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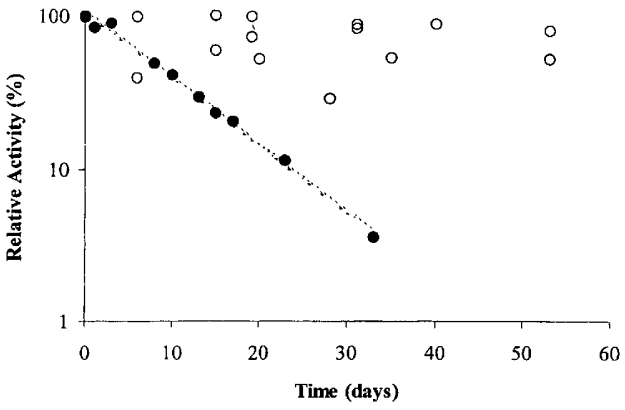
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Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

[Continued on next page]

(54) **Title:** STABLE THREE ENZYME CREATININE BIOSENSOR



(57) **Abstract:** The invention provides methods for preparing a stable, multiple-use three enzyme biosensor for the amperometric determination of creatinine in biological liquids that has a useful lifetime that extends significantly beyond that of presently available amperometric biosensors. The biosensor prepared by the methods of the invention encompasses a plurality of immobilized enzymes that are applied to the biosensor as an enzyme-polymer composition. The enzymes, which can include creatinine amidohydrolase, creatine amidinohydrolase and sarcosine oxidase, are immobilized into the enzyme-polymer composition simultaneously as well as applied to the biosensor simultaneously. Prior to being immobilized, the enzymes can be chemically modified by attaching one or more polyethylene glycol (PEG) chains per enzyme monomer. The polymer component can be provided by a polyurethane membrane. The invention also provides a method of preparing a biosensor that limits the diffusion of silver ions emanating the reference electrode, thereby preventing, contact between the silver ions and the enzymes. Related methods of preparing an enzyme-polymer composition for incorporation into a multiple use three enzyme biosensor for the amperometric determination of creatinine in biological liquids also are provided. The invention also provides multiple-use biosensors and enzyme-polymer compositions prepared by the methods disclosed.

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TSUCHIDA T ET AL: "MULTI-ENZYME MEMBRANE ELECTRODES FOR DETERMINATION OF CREATININE AND CREATINE IN SERUM" QUALITY CONTROL CLIN. CHEM. TRANSACTIONS INTERNATIONAL SYMPOSIUM, vol. 29, no. 1, 1983, pages 51-55, XP001008686 page 51 - page 52	1-25
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAMATO, H. ET AL: "A polypyrrole/three enzyme electrode for creatinine detection" ANAL. CHEM., vol. 67, no. 17, 1 September 1995 (1995-09-01), pages 2776-2780, XP002379867 the whole document	1-6, 12-16,18
A	US 6 241 863 B1 (MONBOUQUETTE, UCLA) 5 June 2001 (2001-06-05) column 3 - column 12	1-25
X	ERLENKÖTTER ANSGAR ET AL: "Biosensors and flow-through system for the determination of creatinine in hemodialysate." ANALYTICAL AND BIOANALYTICAL CHEMISTRY. JAN 2002, vol. 372, no. 2, January 2002 (2002-01), pages 284-292, XP002379868 ISSN: 1618-2642 the whole document	1-6, 12-16,18
P,X	DE 10 2004 003793 A1 (BAYER HEALTHCARE) 11 August 2005 (2005-08-11) the whole document	1-6,8-18
P,X	BERBERICH J A ET AL: "A stable three-enzyme creatinine biosensor. 1. Impact of structure, function and environment on PEGylated and immobilized sarcosine oxidase" ACTA BIOMATERIALIA, ELSEVIER, AMSTERDAM, NL, vol. 1, no. 2, March 2005 (2005-03), pages 173-181, XP004748995 ISSN: 1742-7061 the whole document	1-25
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	BERBERICH J A ET AL: "A stable three-enzyme creatinine biosensor. 3. Immobilization of creatinine amidohydrolase and sensor development" ACTA BIOMATERIALIA, ELSEVIER, AMSTERDAM, NL, vol. 1, no. 2, March 2005 (2005-03), pages 193-199, XP004748997 ISSN: 1742-7061 the whole document	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6241863 B1	05-06-2001	NONE	
DE 102004003793 A1	11-08-2005	NONE	