6;

b) one or more containers comprising flavorings in a percentage by volume less than or equal to 10%.

[0026] The present invention originates from the evidence that the known compositions for electronic cigarettes have a basic pH. The present Applicant has conducted experiments and has verified that such a basic pH is responsible for a certain cytotoxicity towards lung cells (human lung fibroblasts). The Applicant has also discovered that by lowering the pH of the composition to a pH between 7 and 7.6, such toxicity is substantially reduced if not eliminated.

Experimental section

[0027] The cytotoxicity of a composition according to the invention towards compositions of the prior art was evaluated through the MTT test: cell survival test with human fibroblasts of pulmonary origin grown in monolayer for the evaluation of the biocompatibility of smoke with mucous membranes.

[0028] The test aims at evaluating the cytotoxicity of the smoke condensate obtained with electronic cigarettes with pH correction to neutrality according to the invention on a cell culture of human lung fibroblasts, as described in the UNI EN ISO 10993-5 and ISO 4387:2019 standards related to the biological evaluation of medical devices, appropriately modified for the purpose.

[0029] The cytotoxicity of the condensate obtained by simulating the exposure to which the user of the electronic cigarette is subjected is an indicator of the safe use of the device in hand and the overall toxicity thereof towards the respiratory epithelium.

[0030] The method used is that of MTT, which is simple and reproducible, originally developed by Mossman.

[0031] An increase or decrease in viable cells results in a concomitant change in absorbance which can be regarded as an indicator of the degree of cytotoxicity caused by exposure to the test substances. The cells are also examined under a microscope after contact with the sample to evaluate any changes in the morphology thereof, membrane integrity or any lysis phenomena.

[0032] A line of Human Lung Fibroblasts (HPF) was used. Source: Innoprot. The cells are cultured in MEM containing 10% FBS and antibiotics.

[0033] Cigarette smoke is sampled using a smoke machine consisting of a vacuum system and a 100 ml flask connected to an electronic cigarette. The electronic cigarette is positioned vertically on an inert plastic fitting. The positioning allows the metal resistance inside the tank and used to heat the liquid to always remain in contact with the liquid itself, as indicated by the manufacturer.

[0034] The electronic cigarette loaded with the appropriate liquid was left to rest about 20 minutes before aspiration, so that the cotton inside the tank was correctly moistened with liquid, after which the first 2/3 puffs were discarded.

[0035] The flask is connected by silicone tubes to a flow meter inside the instrument, which is in turn connected to a vacuum pump and to a PC with control software. The presence of a flow meter allows the constant setting of the aspiration flow. 30 ml of sterile medium were used for the preparation of the condensates. An aspiration/inhalation simulation is performed with a duration of 3" with a pause lasting 45" between one aspiration and the other, to facilitate the collection of the condensate in the medium, keeping the flask constantly under stirring during the experiment, in accordance with ISO 4387:2019.

[0036] The aspiration flow is regulated at 1 L/min. 150 aspiration/inhalation cycles are performed, equal to 25 traditional cigarettes. The smoke from the electronic cigarette, loaded with the special liquid at the beginning of the session, is continuously sampled for the expected number of cycles. The medium with the condensate obtained from such an experiment was brought to pH 7.5 with lactic acid and subjected to cytotoxicity tests.

[0037] Human lung cells were seeded in 96-well plates and allowed to grow for 24h at 37°C and 5% CO_2. On the second day, the medium was removed and replaced by the freshly prepared condensate from the cigarette to be tested.

[0038] In order to determine the IC_{50} value (Inhibiting Concentration 50) at the end of the experiment, the tested concentrations are the condensate as is and the subsequent serial 1:2 dilutions thereof in medium.

[0039] Each sample was tested in sixfold. Untreated cells were used as blank and as positive control the cells were treated with a surfactant of known toxicity (Sodium Lauryl Sulfate - SLS) dissolved in the culture medium at concentrations

between 0.5 mg/ml and 0.03 mg/ml.

[0040] After 24 hours of incubation with the sample and the control, the cytotoxicity test (MTT) was then performed to evaluate the cell survival percentage.

MTT cell viability tests

[0041] The culture medium is aspirated and the cells incubated in 100 μl/well of a 1 mg/ml solution of MTT, for 2 hours at 37°C. The solution is removed and replaced by 200 μl/well of DMSO with subsequent 30' of incubation at room temperature and stirring at medium speed.

[0042] The absorbance at 570 nm is read with a colorimeter (Tecan INFINITE F200) provided with a plate reader by subtracting the background reading at 650 nm.

[0043] The result is expressed as a percentage of cell viability according to the formula:

$$\%\ \textbf{of cell viability} = [OD\ (570\ nm - 650\ nm)\ product\ tested/OD\ (570\ nm - 650\ nm)\ white] \times 100.$$

[0044] In case it is possible to calculate the IC_{50} value (Inhibiting Concentration 50) which indicates the concentration of the product which inhibits cell viability by 50%.

$$IC50 = Conc_{>50} - (Conc_{>50} - Conc_{<50}) \times (\%_{>50} - 50)/(\%_{>50} - \%_{<50})$$

$Conc_{>50}$ = first concentration which gave a cell viability above 50%

$Conc_{<50}$ = first concentration which gave a cell viability less than 50%

$\%_{>50}$ = first cell viability obtained above 50%
$\%_{<50}$ = first cell viability obtained less than 50%.

[0045] The following acceptability criteria of the method were adopted:

for the blank: the mean OD value of the replicates should be ≥ 0.2, the standard deviation should be ≤ 18%, the mean of two columns of blanks should not differ by more than 15%;
for the positive control (CP): the IC_{50} of the SLS positive control should be 0.10 mg/ml ± 0.03, the standard deviation should be ≤ 18%;
for the sample: standard deviation should be ≤ 18%.

[0046] A reduction in cell viability > 30% is considered a cytotoxic effect.

[0047] The samples used were:

- composition for electronic cigarettes according to the invention, comprising 90% by volume of 50:50 glycerol/propylene glycol mixture, water about 5%, flavorings (apple/kiwi) about 10%, nicotine 8 mg/ml, pH adjuster: acid lactic up to pH 7.5;
- composition for electronic cigarettes of the prior art, comprising 90% by volume of 50:50 glycerol/propylene glycol mixture, water about 5%, flavorings (apple/kiwi) about 10%, nicotine 8 mg/ml, pH 8.9;
- HEETS Turquoise selection cigarette 3172 /20-06 (heated-tobacco cigarette), pH 7.9.

[0048] Figure 1 shows the graph of cell viability vs concentration for the positive control (SLS).

[0049] Figure 2 shows the graph of cell viability vs concentration for the composition of the invention at pH 7.5.

[0050] Figure 3 shows the graph of cell viability vs concentration for the electronic cigarette composition of the prior art (pH 8.9).

[0051] Figure 4 shows the graph of cell viability vs concentration for the HEETS Turquoise selection heated-tobacco cigarette.

[0052] The following IC_{50} values were calculated from the data of such experiments:

- positive control IC_{50} = 0.09 ml/ml
- composition for electronic cigarettes according to the invention IC_{50}»1 ml/ml (non-cytotoxic at the highest concentration tested)
- composition for electronic cigarettes of the prior art IC_{50} = 0.93 ml/ml
- IC_{50} heated-tobacco cigarette = 0.19 ml/ml.

[0053] From the above it is apparent that the composition for electronic cigarettes according to the invention is much less cytotoxic than both the composition of the prior art without acidity adjuster and, to an even greater extent, the heated-tobacco cigarette.

[0054] Therefore, the invention further relates to a pH adjuster acid for use in preventing pulmonary cytotoxicity in subjects intaking vapors by means of electronic cigarettes, in which said pH adjuster acid is added to an electronic cigarette composition comprising a glycerol/propylene glycol mixture in a percentage by volume between 80% and 99%, optionally water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%, optionally nicotine in a percentage by weight/volume not exceeding 0.2%, optionally flavorings in a percentage by volume less than or equal to 15%, preferably less than or equal to 10%, and in which said pH adjuster acid gives said composition a pH between 7.0 and 7.6.

[0055] Said pH adjuster acid is preferably selected from orthophosphoric acid, lactic acid and citric acid and the composition for electronic cigarettes is preferably as defined above.

[0056] It is apparent that only some particular embodiments of the present invention have been described, to which those skilled in the art will be able to make all changes required to adapt it to particular applications, without departing from the scope of protection of the present invention.

Claims

1. A composition for inhalation by means of an electronic cigarette comprising:

- a glycerol/propylene glycol mixture in a percentage by volume between 80% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- optionally, flavorings in a percentage by volume less than or equal to 15%, preferably less than or equal to 10%;
- a pH adjuster,

wherein the composition has a pH between 7.0 and 7.6.

2. The composition according to claim 1, wherein the glycerol/propylene glycol mixture is at a volume ratio between 50:50 and 70:30.

3. The composition according to claim 1 or 2, wherein, when the composition contains flavorings, the percentage by volume of the glycerol/propylene glycol mixture is no greater than 90%.

4. The composition according to any one of claims 1 to 3, wherein the flavorings are selected from fruit, tobacco, mentholated flavorings or mixtures thereof.

5. The composition according to any one of claims 1 to 4, wherein the pH adjuster is an organic or inorganic acid.

6. The composition according to claim 5, wherein the pH adjuster is selected from orthophosphoric acid, lactic acid and citric acid.

7. The composition according to any one of claims 1 to 6, wherein the pH is between 7.2 and 7.6, or between 7.3 and 7.6, or between 7.4 and 7.6, or is of about 7.5.

8. The composition according to any one of claims 1 to 7, wherein the pH adjuster is orthophosphoric acid in a percentage by weight of about 0.1%.

9. A base composition comprising:

- a glycerol/propylene glycol mixture in a percentage by volume between 93% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- a pH adjuster, wherein the base composition has a pH between 7.0 and 7.6.

10. A kit comprising:

a) a container comprising a base composition comprising

- a glycerol/propylene glycol mixture in a percentage by volume between 93% and 99%;
- optionally, water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%;
- optionally, nicotine in a percentage by weight/volume not exceeding 0.2%;
- a pH adjuster, wherein the base composition has a pH between 7.0 and 7.6;

b) one or more containers comprising flavorings in a percentage by volume less than or equal to 10%.

11. A pH adjuster acid for use in preventing pulmonary cytotoxicity in subjects intaking vapors by means of electronic cigarettes, wherein said pH adjuster acid is added to an electronic cigarette composition comprising a glycerol/propylene glycol mixture in a percentage by volume between 80% and 99%, optionally water in a percentage by volume less than or equal to 6%, preferably less than or equal to 5%, optionally nicotine in a percentage by weight/volume not exceeding 0.2%, optionally flavorings in a percentage by volume less than or equal to 15%, preferably less than or equal to 10%, and wherein said pH adjuster acid gives said composition a pH between 7.0 and 7.6.

12. The pH adjuster acid for use according to claim 11, wherein the composition is as defined in any one of claims 2 to 9.

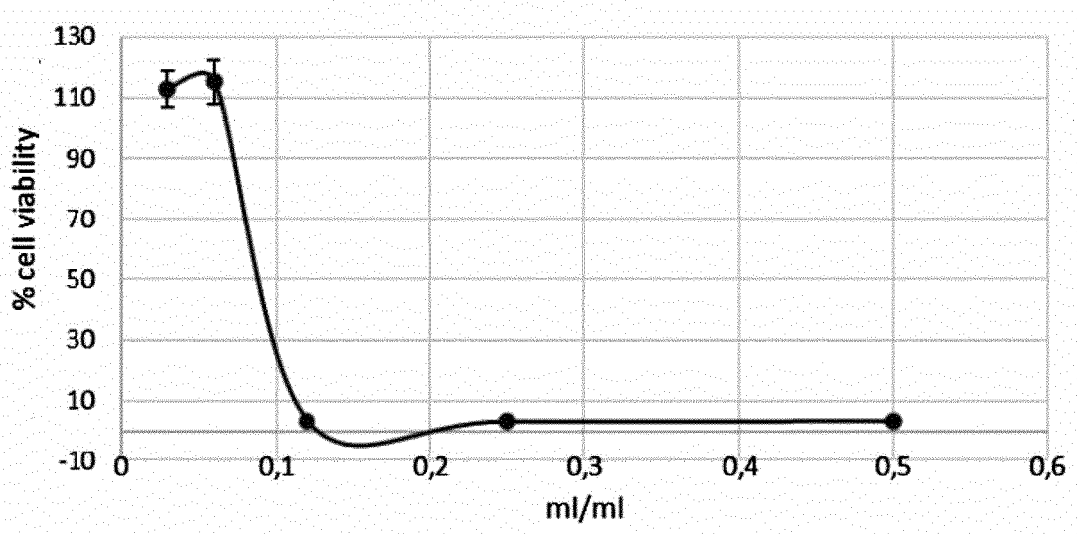

130
110
90
70
50
30
10
-10
% cell viability
0
0,1
0,2
0,3
0,4
0,5
0,6
ml/ml

FIG. 1

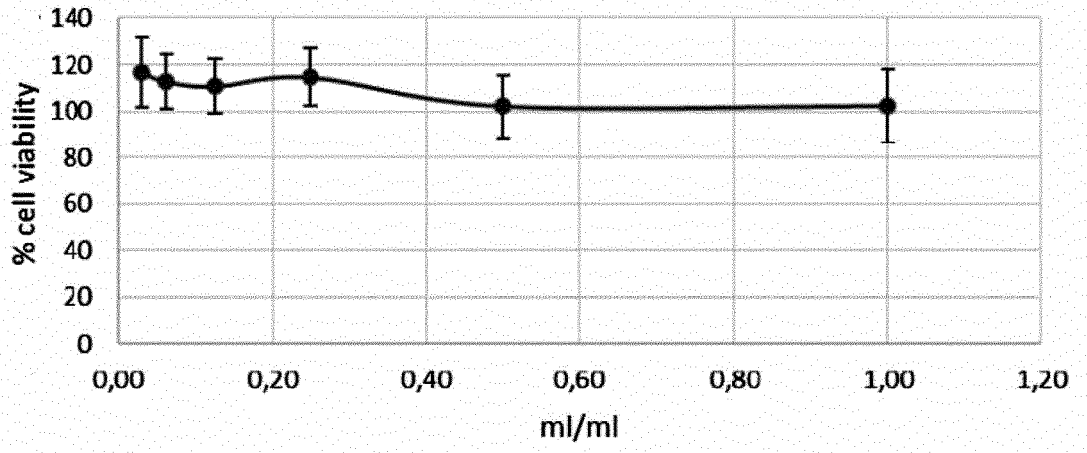
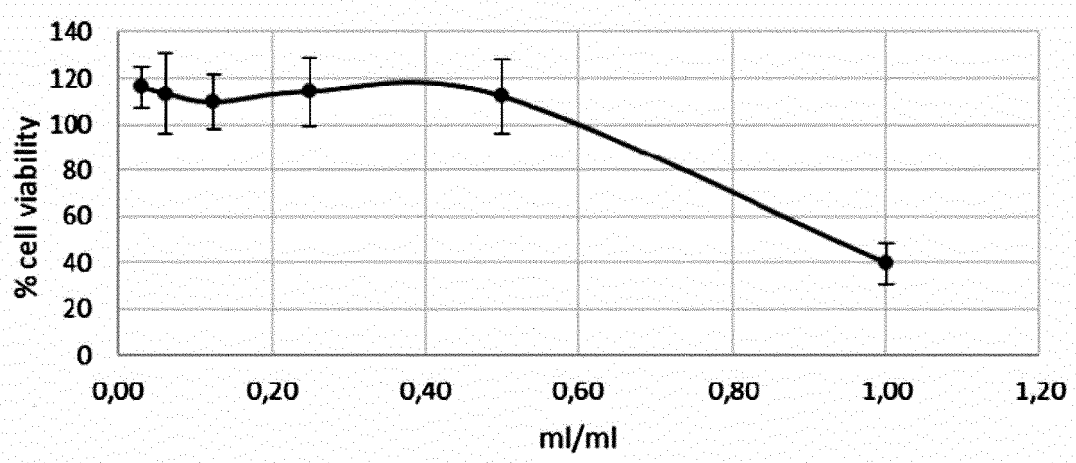

140
120
100
80
60
40
20
0
% cell viability
0,00
0,20
0,40
0,60
0,80
1,00
1,20
ml/ml

FIG. 2


140
120
100
80
60
40
20
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% cell viability
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0,20
0,40
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0,80
1,00
1,20
ml/ml

FIG. 3

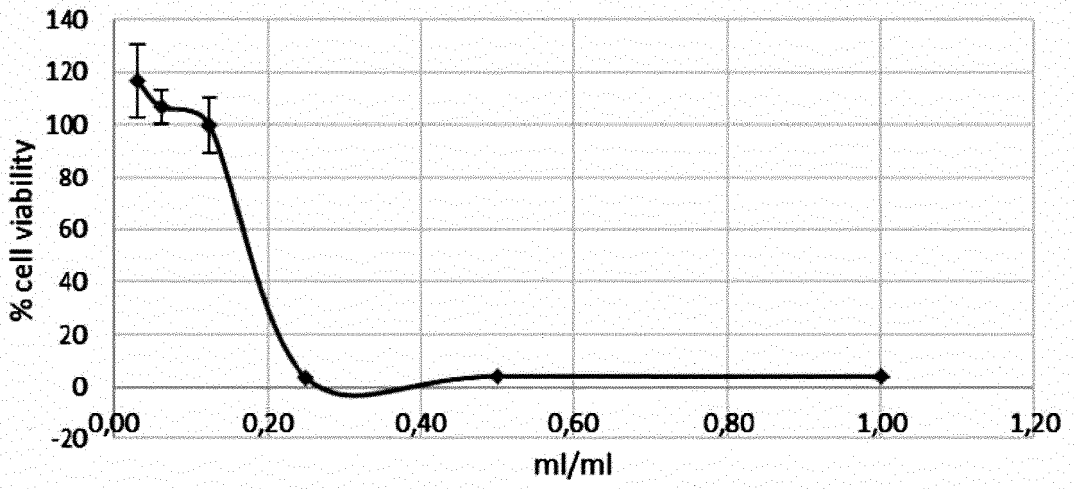
140
120
100
80
60
40
20
0
-20
% cell viability
0,00
0,20
0,40
0,60
0,80
1,00
1,20
ml/ml

FIG. 4

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EUROPEAN SEARCH REPORT

Application Number
EP 21 21 0563

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	CN 110 558 605 A (SHENZHEN WUXIN TECH CO LTD) 13 December 2019 (2019-12-13) * the whole document *	1-7,9, 11,12	INV. A24B15/167 A24B15/28
X	WO 2014/190079 A2 (NJOY INC [US]) 27 November 2014 (2014-11-27) * the whole document *	1-10	
X	WO 2020/239907 A1 (JT INT SA [CH]) 3 December 2020 (2020-12-03) * the whole document *	1-10	
A	US 2014/096781 A1 (SEARS STEPHEN BENSON [US] ET AL) 10 April 2014 (2014-04-10) * the whole document *	10	
A	US 2013/255702 A1 (GRIFFITH JR DAVID WILLIAM [US] ET AL) 3 October 2013 (2013-10-03) * the whole document *	10	
			TECHNICAL FIELDS SEARCHED (IPC)
			A24B

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	8 April 2022	Piret-Viprey, E

CATEGORY OF CITED DOCUMENTS

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08-04-2022

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 110558605 A	13-12-2019	CN 110558605 A	13-12-2019
		WO 2021056526 A1	01-04-2021
WO 2014190079 A2	27-11-2014	EP 3073846 A2	05-10-2016
		US 2014345635 A1	27-11-2014
		US 2022071292 A1	10-03-2022
		WO 2014190079 A2	27-11-2014
WO 2020239907 A1	03-12-2020	CN 113840545 A	24-12-2021
		EP 3975759 A1	06-04-2022
		KR 20220014875 A	07-02-2022
		WO 2020239907 A1	03-12-2020
US 2014096781 A1	10-04-2014	US 2014096781 A1	10-04-2014
		US 2018303169 A1	25-10-2018
		US 2020113233 A1	16-04-2020
		US 2020146347 A1	14-05-2020
		US 2020146355 A1	14-05-2020
US 2013255702 A1	03-10-2013	CN 104349687 A	11-02-2015
		EP 2833744 A1	11-02-2015
		ES 2600171 T3	07-02-2017
		JP 6218803 B2	25-10-2017
		JP 2015512262 A	27-04-2015
		US 2013255702 A1	03-10-2013
		US 2019098938 A1	04-04-2019
		US 2020260784 A1	20-08-2020
		US 2021112860 A1	22-04-2021
		WO 2013148810 A1	03-10-2013

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