

Certified translation from German

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BF 360 B
FN 145825 z

EXTRACT
of Erber Aktiengesellschaft

Effective day: July 25, 1996 ABF

Last registration on June 18, 1996 with the registration number 1
Competent court: Provincial Court of St. Pölten

NAME

1 Erber Aktiengesellschaft

LEGAL FORM

1 Stock corporation

SEAT in

1 Political municipality of Herzogenburg

BUSINESS ADDRESS

1 Industriestraße 21
3130 Herzogenburg

CAPITAL

1 ATS 3,000,000.-

ANNUAL CLOSING

1 December 31

POWER TO REPRESENT

1 If several managing directors are appointed, the corporation shall
be represented by two managing directors jointly.

MANNER OF NOTIFICATION

1 Corporation's notifications shall be made in the Official Journal
of the Vienna Gazette (Amtsblatt zur Wiener Zeitung).

1 Articles of Feb. 19, 1996

1 Contribution agreement of February 19, 1996
Contribution of the company of
Ing. Erich Erber
limited partnership
(FN 20218 h)

001
002



MANAGING DIRECTORS

A Ing. Erich Erber, born April 30, 1953
has represented alone since June 18, 1996

PROCURATOR

B Margarete Erber, born August 21, 1959
has represented alone since June 18, 1996

C Andreas Kern, born November 16, 1963
has represented since June 18, 1996 together
with one more procurator

D Maria Prisching, born April 26, 1962
has represented since June 18, 1996 together
with one more procurator

SUPERVISORY BOARD

E Dr. Kurt Weinreich, born August 31, 1960,
chairman

F Reinhard Brader, born August 23, 1967
vice-chairman

G Martin Josef Pressl, born February 2, 1972
member

----- PERSONS -----

A Ing. Erich Erber, born April 30, 1953,
Dr.-Otto-Tschadek-Straße 13
3100 St. Pölten

B Margarete Erber, born August 21, 1959
Dr.-Otto-Tschadek-Straße 13
3100 St. Pölten

C Andreas Kern, born November 16, 1963,
Wieshofstraße 2
3121 Karlstetten

D Maria Prisching, born April 26, 1962,
Perschlingweg 5
3141 Kapelln

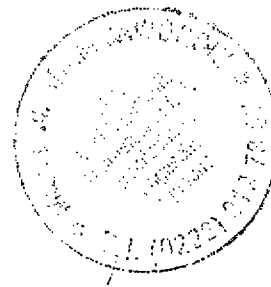
E Dr. Kurt Weinreich, born August 31, 1960,

F Reinhard Brader, born August 23, 1967

G Martin Josef Pressl, born February 2, 1972

----- SUMMARY OF EXECUTIVE ACTS -----

Commercial Court of St. Pölten



1 registered on June 18, 1996 act 28 Fr 1507/96 f
application for new registration of a company obtained on June 5, 1996

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***** END *****

Date of inquiry: July 25, 1996

Made out by RADIO-AUSTRIA

Round seal: Dr. Helmut Scheubrein, notary public in Wien-Alsergrund

Austrian coat-of-arms

Stamp: notary public
as judicial commissioner

/-/ signature

Vienna, November 26, 1996

Wien, 26.11.1996

Die genaue Übereinstimmung der
vorstehenden Übersetzung mit der
vorliegenden Urschrift - Abschrift
- Ablichtung bestätige ich unter
Berufung auf meinen Eid.

Referring to my oath of office, I hereby
certify that the foregoing translation
exactly agrees with the original document
presented to me

Sworn Court Interpreter
Adam Jaworski
Adam Jaworski



BF360B A U S Z U G
FN 145825 z Erber Aktiengesellschaft

Stichtag: 25.07.1996 AB

Letzte Eintragung am 18.06.1996 mit der Eintragsnummer 1
zuständiges Gericht Landesgericht St. Pölten

FIRMA

1 Erber Aktiengesellschaft

RECHTSFORM

1 Aktiengesellschaft

SITZ in

1 politischer Gemeinde Herzogenburg

GESCHÄFTSANSCHRIFT

1 Industriestraße 21
3130 Herzogenburg

KAPITAL

1 ATS 3.000.000

STICHTAG für JAHRESABSCHLUSS

1 31. Dezember

VERTRETUNGSBEFUGNIS

1 Die Gesellschaft wird, wenn mehrere Vorstandsmitglieder
bestellt sind, durch zwei Vorstandsmitglieder gemeinsam
vertreten.

ART DER BEKANNTMACHUNG

1 Die Bekanntmachungen der Gesellschaft erfolgen im Amtsblatt
zur Wiener Zeitung.

1 Satzung vom 19.02.1996

1 Einbringungsvertrag vom 19.02.1996

Einbringung des Betriebes der
Ing. Erich Erber
Kommanditgesellschaft
(FN 20218 h)

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VORSTAND

1 A Ing. Erich Erber, geb. 30.04.1953
vertritt seit 18.06.1996 selbständig

PROKURIST

1 B Margarete Erber, geb. 21.08.1959
vertritt seit 18.06.1996 selbständig

1 C Andreas Kern, geb. 16.11.1963
vertritt seit 18.06.1996 gemeinsam mit
einem weiteren Prokuristen

1 D Maria Prisching, geb. 26.04.1962
vertritt seit 18.06.1996 gemeinsam mit
einem weiteren Prokuristen

AUFSICHTSRAT

1 E Dr. Kurt Weinreich, geb. 31.08.1960
Vorsitzender

1 F Reinhard Brader, geb. 23.08.1967
Stellvertreter des Vorsitzenden

1 G Martin Josef Pressl, geb. 02.02.1972
Mitglied

----- PERSONEN -----

1 A Ing. Erich Erber, geb. 30.04.1953
1 Dr.-Otto-Tschadek-Straße 13
3100 St.Pölten
1 B Margarete Erber, geb. 21.08.1959
1 Dr.-Otto-Tschadek-Straße 13
3100 St.Pölten
1 C Andreas Kern, geb. 16.11.1963
1 Wieshofstraße 2
3121 Karlstetten
1 D Maria Prisching, geb. 26.04.1962
1 Perschlingweg 5
3141 Kapelln
1 E Dr. Kurt Weinreich, geb. 31.08.1960
1 F Reinhard Brader, geb. 23.08.1967
1 G Martin Josef Pressl, geb. 02.02.1972

----- VOLLZUGSÜBERSICHT -----

Landesgericht St. Pölten

1 eingetragen am 18.06.1996 Geschäftsfall 28 Fr 1507/96 f
Antrag auf Neueintragung einer Firma eingelangt am 05.06.1996

INHALTSVERZEICHNIS

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***** ENDE ***** Abfragedatum: 25.07.1996
erstellt über RADIO-AUSTRIA



Helmut Scheuberein
öffentl. Notar
Gerichtskommissär

96 11089 -5

16 DEC 1996

Enzyme-Containing Bactericidal Composition, and Dental and Wound Treatment Preparations Comprising this Composition.

This invention relates to a bactericidal composition of the type containing at least one enzyme which in aqueous milieu and in the presence of oxygen and a substrate may form oxygen peroxide together with a peroxidase and a thiocyanate.

The bactericidal composition according to the invention is generally applicable, but as the composition is primarily developed and tested with respect to preparing a composition for the prevention and treatment of gingivitis and paradentosis, the invention will be more particularly explained in the following with a view to obtaining a solution of this problem.

It is generally accepted that dental plaque depositing on the tooth surfaces as bacterial coatings which contain more or less protein and carbon hydrate substances, epithelial cells and food debris or stimulant debris are sharing the responsibility for caries, dental calculus, gingivitis and paradentosis. Certain of the bacteria synthesize some strongly adhesive carbon hydrates (dextrans) from carbon hydrates of the intaken foodstuffs or stimulants and particularly sucrose constitutes the major basis of the formation of the dextrans. The bacteria in plaques develop organic acids, in particular lactic acid and acetic acid due to the fermentative decomposition and reaction of the particularly decomposable mono- and disaccharides occurring in or developed from intaken foodstuffs and stimulants. The resulting organic acids attack and demineralize the enamel and subsequently the tooth pulp by extracting calcium and phosphate, meaning the outbreak and development of caries. Dental calculus occurs as a cause of a minirali-

zation of dental plaque having a tendency to accumulate calcium and phosphate. If dental plaques are not regularly removed, e.g. by tooth brushing the accumulation of calcium and phosphates will reach a stage where
5 a coherent and wear-resisting network of hydroxy apatite embedding the bacteria develops on the surfaces of the teeth. The progressing dental calculus in which hydroxy apatite amounts to 80% of the total material of dental calculus has a tendency to grow, because the bacteria in
10 dental calculus cannot be removed by ordinary tooth brushing. The clinical correlation between dental calculus and gingivitis has long been known and this correlation has been demonstrated several times. It is likewise known that non-treated gingivitis progresses into para-
15 dentosis as time goes by. Dental calculus develops typically in areas of the teeth that are difficult to clean by tooth brushing. In this context the tooth surfaces facing the spaces between the teeth are particularly important. Dental calculus deposited between the tooth
20 spaces will be spread therefrom and further on to the spaces between the teeth and gingivae, following which the dental calculus under gingivae will be spread from the tooth spaces to other areas of the teeth. In more advanced stages the dental calculus occurs on large
25 areas between gingivae and the neck of the teeth and the organic acids generated by the acids will not only demineralize the teeth but also the bone structures located behind gingivae.

Various ways have been followed in the attempts
30 made so far with a view to confine and inhibit the formation of plaques. These consist principally in either preventing the formation of the dextran gels causing the plaques to attach to the tooth surfaces or in impeding the growth of bacteria by making use of chemical com-
35 pounds acting growth-inhibiting or bactericidally and which in certain cases are produced in situ.

Several methods of preventing the formation of dextran have been suggested. According to the accepted German specification No. 1,467,809 salts of calcium, sodium or magnesium salts of esters of phosphoric acids with sucrose, glucose or lactose can work as cario-static dental hygiene agents. Said chemical compounds are said to derange, thereby confining the formation of dextran gels from sucrose caused by the bacteria. However, the method does not have the intended effect on bacterial coatings in the teeth spaces where plaques are difficult to remove and where adhering food residues most frequently ensure that the bacteria have free access to low-molecular carbon hydrates. Moreover, the method does not have any effect on already developed dental calculus and, therefore, this method is held to be less suited with respect to the inhibition of gingivitis and paradentosis.

According to US patent No. 3,733,399 the formation of dental plaques as a precursor to caries is claimed to be confined by invertase that breaks down the disaccharide sucrose into two monomers: anhydroglucose and fructose. The dextran gels formed in plaques are said only to be synthesized by the bacteria from sucrose and the effect of invertase should apparently be to reduce the amount of sucrose accessible for the bacteria.

The use of invertase together with lactate dehydrogenase is according to US patent No. 4,255,414 likewise claimed to possess an anticaries effect. It is, however, known that cariogenic bacteria, for instance *Streptococcus mutans*, on their own form invertaseous enzymes breaking down sucrose into anhydroglucose and fructose, said bacteria being likely to form dextran gels from anhydroglucose obtained from their own enzymes as well as from invertase applied with a dental hygiene agent. It is further known that dental plaques are also formed if the intaken foodstuffs contain carbon

hydrates, such as starch or lactose, instead of sucrose, and invertase contained in dental hygiene agents cannot be expected to have any action on the plaques formation in those cases. Eventually, invertase does not have any
5 action on already formed plaques and dental calculus and invertase is thus only of minor importance with respect to inhibition and is of no importance in relation to the treatment of gingivitis and parodontosis.

The dextran gels generated in plaques are
10 according to the accepted German patent application No. 1,955,956 proposed to be broken down by dextranases. According to AT patent No. 318,815 it has, however, been found that dextranase was merely capable of breaking down soluble dextran, while in plaques there is some of
15 the insoluble dextran that cannot be broken down. According to US patent No. 4,154,815 dental plaque is confined by making use of zinc in connection with proteases, carbohydrases and/or lipases. Zinc has been found to be active by interference with the plaque structure
20 and the mineralization of plaques, while the enzymes are entitled to decompose the plaques densifying high-polymeric compositions. A clinical experiment referred to in said patent demonstrates that zinc and protease were capable of reducing the amount of plaques on teeth sur-
25 faces, but the experimental report says nothing about the effect in the spaces between the teeth and on dental calculus.

DK patent application No. 5504/78 discloses a caries inhibiting dental hygiene agent in tablet form or
30 powder form, said agent containing only lysozyme as an active component. This reference does not specify the type of lysozyme applied.

According to US patent No. 3,985,869 neither egg white lysozyme nor enzymes obtained by *Streptomyces al-*
35 *bus* or *Streptomyces griseus* or a strain belonging to the genus *Flavobacterium* are capable of lysing carioge-

nic bacteria belonging to the genera Streptococcus and Lactobacillus. According to said reference it is, however, possible by means of four different strains of Streptomyces to produce cell lysing enzymes capable of attacking cariogenic streptococci and lactobacilli. As known, there exists, however, a somewhat cautious attitude with respect to applying enzymes produced from Streptomyces to agents intended for use e.g. in the oral cavity or for wound treatment, because various strains of Streptomyces develop substances, the presence of which is not desired in such agents and which might be difficult to remove from the desired enzymes obtainable from the strains of Streptomyces. Thus, it is generally not desired that for example dental hygiene agents regularly used for long periods of time include antibiotics interfering in the intended microflora balance within the mouth, e.g. in connection with developing resistance.

The accepted German patent application No. 2,027,019 and the parallel US patent No. 4,150,113 deal with a dental hygiene agent containing an addition of oxidoreductase which by oxidative decomposition of a substrate causes hydrogen peroxide to be liberated. This is asserted to provide for obtaining a caries inhibiting effect. The glucose acting as a substrate is said to be obtained from food debris in the mouth containing for example starch or disaccharides, because the dentifrice may as well contain carbohydrases generating glucose from the poly- or disaccharides concerned. According to experiments by which the obtainable effect is rendered probable in said references unphysiologically high concentrations of sucrose were, however, added immediately before the addition of the enzymes and it must be considered improbable that concentrations of hydrogen peroxide sufficient to inhibit or impede the growth of bacteria to a desired degree will be obtained by using

the agent in practice in connection with the intake of foodstuffs more ordinarily made up.

The specification of US patent No. 4,476,108 states that the bactericidal efficacy of hydrogen peroxide intensifies by the addition of peroxidase and electron-donor molecules which by reaction generate free radicals having a high bactericidal activity. Horseradish peroxidase is the preferred peroxidase, while the electron donors are organic molecules among which the following are recited; phenylethylamine, tyrosine, tryptophan, benzoic acid, salicylic acid, hydroquinone, dehydrophenylalanine, vanillin and para-aminobenzoic acid. The presence of hydrogen peroxide is obtained in that the bactericidal agent concerned has a content of hydrogen peroxide, sodium peroxide, methyl peroxide or ethyl peroxide.

EP patent application No. 133,736 relates to dentifrice compositions having an antibacterial activity and are based on the formation of hypothiocyanate (OSCN^-) during utilization. The agents concerned contain an oxidable substrate and an oxidoreductase system specific of said substrate and which in the presence of oxygen in aqueous milieu generates hydrogen peroxide which by reaction with the thiocyanate that is further contained in the agent generates hypothiocyanate under the influence of lactose peroxidase. In a typical embodiment the agents contain for example glucose as an oxidable substrate, which is oxidized with glucose oxidase under the formation of hydrogen peroxide reacting with thiocyanate (SCN^-) added for instance in the form of potassium thiocyanate, and with lactoperoxidase to produce the bacteriostatic hypothiocyanate (OSCN^-).

The agent is proposed to be used in inhibiting caries and for that purpose a bacteriostatic activity may per se provide for obtaining an advantageous effect. For the prevention and treatment of gingivitis and para-

dentosis a bactericidal activity is, however, not sufficient, since bacterial plaques in the spaces between the teeth and in gingival pockets will continue their growth upon decline of the inhibiting efficacy of the hypothiocyanate.

The use of the hypothiocyanate-forming system is therefore supposed to afford only a limited effect on the development of gingivitis and paradentosis and a proper inhibition can hardly be obtained thereby.

10 An effective inhibition and treatment of gingivitis and paradentosis can solely be obtained by extinguishing the bacteria localized between the teeth and in the gingival pockets. None of the above mentioned methods of inhibiting the formation of plaques either by
15 preventing dextrans from forming or by attenuating or possibly extinguishing the bacteria involved proves any properly substantiated effect on plaques occurring between the teeth and in gingival pockets and the primary object of the inventions referred to actually also
20 consists in the inhibition of caries.

There is therefore a need for a bactericidal composition capable of ensuring a high mortality of bacteria, particularly in the areas of the oral cavity where the bacteria give rise to gingivitis or paradentosis.

It has now surprisingly been found that bacteria of the types that are considered to be responsible for the occurrence and progressing of gingivitis and paradentosis, which bacteria as stated above cannot be directly lysed and extinguished by the cell wall decomposing enzyme lysozyme, after having been attenuated with hypothiocyanate (OSCN^-) to a much higher degree are broken down and extinguished by said enzyme.

Use is made of this recognition with respect to
35 the bactericidal composition according to the invention containing at least one enzyme which in aqueous milieu

and in the presence of oxygen and a substrate for the enzyme is capable of generating hydrogen peroxide and a peroxidase and a thiocyanate, the composition according to the invention being characterized in that it further
5 contains lysozyme.

When the bactericidal composition according to the invention, for example in prophylaxis or treatment of gingivitis or paradentosis, is applied in use between the tooth spaces and into the gingival pockets, the hydrogen peroxide forming enzyme will come into contact
10 with food debris and stimulant debris containing substances acting as substrate for the enzyme. This entails oxidation of said substances under the liberation of hydrogen peroxide which by the influence of the peroxidase
15 contained in the composition and naturally available peroxidase is reacted with thiocyanate, thereby generating hypothiocyanate (OSCN^-). This affects the available, undesired bacteria in such a manner that they become sensitive to and killed by the lysozyme contained
20 in the composition and which would not have been able to break down the bacteria, if they had not been attenuated by the hypothiocyanate.

A preferred embodiment of the composition according to the invention is characterized in that the hydrogen peroxide forming enzyme is glucose oxidase.
25

In particular when the composition is to be used in connection with dental hygiene it may be appropriate that it further contains invertase or lactase or both, so that sucrose or lactose present in the oral cavity
30 are decomposed under the formation of glucose serving as substrate for the glucose oxidase. This provides for obtaining not only an appropriate formation of hydrogen peroxide which further results in a formation of hypothiocyanate but also a removal of said undesired disaccharides.
35

However, the composition may in itself contain a substrate, also when used for dental hygiene, in which

case the formation of hydrogen peroxide is not restricted by the amount of substances naturally present in the oral cavity and which act as substrate for the hydrogen peroxide forming enzyme.

5 If a composition according to the invention which contains glucose oxidase, is particularly intended to be used for wound treatment, it is expedient that it further includes glucose or glucose forming combinations of materials as substrate for the glucose oxidase and has a
10 water content not exceeding the lowest value at which the glucose oxidase is active.

 The reactions resulting in the generation of hypothiocyanate attenuating bacteria and causing them to be extinguished by the lysozyme occur only when the composition comes into contact with water, e.g. in the form
15 of blood or lymph and, consequently, the composition is stable until use. The composition may as well contain no glucose, but if so, it is used together with a glucose-containing or -forming agent.

20 When the composition according to the invention is intended to be used for wound treatment, the alternatively hydrogen peroxide forming enzyme may be an amino acid oxidase, in which case the amino acids present in blood and lymph act as substrate.

25 In preferred embodiments of the composition the peroxidase constituting a component of the composition and ensuring the formation of hypothiocyanate from the thiocyanate and the hydrogen peroxide is lactoperoxidase, myeloperoxidase or horseradish peroxidase, all of
30 which enzymes are classified E.C.1.17.1.7 (IUPAC).

 As mentioned, a particular interest is attached to the application of the composition in dental hygiene agents and the invention further relates to dental hygiene preparations that are particularly suitable to ensure
35 a selectively high mortality of bacteria in tooth spaces and in the gingival pockets without affecting the

microflora in the oral cavity. A general affection on the total microflora in the oral cavity is incidentally undesired.

The dental hygiene agents according to the invention may be in the form of toothpicks, tooth thread or miniature brushes, in particular for the prevention and treatment of gingivitis and paradentosis and are characterized in that they are impregnated with the above mentioned composition according to the invention.

10 The impregnation of for example tooth picks may be effected by humidifying them with solutions of the hydrogen peroxide forming enzyme, peroxidase, thiocyanate and lysozyme and stabilizing agents known per se for enzymes followed by drying, for instance freeze dry-
15 ing, of the humidified tooth picks.

Alternatively, the dentifrice may be in the form of a solution or suspension adapted to be introduced into the spaces between the teeth and gingival pockets by means of a brush, tooth thread, toothpicks or the like,
20 said solution containing the hydrogen peroxide generating enzyme, peroxidase, thiocyanate and lysozyme, preferably together with ordinary stabilizing and preserving agents.

Experiments have proven that the bactericidal effect of the composition according to the invention is so high that the comparatively small amount of the components of the composition that may be added by means of impregnated toothpicks will be sufficient to thereby obtain a markable bactericidal effect.

30 Even though it is a particular feature of the invention that the composition by treatment or prophylaxis of gingivitis and paradentosis for example by means of toothpicks is applied locally into gingival pockets and the spaces between the teeth, this composition may advantageously also be used in other dental hygiene agents, such as toothpaste the use of which only affords
35 an inferior localized supply.

Incidentally, the composition according to the invention will obvious also be applicable in combination with other agents for enhancing the intended effect. Thus, the EDTA will be capable of enhancing the effect
5 of the composition on bacteria having a content of calcium compounds in the cell walls.

In case the bactericidal composition is to be used for wound treatment a dressing material may be impregnated with it, or the composition may constitute a
10 component of a vulneary powder.

Even though the bactericidal composition has mainly been described in the preceding in connection with its use for dental care or wound treatment, the composition is principally generally applicable else-
15 where, if an effective bactericidal activity is desired which is gentle to the host organism and has a limited duration and which does not involve the same risk of forming resistant strains of micro organisms as when using antibiotics. In this respect it is emphasized that
20 the composition according to the invention may be prepared by use of such enzymes, all of which are approved for use in foodstuffs.

The quantitaive ratios between the components of the bactericidal composition concerned may vary within
25 a wide range. In view of the fact that the components individually must be considered to be non-toxic there is no risk that an overdose will cause disadvantageous side effect, this particularly applies to dental hygiene agents which in themselves do not contain a substrate
30 for the hydrogen peroxide generating enzyme so that the maximum amount of generated hypothiocyanate is determined by the amount of foodstuff and stimulant residues remaining on the localities concerned in the mouth.

The quantitative composition of various prepara-
35 tions according to the invention that have proven effective will appear from the following examples.

With a view to further characterizing the enzymes used in the examples and the units of quantities therefor, the following is to be noticed.

5 Lactase: β -galactosidase (E.C. 3.2.1.23) is given in the unit L.U. (Lactase unit) defined as the amount of enzyme producing in one minute 1 mg glucose from 6 ml of 5.0% lactose at 20°C and pH 6.5.

10 Invertase: β -fructofuranosidase (E.C. 3.2.1.26) is given in S.U. (Sumner Unit) defined as the amount of enzyme generating in five minutes 1 mg invert sugar from 6 ml of 5.4% sucrose at 20°C and pH 4.7.

15 Glucose oxidase is classified E.C. 1.1.3.4. and is given in the unit GOU (glucose oxidase unit) defined by D. Scott in J. Agri. Food Chem. 153 (1): 727 (1953).

20 Lactoperoxidase is classified E.C. 1.17.1.7. and has the function in the composition concerned to catalyze the reaction $\text{H}_2\text{O}_2 + \text{SCN}^- \rightarrow \text{H}_2\text{O} + \text{OSCN}^-$. This enzyme is given in ABTS units as defined by J.S. Shindler and W.G. Bardsley: (Biochem. Biophys. Res. Commun. 67: 1307 (1975)).

13

Lysozyme, also designated muramidase, is N-acetylmuramylhydrolase (E.C. 3.2.1.17). This enzyme decomposes N-acetylglucosamine and N-acetylmuramic acid from the cell walls. The activity of lysozyme is measured by lysing of *Micrococcus lysodieticus*. As a well defined standard of the activity is difficult to determine, the amount of the lysozyme applied is, however, given in absolute values in the following examples. The material as used had a purity of 99% and was extracted from egg white.

15

EXAMPLE 1

A solution containing the following ingredients per ml was prepared:

	Invertase	900 S U
	Glucose oxidase	45 GOU
20	NaSCN	0.05
	Lacto peroxidase	28 ABTS-units
	Lysozyme	0.03 g
	Sodium EDTA	0.05 mmol

in an aqueous buffer containing 10 to 200 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ at pH 6.0.

With a view to testing the bactericidal effect of the composition thus obtained in the presence of a substrate various organisms belonging to the genera *Streptococcus*, *Lactobacillus*, *Bacteroides*, *Flavobacterium* and *Fusobacterium* were individually cultured in a favourable growth medium. When the organisms had obtained balanced growth with a cellular density of 10^5 to 10^7 cells/ml the above solution was added to the cultures in an amount ranging between 50 to 200 $\mu\text{l}/10$ ml culture. At the same time sucrose was added in an amount ranging between 0.1 and 1% in order to start the reaction eventually resulting in the OSCN⁻.

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The bacterial cultures were subsequently incubated for three days at temperatures of 25, 37, 40 or 45°C, during which intervals the cultures were daily inspected.

- 5 It turned out that in every case the bacterial growth ceased even with an addition of only 50 µl of the solution per 10 ml growth culture.

EXAMPLE 2

- 10 A solution was prepared containing the following ingredients per ml:

	Invertase	900 S U
	Glucose oxidase	45 GOU
	NaSCN	0.05 mmol
15	Lactoperoxidase	28 ABTS-units
	Lysozyme	0.03 g
	Mannitol	0.02 g
	Sodium EDTA	0.05 mmol

in a phosphate buffer at PH 6.0.

- 20 The pique end of toothpicks was immersed into this solution causing that 5 to 7 µl of the solution adhered to each toothpick.

The toothpicks were subsequently freeze-dried.

- With a view to testing the bactericidal effect
25 of the toothpicks thus impregnated, 10^5 bacteria of different strains of the genera Streptococcus and Lactobacillus were incubated on agar plates. The toothpicks that were impregnated with the composition according to the invention were pressed down into the sucrose-
30 containing agar plates which were then incubated for six days, and the growth was daily observed. Tests were carried out by incubation at 25°C as well as at 40°C, and in particular at 37°C. A clear zone had formed around the toothpicks, completely free of bacterial
35 growth in an area varying between 5 and 15 mm reckoned from the toothpick. The size of said clear zone was de-

pendent on the genus of bacteria used. In any case no bacteria colony was observed within the clear zone, even after a six-day growth, meaning that the bacteria in this zone had been extinguished and not only physiologically weakened.

A test was also carried out on the bactericidal effect of the impregnated toothpicks after the toothpicks had been stored in airtight packages at 20°C for three months. The bactericidal effect of said toothpicks appeared to have been preserved completely unabated, thereby drawing the conclusion that the bactericidal composition according to the invention includes a storing stability sufficient for practical use.

For the purpose of comparison toothpicks were impregnated in a similar manner with a solution as that outlined above but with no content of lysozyme. On testing the bactericidal effect of said toothpicks on agar plates results were obtained showing a considerably lower bactericidal effect compared to the effect obtained when lysozyme is also present.

For the purpose of drawing an additional comparison toothpicks were impregnated with an aqueous buffer solution of lysozyme and EDTA in the above quoted amounts but without enzymes and NaSCN. Said latter toothpicks could not prove any significant bactericidal activity by the test carried out.

16

EXAMPLE 3

Preparation of a solution for impregnating tooththread containing the following ingredients:

	Invertase	30000 S U
5	Lactase	23000 L U
	Glucose oxidase	1000 GOU
	NaSCN	5 mmol
	Lactoperoxidase	940 ABTS-units
	Lysozyme	1.5 g
10	Carboxymethylcellulose	0.5 g
	Inositol	1.75 g
	Sodium EDTA	5 mmol
	Na ₂ HPO ₄ to pH	6.5
	Distilled water	ad 100 ml

15

EXAMPLE 4

Preparation of a solution for impregnating tooth thread containing the following ingredients:

	Invertase	90000 S U
20	Lactase	70000 L U
	Glucose oxidase	4500 GOU
	NaSCN	5 mmol
	Lactoperoxidase	940 ABTS-units
	Lysozyme	3 g
25	Mannitol	1 g
	Sorbitol	2 g
	Sodium EDTA	5 mmol
	Na ₂ HPO ₄ to pH	6.0
	Colour and flavourings	
30	Distilled water	ad 100 ml

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EXAMPLE 5

An aqueous solution into which toothpicks, tooththread or miniature brushes shall be immersed immediately before use was prepared from the following ingredients:

	Invertase	1800 S U
	Lactase	1400 L U
	Glucose oxidase	80 GOU
	NaSCN	0.1 mmol
10	Lactoperoxidase	56 ABTS-units
	Lysozyme	60 mg
	Glycerol	20 g
	EDTA	0.1 mmol
	Na ₂ HPO ₄ to pH	6.0
15	Colour and flavourings	
	Distilled water	ad 100 ml

It can be calculated that in use such a solution will be diluted about three times by saliva available in gingival pockets and on the surfaces of the tooth necks. The resulting concentration of the components contained in the composition to about one fourth of the above specified values will be completely sufficient to obtain the desired bacteriostatic activity.

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EXAMPLE 6

An aqueous solution as in Example 5 was prepared but without invertase and colour and flavourings. Gauze wads were drenched with the solution and subsequently dried in vacuum. The resulting wound dressing material was suitable to be used in connection with a vulnerable powder containing besides the components ordinary of such a powder, about 2% by weight of glucose.



P A T E N T C L A I M S

1. A bactericidal composition containing at least one enzyme which in aqueous milieu and in the presence of oxygen and a substrate for the enzyme may form
5 oxygen peroxide together with a peroxidase and a thiocyanate, characterized in that it further contains lysozyme.

2. A composition as claimed in claim 1, characterized in that the hydrogen peroxide forming enzyme
10 is glucose oxidase.

3. A composition as claimed in claim 2, in particular for use in dental care, characterized in that it further contains invertase or lactase or both.

4. A composition as claimed in claim 2, particularly for use in wound treatment, characterized in that it further contains glucose or glucose forming combinations of materials as a substrate for the glucose oxidase and has a water content below the smallest value at which the glucose oxidase is active.

20 5. A composition as claimed in claim 1, in particular for use in wound treatment, characterized in that the hydrogen peroxide generating enzyme is an amino acid oxidase.

6. A composition as claimed in any of the preceding claims, characterized in that the peroxidase is selected among the lactoperoxidase, myeloperoxidase and horseradish peroxidase classified in E.C. 1.17.1.7.

7. A dentifrice in the form of toothpicks, tooth thread or miniature brushes, in particular for the inhibition and treatment of gingivitis and paradentosis, characterized in that they are impregnated with a composition according to any of claims 1 to 3.

8. A dentifrice in the form of a solution or suspension, in particular for the inhibition and treatment
35 of gingivitis and paradentosis and adapted to be applied into the spaces between the teeth and gingival pockets,



characterized in that it is an aqueous solution of a composition according to any of claims 1 to 3.

9. A wound treatment preparation, characterized in that it is a dressing material impregnated with a composition according to claims 4 or 5.

10. A wound treatment preparation, characterized in that it is a vulnerable powder containing a composition according to claims 4 or 5.