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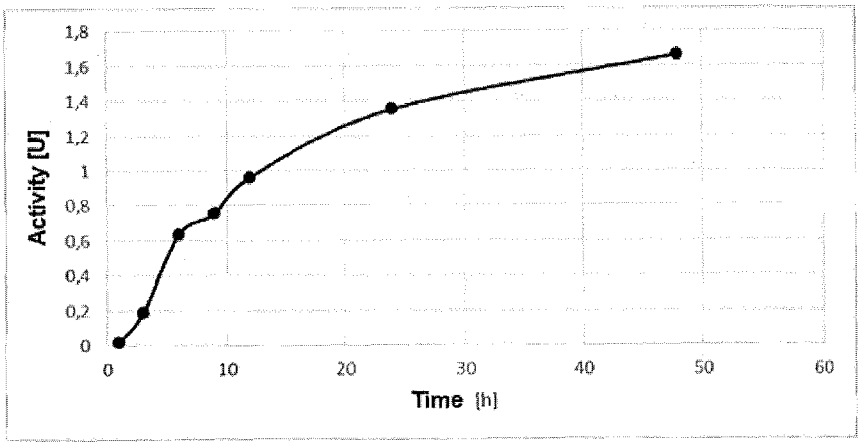

Fig. 1

(57) Abstract: The first object of the invention is an implantological composition comprising cystatin, a carrier, a neutralizing agent, deionized water or buffer, characterized in that it comprises cystatin with specific activity of at least 10 inhibitor units per milligram of protein in the amount of 0.02% (by weight), a carrier substance in the amount of 15.0% to 20.0% (by weight) and a neutralizing agent in the amount of no more than necessary to obtain a suitable pH value, preferably selected from the group comprising: NaOH or TEA, and the composition comprising deionized water or PBS buffer making up to 100% (by weight) of the weight of the composition. The second object of the invention is a use of the implantological composition for coating implants or for local administration to promote osseointegration.

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AN IMPLANTOLOGICAL COMPOSITION COMPRISING CYSTATIN AND ITS USE IN BONE IMPLANTOLOGY

The object of the invention is a pharmaceutical composition comprising cystatin and poloxamer P407 and carbomer 971P or carbomer 974P, as well as other compounds, such as nipagins, and use thereof in bone implantology, in particular for coating implants or for application on an implant fixation site in order to promote effective osseointegration thereof.

Cystatins have inhibitory properties towards proteases involved in bone resorption, they stimulate bone formation and mineralization, they also have immunosuppressive and antimicrobiological activity. Due to all of these properties, coating of TSADs (Temporary Skeletal Anchorage Devices) with a cystatin layer or applying it to the fixation site may at the same time prevent bone resorption events in the vicinity of the TSAD, promote its overgrowth with new bone tissue, inhibit its rejection through an immunological route, as well as prevent microbiological superinfections (Goto T, Yamaza T, Tanaka T.: Cathepsins in the osteoclast. J Electron Microsc (Tokyo), 2003, 52(6):551-8; Everts V, Korper W, Hoeben KA, Jansen ID, Bromme D, Cleutjens KB, Heeneman S, Peters C, Reinheckel T, Saftig P, Beertsen W.: Osteoclastic bone degradation and the role of different cysteine proteinases and matrix metalloproteinases: differences between calvaria and long bone. J Bone Miner Res. 2006, 9, 1399-408; Danjo A, Yamaza T, Kido MA, Shimohira D, Tsukuba T, Kagiya T, Yamashita Y, Nishijima K, Masuko S, Goto M, Tanaka T.: Cystatin C stimulates the differentiation of mouse osteoblastic cells and bone formation. Biochem Biophys Res Commun., 2007, 360, 199-204).

Despite the general relatively high success rate of implantation, failures in implant use in some patients in fact force researchers to search for factors that promote effective bone tissue fusion with implant titanium surface, namely the so-called osseointegration. In case of failures, a total or partial lack of this phenomenon is observed. Osseointegration may also occur solely for a period of time and then decline. The causes of such failures are not always known. These may often depend on factors biological in nature, e.g. the bone may be too sparsely vascularized, or its quality or compactness may prove not to be adequate. Loosening of implant fixation may also involve adverse bone remodeling at the fixation site due to immunological reactions, activation of mechanically stimulated osteoclasts or due to superinfections with microorganisms, which penetrate deep into bone tissue during a surgical procedure.

In the remodeling process, *inter alia* cysteine proteases of the papain family are involved, mainly cathepsin B, L and K, which are secreted into the extracellular matrix by osteoclasts. Cysteine proteases are also utilized by a variety of bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus, Perphyromonas gingivalis*), parasites (*Trypanosoma cruzi, Leishmania donovani*) and fungi (*Candida albicans, Candida parapsilosis* and *Candida tropicalis*) in an invasive process to degrade host tissues and they are essential for proliferation in some viruses (*Polio, Picornavirus*).

In physiological conditions, excessive activity of these enzymes is limited by their endogenous inhibitors called cystatins. Due to this, cystatins are considered as potential pharmaceuticals in therapy of diseases, in the course of which excessive proteolytic activity is found or of diseases associated with an infection with microorganisms. It was also found that cystatins stimulate osteoblast differentiation, as well as formation

and mineralization of bones. Cystatins also have immunosuppressive properties by inhibiting legumain and cathepsin S involved in presentation of antigens mediated by MHCs class II.

Use of cystatins in medicine was the object of patents and patent applications globally. In Polish patent document PAT.217192, a use of cysteine protease inhibitor monomer lyophilizate, being ovocystatin, as an active substance of a pharmaceutical agent used on preventing and/or treating of dementia, including preventing and inhibiting cognitive functions deterioration, especially those progressing along with development of dementia, in particular in Alzheimer's disease and in aging process. In turn, in a Japanese patent application JP2017205111A, a protein composition was disclosed comprising angiogenin and/or its degradation products in an amount of 2 to 15 mg/100 mg and cystatin and/or its degradation products with a mass ratio to angiogenin in range of 0.003 to 0.6. As a result of using the protein composition, bones can be strengthened, and it can thereby prevent and treat various bone diseases, such as osteoporosis, bone fracture, rheumatism and arthritis. In US2918153789A1, the disclosure relates to an oral composition for reducing teeth sensitivity. The oral composition comprises zinc and a copper salt capable of coating and closing dental tubules by protein agglomeration providing in a short period of time an effect on preventing and reducing teeth hypersensitivity symptoms. The composition comprises a foaming agent (an anionic surfactant such as sodium alkyl sulfate and sodium lauryl sulfate), a nonionic surfactant (copolymer (poloxamer) polyoxyethylene-polyoxypropylene, polyoxyethylated hydrogenated castor oil or polyoxyethylene sorbitan) and a fatty acid ester. The foaming agent can be present in the amount of 0.5 to 5% by weight in relation to the total mass of the oral composition and preferably 0.5 to 3.5% by weight. Polish patent PAT.219098 discloses a pharmaceutical composition for treating periodontal diseases in open and inpatient dental treatment, as well as in dental surgery. The pharmaceutical composition is in a gel form with adjusted viscosity. Its active ingredient is a base consisting of an inhibitor of cysteine peptidases derived from egg whites, urea, human and animal amniotic fluid, human and animal placenta and from plants, with an activity of 1-50 inhibitor units, being suspended in 50 g of a polyhydric alcohol and 50 g of a 0.05 molar solution of phosphate buffer with a pH of 6.0 to 7.5, in the amount of 20 to 50% by weight, with its carrier being gelling agents in the amount of 7 to 20% by weight and water making up to 100% by weight. The gelling agents are methyl cellulose, hydroxypropyl methyl cellulose, sodium carboxymethyl cellulose, gelatin, citrus-apple pectin or dextran. It is preferable to use additives in the form of polyhydric alcohols and the Nipagin composition.

Despite high implantation efficacy (93.43%), application failures – particularly unwarranted in the mandible where the cortical plaque should provide for good mechanical retention of TSADs – compel not only the necessity of searching for factors promoting disintegration of mini-implants but also active efforts to form conditions preventing their premature loss. Loosening of TSAD's fixation may be associated with the phenomenon of bone remodeling at the site of the screw by direct activation of mechanically stimulated osteoclasts or indirectly due to superinfections with microorganisms penetrating through an incision in the gingiva deep into bone tissue together with the TSAD. Thus, preparations are being sought that would support bone remodeling, sustain the osseointegration process and inhibit or even thwart superinfections with microorganisms during surgical procedures. In that respect, this kind of preparation should also be easy to use, easy to administer locally, nonirritating to mouth tissues and exhibiting good adherence to implant surfaces or solid tissues. Furthermore, this kind of preparation should be characterized with long term stability. Unexpectedly, the hereinabove problems are solved by the present invention.

The first object of the invention is an implantological composition comprising cystatin, a carrier, a neutralizing agent, deionized water or buffer, characterized in that it comprises cystatin with specific activity of at least 10 inhibitor units per milligram of protein in the amount of 0.02% (by weight), a carrier substance in the amount of 15.0% to 20.0% (by weight) and a neutralizing agent in the amount of no more than necessary to obtain a suitable pH value, preferably selected from the group comprising: NaOH or TEA, and the composition comprising deionized water or PBS buffer making up to 100% (by weight) of the weight of the composition.

In a preferred embodiment of the invention, the carrier substance is poloxamer 407.

In a further preferred embodiment of the invention, the composition comprises a gelling agent in the amount of no more than 0.25% (by weight) of the weight of the composition. The composition may be applied to soft tissues as well as solid tissues and implants, depending on the content of the gelling agent. When it is absent from the composition then the composition shows better adhesive properties towards a soft tissue surface, e.g. epithelium. In contrast, after the addition of a gelling agent, the composition adheres better to solid tissue or implants.

In a further preferred embodiment of the invention, an adhesive properties enhancing substance is carbomer 974P or carbomer 971P.

In a further preferred embodiment of the invention, the composition comprises additives.

In a preferred embodiment of the invention, the additives constitute from 0% to 25% (by weight) of the weight of the composition.

In a further preferred embodiment of the invention, the additives are selected from the group comprising: parabens (nipagins), glycerol, polyoxyethylene glycol 200, propylene glycol.

In a further preferred embodiment of the invention, cystatin is of animal or plant origin or it is human recombinant.

In a further preferred embodiment of the invention, the suitable pH of the composition is 7.40 ± 0.05.

In a further preferred embodiment of the invention, the composition is in a liquid form in temperature of 20°C.

In a further preferred embodiment of the invention, the composition is in a gel form in temperature of 37°C.

The second object of the invention is a use of the implantological composition for coating implants or for local administration to promote osseointegration.

Embodiments of the invention are illustrated on figures, wherein Fig. 1 shows the activity of cystatin released in time from the formulation of the following composition: 17% poloxamer 407 and 0.02% cystatin, in a base of deionized water, Fig. 2 shows the activity of cystatin released in time from the formulation of the following composition: 17% poloxamer 407, 0.1% carbomer 974P and 0.02% cystatin, in a base of deionized water, Fig. 3 shows shear stress as a function of shear rate for the prepared gels in temperature

of 20°C, Fig. 4 shows shear stress as a function of shear rate for the prepared gels in temperature of 37°C, Figs 5-8 show viscosity as a function of temperature for the prepared gels.

The studies show that cystatin at the concentration of 0.2 mg/ml is stable in the conditions of the preparation of the formulation. Also, the presence of poloxamer 407 (at the concentration of 15%-20% w/w) and carbomer 974P (at the concentration of 0.1-0.25% w/w) has no adverse effect on cystatin activity (Example 8). Moreover, it was found that the formulation with the concentration of 0.2 mg/ml is stable for the period of at least 12 months (Example 9). To summarize, both the method of preparation of the formulation and its composition provide a fully active and stable cystatin formulation.

The prepared hydrogels are characterized by an initial (until about 10 hours) rapid release of cystatin, whereas in the following hours the release rate is gradually decreasing (Fig. 1 and 2). Hydrogels based on poloxamer 407 and carbomers 971P/974P can be used as a sustained release form for a biologically active protein such as cystatin.

All the prepared hydrogels in temperature of 20°C were viscoelastic liquids, whereas in human body temperature (37°C) they were in gel form. This is a known property of hydrogels containing poloxamer P407, called temperature dependent thixotropy. These properties are advantageous for using the formulation as an implant coating substance and the high viscosity allows retention of the formulation at the application site. The liquid form of the formulation in 20°C facilitates its application at the implant fixation site. Below (Fig. 3 and 4) shear stress as a function of shear rate is shown, in temperatures of 20°C and 37°C for gels on the basis of deionized water, with the following compositions:

- 17% poloxamer 407 (Examples – composition I).
- 17% poloxamer 407, 0.1% carbomer 974P, sodium hydroxide to obtain pH of 7.4 (Examples – composition II),

In human body temperature (37°), formulations based on poloxamer 407 were in the form of gel. The prepared hydrogels in temperature 37°C show a much higher viscosity than in temperature of 20°C. This is a known property of hydrogels comprising this polymer, called temperature dependent thixotropy. It is caused by the formation of micelles by poloxamer 407 chains as the temperature increases. The transition from sol to gel occurred in the range of 20.5°C to 33°C, and thus above room temperature and below human body temperature, due to which the hydrogels can remain liquid during storage and application and after contacting mucus membranes increase their viscosity. Below (Figs 5, 6, 7 and 8) viscosity as a function of temperature is shown for gels on the basis of deionized water, with the following compositions:

- 17% poloxamer 407 (Examples – composition I).
- 17% poloxamer 407, 0.1% carbomer 974P, sodium hydroxide to obtain pH of 7.4 (Examples – composition II)
- 17% poloxamer 407, 0.25% carbomer 974P, sodium hydroxide to obtain pH of 7.4 (Examples – composition III)
- 15% poloxamer 407 (Examples – composition IV)
- 15% poloxamer 407, 0.175% carbomer 974P, sodium hydroxide to obtain pH of 7.4 (Examples – composition V)

- 20% poloxamer 407 (Examples – composition IV)
- 20% poloxamer 407, 0.175% carbomer 974P, sodium hydroxide to obtain pH of 7.4 (Examples – composition VII)

Example 1. Composition I

Cystatin	0.02 g
Poloxamer 407	17 g
Deionized water	ad 100.0 g

To a weighted portion of the solvent 17 g of poloxamer 407 are added, followed by adding the rest of deionized water to the mass of 99.98 g. The whole mixture is agitated in order to provide wetting of the introduced poloxamer, and then it is placed in temperature of 2-8°C for 24h to obtain a clear solution. After this time, the poloxamer solution is transferred to 0.02 g of freeze dried cystatin, it is mixed until the cystatin is dissolved and a homogenous mixture is obtained having a pH of 7.4. Next, the solution is filtered through a syringe filter with pore diameter of 0.22 µm in aseptic conditions. The filtered solution is sealed in ampoules having a volume of 2 ml in aseptic conditions. The obtained agent is intended for coating of bone implants.

Example 2. Composition II

Cystatin	0.02 g
Carbomer 974P	0.1 g
Poloxamer 407	17 g
10M NaOH solution	q.s.
Deionized water	ad 100.0 g

To about 60 g of deionized water 0.1 g of carbomer 974P is added, and then it is mixed until a homogenous mixture is obtained. Next, with constant agitation, 17 g of poloxamer 407 are added in small portions to the mass of about 77 g. After wetting the introduced poloxamer, the whole mixture is placed in temperature of 2-8°C for 24h for it to dissolve. Then, the mixture is adjusted to pH = 7.40 ± 0.05 using 2-5 drops (as needed) of 10M NaOH solution and with deionized water to the mass of 80 g. After reaching suitable pH, the prepared gel is sterilized in an autoclave in the following conditions: 121°C and overpressure of 1 atm for 15 minutes. After the sterilization process, the gel is cooled and then in aseptic conditions 20 g of 17% poloxamer 407 solution comprising 0.02 g of freeze dried cystatin, filtered through a syringe filter with pore diameter of 0.22 µm, is added therein. The whole mixture is agitated until a homogenous mixture is obtained. The ready solution is sealed in ampoules having a volume of 2 ml in aseptic conditions. The obtained agent is intended for coating of bone implants.

Example 3. Composition III

Cystatin	0.02 g
Carbomer 974P	0.25 g
Poloxamer 407	17 g
10M NaOH solution	q.s.
Deionized water	ad 100.0 g

To about 60 g of deionized water 0.25 g of carbomer 974P is added, and then it is mixed until a homogenous mixture is obtained. Next, with constant agitation, 17 g of poloxamer 407 are added in small portions to the mass of about 77 g. After wetting the introduced poloxamer, the whole mixture is placed in temperature of 2-8°C for 24h for it to dissolve. Then, the mixture is adjusted to pH = 7.40 ± 0.05 using 2-5 drops (as needed) of 10M NaOH solution and with deionized water to the mass of 80 g. After reaching suitable pH, the prepared gel is sterilized in an autoclave in the following conditions: 121°C and overpressure of 1 atm for 15 minutes. After the sterilization process, the gel is cooled and then in aseptic conditions 20 g of 17% poloxamer 407 solution comprising 0.02 g of freeze dried cystatin, filtered through a syringe filter with pore diameter of 0.22 µm, is added therein. The whole mixture is agitated until a homogenous mixture is obtained. The ready solution is sealed in ampoules having a volume of 2 ml in aseptic conditions. The obtained agent is intended for coating of bone implants.

Example 4. Composition IV

Cystatin	0.02 g
Poloxamer 407	15 g
Deionized water	ad 100.0 g

To a weighted portion of the solvent 15 g of poloxamer 407 are added, followed by adding the rest of deionized water to the mass of 99.98 g. The whole mixture is agitated in order to provide wetting of the introduced poloxamer, and then it is placed in temperature of 2-8°C for 24h to obtain a clear solution. After this time, the poloxamer solution is transferred to 0.02 g of freeze dried cystatin, it is mixed until the cystatin is dissolved and a homogenous mixture is obtained having a pH of 7.4. Next, the solution is filtered through a syringe filter with pore diameter of 0.22 µm in aseptic conditions. The filtered solution is sealed in ampoules having a volume of 2 ml in aseptic conditions. The obtained agent is intended for coating of bone implants.

Example 5. Composition V

Cystatin	0.02 g
Carbomer 974P	0.175 g

Poloxamer 407	15 g
10M NaOH solution	q.s.
Deionized water	ad 100.0 g

To about 60 g of deionized water 0.175 g of carbomer 974P is added, and then it is mixed until a homogenous mixture is obtained. Next, with constant agitation, 15 g of poloxamer 407 are added in small portions to the mass of about 77 g. After wetting the introduced poloxamer, the whole mixture is placed in temperature of 2-8°C for 24h for it to dissolve. Then, the mixture is adjusted to pH = 7.40 ± 0.05 using 2-5 drops (as needed) of 10M NaOH solution and with deionized water to the mass of 80 g. After reaching suitable pH, the prepared gel is sterilized in an autoclave in the following conditions: 121°C and overpressure of 1 atm for 15 minutes. After the sterilization process, the gel is cooled and then in aseptic conditions 20 g of 15% poloxamer 407 solution comprising 0.02 g of freeze dried cystatin, filtered through a syringe filter with pore diameter of 0.22 µm, is added therein. The whole mixture is agitated until a homogenous mixture is obtained. The ready solution is sealed in ampoules having a volume of 2 ml in aseptic conditions. The obtained agent is intended for coating of bone implants.

Example 6. Composition VI

Cystatin	0.02 g
Poloxamer 407	20 g
Deionized water	ad 100.0 g

To a weighted portion of the solvent 20 g of poloxamer 407 are added, followed by adding the rest of deionized water to the mass of 99.98 g. The whole mixture is agitated in order to provide wetting of the introduced poloxamer, and then it is placed in temperature of 2-8°C for 24h to obtain a clear solution. After this time, the poloxamer solution is transferred to 0.02 g of freeze dried cystatin, it is mixed until the cystatin is dissolved and a homogenous mixture is obtained having a pH of 7.4. Next, the solution is filtered through a syringe filter with pore diameter of 0.22 µm in aseptic conditions. The filtered solution is sealed in ampoules having a volume of 2 ml in aseptic conditions. The obtained agent is intended for coating of bone implants.

Example 7. Composition VII

Cystatin	0.02 g
Carbomer 974P	0.175 g
Poloxamer 407	20 g
10M NaOH solution	q.s.
Deionized water	ad 100.0 g

To about 60 g of deionized water 0.175 g of carbomer 974P is added, and then it is mixed until a homogenous mixture is obtained. Next, with constant agitation, 20 g of poloxamer 407 are added in small portions to the mass of about 77 g. After wetting the introduced poloxamer, the whole mixture is placed in temperature of 2-8°C for 24h for it to dissolve. Then, the mixture is adjusted to pH = 7.40 ± 0.05 using 2-5 drops (as needed) of 10M NaOH solution and with deionized water to the mass of 80 g. After reaching suitable pH, the prepared gel is sterilized in an autoclave in the following conditions: 121°C and overpressure of 1 atm for 15 minutes. After the sterilization process, the gel is cooled and then in aseptic conditions 20 g of 20% poloxamer 407 solution comprising 0.02 g of freeze dried cystatin, filtered through a syringe filter with pore diameter of 0.22 µm, is added therein. The whole mixture is agitated until a homogenous mixture is obtained. The ready solution is sealed in ampoules having a volume of 2 ml in aseptic conditions. The obtained agent is intended for coating of bone implants.

Example 8. Compositions of the formulations and their anti-papain activity

	Composition	Cystatin activity
Composition 1	17% poloxamer 407 0.02% cystatin	1.246 U/ml
Composition 2	17% poloxamer 407 0.1% carbomer 974P 0.02% cystatin .NaOH until pH of 7.4	1.167 U/ml
Composition 3	17% poloxamer 407 0.25% carbomer 974P 0.02% cystatin .NaOH until pH of 7.4	1.197 U/ml
Composition 4	15% poloxamer 407 0.02% cystatin	1.211 U/ml
Composition 5	15% poloxamer 407 0.175% carbomer 974P 0.02% cystatin .NaOH until pH of 7.4	1.259 U/ml
Composition 6	20% poloxamer 407 0.02% cystatin	1.270 U/ml
Composition 7	20% poloxamer 407 0.175% carbomer 974P 0.02% cystatin	1.222 U/ml

	.NaOH until pH of 7.4	
Reference composition	0.02% cystatin (aqueous solution)	1.251 U/ml

Example 9. Stability of an example formulation in the period of 12 months.

Gel composition	Cystatin activity at time t=0	Cystatin activity after 12 months
17% poloxamer 407 0.02% cystatin	1.246 U/ml	1.358 U/ml
17% poloxamer 407 0.1% carbomer 974P 0.02% cystatin NaOH until pH of 7.4	1.167 U/ml	1.160 U/ml
17% poloxamer 407 0.25% carbomer 974P 0.02% cystatin NaOH until pH of 7.4	1.197 U/ml	1.250 U/ml
15% poloxamer 407 0.02% cystatin	1.211 U/ml	1.201 U/ml
15% poloxamer 407 0.175% carbomer 974P 0.02% cystatin NaOH until pH of 7.4	1.259 U/ml	1.265 U/ml
20% poloxamer 407 0.02% cystatin	1.270 U/ml	1.243 U/ml
20% poloxamer 407 0.175% carbomer 974P 0.02% cystatin NaOH until pH of 7.4	1.222 U/ml	1.182 U/ml

Claims

1. Implantological composition comprising cystatin, a carrier, a neutralizing agent, water or buffer, characterized in that it comprises cystatin with specific activity of at least 10 inhibitor units per milligram of protein in the amount of 0.02% (by weight), a carrier substance in the amount of 15.0% to 20.0% (by weight) and a neutralizing agent in the amount of no more than necessary to obtain a suitable pH value, preferably selected from the group comprising: NaOH or TEA, and the composition comprising deionized water or PBS buffer making up to 100% (by weight) of the weight of the composition.

2. The composition of claim 1, characterized in that the carrier substance is poloxamer 407.

3. The composition of claim 1 to 2, characterized in that the composition comprises a gelling agent in the amount of no more than 0.25% (by weight) of the weight of the composition.

4. The composition of claim 1 to 3, characterized in that an adhesive properties enhancing substance is carbomer 974P or carbomer 971P.

5. The composition of claim 1 to 4, characterized in that the composition comprises additives.

6. The composition of claim 1 to 5, characterized in that the additives constitute from 0% to 25% (by weight) of the weight of the composition.

7. The composition of claim 1 to 6, characterized in that the additives are selected from the group comprising: parabens (nipagins), glycerol, polyoxyethylene glycol 200, propylene glycol.

8. The composition of claim 1 to 7, characterized in that cystatin is of animal or plant origin or it is human recombinant.

9. The composition of claim 1 to 8, characterized in that the suitable pH of the composition is 7.40 ± 0.05.

10. The composition of claim 1 to 9, characterized in that the composition is in a liquid form in temperature of 20°C.

11. The composition of claim 1 to 10, characterized in that the composition is in a gel form in temperature of 37°C.

12. Use of the implantological composition as defined in claim 1 to 11 for coating implants or for local administration to promote osseointegration.

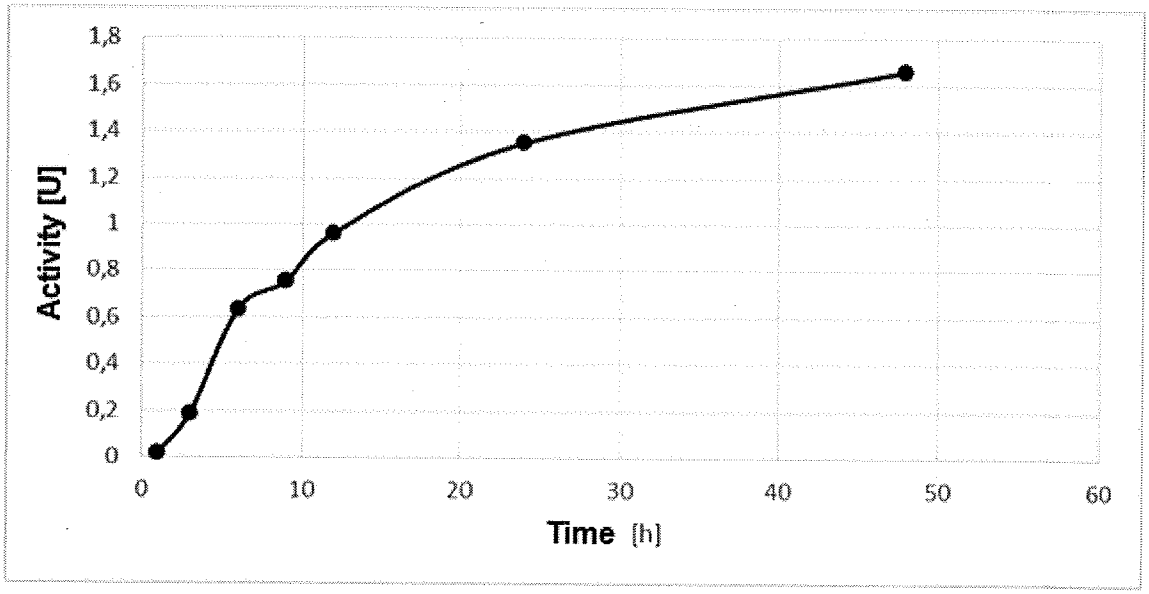
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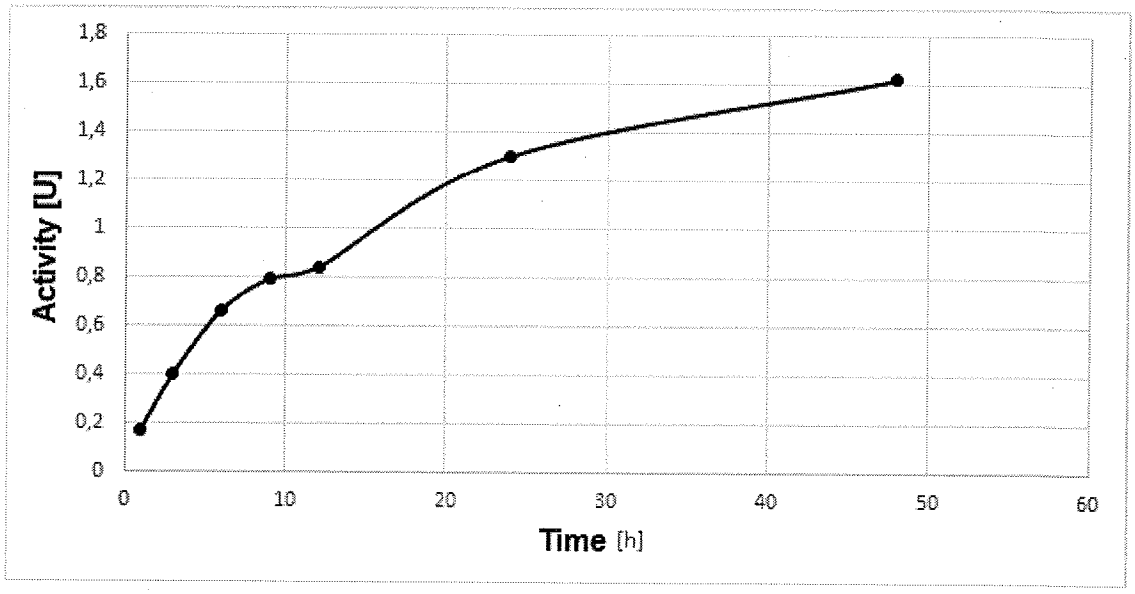
Fig. 1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Time [h]

Fig. 2

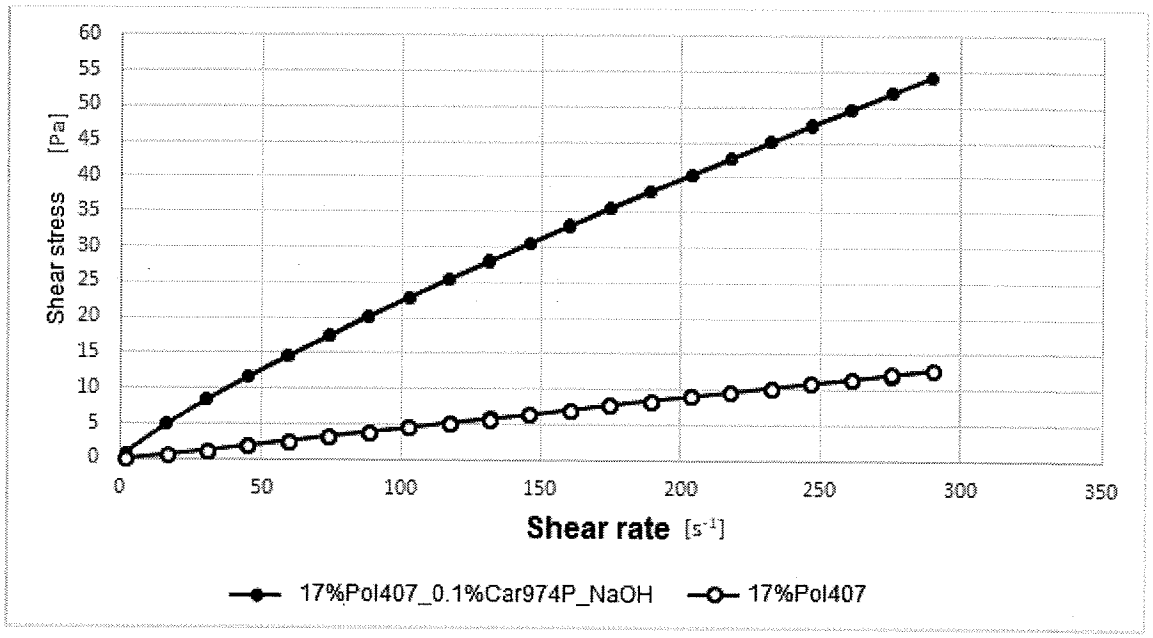
Shear stress [Pa]
60
55
50
45
40
35
30
25
20
15
10
5
0
0
50
100
150
200
250
300
350
Shear rate [s⁻¹]
17%Pol407_0.1%Car974P_NaOH
17%Pol407

Fig. 3

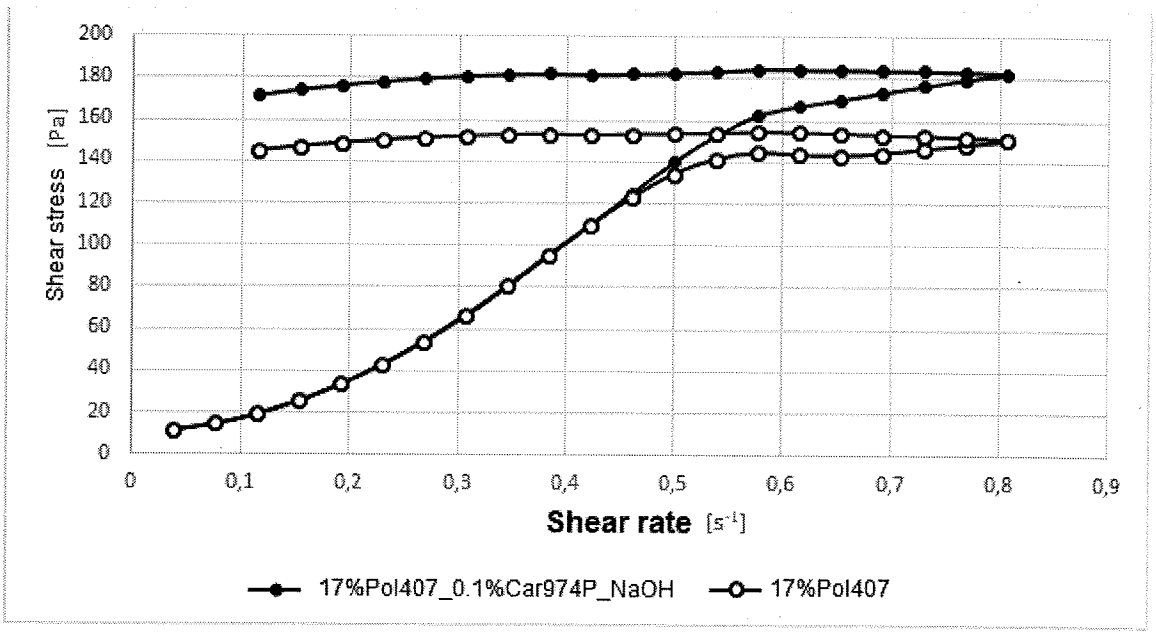
Shear stress [Pa]
200
180
160
140
120
100
80
60
40
20
0
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
Shear rate [s⁻¹]
17%Pol407_0.1%Car974P_NaOH
17%Pol407

Fig. 4

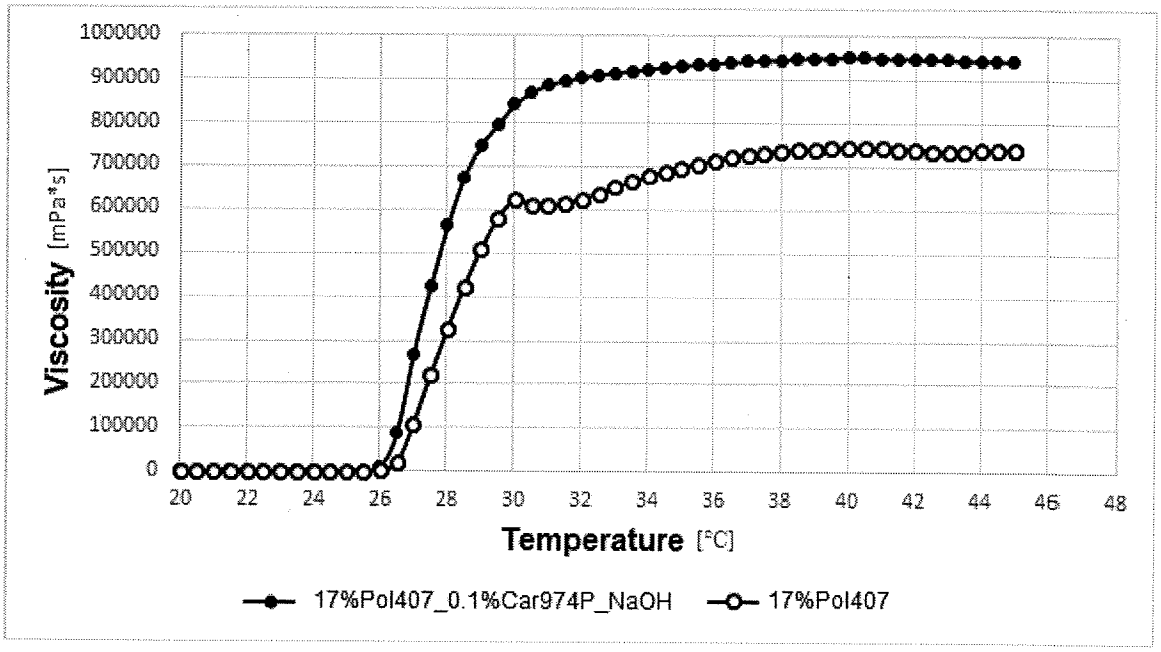
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Viscosity [mPa*s]
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
Temperature [°C]
17%Pol407_0.1%Car974P_NaOH
17%Pol407

Fig. 5

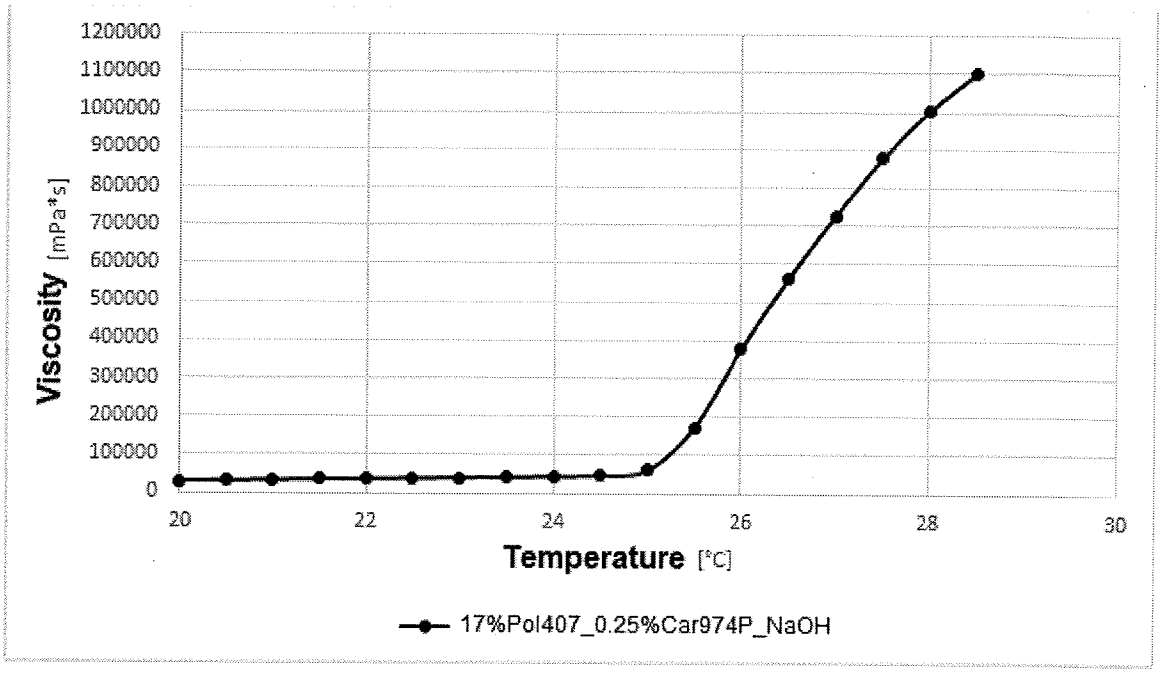
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Viscosity [mPa*s]
20
22
24
26
28
30
Temperature [°C]
17%Pol407_0.25%Car974P_NaOH

Fig. 6

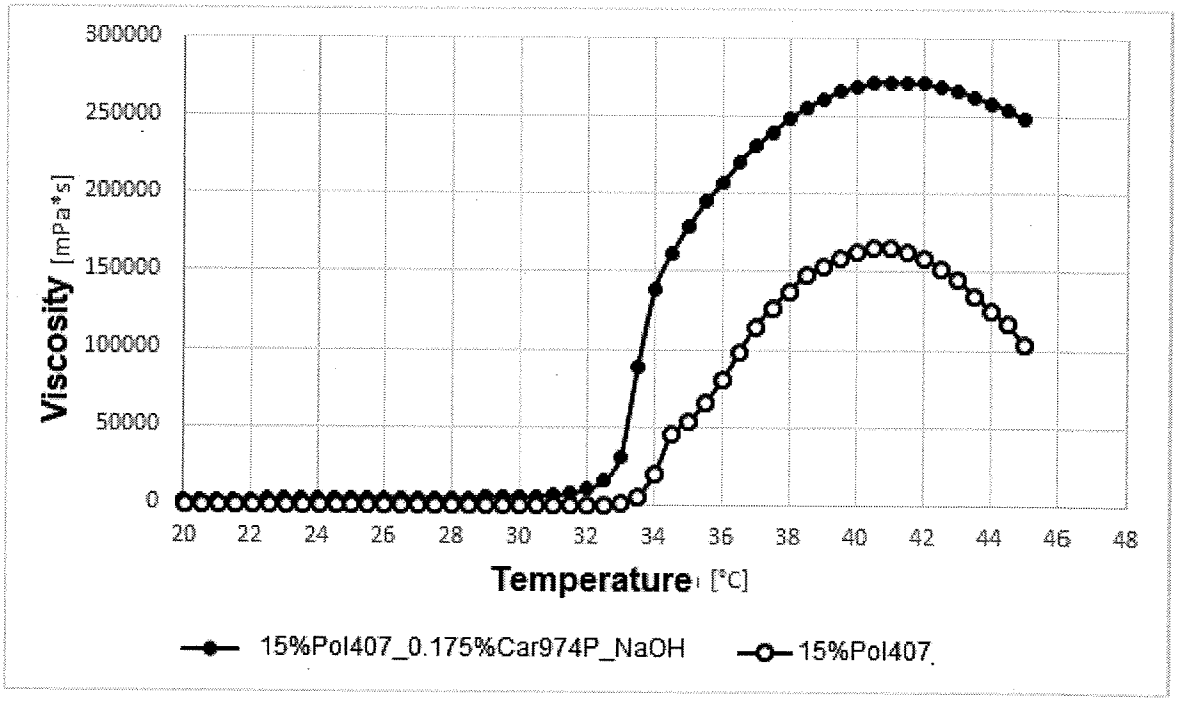
300000
250000
200000
150000
100000
50000
0
Viscosity [mPa*s]
20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
Temperature [°C]
15%Pol407_0.175%Car974P_NaOH
15%Pol407

Fig. 7

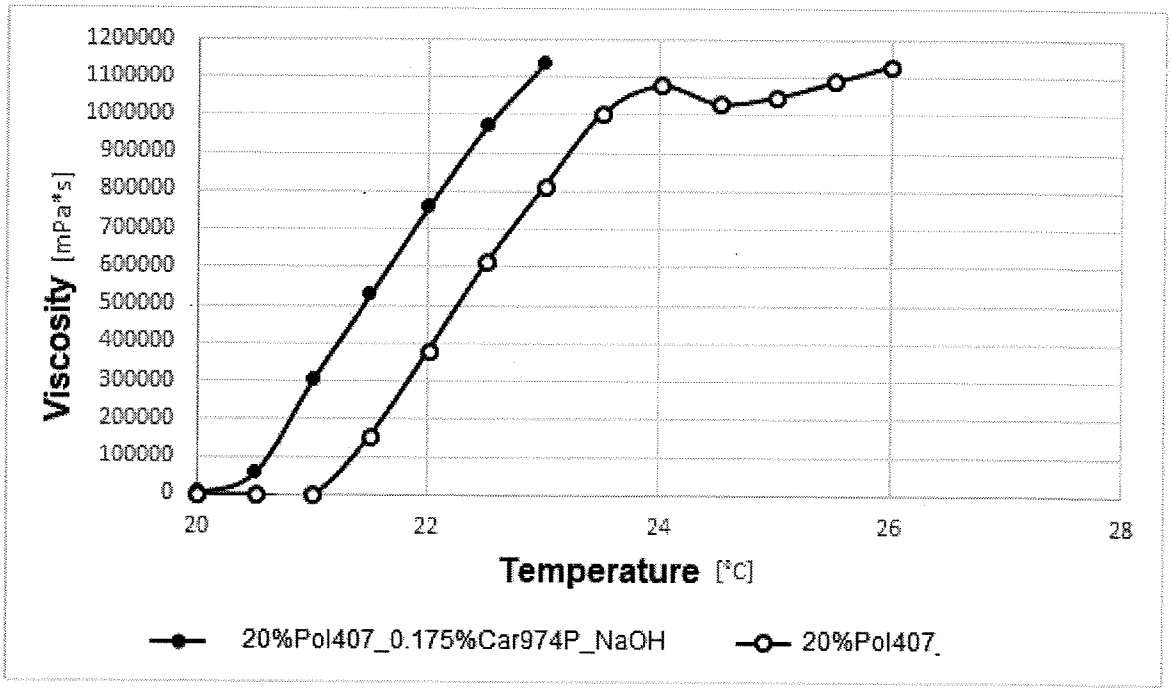
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Viscosity [mPa*s]
20 22 24 26 28
Temperature [°C]
20%Pol407_0.175%Car974P_NaOH
20%Pol407

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/PL2019/050069

A. CLASSIFICATION OF SUBJECT MATTER
A61L27/28 (2006.01), A61L27/54 (2006.01), A61K38/55 (2006.01)

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)
A61L27/28, A61L27/52 , A61L27/54, A61K38/55, A61K47/34

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Epodoc&WPI via Epoquenet, Database of PPO, Internet (Google)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	PL219098 B1 (UNIWERSYTET MEDYCZNY IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU, Wrocław, PL), 2015-03-31, claim 1	1 2-12
A	PL 218970 B1 (UNIWERSYTET MEDYCZNY IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU, Wrocław, PL), 2015-02-27, claim 1	1-12
A	PL 195665 B1 (VALEANT SPÓŁKA Z OGRANICZONĄ ODPOWIEDZIALNOŚCIĄ SPÓŁKA JAWNA, Rzeszów, PL), 2007-10-31, claims 1-3	1-12
Y A	WO0156627A1 (AM PHARMA BV; NIEUW AMERONGEN ARIE VAN; T HOF WILLEM VAN; VEERMAN ENGELMUNDUS CORN), 2001-08-09, abstract	12 1-11

☐ 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
"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
16 Mar 2020 (16.03.2020)	16 Mar 2020 (16.03.2020)

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International application No.
PCT/PL2019/050069

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
PL219098 B1	2015-03-31	.....	.....
PL218970 B1	2015-02-27	.....	.....
PL195665B1	2007-10-31	.....	.....
WO0156627A1	2001-08-09	.....	.....