

UNITED STATES PATENT AND TRADEMARK OFFICE

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

Ex parte FRANCK ZAL, ANDRE TOULMOND and
FRANCOIS LALLIER

Appeal 2007-3888
Application 10/296,982
Technology Center 1600

Decided: February 27, 2008

Before TONI R. SCHEINER, ERIC GRIMES, and JEFFREY N.
FREDMAN, *Administrative Patent Judges*.

GRIMES, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a blood substitute composition and methods of using it. The Examiner has rejected the claims as anticipated or obvious. We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

BACKGROUND

The Specification discloses “the use of a high molecular weight extracellular haemoglobin as a blood substitute” (Spec. 1). Extracellular

hemoglobins, found in annelids, “are giant biopolymers, made up of approximately 200 polypeptide chains” (*id.* at 3).

The Specification states that the extracellular hemoglobin of the nightcrawler (*Lumbricus terrestris*) has been studied as a blood substitute but “would not be suitable, firstly due to probable disturbance of the vasodilation and/or vasoconstriction due to the absence of free cysteine residues . . . and, secondly, this haemoglobin presents too weak an affinity with oxygen, i.e. a high P_{50} ” (*id.*).

The Specification discloses that extracellular hemoglobin isolated from the lugworm (*Arenicola marina*) has advantageous properties for use as a blood substitute. It has a P_{50} of 6.4 mm Hg, which compares favorably with that of human hemoglobin (Spec. 11) and it possesses free cysteine residues that allow it to transport NO and SNO, an activity shared by vertebrate hemoglobins (*id.* at 12-13).

The Specification provides a working example in which *A. marina* hemoglobin was used as a blood substitute in mice (*id.* at 14). The Specification reports that “there are no behavioural or physiopathological effects in these mice partially transfused with haemoglobin of *Arenicola marina*” (*id.*).

DISCUSSION

1. CLAIMS

Claims 1-17 and 21-25 are pending and on appeal. The claims subject to each rejection have not been argued separately and therefore stand or fall together. 37 C.F.R. § 41.37(c)(1)(vii). Claims 1, 2, and 23 are representative and read as follows:

1. A method for substituting blood comprising substituting said blood with an extracellular haemoglobin having a molecular weight of approximately 3 to approximately 4 million Daltons, comprising chains of polymerised globins, containing free cysteines binding to NO and/or SNO groups, and having a P₅₀ of approximately 6 to approximately 7 mm Hg.

2. A blood substitute mixture comprising a physiologically acceptable buffer for a vertebrate and an extracellular haemoglobin as a blood substitute, said extracellular haemoglobin having a molecular weight of approximately 3 to approximately 4 million Daltons, comprising chains of polymerised globins, containing free cysteines binding to NO and/or SNO groups, and having a P₅₀ of approximately 6 to approximately 7 mm Hg.

23. The blood substitute mixture of claim 2, wherein said buffer comprises Hepes.

2. ANTICIPATION

Claims 2-13 stand rejected under 35 U.S.C. § 102(b) as anticipated by Zal (1997)¹ as evidenced by Zal (1996).² The Examiner finds that the *Arenicola marina* extracellular hemoglobin disclosed by Zal (1997) meets all the limitations of the extracellular hemoglobin recited in claim 2 (Answer 4-5 and 12-14). The Examiner also finds that the composition taught by Zal (1997) includes a “physiologically acceptable buffer for a vertebrate,” as required by claim 2, because it includes the buffer bis-tris propane (Answer

¹ Zal et al., “Quaternary structure of the extracellular haemoglobin of the lugworm *Arenicola marina*,” Eur. J. Biochem., Vol. 243, pp. 85-92 (1997). Both the Examiner and Appellants cite to the full-text reference (Answer 4-5; App. Br. 8) even though only the abstract of the Zal (1997) paper is of record. To complete the record, we have entered the full-text paper into the official Image File Wrapper.

² Zal et al., “The multi-hemoglobin system of the hydrothermal vent tube worm *Riftia pachyptila*,” Journal of Biological Chemistry, Vol. 271, pp. 8869-8874 (1996).

5, citing the disclosure in Zal (1996) that bis-tris propane was selected for its pKa of 6.8, close to vascular pH).

We agree with the Examiner's reasoning and conclusion.

Appellants argue that bis-tris propane is not a physiologically acceptable buffer (App. Br. 6). In support of this argument, Appellants have submitted a declaration under 37 C.F.R. § 1.132 by inventor Franck Zal. Dr. Zal declares that a bis-tris propane buffer "is not a physiologically acceptable buffer, as evidenced by the attached product data sheets" (Zal Declaration, filed Jan. 17, 2006, p. 2).

The product data sheets referred to in the declaration are from Sigma and Research Organics, Inc. The Sigma product information sheet for BIS-TRIS propane states, under the heading "Precautions and Disclaimer": "For Laboratory Use Only. Not for drug, household or other uses." The information on Bis Tris propane from Research Organics states: "Hazard: May be Harmful, May be Irritating – See MSDS [Material Safety Data Sheet]."

The Examiner's response to this argument is that the warnings included in the Sigma and Research Organics sheets indicate only that those companies sell chemicals for research use, not for use in pharmaceuticals (Ans. 11-12). Thus, the Examiner finds that the evidence included with the Zal Declaration does not show that bis-tris propane per se is physiologically unacceptable (*id.*).

We conclude that the weight of the evidence in the record supports the Examiner's position rather than Appellants'. Zal (1997) refers to Zal (1996) for details of the buffer used (Zal (1997) 86, left-hand col.: "The column

was equilibrated with a saline buffer as described [in Zal (1996)]”). Zal (1996) states that a previously used buffer was modified by substituting bis-tris propane for HEPES because “the pK of bis-tris-propane (6.8) [is] closer to the vascular and coelomic pH (~7)” (Zal (1996) 8870, left-hand col.). The buffer disclosed by Zal (1996) is virtually identical to the buffer used in the instant Specification for purifying *A. marina* hemoglobin except for the buffering agent used (bis-tris propane in Zal (1996), HEPES in the Specification) (Spec. 9: 26-30).

Zal (1997) describes the bis-tris propane-containing buffer as a “physiological” buffer: Zal (1997) determined the molecular weight of *A. marina* hemoglobin using multi-angle laser-light scattering (MALLS) analysis performed “directly on-line with the FPLC system” used to purify the hemoglobin (Zal (1997) 86, left-hand column). Zal (1997) states that one of the advantages of MALLS analysis is that it “can be performed directly on-line with an FPLC system using physiological buffers, thus avoiding sample deterioration and allowing sample recovery” (*id.* at 90, left-hand column).

Based on the teaching of Zal (1996) that bis-tris propane has a pK (and therefore buffering capacity) at pHs near 7, and Zal (1997)’s characterization of bis-tris propane as a “physiological buffer”, the evidence of record supports the Examiner’s conclusion that bis-tris propane is a physiologically acceptable buffer.

Appellants have not presented persuasive evidence that bis-tris propane is not a physiologically acceptable buffer. Whether a buffer is physiologically acceptable depends on the properties of the compound, not

the source from which the compound was purchased. We agree with the Examiner that Appellants' reliance on product data sheets from Sigma and Research Organics is misplaced, since those companies sell products only for research use. Appellants have provided no evidence to show that *any* chemical (including, for example, NaCl or HEPES) sold by those companies would be sold as "suitable for pharmaceutical use" or the equivalent. Thus, the evidence submitted in support of the Zal Declaration does not show that bis-tris propane is not a physiologically acceptable buffer. Dr. Zal's assertion that it is not physiologically acceptable is unsupported by persuasive evidence, and therefore we do not find the assertion credible.

Appellants also argue that Zal (1997) does not disclose an extracellular hemoglobin with a P_{50} of 6 to 7 mm Hg: "The ZAL et al[.] article seeks merely to study the structure of *Arenicola marina* haemoglobin as it occurs in nature. . . . [I]n nature, the P_{50} of the recited lugworm haemoglobin is between 2 and 3." (App. Br. 5.) Appellants also argue that the Specification teaches that P_{50} is measured at 37° C, and "[g]iven that oxygen affinity is known to be temperature dependent and given that there is no teaching in the ZAL article of studying lugworm hemoglobin at such temperatures, there is no basis to assume" that the prior art hemoglobin had a P_{50} of 6-7 mm Hg (*id.* at 9).

These arguments are not persuasive. Zal (1997) purified *A. marina* hemoglobin to homogeneity (Zal (1997), sentence bridging pages 86 and 87). Zal (1997) discloses a preparation of homogeneous *A. marina* hemoglobin in a buffer virtually identical to the buffer used in the instant Specification, with the substitution of bis-tris propane for HEPES, not *A.*

marina hemoglobin “as it occurs in nature.” Appellants’ implication that the *A. marina* hemoglobin disclosed by Zal (1997) would be expected to have a P_{50} of 2-3 mm Hg is therefore unsupported by the evidence.

With regard to temperature, Appellants have provided no evidence that *A. marina* hemoglobin does not have a P_{50} in the range of 6-7 mm Hg at, for example, room temperature. In any case, Zal (1997) teaches that the *A. marina* hemoglobin was “incubated at 100° C for 90 s” during preparation for electrophoresis (Zal (1997) 86, left-hand col.); the hemoglobin taught by Zal (1997) therefore must have been at 37° C at some point. In short, Appellants have not provided a reasonable basis for concluding that the P_{50} of the *A. marina* hemoglobin disclosed by Zal (1997) would be different from the P_{50} of the *A. marina* hemoglobin exemplified in the instant Specification.

Finally, Appellants argue that “Claims 2-13 are further distinct from the disclosures of the Zal articles as regards the recitation of free cysteines. . . . Contrary to the assertions of the Official Action, the ZAL et al[.] 1997 article at pg. 87 does not disclose free cysteines are present and that they bind to NO and/or SNO groups.” (App. Br. 8.)

This argument is also not persuasive. We agree with the Examiner that Zal (1997) discloses the presence of free cysteines in *A. marina* hemoglobin. Zal (1997) states that the polypeptide chains designated a1, a2, and d each have one free cysteine, or Cys, residue. (Zal (1997) 88, right-hand column: “chains a1 and a2 possess three Cys, . . . one is free. Also, . . . chain d [has] one free Cys.”)

Zal (1997) does not state that the free cysteines are bound to NO or SNO molecules but, as the Specification makes clear, that binding only occurs when the *A. marina* hemoglobin is in a vertebrate blood vessel. The Specification states that “[i]n addition to its role as a transporter of oxygen, the haemoglobin of vertebrates plays an important role in the transport of NO and SNO. . . . [O]nly the haemoglobins belonging to marine worms colonising environments rich in hydrogen sulphide had the sites (presence of free cysteines on the globin-type chains) necessary to perform this function.” (Spec. 12-13.) Thus, the claim limitation requiring “free cysteines binding to NO and/or SNO groups” only requires that the hemoglobin have free cysteines capable of binding NO or SNO, not that those molecules are actually bound to hemoglobin in the claimed composition.

In summary, we agree with the Examiner that the composition disclosed by Zal (1997) reasonably appears to meet all the limitations of claim 2. We affirm the rejection of claim 2 under 35 U.S.C. § 102(b). Claims 3-13 fall with claim 2.

3. OBVIOUSNESS I

Claims 1-17, 21, and 22 stand rejected under 35 U.S.C. § 103 as obvious in view of Zal (1997), Hirsch,³ and Anderson.⁴ The Examiner

³ Hirsch et al., “A first evaluation of the natural high molecular weight polymeric *Lumbricus terrestris* hemoglobin as an oxygen carrier,” *Artif. Cells, Blood Substitutes, Immobilization Biotechnol.*, Vol. 25, pp. 429-444 (1997). As with Zal (1997), the Examiner cites to the full-text reference (see the Answer, page 8) even though only the abstract of the Hirsch paper is of record. To complete the record, we have entered the full-text paper into the official Image File Wrapper.

⁴ Anderson et al., U.S. Patent 6,184,356, issued Feb. 6, 2001.

relies on Zal (1997) for disclosure of the composition recited in the rejected claims, but notes that Zal (1997) “does not suggest using the compositions disclosed therein as a blood substitute” (Answer 7). The Examiner finds that Hirsch discloses “the use of hemoglobin from *Lumbricus terrestris* (another annelid) as an oxygen carrier in blood” (*id.* at 8). The Examiner finds that Anderson teaches that “use of naturally occurring haemoglobins from a variety of organisms including annelids was well known,” and that Anderson “suggests that it is desirable to use large molecular weight haemoglobins because they have reduced oncotic pressure; a recognized problem in the art of using haemoglobins as blood substitutes” (*id.*).

The Examiner concludes that it would have been obvious to use the extracellular hemoglobin disclosed by Zal (1997) as a blood substitute in the method disclosed by Hirsch because “those of skill in the art were actively seeking new hemoglobin proteins for use as blood substitutes,” and the high molecular weight of the *A. marina* hemoglobin would have been expected to result in “reduced oncotic pressure” (*id.*).

We agree with the Examiner that the cited references support a prima facie case of obviousness. Zal (1997) is discussed above. Hirsch describes an experiment in which extracellular hemoglobin from *Lumbricus terrestris* (nightcrawler) was used as a blood substitute in mice and rats (Hirsch 432). Hirsch discloses that the “[m]ice and a rat model partially exchanged with LtHb [*L. terrestris* hemoglobin] showed no apparent behavioral and physical changes” (*id.* at 429). Hirsch concludes that the data “should draw attention to the natural extracellular hemoglobins or oxygen carriers in the search for blood substitutes” (*id.* at 442).

Anderson discloses that *L. terrestris* hemoglobin has a molecular weight of 3600 kDa and that “[o]ther invertebrate hemoglobins are also large multi-subunit proteins” (Anderson, col. 5, ll. 57-65). Anderson also teaches that hemoglobins with increased molecular weight (e.g., by polymerizing free hemoglobin) provides increased half-life and reduced oncotic pressure (*id.* at col. 7, l. 65 to col. 8, l. 2) and that reduced oncotic pressure is a desirable property in blood substitutes (*id.* at col. 9, ll. 33-45).

We agree with the Examiner that a person of ordinary skill in the art would have considered it obvious, based on the cited references, to use the *A. marina* extracellular hemoglobin taught by Zal (1997) as a blood substitute. Such a use would have been suggested by Hirsch’s disclosure of the successful use of the similar extracellular hemoglobin of *L. terrestris* as a blood substitute in rats and mice, and by Anderson’s disclosure that high molecular weight hemoglobins have the desirable properties of increased half-life in the blood stream and reduced oncotic pressure.

Appellants argue that the cited references would not have provided a reasonable expectation of success and support, at best, an “obvious to try” rationale (App. Br. 11).

This argument is not persuasive. Hirsch discloses the successful use of *L. terrestris* extracellular hemoglobin as a blood substitute in mice and rats. This teaching would have provided an ample basis for a person of ordinary skill in the art to reasonably expect that the extracellular hemoglobins of other annelids, such as the *A. marina* hemoglobin taught by Zal (1997), could also be used successfully in blood substitute compositions.

3. OBVIOUSNESS II

Claims 23-25 stand rejected under 35 U.S.C. § 103 as obvious in view of Zal (1997), Hirsch, Anderson, and Taylor.⁵ Claims 23-25 require that the claimed method or composition contain HEPES. The Examiner relies on Zal (1997), Hirsch, and Anderson for the teachings discussed above, and finds that Taylor teaches that “sulfonic acids such as Hepes were routinely used in blood substitutes (Col. 12, lines 10-52) and that the choice of buffer depends on the particular circumstances wherein the blood substitute is used” (Ans. 9).

The Examiner concludes that

those of skill in the art developing and characterizing haemoglobins for use as blood substitutes would have chosen a buffer such as Hepes, for the hemoglobin solution, that had a buffering capacity at physiological pH. Moreover, it was well within the ordinary skill of the art to select the appropriate buffer for the conditions at hand as evidenced by Taylor.

(*Id.* at 10.) We agree with the Examiner’s reasoning and conclusion.

Appellants argue that “[t]here is also no evidence suggesting that HEPES is an art recognized equivalent for the buffer (BTP) taught by ZAL et al. As already noted, the evidence of record establishes that BTP is not a physiologically acceptable buffer.” (App. Br. 12.)

This argument is not persuasive. For the reasons discussed above, we conclude that a preponderance of the evidence of record supports the Examiner’s position that bis-tris propane (BTP) is a physiologically acceptable buffer. Taylor shows that HEPES was established as a buffering agent in blood substitutes, and therefore those of skill in the art would have

⁵ Taylor, U.S. Patent 5,514,536, issued May 7, 1996.

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considered it obvious to substitute HEPES for the bis-tris propane in the blood substitute composition and method suggested by Zal (1997), Hirsch, and Anderson.

SUMMARY

We affirm the rejection of claims 2-13 as anticipated by Zal (1997) and the rejection of claims 1-17 and 21-25 as obvious in view of the references cited by the Examiner.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED

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