(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 January 2010 (28.01.2010)

PCT

(10) International Publication Number
WO 2010/010582 A1

(51) International Patent Classification:
C12Q 1/18 (2006.01)

(21) International Application Number:
PCT/IT2008/000492

(22) International Filing Date:
21 July 2008 (21.07.2008)

(25) Filing Language: Italian

(26) Publication Language: English

(71) Applicants *(for all designated States except US)*: **HUMANITAS MIRASOLE S.P.A.** [IT/IT]; Via Manzoni, 56, I-20089 Rozzano (IT). **POLITECNICO DI MILANO** [IT/IT]; Piazza Leonardo da Vinci, 32, I-20133 Milano (IT). **ISTITUTO DI RICERCHE CHIMICHE E BIOCHIMICHE GIULIANA RONZONI** [IT/IT]; Via G. Colombo, 81, I-20133 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GRAZIANI, Giorgio** [IT/IT]; c/o HUMANITAS MIRASOLE S.p.A., Via Manzoni, 56, 1-20089 Rozzano (IT). **MONTANELLI, Alessandro** [IT/IT]; c/o HUMANITAS MIRASOLE S.p.A., Via Manzoni, 56, I-20089 Rozzano (IT). **MELONE, Lucio** [IT/IT]; c/o POLITECNICO DI MILANO, Piazza Leonardo da Vinci, 32, I-20133 Milano (IT). **VISMARA, Elena** [IT/IT]; c/o POLITECNICO DI MILANO, Piazza Leonardo da Vinci, 32, I-20133 Milano (IT). **TORRI, Giangiacomo** [IT/IT]; c/o Istituto di Ricerche Chimiche e Biochimiche Giuliana Ronzoni, Via G. Colombo, 81, I-20133 Milano (IT).

(74) Agents: **LONG, Giorgio** et al.; Jacobacci & Partners S.p.A., Via Senato, 8, I-20121 Milano (IT).

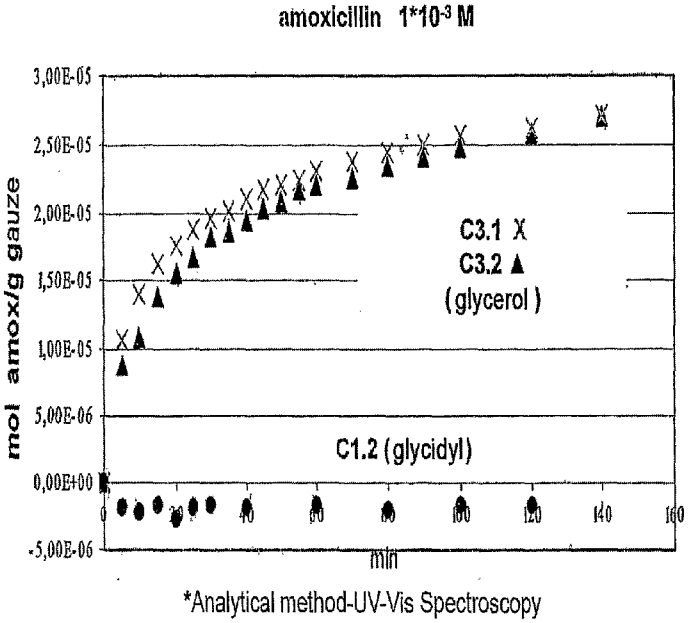
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG,

[Continued on next page]

(54) Title: ANTIBIOGRAM METHOD AND KIT

(57) Abstract: The present invention regards a method and related kit for carrying out a diagnostic antibiogram test. In particular, the present invention regards a kit for carrying out a clinical antibiogram analysis comprising one or more supports made of hydrocarbon material con functionalised with monomers chosen from among glycidyl methacrylate (GMA) and its diol derivative, acrylic acid and its derivatives, N-vinylpyrrolidone, acrylamide and its derivatives and vinyl acetate, said support being loaded with a known and predefined quantity of an antibiotic agent.

Graph 1- Affinity amoxicillin-gauzes and adsorption kinetic T=25°C*



*Analytical method-UV-Vis Spectroscopy

FIG. 1

WO 2010/010582 A1

SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

— *with international search report (Art. 21(3))*

DESCRIPTION

"Antibiogram method and kit"

Field of the invention

The present invention regards a method and related application to a kit for carrying out a diagnostic antibiogram test.

State of the art

The antibiogram is an "in vitro" diagnostic exam used for evaluating the sensitivity of a bacterial microorganism of a biological sample under examination towards specific antibiotics and in particular for evaluating its resistance R or sensitivity. The object of the test is to allow, on the basis of detected sensitivities, choosing the most effective antibiotic therapy to subject the patient to which the examined biological sample belongs.

If a growth inhibition is created on the culture medium in which the microorganism of the analysed sample is made to grow, this is an indication of the sensitivity of the bacteria to the tested antibiotic; if instead the growth of the bacteria colonies is not stopped, the microorganism is considered resistant to the antibiotic.

The methodology for carrying out the antibiogram is internationally laid down by NCCLS (National Committee for Clinical Laboratory Standards), so as to standardise the analysis as a function of the purity of the bacterial

strain to be tested, of the density/concentration of the inoculation, of the incubation conditions, of the result reading method and of the biological and clinical criteria for interpreting the result itself.

There are various methods for carrying out the antibiogram. The first is the dilution method, with which the bacteria resistance/sensitivity is evaluated at increasing concentrations of a single antibiotic. The object of this test is to evaluate the MIC (minimum inhibitory concentration). The analysis is generically conducted in liquid phase, preparing various dilutions of the antibiotic in different test tubes containing liquid culture medium. Every test tube is then inoculated with equal quantities of bacterial culture and, after incubation at 37°C for 24 hours, the turbidity of the solution is evaluated in the various test tubes. The first test tube, in dilution order, which contains a clear solution is that which has the lowest inhibitory concentration of antibiotic (MIC).

There is also a test on the market on solid state (E-Test of AB Biodisk) which employs a strip impregnated with increasing quantities of antibiotic along its length. Such device is placed on culture medium (agar) on which the bacterial colonies are present and then, after incubation, the point is evaluated at which the

inhibition zone intersects the strip. A predefined scale present on the strip indicates the antibiotic concentration in that point.

A different antibiogram test uses the so-called diffusion method (Bauer - Kirby method). This analysis type permits determining the sensitivity of a microorganism towards numerous antibiotics placed on solid medium plates, by means of applying the antibiotics on solid medium inoculated/spread with the microorganism to be analysed. In practice, discs of a support containing the antibiotic in a known quantity are deposited on the solid medium, this is incubated for 24 hours and the diameter of the inhibition zone which forms is determined. On the basis of values predefined for every antibiotic, the measurement of the diameter of the inhibition zone which forms around the disc permits establishing the sensitivity, resistance or intermediate condition of the microorganism placed in the medium towards the specific antibiotic.

In all the above-described cases, a correct determination of the antibiogram very much depends on the precision with which the solutions or discs or solid strips are prepared with known antibiotic concentrations. In particular, in the case of solid supports impregnated with antibiotic, it is on one hand necessary to prepare

perfectly titrated solutions of antibiotic, on the other hand necessary to ensure that the quantity of antibiotic adsorbed on the support is actually the desired amount and that its adsorption occurs in a repeatable manner. This is not always easy and there can be evaluation errors that can then affect the doctor's clinical choices.

Summary of the invention

The object of the present invention is therefore that of overcoming such drawbacks by providing a method and kit related thereto which ensure a high precision and repeatability of results.

The invention was inspired by the discovery that materials containing at least one hydrocarbon portion, and in particular polysaccharide materials, functionalised with synthetic monomers as will be outlined below, are capable of binding active molecules such as antibiotics of various nature according to specific kinetics. The main parameters which influence the binding kinetics are the concentration of the antibiotic solution, which is put in contact with the substrate, and the contact time between the substrate and such solution.

It is therefore possible to calculate the following calibration curves: "bound antibiotic

quantity"/"antibiotic solution concentration" and "bound antibiotic quantity"/"time of contact", which permit carrying out the step of impregnating the substrate with the antibiotic in a very precise, repeatable and controlled manner.

Brief description of the figures

Figure 1 shows a graph of drug-substrate affinity versus contact time;

Figure 2 shows a graph of drug-substrate affinity versus contact time, at different concentrations from those of figure 1;

Figure 3 shows a drug-substrate adsorption isotherm at different drug concentrations.

Detailed description of the invention

The present invention therefore regards a substrate made of a material having at least one hydrocarbon portion, preferably being a hydrocarbon substrate, functionalised with a monomer capable of binding antibiotic molecules, loaded or saturated with said antibiotic molecules.

A further object of the invention is a method for carrying out an antibiogram that provides for the use of a substrate as described above.

A further object of the invention is a kit for carrying out the aforesaid method.

With the term "hydrocarbon material", both a material

entirely composed of hydrocarbon chains and/or rings and a material which comprises only one hydrocarbon portion will be intended from hereon.

The hydrocarbon material according to the invention is preferably chosen from among polysaccharide material, a polyamide, a polyester, polypropylene or another polyolefin.

The polysaccharide material is preferably selected between woven or non-woven cellulose or its derivative such as cellulose acetate.

The preferably used monomers in the invention are chosen from among glycidyl methacrylate (GMA) and its derivatives, acrylic acid and its derivatives, N-vinylpyrrolidone, acrylamide and its derivatives and vinyl acetate. The preferred monomer is that which derives from the functionalisation of the substrate with glycidyl methacrylate and subsequent opening of the epoxy ring to give a (3-glycerol-O-carbonyl)-2-propyl-functionalised substrate.

The method for functionalising the hydrocarbon material of the invention is described in the scientific publication Vismara et al., European Polymer Journal 41 (2005), 1787-1797 and in the European patent EP 0 242 172.

In practice, the hydrocarbon material can be treated with

both chemical (for example Fenton's reagent) and physical (for example electron beam technology) radical initiators so as to form radicals in the substrate which are sufficiently stable over time so that they can then react with the above-defined monomers. If such monomers are glycidyl methacrylate molecules, one obtains a substrate functionalised with glycidylcarbonyl-2-propyl groups which, by treatment in strongly ionic aqueous environment, are converted into (3-glycerol-O-carbonyl)-2-propyl groups for the opening of the epoxy ring, giving the corresponding diol.

As shown in diagram 2 below, the radical reaction can be quenched by a second monomer molecule, thus forming dimer moieties.

The hydrocarbon material thus functionalised with the above-defined monomers is then immersed in a solution containing an active ingredient with antibiotic activity and is subjected to stirring for a time generally in the range of 1 - 96 hours, as required. In this manner, the active ingredient is then adsorbed on the substrate and bound by the above-defined monomer or dimer moieties with bond kinetics that depend both on the concentration of the active ingredient solution and on the contact time with such solution.

Once the step of stirring and contact with the active

ingredient solution is terminated, the substrate is washed with deionised water and then dried, so as to eliminate the active ingredient not bound to the monomer or dimer moieties.

The quantity of active ingredient retained by the substrate can be precisely derived, as a function of the concentration of the active ingredient solution and the contact time therewith, by extrapolating the graphs reported in figure 1, 2 and 3. It can also be confirmed by weighing the substrate before or after the contact with the active ingredient solution.

Preferred examples of active ingredients with antibiotic activity usable for the purposes of the present invention include penicillin, aminoglycosides, quinolonic antibiotics, tetracycline, chloramphenycol, sulfacetamide, sulfametazine, sulfadiazine, sulfamerazine, sulfamethizole, erythromycin, clarithromycin, rifamycin, rifampycin, ceftazidime, amoxicillin, amoxicillin trihydrate, clavulanic acid, vancomycin hydrochloride, teicoplanin, linezolide.

The material of the invention is therefore extremely versatile, capable of adsorbing active ingredients of very different molecular weight and structure, such as for example clavulanic acid (199.16 PM) and vancomycin hydrochloride (1485.71 PM).

EXPERIMENTAL PART

Functionalising cellulose with glycidyl methacrylate monomer

The process of functionalising polysaccharides via radical reaction, both with initiators of physical and chemical type, is noted and reported both in scientific publications (Vismara et al., European Polymer Journal 41 (2005) 1787-1797) and in patents (Vismara et al., 2002, European Patent application EP02425172).

Diagram 1 shows the activation mechanisms of cellulose substrates by means of electron beam or Fenton's reagent with formation of non-selective radicals that are sufficiently stable over time so to react with the substrate, as shown in diagram 2. The experimental parameters related to such processes are amply described in the abovementioned publications. The same reactions are applicable to the other hydrocarbon substrates according to the invention.

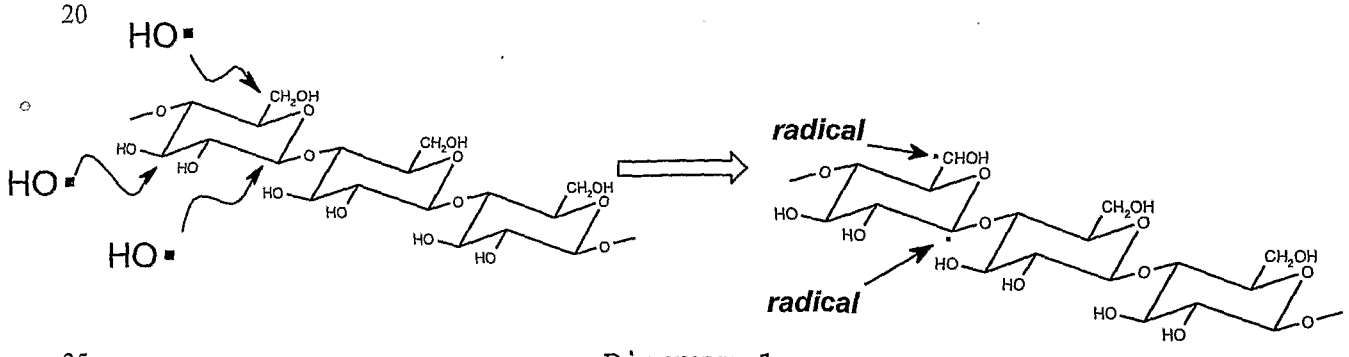


Diagram 1

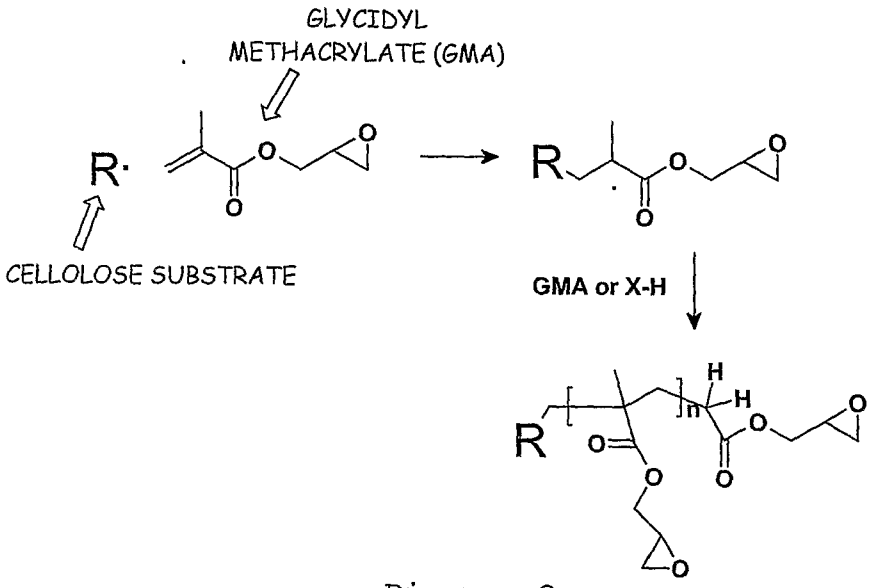


Diagram 2

It must be noted that, from the structural standpoint, the functionalisation of a hydrocarbon substrate with Fenton's reagent or with electron beam leads to different results. In fact, Fenton's reagent can cause the formation of radicals in every point of the molecular mass of the substrate, while the electron beam only acts on the substrate surface. This means that, according to the technology employed, the monomer or dimer moiety will be differently exposed towards the outside, which could lead to different active ingredient release modes. It is thus important that the methodology for the preparation of the functionalised substrate is standardised, so as to provide substrates with comparable reactivity.

As said above, the substrate functionalised with the

glycidyl methacrylate provides, after treatment in strongly ionic aqueous environment, the respective diol, according to diagram 3:

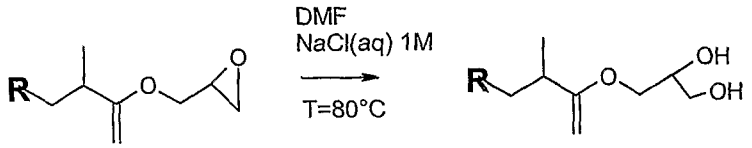


Diagram 3

EXAMPLE - Opening the epoxy ring

1 g of cellulose material functionalised with glycidyl methacrylate as described above is inserted in a one-neck 250 ml flask equipped with a reflux condenser, in which 100 ml of anhydrous dimethylformamide (DMF) is loaded. The system is then placed under stirring (magnetic stirrer) for 2 hours at room temperature, then it is heated to 80°C; once this temperature is reached, 200 ml of 1 M NaCl aqueous solution are added. After 18 hours at 80°C under stirring, the flask is cooled, the liquid is decanted, deionised water is added (about 100 ml) and the resulting mixture is magnetically stirred for 30 min at 80°C in an oil bath. The operation is repeated 3 times, then the substrate is washed 3 times (20 min per wash) with 100 ml of acetone and is left to dry in air until a constant weight is attained.

Impregnation of the functionalised substrate with active

ingredients with antibiotic activity

Solution A: 500 mg of RIFOCIN® were diluted with 20 ml of deionised water in a 100 ml flask, reaching a rifamycin concentration of 2.39E-2 M.

Solution B: analogously, a solution was prepared of 2.39E-2 M ceftazidime.

Solution C: analogously, a solution was prepared of 2.39E-2 M amoxicillin.

3 samples of 2 cm x 2 cm cellulose substrate were prepared, functionalised with open glycidyl methacrylate to form the diol derivative (SD) and were dried at 55°C for 1 hour. The samples were inserted in the four flasks containing the solutions A, B and C and were placed under stirring on a stirrer JULABO SW22 at 100 rpm for 72 hours at room temperature. After having been removed from the respective solutions, the samples were dried on filter paper, washed with about 200 ml of cold deionised water (T=5-10°C), filtered and dried once again on filter paper. Then the samples were placed under vacuum for at least 24 hours. It is necessary to wash the samples treated with amoxicillin well, since the drug forms suspensions in water.

Evaluation of the antibacterial activity of the substrates impregnated with antibiotics

The samples prepared in the preceding point were

subjected to a blind study, with a laboratory method of the type used in microbiological clinical analysis laboratories for the evaluation of the sensitivity/resistance of bacteria agents to the antibiotics (Kirby - Bauer method).

The method comprises:

- Spreading a culture broth, with the presence of bacteria in standardised concentration, on Petri dishes with a culture medium composed of agar added with substances that favour bacterial growth (Mueller-Hinton medium);
- Depositing, on such bacteria culture, a disc of blotting paper impregnated with antibiotic;
- Observing the presence/absence of bacterial growth inhibition zones around the discs impregnated with antibiotic and measuring their diameter;
- Determining the presence of a bacterial growth inhibition zone and its diameter allows judging the sensitivity of the bacteria colony being studied to that particular antibiotic agent.

In the specific case, the following were prepared:

- Standardised cultures of bacteria strains (0.5 McFarland): strain 1, Gram+ Staphylococcus aureus ATCC 29213; strain 2, Gram- Escherichia coli ATCC 25922;

- Discs of the substrate impregnated with antibiotics (solutions A, B and C) prepared as stated above.

The test was conducted as stated above (two different sessions: 17/01/2007 and 19/01/2007), and the obtained results are reported in table 1.

Table 1 - Determination of inhibition zones (expressed in cm)

Sample	**Description**	**17/01/07 Strain 1**	**19/01/07 Strain 1**	**17/01/07 Strain 2**	**19/01/07 Strain 2**
1	SD	NO	NO	NO	NO
2	SD + amoxicillin	2.5	N.D.	2.5	2.0
3	SD + ceftazidime	2.5	3.0	2.0	2.0

The results are in line with the expected sensitivity of the various tested antibiotic agents to the analysed strains, which demonstrates a regular release of the antibiotic by the functionalised substrate according to the invention.

In Table 2, the qualitative data are reported with regard to a second series of experiments, in which "YES" corresponds to the presence of a bacterial growth

inhibition zone and "NO" indicates the absence of inhibition zone.

Table 2 - Determination of the presence/absence of bacterial growth inhibition zones

Sample	Description	Bacterial suspension (MF)					
		0.5		0.05		0.005	
		Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2
5	SD + rifamycin	YES	NO	YES	NO	YES	NO
3	SD + ceftazidime	NO	YES	NO	YES	NO	YES

Also in this case, the results are in accordance with the normal behaviour of the tested antibiotics.

The functionalising of the substrate with the monomers of the invention depends on the so-called "molar substitution" (MS), which indicates the average number of monomer/dimer units for every glycoside ring, as determined by means of FT-IR spectroscopy and/or by the weight increase of the final material. The MS can vary between 0 and 2, preferably between 0.2 and 1.

For the purposes of the invention, it will therefore be

appropriate that the MS is homogenous and standardised.

In fact, it was seen that for a given MS, the reaction kinetics which lead to the bond of the active ingredient with the functionalised substrates of the invention, particularly the substrate functionalised with (3-glycerol-O-carbonyl)-2-propyl groups, is such to permit the preparation of substrates loaded with precise and predictable amounts of the active ingredient, as a function i) of the concentration of the active principle solution with which the functionalised substrate comes into contact and ii) the contact time between the substrate and such solution.

Figures 1 and 2 show the adsorption kinetics of amoxicillin (amox mol/substrate g) versus the contact time, at two different concentrations of amoxicillin (10^{-3} M and 10^{-4} M, respectively). In both cases, the substrate functionalised with (3-glycerol-O-carbonyl)-2-propyl binds the antibiotic in a time-dependent manner, according to a predefined kinetic function.

Figure 3, on the other hand, shows the adsorption kinetics of amoxicillin (amox mol/substrate g) versus the molar concentration of amoxicillin, after a given contact time. Also in this case, the adsorption kinetics of the active ingredient on the functionalised substrate follows an adsorption function predefined for the glycerol

derivative.

Object of the present invention is then a kit for carrying out a clinical antibiogram analysis comprising one or more supports made of a hydrocarbon material functionalised with the above-defined monomers, chosen from among glycidyl methacrylate (GMA) and its diol derivative, acrylic acid and its derivatives, N-vinylpyrrolidone, acrylamide and its derivatives and vinyl acetate, said support being loaded with a known and predefined quantity of an antibiotic agent.

Preferably, the kit of the invention comprises a first series of supports as defined above, each loaded with a different quantity of the same antibiotic, said different quantities preferably being decimal scale multiples.

Preferably, the kit of the invention comprises at least a further series of supports as defined above, every support of said at least one further series being loaded with a different quantity of an antibiotic that is different from the antibiotic of said first series, said different quantities being preferably decimal scale multiples.

The antibiotic agents will for example be chosen from among those indicated above.

The supports for the inventive kit can be prepared from hydrocarbon material chosen from the group comprising

polysaccharide material, a polyamide, a polyester, polypropylene or other polyolefin, according to the methods described above.

This kit can allow carrying out analytical tests both substituting the dilution method in liquid or solid form, and substituting the Kirby - Bauer method, with high reliability and repeatability of results, due to the fact that the substrate of the invention allows retaining, in a stable and enduring manner, precise quantities of antibiotic agent.

Forming a further object of the invention is a method for carrying out an antibiogram which comprises the passages of:

- Spreading a culture broth, with the presence of bacteria in standardised concentration, on Petri dishes with a culture medium composed of agar added with substances which favour bacterial growth;
- Depositing, on such bacterial culture, one or more supports loaded with antibiotic as defined in the analysis kit described above and incubating for a predefined time;
- Observing the presence/absence of bacterial growth inhibition zones around the discs impregnated with antibiotic and measuring their diameter,

where the determination of the presence of a bacterial

growth inhibition zone and its diameter allows judging the sensitivity of the bacterial colony being studied to the tested antibiotic agent or antibiotic agents.

5

CLAIMS

1. Kit for carrying out a clinical antibiogram analysis comprising one or more supports made of hydrocarbon material functionalised with monomers chosen from among glycidyl methacrylate (GMA) and its diol derivative, acrylic acid and its derivatives, N-vinylpyrrolidone, acrylamide and its derivatives and vinyl acetate, said support being loaded with a known and predefined quantity of an antibiotic agent.

2. Kit according to claim 1, wherein said hydrocarbon material is chosen from among polysaccharide material, a polyamide, a polyester, polypropylene or other polyolefin.

3. Kit according to claim 2, wherein said polysaccharide material is chosen from among woven or non-woven cellulose or its derivative such as cellulose acetate.

4. Kit according to any one of the claims 1 - 3, wherein said antibiotic agent is chosen from among penicillin, aminoglycosides, quinolonic antibiotics, tetracycline, chloramphenycol, sulfacetamide, sulfametazine, sulfadiazine, sulfamerazine, sulfamethizole, erythromycin, clarithromycin, rifamycin, rifampycin, ceftazidime, amoxicillin, amoxicillin trihydrate, clavulanic acid, vancomycin

hydrochloride, teicoplanin, linezolide.

5. Kit according to any one of the claims 1 - 4, wherein said one or more supports are made of hydrocarbon material functionalised with (3-glycerol-O-carbonyl)-2-propyl groups.

6. Kit according to any one of the claims 1 - 5, wherein said kit comprises a first series of said supports, each one loaded with a different quantity of the same antibiotic.

7. Kit according to any one of the claims 1 - 6, wherein said kit comprises at least one further series of said supports, every support of said at least one further series being loaded with a different quantity of a same antibiotic which is different from the antibiotic of said first series.

8. Kit according to claim 6 or 7, wherein said different antibiotic quantities are decimal scale multiples.

9. Kit according to any one of the claims 1 - 8, wherein said supports have the form of discs for antibiogram.

10. Kit according to any one of the claims 1 - 9, wherein said supports are obtained by means of functionalising said hydrocarbon material with said monomers via radical reaction with chemical initiator

such as Fenton's reagent or physical initiator such as electron beam.

11. Kit according to claim 10, wherein said hydrocarbon material is made to react by means of said radical reaction with glycidyl methacrylate and the reaction product is treated in strongly ionic aqueous environment so to give said (3-glycerol-O-carbonyl)-2-propyl derivative.

12. Kit according to claim 10 or 11, wherein said hydrocarbon material functionalised with said monomers is loaded with antibiotic agent by means of contact with an antibiotic agent solution, in which the quantity of loaded antibiotic agent depends on the concentration of said solution and on the contact time with said solution.

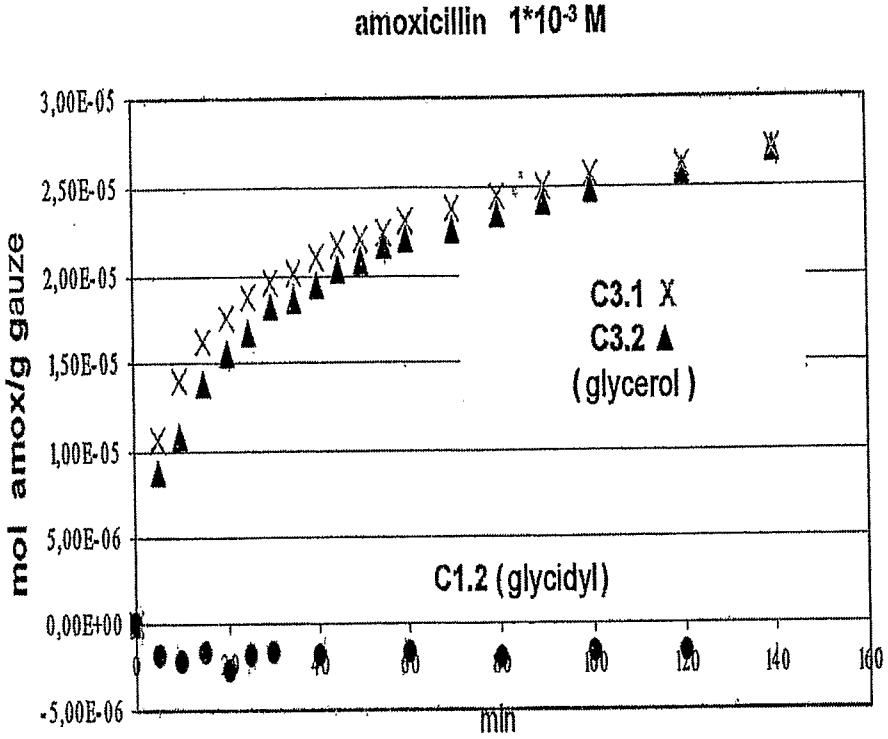
13. Method for carrying out an antibiogram which comprises the passages of:

- Spreading a culture broth, with the presence of bacteria in standardised concentration, on Petri dishes with a culture medium composed of agar added with substances that favour bacterial growth;
- Depositing, on such bacterial culture, one or more supports loaded with antibiotic as defined in any one of the claims 1 - 12 and incubating for a predefined time;

- Observing the presence/absence of bacterial growth inhibition zones around discs impregnated with antibiotic and measuring their diameter,

where the determination of the presence of a bacterial growth inhibition zone and its diameter allows judging the sensitivity of the bacteria colony being studied to the tested antibiotic agent or antibiotic agents.

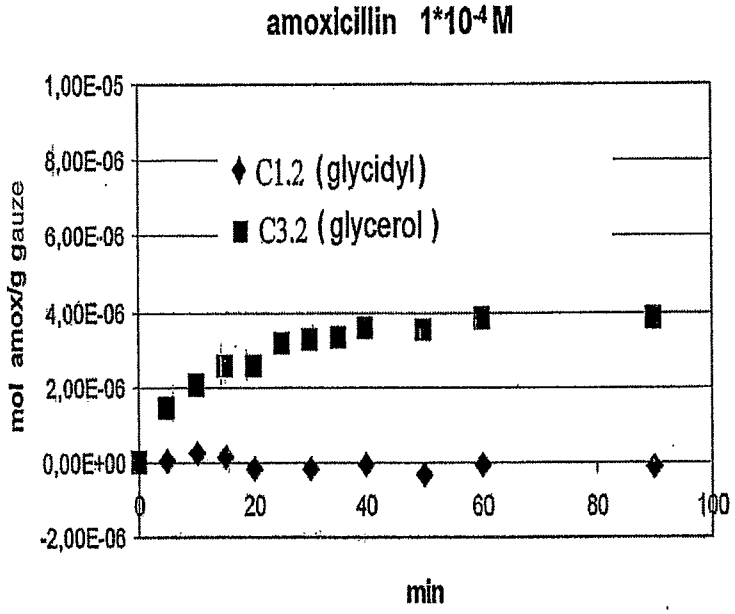
Graph 1- Affinity amoxicillin-gauzes and adsorption kinetic T=25°C*



*Analytical method-UV-Vis Spectroscopy

FIG. 1

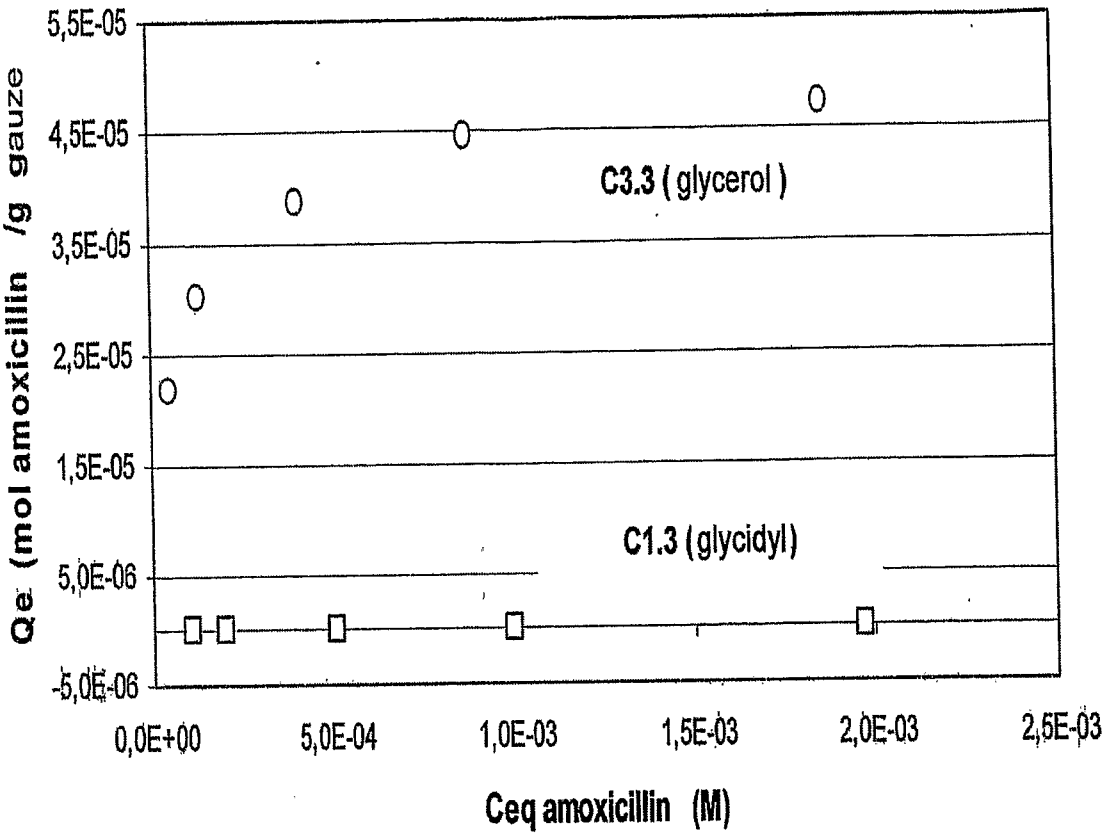
Graph 2- Affinity amoxicillin-gauzes and adsorption kinetic T=25°C*



*Analytical method-UV-Vis Spectroscopy

FIG. 2

Graph 3- Adsorption isotherm amoxicillin gauzes at 25°C



*Analytical method-UV-Vis Spectroscopy

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IT2008/000492

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/18

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)
C12Q

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 practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEE, MOON KYU ET AL: "The study of antibiotic drug-loaded polymer films for the prevention of the infection of external fixation devices." KEY ENGINEERING MATERIALS , 342-343(ADVANCED BIOMATERIALS VII), 533-536 CODEN: KEMAEY; ISSN: 1013-9826, 2007, XP008103357 abstract page 535	1-13
Y	FR 2 574 090 A (GENERAL DIAGNOSTICS FRANCE) 6 June 1986 (1986-06-06) page 2, line 3 - line 9 page 5; examples 2-12 page 7; examples 13-20	1-13

-/--

[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 document 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	Date of mailing of the international search report
10 March 2009	19/03/2009

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	Griffith, Gerard

INTERNATIONAL SEARCH REPORT

International application No
PCT/IT2008/000492

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ALBERTI A ET AL: "Electron beam irradiated textile cellulose fibres" EUROPEAN POLYMER JOURNAL, PERGAMON PRESS LTD. OXFORD, GB, vol. 41, no. 8, 1 August 2005 (2005-08-01), pages 1787-1797, XP004935559 ISSN: 0014-3057 cited in the application page 1788, column 1	1-13
A	GB 2 128 737 A (BEECHAM GROUP PLC) 2 May 1984 (1984-05-02) the whole document	1-13
A	US 4 714 675 A (WAKO PURE CHEMICAL INDUSTRIES, LTD.) 22 December 1987 (1987-12-22) examples 1-12	1-13
A	BEECHING, N. J. ET AL: "Comparative in vitro activity of antibiotics incorporated in acrylic bone cement." JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY , 17(2), 173-84 CODEN: JACHDX; ISSN: 0305-7453, 1986, XP008103356 abstract page 175	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IT2008/000492

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2574090 A	06-06-1986	IT 1184001 B	22-10-1987
GB 2128737 A	02-05-1984	NONE	
US 4714675 A	22-12-1987	JP 1723662 C	24-12-1992
		JP 4009518 B	20-02-1992
		JP 58179499 A	20-10-1983