

UNITED STATES PATENT AND TRADEMARK OFFICE

**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Ex parte MARK T. MARTIN

Appeal No. 2001-0940
Application No. 08/467,712

ON BRIEF

Before WINTERS, WILLIAM F. SMITH, SCHEINER, Administrative Patent Judges.

SCHEINER, Administrative Patent Judge.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 12-17, all the claims remaining in the application. Claim 12 is representative of the subject matter on appeal and reads as follows:

12. A method for the determination of the concentration of an enzyme substrate present in a specimen, comprising the steps of:

(a) contacting said specimen with an oxidoreductase conjugate under conditions which permit the oxidation of the enzyme substrate, said oxidoreductase conjugate comprising a co-factor and a species capable of generating an electrochemiluminescent signal separately linked in close proximity to an active site of said oxidoreductase in a manner which permits their electrochemical interaction with each other and a substrate also in close proximity to the active site;

(b) measuring the change in electrochemiluminescence from a base reading; and

(c) determining the concentration of enzymatic substrate based on the measured change in electrochemiluminescent signal.

The references relied on by the examiner are:

Yomo et al. (Yomo), "Preparation and kinetic properties of 5-ethylphanazine-glucose-dehydrogenase-NAD⁺ conjugate, a semisynthetic glucose oxidase," Eur. J. Biochem., Vol. 200, pp. 759-766 (1991)

Claims 12-17 stand rejected under 35 U.S.C. § 103 as unpatentable over Nacamulli and Yomo.

We reverse the rejection.

DISCUSSION

The present invention is directed to an electrochemiluminescence-based assay for determining the concentration of a substrate of an oxidoreductase. As explained in the specification, the oxidoreductase used in the assay "is not present in its natural form, but [] has been chemically modified so that it has two unnatural appendages," converting the enzyme into a chemiluminescent biosensor. Specification, page 5. "One appendage is a covalently attached [enzyme cofactor, e.g. NAD] . . . specifically attached in a way that it can bind in the active site of the enzyme and function as a redox reagent as part of the natural enzyme mechanism" and "[t]he second appendage is an [electrochemiluminescent] label," e.g., Ru(bpy)₃²⁺, also attached near the active site of the enzyme. Id.

According to the specification, during the course of an assay, analyte (i.e., an oxidoreductase substrate) is oxidized in the presence of the biosensor, which converts the NAD⁺ containing appendage to NADH. The Ru(bpy)₃²⁺ and the NADH are oxidized at the surface of an electrode, forming Ru(bpy)₃³⁺ and NADH[•] (a radical). The NADH[•] spontaneously loses a hydrogen, forming NAD[•]. The NAD[•], a strong reductant, reacts with Ru(bpy)₃³⁺, a strong oxidant, to form the excited state of the label, Ru(bpy)₃^{2+*}. The label decays to the ground state through a normal fluorescence mechanism, emitting a

photon having a given wavelength. Specification, page 9. This process regenerates the original form of the electrochemiluminescent label, which cycles repetitively through the reaction sequence, emitting multiple photons during each measurement period. Specification, page 10. The intensity of the observed luminescence is proportional to the concentration of the analyte. Id., page 11.

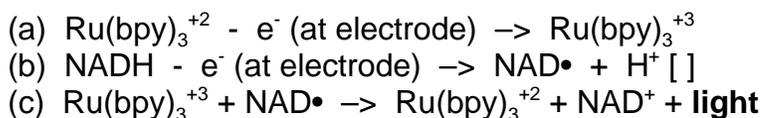
Obviousness

According to the examiner, “Nacamulli [teaches] methods for determining the concentration of an enzyme substrate in a specimen by contacting said specimen with an oxidoreductase . . . plus the cofactor NAD and an [electrochemiluminescent] substance $\text{Ru}(\text{bpy})_3^{2+}$ then measuring the change in the chemiluminescent signal produced by the reduction of the $\text{Ru}(\text{bpy})_3^{2+}$ by the NADH generated from the reaction between the substrate and the enzyme. The rate of signal generation is measure[d] simultaneously with the addition of the reagents” and “ $\text{Ru}(\text{bpy})_3^{2+}$ is recycled with voltage pulses.” Paper No. 4, page 5.¹ We note, however, that the examiner’s characterization of the reference is factually inaccurate - Nacamulli does not measure substrate concentration, rather, the rate of the enzymatic reaction is measured using known initial concentrations of all the reactants. In addition, we note that the electrochemiluminescent reaction is “slowed down . . . by using narrow voltage pulses,” apparently to “provide for better conditions for rate measurements.” Nacamulli, column 5, lines 5-23 and column 6. In any case, the examiner concedes that Nacamulli does not describe “a ternary complex,” i.e., the modified oxidoreductase biosensor required by the claims, and relies on Yomo to make up this difference. Paper No. 4, page 5.

¹ The Answer refers to Paper No. 9 (final rejection mailed December 22, 1997) for the statement of the rejection. Paper No. 9, in turn, refers to Paper No. 4 (non-final office action, mailed March 7, 1997).

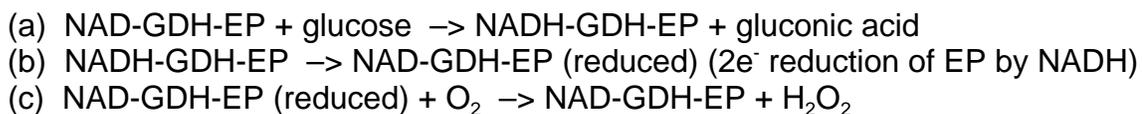
Yomo describes a “ternary conjugate” comprising an oxidoreductase modified by conjugation with NAD and ethylphenazine (EP). As explained by the examiner, “[t]he conjugate can be cycled from a state where the bound NAD is reduced to NADH, followed by reoxidation of the NADH by EP, whereupon the reduced EP can be reoxidized and detected with [a chromogen].” Paper No. 4, page 5.

Appellant argues that the reactions of the present invention [and Nacamulli] “are vastly different” from the reaction described by Yomo. Brief, page 12. In particular, appellant argues that both the present invention and Nacamulli “measure the reaction rates of oxidoreductases that generate NADH through an [electrochemiluminescence]-generating reaction of reactive intermediates formed from NADH and Ru(bpy)₃ at an oxidizing electrode” and “[t]he mechanism for this process may be represented as follows:”



Reply Brief, page 13.

Yomo, on the other hand, produces a semisynthetic glucose oxidase by forming a ternary complex comprising glucose dehydrogenase, NADH and EP, in which, according to appellant, EP acts as an electron mediator and “accepts two electrons from NADH and transfers them to molecular oxygen, causing the ternary complex to function as a glucose oxidase . . . as follows:”



Reply Brief, pages 13 and 14.

According to the examiner, however, “it would have been prima facie obvious . . .

to utilize a ternary conjugate of oxidoreductase, NAD and [an electrochemiluminescent] substance” in Nacamulli’s assay “because of the better kinetic properties of the conjugate and lower cofactor/[electrochemiluminescent] reagent requirements.” Paper No. 4, page 5. While the examiner does not identify anything in particular in either reference which supports this assertion of better kinetic properties, we note that Yomo does observe rate acceleration due to conjugation of the reaction components. Yomo, page 763.

Nevertheless, the examiner does not dispute appellant’s characterization of the reactions described in Nacamulli and Yomo, nor does the examiner dispute appellant’s assertion that Ru(bpy)₃ and EP “have completely different functionalities” (Reply Brief, page 14) in the reactions. In our view, the fact that Yomo observed rate acceleration (presumably, the “better kinetic properties” referred to by the examiner) due to conjugation of the components of one enzymatic reaction would not have prompted one skilled in the art to conjugate the components of Nacamulli’s reaction, given the differences between these two reactions, and the fact that Nacamulli intentionally slows the rate of reaction. We agree with appellant that “[o]ne of ordinary skill in that art need only juxtapose the two mechanisms of action for Ru(bpy)₃ in Nacamulli and EP in the ternary complex of Yomo to realize that one is not a reasonable substitute for the other” (Reply Brief, page 14) and that “the prior art of record does not teach or suggest . . . ternary complexes that comprise [electrochemiluminescent] signaling molecules” (Id., page 13).

An adequate showing of motivation to combine requires “evidence that ‘a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.’” Ecolochem, Inc. v. Southern Calif. Edison Co., 227 F.3d 1361, 1375, 56 USPQ2d 1065, 1075 (Fed. Cir. 2000) (quoting In re Rouffet, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1456 (Fed. Cir. 1998)). Thus, “[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.” In re Fine, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988).

In our judgment, on this record, the only reason or suggestion to modify the teachings of the references in the manner proposed by the examiner comes from appellant’s specification. Accordingly, we find that the examiner’s burden of establishing a prima facie case of obviousness has not been met and the rejection of

claims 12-17 under 35 U.S.C. § 103 is reversed.

REVERSED

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Sherman D. Winters)	
Administrative Patent Judge)	
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William F. Smith)	APPEALS AND
Administrative Patent Judge)	
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Administrative Patent Judge)	

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