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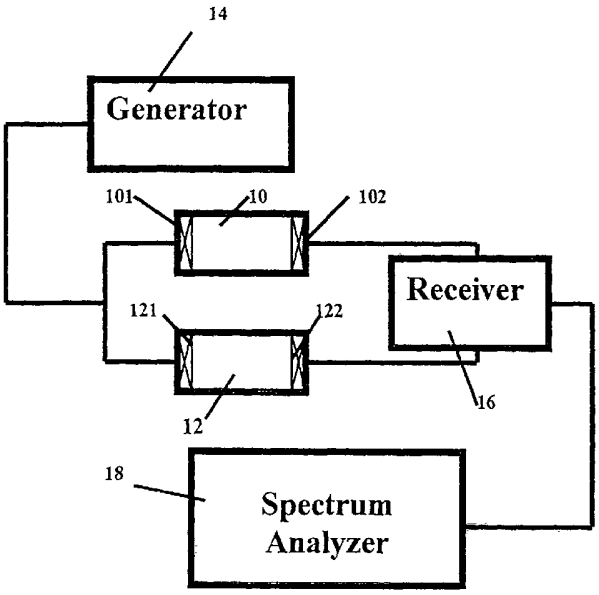
(54) Title: THE ACOUSTICAL METHODS AND APPARATUS FOR IDENTIFICATION AND SELECTIVE TREATMENT OF A CELLULAR SYSTEM



(57) Abstract: The problem solved includes identification of frequencies of ultrasonic oscillations characterizing a certain type of cellular structures (i.e. the characteristic frequencies). These frequencies are registered by means of a measuring system characterized by a complex type of intrinsic resonances comprising the natural oscillations of both the cellular system and that of the media interacting with the latter. For the purpose of selective influence on a certain component of cellular systems it is proposed to expose the latter ones to ultrasonic waves of a significantly higher intensity at the aforesaid characteristic frequencies until the desired effect (up to the complete inactivation) is produced. The produced and the apparatus for implementation of the method are described.

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DESCRIPTION OF INVENTION

THE ACOUSTICAL METHODS AND APPARATUS FOR IDENTIFICATION AND SELECTIVE TREATMENT OF A CELLULAR SYSTEM

FIELD OF THE INVENTION

The group of inventions relates to biotechnology, in particular, to microbiology and cell biology, and can be used for identification and treatment of various cellular systems in medicine, veterinary medicine, in timber and food industries and other fields of science and industry.

BACKGROUND OF THE INVENTION

The interaction of ultrasound with biological medium is an object of numerous investigations and technological applications. The ultrasound is a unique tool permitting, on the one hand, to determine characteristics of biological systems, and on the other hand, to produce a desired effect on them for the purposes of medicine and technology. The term "ultrasound" in this description implies the acoustic waves in the frequency range from 1 kHz to hundreds of MHz.

One of the structural and functional units of biological medium is the cell, so assaying mechanisms of ultrasonic effect along with recommendations on the technology of said effect is the substance of many inventions. It should be taken into account that the dimensions of biological systems are small, from tens of nanometers

to fractions of micrometers. In most cases the systems have complex composition, structure and specific functions, which determine their responses to external stimuli. The response of a biological structure, in this case of the bio-membrane, depends on composition and physical condition of individual membrane components and on the parameters of the immediate environment of the membrane. That is, the response of a bio-membrane is determined by its molecular composition, the charge of polar groups of membrane molecules, the so-called "bound" water, the presence of various trans-membrane gradients and other factors. At that, an important characteristic of biological membranes is the surface pressure, and its evaluation allows obtaining a more complete information on the intra-cell processes (see, for example, **SU 1465768 A1**, Semenov S.N. et al. 15.03.1989) [1].

Besides, the biological structures display vital activity in liquid media, and constitute, to a certain extent, liquid media themselves. The media based on water in some concentration are characterized by the following acoustical parameters: the velocity of ultrasound not exceeding 1500 m/s and small attenuation in the kilohertz range of ultrasonic waves.

There are different known mechanisms of interaction of cellular structures and ultrasound, the "strong" and the "weak" modes of ultrasonic influence on the medium being considered. The "strong" ultrasound is generally used for disintegration of structures, production of emulsions and dispersions, and other technological purposes usually characterized by producing cavitation in liquid media. It is the cavitation that constitutes the source of high pressure necessary for the destructive physical and chemical

treatment. The "weak" ultrasound does not, as a rule, bring about physical or chemical transformations and is used for the purpose of measuring parameters of biological objects or affecting them without causing the irreversible biological destruction.

For instance, there is a known method of ultrasonic treatment of biological structures in liquid suspensions. According to it, the complex-shape oscillations are generated in the walls of a container with liquid suspension. By means of this technique through regulation of the amplitude of ultrasonic oscillations the biological structures (cells) are distributed over the length and the radius direction of the container according to the desired law (**WO 91/13674 A1**, Whitworth G., B01D 43/00..., 19.09.1991) [2], which subsequently allows to make their selection.

Another method of the "weak" ultrasonic treatment may be used for agglutination of particles or cells via cross-bridging molecules in the immuno-agglutination assays. According to it, the biological suspension is placed in the field of standing ultrasonic waves, this resulting in sedimentation of its components (**WO 93/19367 A2**, Coakley W.T., et al., G01N 33/541, 33/53, 29/02... 30.09.1993) [3].

There is a known acoustic filter for separation and recycling of suspended particles. An apparatus is described that allows, by means of a multi-frequency resonator with two ultrasonic emitters, to separate with high efficiency the components of liquid biological media such as mammalian, bacterial and plant cells or aggregates in a flow-type acoustic reactor (**WO 95/01214 A1**, Trampler F. Et al., B01D 21/00, 43/00, 12.01.1995) [4].

The most close to the present inventions are the methods implying the registration of characteristics of biological objects and

subsequent effect on them with the purpose of altering the state of the objects.

A device is known, which implements the method for treating morbid cells in the living body by means of sonic and/or ultrasonic waves. For the selective destruction of cells, mainly tumor cells, the ultrasonic radiation with natural frequency spectrum (of 1kHz to 10 MHz) for the kind of cells being treated is used, which destroys said cells (**EP 0774927 B1**, Theuer E., A61B 19/00, 19.08.1998) [5]. For registration of frequency spectrum an acoustic tract is used comprising an electric generator connected to a wideband ultrasonic emitter. The emitter generates in the examined medium ultrasonic oscillations, their spectrum being registered by an acoustic receiver connected to the spectrum analyzer. The emitter can generate acoustic field of intensity up to 300 W/cm^2 and can operate in the pulse mode.

There is also a known method of determining *in vivo* or *in vitro* the acoustic parameters of biological structures, such as viruses, bacteria, fungi, tissue masses, worms, arthoropods and others, using the resonance frequencies of these objects as characteristic parameters. The resonance frequencies are determined by the methods of acoustic spectroscopy. An acoustic tract is used to feed the ultrasound of variable frequency into a biological structure and to register the acoustic resonances of the object as a single integral system (for example, of a fish). To produce a selective effect on the chosen systems, in particular, for their destruction or inactivation, they are subjected to ultrasound at frequencies corresponding to the aforesaid resonances (**WO 00/15097 A3**, Brooks J.H.J., Abel A.E., 23.03.2000) [6].

However, the above-mentioned references [5], [6] place the emphasis on measurement of the resonance acoustical characteristics of biological structures and recommend subjecting structures to ultrasonic frequencies corresponding to those of their acoustic resonance. However, as it follows from reference [5], the intrinsic frequencies of microbiological objects, for example, bacteria and viruses, lie in the extreme high-frequency ultrasonic range (of the order of 1GHz).

In this range of wavelength in liquids (for liquid is the immersion medium for examined structures), the ultrasound oscillations are completely damped at the distances as small as several micrometers, and the associate mass of the immersion medium itself should be also taken into account in the oscillatory process. This circumstance causes considerable difficulties in implementation of the above-mentioned known methods [5], [6], requiring thereby new solutions. In the prior art there exist no suggested solutions embodying a technology allowing to identify biological objects using substantially lower ultrasonic frequencies and to produce selective effect on their cellular system.

SUMMARY OF THE INVENTION

The invention provides a method for identification of frequencies of ultrasonic oscillations characterizing a certain type of biological cellular system (here, the biomembranes, for instance, the cytoplasmatic membranes). These frequencies, hereinafter called characteristic (natural) frequencies, are registered with the measuring system, possessing a complex type of natural resonances including also the natural oscillations of the cellular

system. For the selective influence on a certain component, until its complete inactivation, it is proposed to subject the cellular system to ultrasonic oscillations on the characteristic frequencies and with the increased intensity compared to the one used in measurements of characteristic frequencies.

The claimed group of inventions, unified by the common inventive idea, comprises acoustic methods for identification and selective influence on cellular systems, and an apparatus for application of the aforesaid methods.

The acoustical method of identification of a cellular system consists in generation of ultrasonic oscillations in the medium containing the cellular system, in registration and analysis of spectra of ultrasonic oscillations propagating through the aforesaid medium, and in determining the identification features of the cellular system in the spectra.

The method is characterized in that generation and registration of ultrasonic oscillations are performed with the measuring apparatus including the reference and the measuring channels connected according to differential scheme and balanced so that, if both channels contain the same medium, the output signal spectrum would have no resonance peaks. During identification the examined medium is introduced into the measuring channel, the differential frequency spectrum, natural for the examined medium with respect to the reference one, is registered, the resonance peaks are detected in the aforesaid frequency spectrum, and their respective frequencies are identified as the characteristic frequencies of the cellular system and/or its components in the examined medium.

The method can be characterized in that generation, reception and analysis of frequency spectra are performed in the frequency range from 10 kHz to 800 MHz. The media containing cellular systems can be introduced into the measuring device in the form of mixtures based on aqueous solutions, mainly the physiological solution. A flow-type measuring device can be employed.

The method can also be characterized in that the medium containing the cellular system is introduced into the measuring device in the form of an object of biological tissue. The object of biological tissue used as the medium containing the cellular system is brought into acoustic bond with the measuring device placed outside and/or inside the aforesaid object of biological tissue.

The acoustical method of selective influence on a cellular system consists in generation of ultrasonic oscillations in the medium containing the cellular system and placed into a measuring device supplied with the means for regulation of frequency and intensity of ultrasonic oscillations.

The method can be characterized in that the ultrasonic oscillations in the medium containing the cellular system are generated on the characteristic frequencies of ultrasonic oscillations of the cellular system and/or its components in the examined medium, at that, the intensity of ultrasonic oscillations is chosen to be above the empirically found threshold value of intensity, causing the irreversible destruction of the cellular system or its components.

The intensity of ultrasonic oscillations is chosen according to the condition $P_{treatment} = (10 \div 15)\ P_{measurement}$ where $P_{treatment}$ is the intensity of ultrasonic oscillations excited inside the medium in the process of selective treatment, and $P_{measurement}$ is the intensity of

ultrasonic oscillations used in determining the characteristic frequencies.

Another object of the claimed group of inventions is an apparatus for identification and selective treatment of cellular systems. It includes a measuring device for placing a medium containing cellular systems, which device comprises the means for generation and reception of ultrasonic oscillations, connected respectively to a generator of electric oscillations and a receiver with its output connected to the means for spectral analysis of ultrasonic oscillations and identification of characteristic frequencies. The measuring device includes measuring units intended for the reference medium and the examined medium, the receiver has differential inputs. The means for generation and reception of ultrasonic oscillations are embodied in the form of the wideband emitting and receiving piezoelectric transducers, the emitting ones being connected in parallel to the generator of electric oscillations, and the receiving ones related to the reference medium and the examined medium being connected to the differential inputs of the receiver.

The apparatus may include an electric generator embodied as a sweep generator and a controllable attenuator. The piezoelectric transducers can be manufactured of ceramics on the basis of solid solutions of barium/lead zirconate/titanate, the measuring units for the reference medium and the examined medium can be manufactured in the form of cuvettes, in particular, the flow-type ones. The emitting and receiving piezoelectric transducers can be placed outside and/or inside the object of biological tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

The object of the invention is better understood in connection with the drawings, wherein:

FIG. 1 is a schematic drawing of the experiment and of the measuring apparatus;

FIG. 2 shows experimental data obtained on the solutions:

Graph A relates to the ***Bac. anthracoides*** culture;

Graph B relates to the ***Bac. thurindiensis*** culture;

Graph C relates to the mixture of ***Bac. anthracoides*** + ***Bac. thurindiensis*** cultures;

FIG. 3 shows the experimental dependence of the amplitude of the output signal (A) on concentration (C) for both cultures, measured on the natural frequencies.

FIG. 4 shows the experimental dependence of the characteristic-frequency signal amplitude on the time of ultrasonic treatment of the cellular system.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The acoustical identification of various cellular systems and subsequent selective treatment thereof are carried out using the apparatus shown in the block diagram (see FIG. 1).

The apparatus comprises a measuring device including two channels, the reference one and the measuring one, being represented, for example, by the cuvettes 10, 12 of identical dimensions (their length along the direction of ultrasound

propagation constituting 60 to 100 mm). At the butt ends of each cuvette the ultrasonic transducers 101, 102, 121 and 122, constituting the measuring basis, are placed. The ultrasonic transducers 101 and 102 along with cuvette 10 form the reference acoustic tract; the ultrasonic transducers 121 and 122 along with cuvette 12 form the measuring tract and the tract for treating the cellular systems. The object of measurement (or treatment) can be a cellular system, for example, suspension of cells placed into the cavity of cuvettes 10 and 12 and being in the acoustic contact with the aforesaid transducers 101, 102, 121 and 122. If an object of biological tissue (either a biological object as a whole, or individual organ or else a fragment of an organ) is used as a medium containing the cellular system, the ultrasonic transducers 101, 102, 121 and 122 are placed outside and/or inside the aforesaid object providing the acoustical bond with the latter.

The scheme of similar measurements is well known and described in classic books on acoustic technologies (cf., e.g., "PHYSICAL ACOUSTIC. Principles and Methods" / ed. W.P.Mason, vol. 1: Methods and Devices, part A. - Academic Press, NY, ch. 4, 1964). [7].

In the measurements, the reversible ultrasonic transducers are used, permitting to effect both generation and registration of ultrasonic oscillations. As the transducers, the known wideband piezoelectric ceramic transducers are used, embodied out of barium/lead zirconate/titanate solid solutions. Such transducers are easily accessible and are widely used for both scientific and applied purposes in the frequency range of 1 kHz to tens of MHz (cf., e.g., the monograph "Ultrasonic Transducers"/ ed. Y.Kikuchi. - Corona

Publishing Company, LTD, Tokyo, 1969) [8]. Such piezoelectric transducers are peculiar in that they can be placed over the surface of a cuvette, for example, pasted up with the help of the compound. At the measurements of an object of biological tissue they can be fastened directly on the object or introduced into its bulk.

The measuring tract shall comprise a thermostatic system for the examined liquids and biological objects, while the cuvettes, for the convenience of manipulation, can be embodied as the flow-type ones and supplied with corresponding pumping systems (not shown in the FIG. 1).

To provide generation of ultrasonic oscillations the ultrasonic transducers are connected to the electrical generator 14 equipped with the means of frequency regulation (in the range from 10 kHz to 800 MHz) and of output power (from milliwatts to tens of Watts). Such generator 14 may be a sweep generator equipped also with the controllable attenuator. Reception and registration of ultrasonic oscillations are carried out by means of the receiver 16 equipped with two differential inputs and functioning as a transducer amplifier. The output of the receiver 16 is connected to the input of the spectrum analyzer 18.

The transducers 101 and 121 are connected to generator 14 in parallel, while the transducers 102 and 122 are connected to the input of receiver 16 in opposite to provide the differential measurement mode. For better understanding of the main point of the invention, the description of implementation of the proposed method is given below for the case of liquid media, the described routine, however, having no peculiarities in the case of a biological object.

The measuring system is considered ready for measurements when both cuvettes are filled with the same substance, for example, with physiological solution, and the output signal of spectrum analyzer 18 (the frequency response function of the measuring tract) does not show pronounced oscillations with respect to the "grass" while scanning the frequency of generator 14.

As it follows from the problem solved by the claimed invention, the methods of identification and selective effect provide for two procedures, differing, however, in technology and operating characteristics of ultrasonic field effect (cf. the above mentioned "weak" and "strong" modes of ultrasonic effect on the medium).

The first procedure consists in identification of cellular systems. For this purpose, registration of frequency characteristics of the medium placed in cuvettes is carried out using the "weak" ultrasound mode on the known mixtures. The measured spectra are compared to the types of cellular systems, the acoustic "portrait" of the latter being determined.

The second procedure consists in using the acoustic "portraits", that is, the frequency characteristics registered according to the first procedure, for the selective treatment of cellular systems with ultrasound of higher intensity (the "strong" ultrasound mode). It is evident that the terms "weak" and 'strong" have no permanent meaning, however, taking into account the previous analysis along with subsequent explanations and recommendations, the choice of modes would not present any difficulties to the specialists.

To effect the first procedure of identification of cellular systems the following operations are to be performed:

A. Cuvettes 10 and 12 are filled with physiological solution. A known cell culture (for example, the ***Bac. anthracoides***) with known concentration of cells (for example, 1.10^7 cells/ml) is prepared.

A known quantity, e.g. 20 ml, of the prepared cell culture is introduced into the measuring cuvette 12 and the corresponding frequency response function is registered with the spectrum analyzer 18 (see FIG. 2, A). The peaks are detected in the aforesaid frequency response function, and the corresponding frequencies are identified as characteristic frequencies F_1, F_2, inherent to the above mentioned known cell culture.

Then the concentration of the aforesaid known cell culture is increased (for example, by adding dosed quantities of the solution into the cuvette 12) and the dependence of signal amplitude on the concentration of cell culture is registered at the aforesaid characteristic frequencies F_1, F_2 (see FIG. 3).

B. The cuvettes 10 and 12 are filled with physiological solution. Another known cell culture (for example, the ***Bac. thurindiensis***) at known concentration (for example, 1.10^7 cells/ml) is prepared. The measurements entirely similar to those of A are conducted. The results are shown in FIG. 2, B. In this case the ***Bac. thurindiensis*** cell culture can be demonstrated to have a single pronounced characteristic frequency F_3.

Further, similar to the previous operation described in A, the concentration of the ***Bac. thurindiensis*** cell culture in the examined solution is increased, and the dependence of the amplitude of the aforesaid characteristic peak (at the frequency F_3) on the cell culture concentration is registered (see FIG. 3).

It can be seen that in both cases shown in FIG. 3 the "concentration – amplitude" dependence is linear. This circumstance can serve as a basis for quantitative determination of cell culture concentration in the unknown specimens by means of comparison with the reference.

C. A mixture of cultures examined in A and B is prepared. Further the same measurement procedure is followed as was used for individual cell cultures.

Cuvettes 10 and 12 are filled with physiological solution, then a known quantity of mixture of the ***Bac. anthracoides*** and the ***Bac. thurindiensis*** cultures is introduced into cuvette 12, and the peaks on the frequency response function are registered (see FIG. 2, C). It can be seen that the registered peak frequencies are equal to F_1, F_2 and F_3, i.e. the same values that were obtained separately in the experiments A and B.

Further, the dependence of amplitude of the characteristic peak on the time of ultrasonic treatment of the cellular system is registered. The FIG. 4 represents such dependence for one of the cell cultures, the ***Bac. thurindiensis***, measured at the characteristic frequency F_3. The amplitude A of the output signal can be seen to diminish with the treatment time. These results permit to make in future a choice of the mode of selective influence on cellular systems, aimed at changing their life condition, for example, affecting selectively the individual processes of vital activity of cells.

The second procedure, the acoustical method of selective treatment itself, consists, as it was mentioned above, in the ultrasonic treatment at the identified characteristic frequencies corresponding to the maximal attenuation of ultrasound in the

medium. For such treatment ultrasound of higher intensity is used (the above-mentioned "strong" ultrasound mode). For a given type and size of the cuvettes (during the analysis of a biological object it is the distance between the transducers of the measuring device) and a given type of media the threshold value of intensity of ultrasonic oscillations is empirically determined, which would not cause an irreversible destruction of the chosen cellular system or its components. During the selective treatment procedure the intensity $P_{treatment}$ is chosen to be above the determined threshold value of the "weak" ultrasound intensity, which is usually applied at the acoustical spectroscopy of biological media.

For this purpose, the above-mentioned cellular system (within the framework of the experimental scheme of FIG. 1) is exposed to the ultrasound at one of the identified absorption frequencies. For example, the exposure is carried out at the frequency F_1 (see FIG. 2). The ultrasound intensity $P_{treatment}$ is set higher than that used for measurements:

$$P_{treatment} = (10 \text{ to } 15)\ P_{measurement},$$

The treatment is carried out for the predetermined time interval, preferably 15 minutes.

Thus, for the ***Bac. anthracoides*** culture the ultrasonic treatment at the frequency F1 for 20 minutes has demonstrated the inactivation of the culture: the growth rate of the culture after inoculation on the nutrient medium decreases compared to the unprocessed sample specimen. Presented below are the results obtained for both cellular systems examined. The experiments show that the aforesaid modes of ultrasonic treatment of mixtures of cell cultures suppress the growth rate of the ***Bac. anthracoides*** culture

only, while in the case of the ***Bac. thurindiensis*** culture this phenomenon does not take place. The degree of activity is marked according to the admitted scale of qualitative changes (according to the number of +).

Cell structure	Processed cells	Reference
Bac. anthracoides	+	+ + +
Bac. thurindiensis	+ + +	+ + +

The experimental data presented show that the claimed ultrasonic methods allow to identify reliably the cellular systems and to produce the selective effect on the components of cell culture mixtures, thus opening new opportunities for biotechnology.

INDUSTRIAL APPLICABILITY

The method can be implemented with employing equipment and techniques known in biotechnology and ultrasonic technology.

CLAIMS

1. The acoustical method for identification of a cellular system, consisting in:

generation of ultrasonic oscillations in the medium containing a cellular system;

registration and analysis of spectra of ultrasonic oscillations propagating in the aforesaid medium,

and detection of identification features of the cellular system in the spectra,

differing in that

generation and registration of ultrasonic oscillations are performed with the measuring apparatus including the reference and measuring channels connected according to differential scheme and balanced so that, if both channels contain the same medium, the output signal spectrum has no resonance peaks;

in the process of identification the examined medium is introduced into the measuring channel, and the differential frequency spectrum, natural for the examined medium with respect to the reference one, is registered;

the resonance peaks are detected in the aforesaid frequency spectrum, and their respective frequencies are identified as the characteristic frequencies of the cellular system and/or its components in the examined medium.

2. The method according to claim 1 **differing in that** generation, reception and analysis of frequency spectra of ultrasonic

oscillations are performed in the frequency range from 10 kHz to 800 MHz.

3. The method according to claims 1 or 2 **differing in that** the media containing cellular systems are introduced into the measuring device in the form of mixtures based on aqueous solutions, mainly the physiological solution.

4. The method according to any of claims 1 - 3 **differing in that** the flow-type measuring device is employed.

5. The method according to claims 1 or 2 **differing in that** the medium containing the cellular system is introduced into the measuring device in the form of an object of biological tissue.

6. The method according to claims 1 or 2 **differing in that** the object of biological tissue used as the medium containing the cellular system is brought into acoustic bond with the measuring device placed outside and/or inside the aforesaid object of biological tissue.

7. An acoustical method for selective effect on a cellular system consisting in generation of ultrasonic oscillations in the medium containing the cellular system and placed into a measuring device supplied with the means for regulation of frequency and intensity of ultrasonic oscillations,

differing in that

the ultrasonic oscillations in the medium containing the cellular system are generated on the characteristic frequencies of ultrasonic oscillations of the cellular system and/or its components in the examined medium,

at that, the intensity of ultrasonic oscillations is set to be above the empirically found threshold value of intensity causing the irreversible destruction of the cellular system or its components.

8. The method according to claim 7 **differing in that** the intensity of ultrasonic oscillations is chosen according to the condition

$$P_{treatment} = (10 \div 15)\ P_{measurement}$$

where $P_{treatment}$ is the intensity of ultrasonic oscillations excited inside the medium in the process of selective treatment, and $P_{measurement}$ is the intensity of ultrasonic oscillations used in determining the characteristic frequencies.

9. The method according to claims 7 or 8 **differing in that** generation of ultrasonic oscillations on characteristic frequencies is carried out in the frequency range of 10 kHz to 800 MHz.

10. The method according to any of claims 7 - 9 **differing in that** the media containing the cellular systems are introduced into the measuring device in the form of mixtures based on aqueous solutions, mainly on the physiological solution.

11. The method according to any of claims 7 - 10, **differing in that** the flow-type measuring device is employed.

12. The method according to claims 7 or 8 **differing in that** the medium containing the cellular system is introduced into the measuring device in the form of an object of biological tissue.

13. The method according to claims 7 or 8 **differing in that** the object of biological tissue used as a medium containing the cellular system is brought into acoustic bond with the measuring device by placing it outside and/or inside the aforesaid object of biological tissue.

14. The apparatus for identification and selective treatment of cellular systems, including:

a measuring device for placing a medium containing cellular systems, which device comprises the means for generation and reception of ultrasonic oscillations, connected respectively to a generator of electric oscillations and a receiver with its output connected to the means for spectral analysis of ultrasonic oscillations and identification of characteristic frequencies,

differing in that

the measuring device includes measuring units intended for the reference medium and the examined medium,

the receiver has differential inputs,

the means for generation and reception of ultrasonic oscillations are embodied in the form of the wideband emitting and receiving piezoelectric transducers,

at that, the emitting piezoelectric transducers are connected in parallel to the generator of electric oscillations, and

the receiving piezoelectric transducers related to the reference medium and the examined medium is connected to the differential inputs of the receiver.

15. An apparatus according to Claim 14 **differing in that** the electric generator is embodied as a sweep generator and has a controllable attenuator.

16. An apparatus according to Claim 14 or 15 **differing in that** the piezoelectric transducers are manufactured of ceramics on the basis of solid solutions of barium/lead zirconate/titanate.

17. An apparatus according to Claim 14 - 16 **differing in that** the aforesaid measuring units for the reference medium and the examined medium are manufactured in the form of cuvettes.

18. An apparatus according to Claim 14 - 16 **differing in that** the aforesaid measuring units for the reference medium and the examined medium are manufactured in the form of the flow-type cuvettes.

19. An apparatus according to Claim 14 - 16 **differing in that** the aforesaid emitting and receiving piezoelectric transducers are placed outside and/or inside the object of biological tissue.

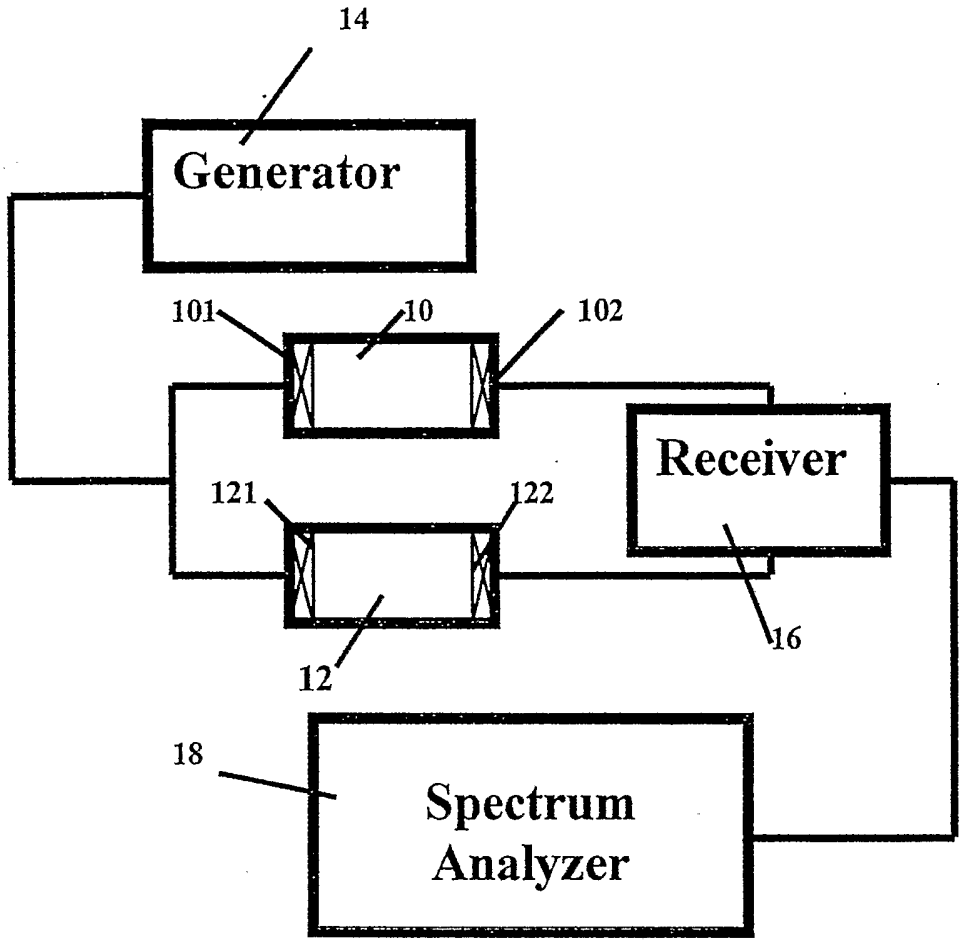
14
Generator
101
10
102
Receiver
121
122
16
12
18
Spectrum
Analyzer

FIG. 1

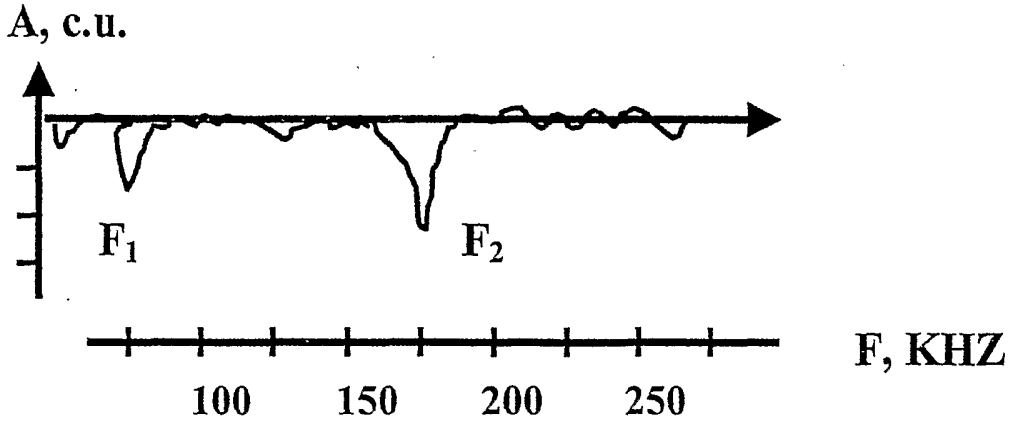

FIG. 2, A

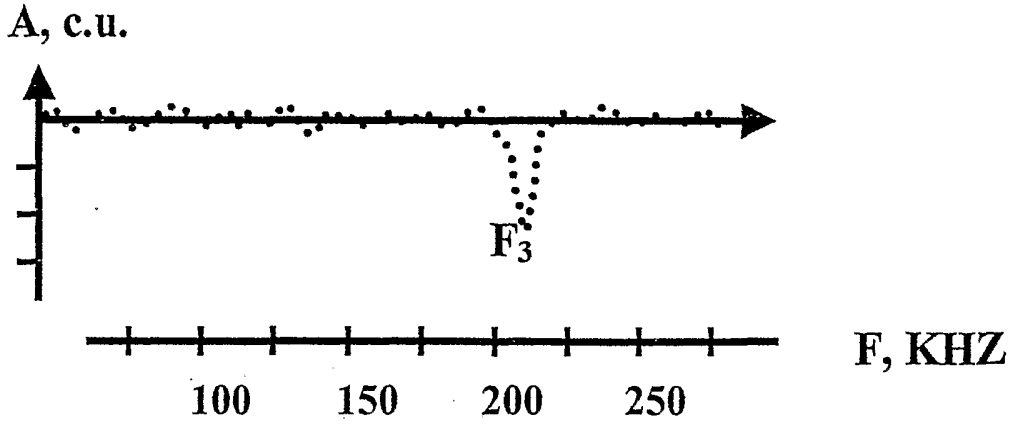

FIG. 2, B

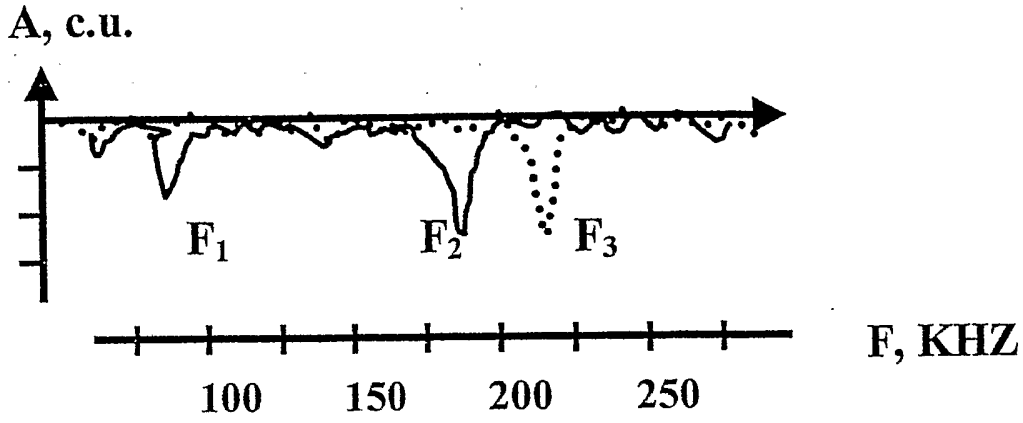

FIG. 2, C

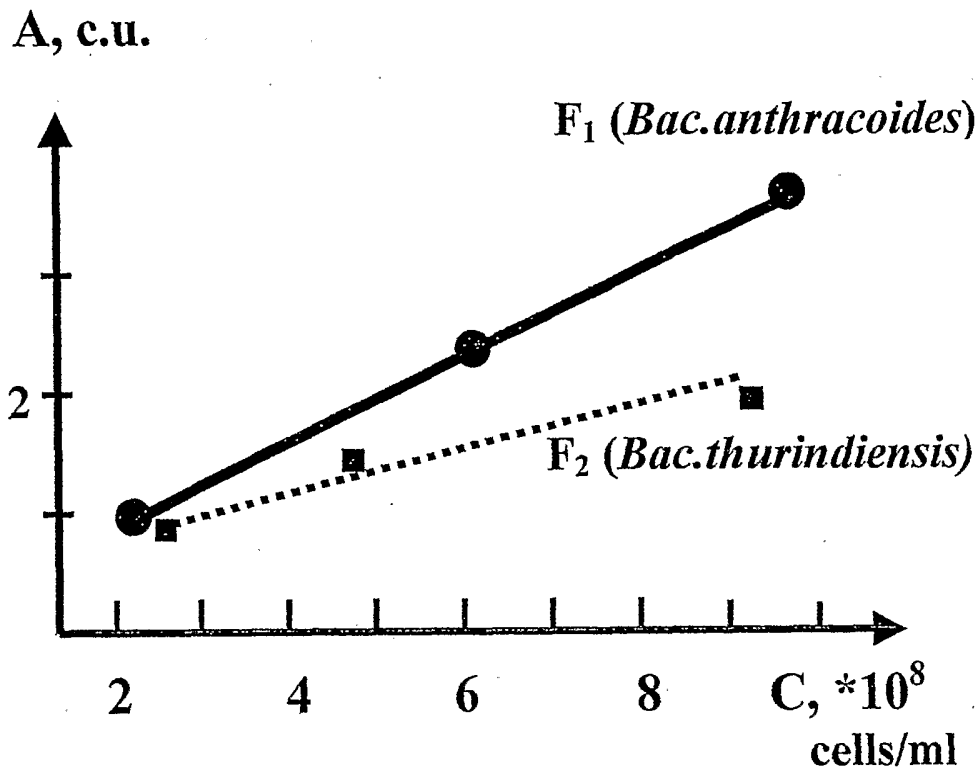
A, c.u.
F1 (Bac.anthracoides)
2
F2 (Bac.thurindiensis)
2
4
6
8
C, *10^8
cells/ml

FIG. 3

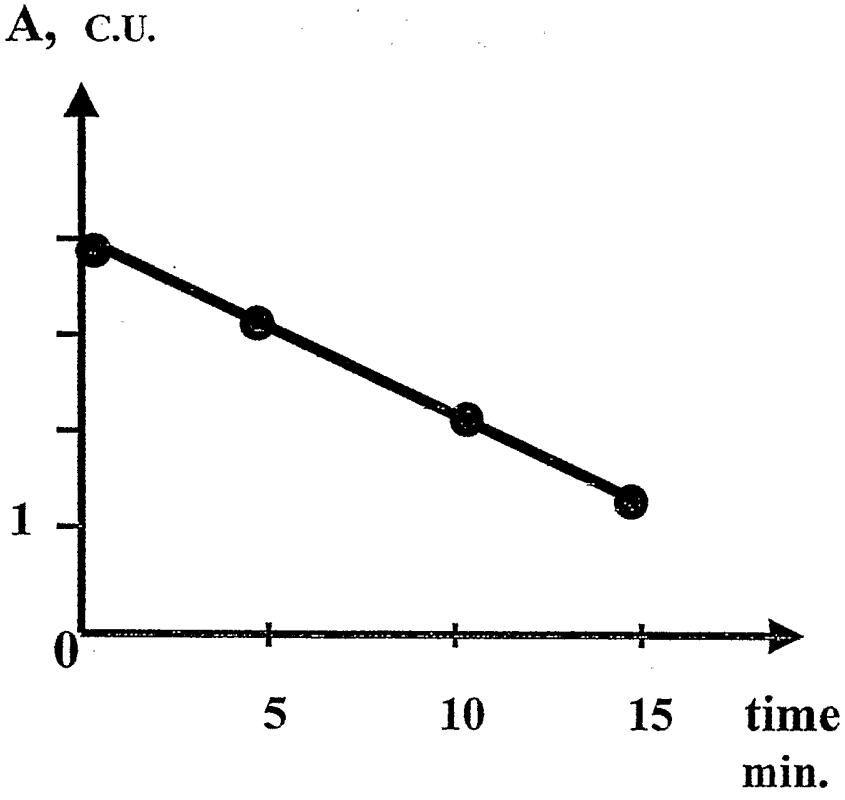

FIG. 4

INTERNATIONAL SEARCH REPORT

PCT/CZ 01/00046

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N29/04 G01N29/02

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)
IPC 7 G01N

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, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00 15097 A (BERKSHIRE LAB INC ;ABEL ALBERT E (US); BROOKS JULIANA H J (US)) 23 March 2000 (2000-03-23) cited in the application	1,14
A	page 4, line 11 -page 5, line 26 page 8, line 6 - line 16 page 9, line 24 -page 10, line 10 page 20, line 29 -page 21, line 23 figure 2; example 2; table 1	7
Y	WO 89 03035 A (HEALTH LAB SERVICE BOARD) 6 April 1989 (1989-04-06)	1,14
A	page 1 -page 8	7
	-/--	

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"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
5 June 2002	12/06/2002

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, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Joyce, D

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EGGERS F ET AL: "BROAD-BAND ULTRASONIC MEASUREMENT TECHNIQUES FOR LIQUIDS" MEASUREMENT SCIENCE AND TECHNOLOGY, IOP PUBLISHING, BRISTOL, GB, vol. 7, no. 1, 1996, pages 1-19, XP000551465 ISSN: 0957-0233 the whole document	1,7,14
A	US 5 952 560 A (CUSACK PETER JOHN ET AL) 14 September 1999 (1999-09-14) column 2, line 55 -column 6, line 54	1,7,14
A	WO 98 01394 A (CORDEMANS DE MEULENAER ERIC ;CANIVET YVES (BE); HANNECART BAUDOUIN) 15 January 1998 (1998-01-15) the whole document	1,7,14
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