美国健康医疗仪器国际公司

35 Weaver Street, *Scarsdale*, New York 10583. U.S.A.

血氧分析仪--Hemox Analyzer

美国 TCS Scientific Corp 产品

Introduction

The HEMOX ANALYZER is an automatic system for the recording of blood oxygen equilibrium curves and related phenomena. The recording can be performed in the association or dissociation modes, utilizing fresh whole blood or hemolysate. A recording can be performed with as little as 2 micro liter of blood, but for routine measurement it is best to use 30 to 50 micro liters.

The operating principle of the HEMOX ANALYZER is based on dual wavelength spectrophotometry for the measurement of the optical properties of hemoglobin and a Clark electrode for measuring the oxygen partial pressure in millimeters of mercury. The resulting signals from both measuring systems are fed to the X Y recorder or PC, which plots the resulting curve .

In the new Model B the blood sample (30 to 50 ul) is added to the HEMOX Solution or other buffer contained in a sample tube for transfer into the cuvette. The cuvette, which is an optical sample cell containing the oxygen electrode and thermistor probe, is mounted in a cuvette holder. Also contained in the ccuvette is a magnetic stirring bar for rapid stirring of the sample buffer mixture. The cuvette contains two (2) stoppers on the top, one of which is used for sample intake, while the other one supplies vacuum to the cuvette chamber. After the plotting of the curve, the sample is removed through the bottom outlet port to drain.

Front Panel Display

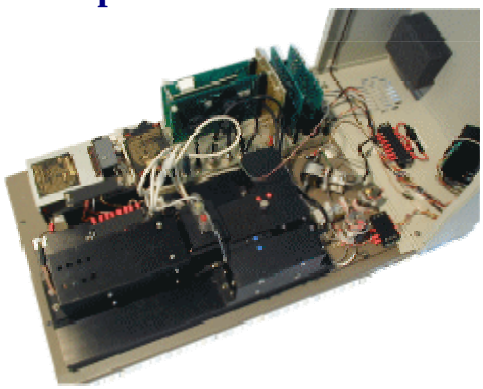
After the sample buffer solution has been drawn into the cuvette, the temperature of the mixture has to equilibrate until it has reached 37°C. Than the sample is oxygenated with air or oxygen, supplied from an outside air tank. After the sample has been completely oxygenated and the pO2 has been adjusted to the correct value, the ploting of the deoxygenation curve can begin by switching the AIR/NITROGREN switch to NITROGEN. The completion of the curve is reached when the pO2 approaches zero.

The time for completing the plotting of the curve is approximately 30 minutes but may be reduced by increasing the stirring speed and the flow of nitrogen. However, there are limitation to the plotting speed due to the saturation equilibrium between the photometric and the pO2

四川省, 成都市上东大街 36 号, 新和•春禧时代 12-11, 中国, 四川省. 邮编 610016 www.ahmedical.com.
USA: 01-914- 498-4158; 中国 电话及传真: **028-86653817;** 手机: 13618077026; 电子邮件: ahmedical@verizon.net;

systems. The measurement is normally carried out at a temperature of 37°C to simulate physiological conditions. However, the temperature control system permits measurement at other temperatures in conjunction with the built in cooling coil connected to an external cooling bath (supplied by the customer).

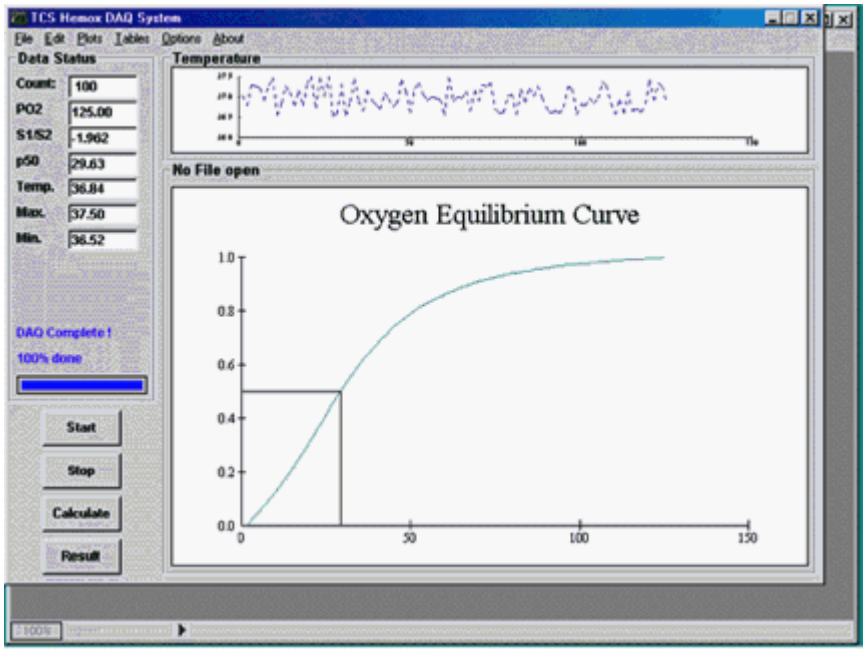
Description



The HEMOX Analyzer consists of the main console which is a sheet metal housing, upon the base of which the optical and electronic components are mounted. Also contained in the housing is the gas selection valve system and the pumping system for sample intake and removal.

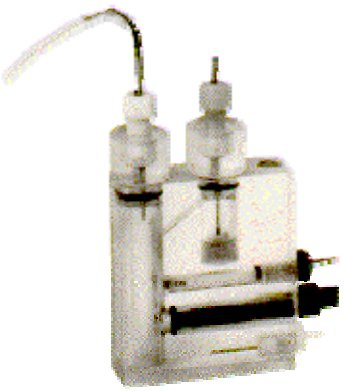
The housing contains in its front section the optical bench with light source, lens system, cuvette holder and the photo multiplier detectors. The rear part of the housing contains the power supplies and the electronic chassis, including the four PC boards. The housing cover has a small hinged lid for easy access to the sample cuvette for sample loading and routine maintenance of the cavity and the pO2 electrode.

The complete top cover of the housing is hinged on the right side of the base and can be swung open by removal of two nuts, located under the base of the housing. All utilities, like main power, gases and optional cooling water, enter from the rear of the instrument. Also the main fuse (2.0 amps.) is located in the rear. All operational controls are front panel mounted for easy access and convenience of adjustment.



氧亲和力测定

应用 Hemox analyzer 测量： Hb 样品的氧解离曲线，Hb 样品的氧亲和力（P50 ）
和协同效应（ Hill 系数）由仪器分析软件自动算出。



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P02 Measurement

The oxygen concentration of an aqueous solution is very easily measured with a CLARK oxygen electrode. This method of measurement not only is accurate, but also reliable and reproducible. The HEMOX ANALYZER uses such a CLARK electrode for determining the oxygen concentration directly in the sample cavity where the absorbance is also being monitored.

Under normal conditions the oxygen concentration or, as it is commonly called, the oxygen partial pressure, under athmospheric conditions of 760 mm of mercury, is 158 mm of mercury for the sample in the cavity. This saturation point is used for full scale calibration of the recorder prior to starting the plotting of the curve.

When the oxygen is being replaced by an inert gas, as for for instance nitrogen, argon or helium, in a continous procedure, hemoglobin becomes deoxygenated according to the following equation: [HbO] <----> Hb + O

The figure to the right shows a typical association curve of normal blood which was obtained with the HEMOX ANALYZER. The curve recording was started with the blood sample in the deoxygenated state and was then slowly oxygenated. When the x axis reading is approx. 1.5 mm Hg, i.e. the oxygen partial pressure has reached almost zero, and the hemoglobin has been almost completely deoxygenated, the recording of the curve can be started, i.e. the oxygenation can begin, by flipping the gas selector switch into the "AIR" position and setting the pen to to the starting position.

In addition to plotting association curves with the instrument, it is also possible to plot the dissociation curve by starting with the sample in the oxygenated state and slowly deoxygenating it. However, in order to obtain a hysteresis free recording of both curves, the gas flow and stirring speed must be critically adjusted, resulting in a much longer plotting time of one hour or more.

The new Model B has been adjusted specifically for the recording of the association curve. If it is desired to record the dissociation curve, it will require not only adjusting of the stirring speed and gas flow, but also a change of the time constant in the amplifier circuit.

Hemox Analytical Software

The Software

The Hemox Analytical Software (HAS) is a newly developed product by TCS Scientific Corporation. This product offering will directly interface a PC to the Hemox Analyzer utilizing the same output cables currently connecting the Hemox Analyzer to the X-Y Chart Recorder. The software collects real time data points using an A/D Data Acquisition Module (DAQ). The DAQ comes as part of the product. The HAS reads the data points captured via the DAQ and plots the Oxygen Equilibrium Curve in both oxy and deoxy state. The estimate p50, PO2, S1/S2 (logPO2), and Temperature (temperature points are collected only with the newest model of the Hemox Analyzer) are displayed in real-time. When the test run has completed the estimated p50 will do a regression analysis to calculate the actual p50. The user has the option of viewing numerous other results such as the Hill Plot and Hill Derivative Curve. Convergence of Adair constants and a data table with p0 through p100 is also viewable. The user can set the cooperativity range with a preference section and can also choose how to smooth the curve fitting process. They can also choose linear or logarithmic modes along with overall or stepwise processing.

The realtime plot of the Oxygen Equilibrium Curve can be collected from both the de-oxygenation state and the oxygenation state. The user can start/stop data capture manually or programatically via starting and ending PO2 (pressure points).

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HEMOX Analyzer Specifications

ACCURACY P50: + 1 mm Hg at controlled pH of 7.40 +.01 and a temperature of 37.0°C

SAMPLE SIZE: Selectable from 5 to 50 microliters of whole blood or hemolysate.

PLOTTING TIME: 30 minutes or less for a complete dissociation or association curve.

PHOTOMETRIC SYSTEM: Dual Wavelength Photometer, split beam type, dual photomultiplier detector with log/ratio converter for linear concentration read out, PC-Board circuitry, front panel controls. p02 METER: The CLARK Electrode system has a digital readout meter with 0.1 mm Hg readability.

GAS-SYSTEM: Multi Gas System for use with commercial air and nitrogen tanks, featuring one 2 way and one 3 way solenoid selector valves for gas selection by front panel control.

SAMPLE SYSTEM: Built in Pressure-Vacuum Pump for sample filling and flushing of the cuvette, controls located on front panel.

TEMPERATURE CONTROL: Electric cuvette heating system for operation at 37°C, precision proportional temperature controller with thermistor control, digital temperature read out of sample chamber temperature with accuracy of 0.2°C.

Biorheological Properties of Reconstructed Erythrocytes and its Function of Carrying-Releasing Oxygen

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Xiang Wang[1†], Wei Gao[1], Weiyan Peng[1], Jiaxin Xie[1] and Yaojin Li[1]

[1]College of Bioengineering, Chongqing University, Chongqing, P.R. CHINA

[†]*Correspondence*: Xiang Wang, College of Bioengineering, Chongqing University, Chongqing, 400044, P.R. China xwangchn@vip.sina.com

Erythrocyte shape and biomechanical properties have close relation to its physiological function. In this research the erythrocyte was reconstructed with natural structure protein and lipids based on cellular mechanics and hemorheology concepts. The biomechanical properties of the reconstructed erythrocyte were determined with the micropipette aspiration system. The shapes of reconstructed erythrocyte were obtained with electron scanning microscope. **The oxygen carrying-releasing function was analyzed with the HEMOX analyzer from TCS,** the experimental results indicated that the reconstructed erythrocytes were similar to the natural erythrocyte: having biconcave disc shape, good deformability and carrying-releasing oxygen function.

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TCS Customers: Hemox Analyzers are installed at leading hospitals and research centers worldwide.

- Abbott Laboratories (USA)
  Auckland University (Australia)
  Baxter Healthcare Corp. (USA)
  Cambridge University (England)
  Centers for Disease Control (USA)
  Children's Hospital of Philadelphia/University of PA (USA)
  Hospital Maggiore (Italy)
  INSERM (France)
  Jordan University Hospital (Jordan)
  Hospital San Carlos (Spain)
  Mayo Clinic (USA)
  National Institutes of Health (USA)
  Oak Ridge National Laboratory (USA)
  Osaka Medical School (Japan)
  Rockefeller University (USA)
  Somatogen (USA)
  Tokyo University (Japan)
  Uniformed Services University of the Health Sciences (USA - Military)
  University of Geneva (Switzerland)
  University of Lisbon (Portugal)
  Upjohn Company (USA)
  Wellcome Research Laboratories (England)
  Westmead Hospital/University of Sydney (Australia)

http://www.stanfordlab.com/LabTestGuide/Search.aspx?TestName=O **Stanford Hospital Clinical Lab Test Directory**

Oxygen Dissociation P50, RBC — Order Code: 11986R

Specimen Type:	Whole blood
Container Type:	Green-top tube (sodium heparin)
Required Volume:	5 mL
Minimum Volume (Pediatric):	1 mL
Methodology:	**Hemox-Analyzer** (Measures and Plots 02 Saturation
Standard Run Times:	Mon-Sat
Turnaround Time:	3 days
Special Handling:	Specimens must arrive within 72 hours of draw. Draw blood in a green-top (heparin) tube(s), refrigerate specimen immediately after draw, and send 5 mL of fresh heparinized whole blood refrigerated. Do not transfer blood to other containers. Include a control specimen drawn at the same time from a normal, unrelated, non-smoking individual: draw blood in a green-top (heparin) tube(s), refrigerate specimen immediately after draw, and send 5 mL of fresh heparinized whole blood refrigerated. Do not transfer blood to other containers. Label clearly on outermost label normal control. Rubber-band patient specimen and control vial together.

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血红蛋白在膜分离过程中的氧化规律和抗氧化探索

石晓东[1,1], 路秀玲[1], 郑春杨[1], 于鹏展[1], 索晓燕[1], 王永权[1], 苏志国[1]

(1. 中国科学院过程工程研究所生化工程国家重点实验室，北京 100080; 2. 北京科技大学土木与环境工程学院，北京 100083)

摘 要：脱离了红细胞的血红蛋白(Hb)在溶液中易被氧化成高铁血红蛋白(MetHb)，失去载氧活性. 实验发现，当对红细胞裂解液进行微孔膜分离时，高铁血红蛋白增加不多；但在用层析法去除超氧化物歧化酶等其他红细胞蛋白后再进行超滤膜浓缩血红蛋白时，则出现较多的血红蛋白被氧化成高铁血红蛋白的现象，其氧化反应随超滤过程膜表面流体切向速度的增加而加快，随溶液温度的增加而加快. 抗氧化剂的存在能有效地降低高铁血红蛋白的生成，谷胱甘肽(GSH)、半胱氨酸、N-乙酰半胱氨酸、亚硫酸钠、抗坏血酸(Vc)在溶液中和超滤过程中都能起到对血红蛋白载氧活性的保护作用. 其中 Vc 的效果最好，最适加入量(摩尔比)Vc/Hb=8, pH 8. 将抗氧化的优化条件整合到从红细胞裂解液开始到超滤浓缩血红蛋白的整个流程，在离子交换层析后，添加 Vc 作为抗氧化剂进行超滤浓缩，Hb 活性得到了很好的保护，MetHb 的含量控制在 2.3%，成功地制备了低 MetHb 含量的纯化血红蛋白.

关键词：血红蛋白；膜分离；载氧活性；高铁血红蛋白；抗氧化剂
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1 前 言

以血红蛋白(Hb)为基础的红细胞代用品能够缓解血源紧缺及病毒污染等问题，已成为国际研究的热点[1]. 膜分离是大量制备高纯度血红蛋白的重要步骤. 通常血红蛋白经过微滤(MF)膜去除细胞碎片和超滤(UF)膜去除小分子杂质之后，需采用层析进一步纯化. 层析后的溶液仍需用超滤浓缩更换缓冲液，以用于后续的血红蛋白修饰[2]. 但膜分离过程往往会造成蛋白质的失活，是一个亟待解决的问题.

血红蛋白是有 4 个亚基和 4 个卟啉环的复杂蛋白质，在脱离红细胞后容易解聚成为αβ二聚体，同时卟啉环中的二价铁容易氧化成三价铁，使血红蛋白变为无载氧活性的高铁血红蛋白(MetHb). 在膜分离过程中，血红蛋白溶液受到泵送或搅拌，在膜表面作切向流动，由此形成的剪切力、局部温升[3]和溶液中氧传递速度增加及溶氧量增加、蛋白与膜表面的物理、化学相互作用[4]等均不利于其活性的保持. 对溶液以惰性气体保护来达到脱氧目的在膜分离过程中不容易实现，膜分离过程中蛋白质失活和抗失活的研究目前还处于探索阶段，迄今为止尚未见到有关的文献.

本工作首先研究了膜分离过程中血红蛋白的氧化规律，然后考察了不同还原剂对血红蛋白抗氧化的作用，所选的还原剂包括还原型谷胱甘肽(GSH)、半胱氨酸(Cys)、N-乙酰半胱氨酸(N-A-Cys)、亚硫酸钠和维生素 C(Vc). 对抗氧化的过程进行了优化，探索了还原剂加入量、溶液 pH 值等的影响，测量了血红蛋白的载氧曲线和希尔(Hill)系数以及高铁血红蛋白的含量.

2 材料与方法

2.1 材料

2.1.1 原料及试剂

新鲜牛血采自当地屠宰场，加入适量的柠檬酸钠抗凝. 还原剂 GSH, Cys, N-A-Cys 为分析纯试剂，Sigma 公司产品；亚硫酸钠、Vc 为国产分析纯试剂，其余化学试剂均为分析纯. 实验用水是由美国 Millipore 公司 RiOs 超纯水系统处理得到的超纯水.

2.1.2 仪器

血氧分析仪 Hemox Analyzer, 美国 TCS Scientific Corp 产品. Ultraspec 2000 型 UV/Vis 紫外可见分光光度计，瑞典 GE Healthcare 公司产品. Φ72 型 pH 计，美国 Beckman 公司产品. 膜分离系统为美国 Millipore 公司产品，包括 Labscale 错流超滤系统和 Pellicon 2 错流过滤系统. 真空冷冻干燥机为美国 Labconco 公司产品，容量 8 L. G25 脱盐柱为瑞典 Pharmacia 公司.

2.1.3 膜材料与规格

实验选用的微滤(孔径为 0.45 μm, 0.1 m^2)和超滤膜(10 kD, 0.005 m^2)均为美国 Millipore 公司产品，膜材料均为聚醚砜.

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作者简介：石晓东(1980-)，男，河北省内丘县人，硕士研究生，生物化工专业；苏志国，通讯联系人，Tel: 010-62561817, E-mail: zgsu@home.ipe.ac.cn.