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(54) Title: JOSAMYCIN-BASED PHARMACEUTICAL COMPOSITION AND A PROCESS FOR PREPARING THE SAME

(57) Abstract: The present invention relates to the field of pharmacology and medicine, particularly to an antimicrobial controlled-released drug composition based on josamycin, containing the antibiotic, a copolymer of lactic and glycolic acid, D- mannitol, and polyvinyl alcohol in dimethyl sulfoxide.
Josamycin-based pharmaceutical composition and a process for preparing the same

The present invention relates to the field of pharmacology and medicine, particularly to generation of controlled-release drug compositions based on josamycin, containing submicron size particles and having antimicrobial activity.

Josamycin is a macrolide antibiotic. Its chemical name is "3-acetate-4B-(3-methylbutanoate) leukomycin V". The compound also exists as propionate. The compound is produced by actinomycetes Streptomyces narbonensis var. josamyceticus.

Josamycin is known to have a broad spectrum of activity [1]. This antibiotic acts on gram-positive (Staphylococcus spp., producing and not producing penicillinase, Streptococcus spp., including Streptococcus pyogenes and Streptococcus pneumoniae, Bacillus anthracis, Corynebacterium diphtheriae) and gram-negative (Neisseria gonorrhoeae, Neisseria meningitidis, some kinds of Shigella, Haemophilus influenzae, Bordetella pertussis) bacteria, intracellular microorganisms (Mycoplasma spp., including Mycoplasma hominis, Mycoplasma pneumoniae, Chlamydia spp., including Chlamydia trachomatis, Chlamydia pneumoniae, Ureaplasma urealyticum, Legionella pneumophila), and certain anaerobes (Peptococcus, Peptostreptococcus, Clostridium perfringens, Bacteroides fragilis). Josamycin is even effective in case of resistance to erythromycin. Resistance to josamycin occurs less frequently than to other macrolide antibiotics. When creating high concentrations in an inflammation nidus the drug has bactericidal effect. The action mechanism of josamycin is based on reversible binding to the 50S ribosomal subunit, which inhibits protein synthesis and proliferation of microbial cells.
Josamycin is well distributed in the body and accumulated in various tissues: in lung, lymph
tissue of palatine tonsils, organs of urinary tract, skin and soft tissues [1]. Especially high
concentrations are determined in lungs, tonsils, saliva, sweat and tear liquid. Josamycin con-
centration in human polymorphonuclear leukocytes, monocytes and alveolar macrophages
is about 20 times higher than in other cells. Josamycin is biotransformed in the liver to less
active metabolites. It is excreted mainly with the bile, the excretion in the urine being less
than 20%.

Josamycin is used to treat infections of the upper respiratory tract and ENT-organs (such as
pharyngitis, tonsillitis, paratonsillitis, otitis media, sinusitis, laryngitis), diphtheria, scarla-
tina, infections of the lower respiratory tract (such as acute bronchitis, bronchopneumonia,
pneumonia, including atypical forms, whooping cough, psittacosis), oral infections (such as
gingivitis and paradontium disease), skin and soft tissue infections (such as pyoderma, boils,
anthrax, erysipelas, acne, lymphangitis, lymphadenitis), infections of the urinary tract and
genital organs (such as urethritis, prostatitis, gonorrhea, syphilis, venereal lymphogranu-
loma), Chlamydia, mycoplasma (such as ureaplasma) and mixed infections of the urinary
tract and genitals. Since 2012 josamycin has been included in the list of vital and essential
drugs.

Josamycin, as the majority of antibiotics used in therapy of various infectious diseases, has
many serious drawbacks caused by the low selectivity of action and, consequently, increased
toxicity. When introducing the drug in the body only a small part of it enters the target organ
or cell. Considerable part of the injected drug undergoes biotransformation, without showing
antibacterial action. In this connection, a need exists to introduce an excess amount of the
antibiotic, which leads to serious toxic effects. From the digestive tract organs: lack of ap-
petite, nausea, heartburn, vomiting, disbacteriosis and diarrhea, flatulence, coated tongue,
abdominal cramps, abnormal liver function, transient elevation of liver transaminase (AST,
ALT) activity, compromised bile outflow and jaundice. Others: swelling of feet, transient
dose-related hearing loss, candidiasis, allergic skin reactions (hives, rash) and, very rarely -
fever and general malaise.

To solve the above problems it is necessary to solve the following tasks: to improve drug
efficacy and selectivity of its action and thus to reduce the therapeutic dose and a toxic effect
on the patient, as well as to create fine-dispersed form of the drug, convenient also for injection use. Numerous literature data and the results of our previous studies on a number of anti-tuberculosis antibiotics \([2 - 5]\) show that the inclusion of drug substances in polymer capsules can successfully solve the above problems. Polymer-containing systems provide intracellular drug localization in alveolar macrophages in effective amounts for treatment and have a prolonged effect by adjusting the rate of substance release from the polymer matrix. Furthermore, drug forms with delayed release of active compounds can reduce drug dosage frequency to once a day. Reduction of toxic effects is thus achieved. It should be noted that up to date josamycin was not obtained in a polymer composition to solve the above problems.

Earlier E.S. Severin \emph{et al.} \([6]\) have developed and patented in the Russian Federation a new method for producing controlled-action medicament based on cycloserine, pyrazinamide, and isoniazid containing nanoparticles. These preparations presented solutions of the above antibiotics, polymer, emulsion stabilizer and diuretic in dimethyl sulfoxide (DMSO). As already indicated, in medical practice DMSO is used in treatment of rheumatoid arthritis, Bechterew's disease, discoid lupus erythematosus, thrombophlebitis, eczema, furunculosis, amyloidosis, etc. \([8, 9]\). Together with disulfiram DMSO leads to a prolonged sensitizing effect in alcohol-dependent patients \([10]\) by subcutaneous administration.

Josamycin is currently available in the form of coated tablets (500 mg), dispersible tablets (1 g), suspension for taking internally (in 5 ml - 150 mg). All of the above-mentioned forms are suitable for oral administration. These dosage forms of antibiotics are suitable to be taken by the patients. However, they create the conditions for uncontrolled admission and self-medication. Mode of the treatment process and the patient recovery process are often disturbed. In this case, effectiveness of treatment depends on drug dosage frequency during a day. The following data were published: only 37.7% of patients follow the dosage regimen in case of a 3-fold drug intake, 68.9% - in case of a 2-fold intake and 79.6% - in case of a single intake \([11]\). This also leads to the rapid development of resistance to the drug used. In this regard, as an alternative for drugs for oral administration it is necessary to create formulations of injectable antibiotics, which may be controlled by a physician.
In order to solve the problems associated with josamycin toxicity and to study the possibility of extending of the method for the preparation of polymer capsules with other classes of antibiotics, in particular macrolides, the present inventors have carried out studies using said antibiotic.

Using changes in structural order of dimethylsulfoxide (DMSO) at a temperature range from 20 to 60 °C and its high activity in coordination solvation [7], the present inventors have surprisingly found such ratios of josamycin, PLGA (50/50), D-mannitol, polyvinyl alcohol and DMSO under which a fluid, homogeneous and transparent system is formed, which is stable under room temperature conditions. When diluting the drug composition obtained with water in a ratio of 1:5 to 1:20 (w/w), preferably 1:20, a stable opalescent suspension containing particles of size of about 200 - 300 nm (100%) is formed.

While compositions containing more simple antibiotics, such as cycloserine, have been developed, it is not a simple task to obtain a nanoparticle composition of josamycin, which is a macrolide antibiotic. Josamycin has severe solubility problems. Therefore, till now, josamycin drugs are available only in insoluble forms, i.e. as tablets and coarse suspension.

The present inventors have succeeded in producing polymer-containing forms of josamycin, and the method of the present invention is characterized by simplicity of its realization. The method leads to creation of a macrolide antibiotic composition with high level of specific activity and duration of action. The composition obtained on the basis of josamycin can be used by both oral and injection method of administration.

Thus, a new drug composition based on josamycin, for purposes of the present invention is in the form of polymeric capsules containing the active substance immobilized on the biodegradable polymer carrier (a copolymer of lactic and glycolic acids - PLGA), and nonionic surfactant (polyvinyl alcohol) and an osmotic diuretic (D-mannitol).

The inclusion of josamycin in nanoparticles of polymeric carriers based on biodegradable polymers in combination with plasticizers and cryoprotectants allows achieving highly selective and controlled release of josamycin from nanoparticles. As an additional advantage
it is possible to get completely rid of the body of the polymer matrix, as a result of biodegradation.

This kind of polymer-containing system provides intracellular josamycin localization directly in the macrophages, providing a prolonged action due to the regulation of the substance release rate from the polymer matrix.

To evaluate the antimicrobial activity of the composition as obtained, we have used a more precise method of double serial dilutions of the antibiotic in a liquid medium (broth) in accordance with the recommendations of NCCLS [11], instead of the antibiotic diffusion in the agar (disc-diffusion method) in accordance with the recommendations of NCCLS [12].

Studies of antibacterial activity have shown that the composition as obtained in the form of polymer-based capsules based on josamycin has either higher or similar antibacterial activity compared to free antibiotics against gram-positive, gram-negative and against atypical bacteria. Significantly higher antibacterial activity compared to the free substance (8-fold) was observed in the drug against Enterococcus faecalis.

Results of investigation of the in vivo specific activity of the composition, on the model of staphylococcal sepsis of mice, showed that free josamycin substance is 1.5-fold less efficient than the josamycin composition according to the present invention.

Thus, a new medicament based on josamycin, low toxic drug having a wide range of high-performance, anti-bacterial action was obtained. Presence of high antimicrobial activity of the new formulation against Staphylococcus aureus is especially noteworthy. Staphylococci are extremely common representatives of the microflora of human skin and mucous membranes. Staphylococci cause many infections, including superficial and deep pyogenic infections, intoxication and urinary tract infections. In the United States, they are the leading cause of sepsis, postoperative wound infection and endoprosthesis infection. Among the hospital infectious agents they are on the second place at frequency. In addition, staphylococci are one of the leading causes of microbial food poisoning. The most important for a human
staphylococcus that causes a variety of diseases, is *Staphylococcus aureus*. It is a resistant infectious agent, easily acquiring resistance against antimicrobial drugs.

The following types of staphylococcal infections are distinguished:

- Food poisoning caused by food contamination with staphylococcus toxins;
- Staphylococcal infection, with lesions of the skin integuments (the skin) and mucous membranes - "superficial infection";
- Staphylococcal infection with visceral involvement;
- Septic forms of staphylococcal infection - "blood poisoning."

Despite the fact that these infections occur in people of any age, it is the severest for children and the elderly, especially those who suffer from chronic diseases. Primary staphylococcal pneumonia usually occurs in children and rarely in adults. Acute staphylococcal osteomyelitis is registered only in children, the superficial staphylococcal pyoderma - most often in infants, whereas abscess formation occurs mainly in adults. Thus, development of new drugs or new forms of already known drugs of high antimicrobial activity, in particular against *Staphylococcus aureus*, is an urgent problem.

The nano-josamycin composition as obtained according to the present invention solves this problem, since it has more effective antimicrobial effect compared with the free substance, particularly in regard to *Staphylococcus aureus*.

Technical result is achieved solely by the ability of polymeric carriers based on biodegradable polymers in combination with plasticizers and cryoprotectants to implement highly selective and dosed release of the active substance into the organs-target-cell and then to be completely excreted. The obtained therapeutic effect is persistent as achieved by long stable maintaining of a drug therapeutic dose. A technologically simple method for its preparation is proposed. Ability to dilute the drug with water in a ratio of from 1:5 to 1:20 (w/w) may be used to adjust the dosage of the drug, depending on the individual patient characteristics.

The invention is further illustrated by the following examples.
Example 1. A method of preparing a drug composition based on josamycin

In a three-necked glass flask equipped with a stirrer driven by an electric motor, a thermometer and an air (reflux) refrigerator at 20 - 25 °C the ingredients listed in Table 1 at the indicated percentages were loaded successively. The mixture was stirred and heated on a heating mantle at 50 - 60 °C until complete dissolution of the solids, then cooled to room temperature for 20 - 30 minutes. The finished drug was stored in a sealed container of orange glass. Below is a composition of the drug which was obtained.

The above composition is a transparent homogeneous liquid. The composition is stable when stored over a year. When 20 parts of water is added to 1 part of the josamycin composition, i.e. the composition is diluted with water in a ratio of 1:20 (w/w), a stable opalescent suspension is formed. The particle size determination thereof is given in Example 2.

Table 1. Drug composition

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of component</th>
<th>wt %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Josamycin</td>
<td>2.95 - 3.05</td>
</tr>
<tr>
<td>2</td>
<td>PLGA 50/50</td>
<td>2.95 - 3.05</td>
</tr>
<tr>
<td>3</td>
<td>D-Mannitol</td>
<td>2.95 - 3.05</td>
</tr>
<tr>
<td>4</td>
<td>Polyvinyl alcohol</td>
<td>1.45 - 1.55</td>
</tr>
<tr>
<td>5</td>
<td>Dimethylsulfoxide</td>
<td>the rest</td>
</tr>
</tbody>
</table>

Note. The ratio of the components in the drug composition was chosen based on:
- an antibiotic therapeutic dose;
- drug homogeneity in the temperature range of 20 - 25 °C;
- stability of the main quality indicators in storage.

Example 2. Determination of the particle size of the drug composition in case of diluting with water

Determination of the size and size distribution of particles in the fractions was performed by photon correlation spectroscopy using Malvern Zetasizer ZS instrument (Malvern, United Kingdom). The drug pre-mixed with water in a ratio of 1:20 (w/w) was added to the cuvette
(3 ml) in an amount of 1.3 ml and then measured. The size of the formed particles was in a narrow range of 200 - 300 nm.

Example 3. Evaluation of the drug antimicrobial activity

Determination of the antimicrobial activity of the preparation sample against test cultures of gram-positive and gram-negative microorganisms was carried out by the serial microdilution method in a liquid medium in accordance with the recommendations NCCLS [11] at the visual registration of visible growth. Dynamic measurement of absorbance was performed by using a multichannel spectrophotometer Bioscreen (Labsystems) at a wavelength of 610 nm at 20 minute intervals. Plates with bacterial suspensions were incubated at 37 °C in a thermostatic device module. The initial concentration of microorganisms was 5 x 10^5 CFU/ml. The antimicrobial activity of the drug was determined by the values of the minimal inhibitory concentration (MIC) of the microorganism growth. As the test cultures were used reference strains - Staphylococcus aureus (ATCC 29213), Methicillin-resistant Staphylococcus aureus MRSA (ATCC 43300), Enterococcus faecalis (ATCC 25922), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853). The results are summarized in Table 2.

Table 2. The antimicrobial activity of the composition based on josamycin.

<table>
<thead>
<tr>
<th>PREPARATION</th>
<th>Minimal inhibitory concentration, µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus ATCC 29213</td>
</tr>
<tr>
<td></td>
<td>Methicillin-resistant Staphylococcus</td>
</tr>
<tr>
<td></td>
<td>aureus ATCC 43300</td>
</tr>
<tr>
<td></td>
<td>Enterococcus faecalis ATCC 25922</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli ATCC 25922</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa ATCC 27853</td>
</tr>
<tr>
<td>Josamycin (substance*)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>&gt;128</td>
</tr>
<tr>
<td>Josamycin (composition**)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

* Josamycin substance, which was diluted according to GOST R ISO 20776-1-2010

** Josamycin composition of the present invention
Example 4. Evaluation of the antimicrobial activity of the drug *in vivo.*

The study of the antimicrobial activity of the josamycin composition of the present invention *in vivo* was performed on the sepsis model caused by *Staphylococcus aureus* (strain 10, adapted to mice) after intravenous injection process. As a comparison josamycin substance (diluted in 1% starch gel) was used in an equivalent amount.

In the experiments female mice of colony SHK weighing 22 - 25 g were used. The animals were kept in a vivarium on a standard diet of briquetted feed with free access to drinking water. After 2-week quarantine healthy animals were used in experimental work. Initially lethal dose (WiN) of the staphylococcus for the given line of mice of certain weight by intravenous way of infection was determined. Accounting for the death of the mice was performed daily for 10 days. The lethal dose was 3x10⁷ CFU/mouse.

To determine the relative effectiveness of the test drugs (josamycin composition and josamycin substance) the mice were infected intravenously with *Staphylococcus aureus* in a lethal dose at volume of 0.25 ml. 30 min after the infection josamycin substance (diluted in 1% starch) or the josamycin composition (in suspension) was orally administered to the mice in 4 doses each. As a control group there was a group of untreated animals infected with *Staphylococcus aureus* (lethal dose). The animals were observed for 14 days, the daily death was taken into account. The experimental results are presented in Table 3.
Table 3. Determination of the specific activity of josamycin composition as compared with the josamycin substance on the staphylococcal sepsis model of mice.

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Doses (mg/kg)</th>
<th>Days of experiment (numerator-dead animals, denominator-survivors)</th>
<th>% loss</th>
<th>% survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Josamycin (composition)</td>
<td>100</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0/5 0/5 0/5 0/5 1/4 1/4 1/4 1/4 1/4</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0/5 0/5 1/4 1/4 1/4 1/4 1/4 1/4 1/4</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0/5 1/4 2/3 2/3 2/3 2/3 2/3 2/3 2/3</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0/5 1/4 2/3 3/2 3/2 3/2 3/2 3/2 3/2</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Josamycin (substance)</td>
<td>100</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0/5 0/5 0/5 0/5 1/4 1/4 1/4 1/4 1/4</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0/5 0/5 1/4 1/4 2/3 2/3 2/3 2/3 2/3</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0/5 1/4 2/3 3/2 3/2 3/2 3/2 3/2 3/2</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0/5 1/4 2/3 3/2 3/2 3/2 3/2 3/2 3/2</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Control doses St. aur.</td>
<td></td>
<td>0/5 1/4 3/2 4/1 5/0 5/0 5/0 5/0 5/0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Determination of the sensitivity of microorganisms to antimicrobial agents was carried out according to the methodical instructions MUK 4.2.1890-04 approved and enacted by the Chief State Sanitary Doctor of the Russian Federation G.G.Onischenko 4.3.2004.
Results of specific activity studies of the compositions in vivo on staphylococcal sepsis model of mice showed that both test drugs had pronounced efficacy. Effectiveness of josamycin substance is significantly weaker than josamycin composition (1.5 times). Half amount of the effective dose (ED50) for josamycin substance is 5.6 mg/kg, while the ED50 value for the new josamycin composition is 3.5 mg/kg.

**Example 5.** Toxicty assessment of the composition based on josamycin

The study was carried out on male mice of SHK line weighing 28 - 30 g for 6 groups of 6 mice each, from which 4 groups - experimental, 2 - control. When testing, all mice were injected intragastrically by an atraumatic tip with doses (400, 470, 500 and 700 mg/kg) in a form of suspension in water in amount of 0.5 ml every 30 minutes.

At the post-mortem dissection of experimental animals, changes of the size and shape of the heart and kidney, the surface of the lung, and spleen were not found. While chest and abdominal cavity examination any disorders of internal organ position were not observed. The heart muscle was brownish, dense. The surface of the lung was pale pink color, the lung deflated at the opening of the chest. It should be noted that any changes of the size and shape of the liver, where the biotransformation of josamycin to less active metabolites occurs, were not revealed. Liver tissue was brownish with large light-brown spots. Consistency of the organ was dense. The size and shape of the kidneys were not different from controls. Organ surface was smooth, uniform grayish-brown color.
References


2. Fang Jia-Hwa, Mannohman Singh, O'Heygan Derek, Maninder Horn. Compositions of microparticles and methods for their preparation. / Patent RU № 2257198 - 2005 (WO02/26212)


8. Instruction on medical use of the drug DIMEXIDU. Registration number: PN 003411/01 dated 22.04.2005.


Claims

1. A pharmaceutical composition, characterized in that it comprises josamycin, a copolymer of lactic and glycolic acid (PLGA 50/50), D-mannitol, polyvinyl alcohol (PVA) and dimethyl sulfoxide (DMSO), with the following component ratios, wt %:

<table>
<thead>
<tr>
<th>Component</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Josamycin</td>
<td>2.95 - 3.05</td>
</tr>
<tr>
<td>PLGA 50/50</td>
<td>2.95 - 3.05</td>
</tr>
<tr>
<td>D-mannitol</td>
<td>2.95 - 3.05</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>0.95 - 1.00</td>
</tr>
<tr>
<td>DMSO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>the rest.</td>
</tr>
</tbody>
</table>

2. The pharmaceutical composition according to claim 1, characterized in that it has antimicrobial activity and that it, when diluted with water in a ratio of 1:20 (w/w), forms a suspension of submicron particles of size 200 - 300 nm.

3. The pharmaceutical composition according to claim 2, characterized that it has in vivo antimicrobial activity in a model of staphylococcal sepsis.

4. A method of preparation of the pharmaceutical composition according to claim 1, characterized by the successive steps of

- loading a vessel with josamycin, PLGA 50/50, D-mannitol, PVA and DMSO,
- heating the mixture to the temperature of 50 - 60 °C,
- stirring until complete dissolution of the solids, and
- cooling to room temperature.

5. The method according to claim 4, characterized by a further step of

- adding water to the resulting mixture in a ratio of 1:20 (w/w), to obtain a suspension of submicron particles of size 200 - 300 nm.
### A. CLASSIFICATION OF SUBJECT MATTER

**IPC:** see extra sheet  
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:** A61 K, A61 P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, PAJ, WPI data, CHEM ABS Data, EMBASE, MEDLINE

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>JPS5271489 A, MORIYAMA K ET AL, 1977-06-1 4, abstract, retrieved from WPI database; abstract</td>
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<tr>
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<td>CN1 01380291 A, KONG Q, 2009-03-1 1, abstract, retrieved from WPI database; abstract</td>
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<td>A</td>
<td>US 20020064547 A1 (CHERN REY T ET AL), 30 May 2002 (2002-05-30); claims; See paragraphs [0022], [0037], [0062]</td>
<td>1-5</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
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