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AUTOMATIC ACQUISITION AND COMPUTATION OF DATA FROM THE RADIOMETER DISSOCIATION CURVE ANALYZER

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The whole oxyhemoglobin dissociation curve of blood can be generated using the Radiometer Dissociation Curve Analyzer (model DCA-1), which measures oxygen pressure, oxygen content and pH simultaneously. Thereby the dissociation curve at standard physiological conditions of pH 7.4 and temperature 37°C derived graphically. In this study two methods of acquisition and computation of data, OFF-line and ON-line, are described and compared.

Oxyhemoglobin dissociation curve Radiometer DCA-1 ON-line and OFF-line Acquisition and computation

1. Introduction

The oxyhemoglobin dissociation curve (ODC) represents the graphic relationship between oxygen tension (P_{O_2}) and oxygen saturation of hemoglobin (S_{O_2}). It is well known, that the oxygenation of hemoglobin, i.e., the shape and position of the ODC, is influenced by pH, P_{CO_2}, temperature, ionic strength, carboxyhemoglobin and methemoglobin [1]. Recently was discovered, that also several phosphorylated intermediates of red cell metabolism, particularly 2,3-diphosphoglycerate (DPG) and adenosinetriphosphate (ATP) [2, 3], affect the binding of oxygen to hemoglobin.

To ascertain the position of the ODC it is usual to determine the standard P_{50} value (P_{O_2} at 0.50 saturation corrected to pH 7.4 and 37°C) and for the shape of the curve the *n*-value of the Hill formula [4]. Mostly these parameters are derived from multiple point determinations of saturation (or oxygen content) at specific values of P_{O_2} at 37°C and corrected for deviations from standard pH [5]. Recently a method for generation of the entire ODC from a sample of whole blood was described [6]. A device, the model DCA-1 Dissociation Curve Analyzer, based here upon is now available from Radiometer (Copenhagen). Results have to be derived graphically from the recorded curve.

The aim of the present paper is to describe two methods of computation, OFF-LINE and ON-LINE, of Dissociation Curve Analyzer data.

2. Principle of determination of the ODC with the DCA-1

The Dissociation Curve Analyzer consists of a stainless steel two-compartment cuvette, containing two P_{O_2} electrodes, a pH sensitive glass electrode, the KCl-bridge of a calomel electrode and a stirrer bar. The cuvette is placed in a thermostat bath, which also includes gas humidifiers. Three measuring instruments (digital Acid-Base Analyzers PHM 72 from Radiometer) for the electrodes and a X, Y_1, Y_2-recorder (Honeywell, Model 540) are needed in addition. The whole ODC of a blood sample is obtained by determining continuously and simultaneously oxygen content and P_{O_2} in blood, that, after complete deoxygenation with a N_2–CO_2 gas mixture, is exposed to pure oxygen. As the oxygen enters the blood, the increase of its P_{O_2} is measured by a P_{O_2} electrode connected to the X-axis of the recorder, and simultaneously the

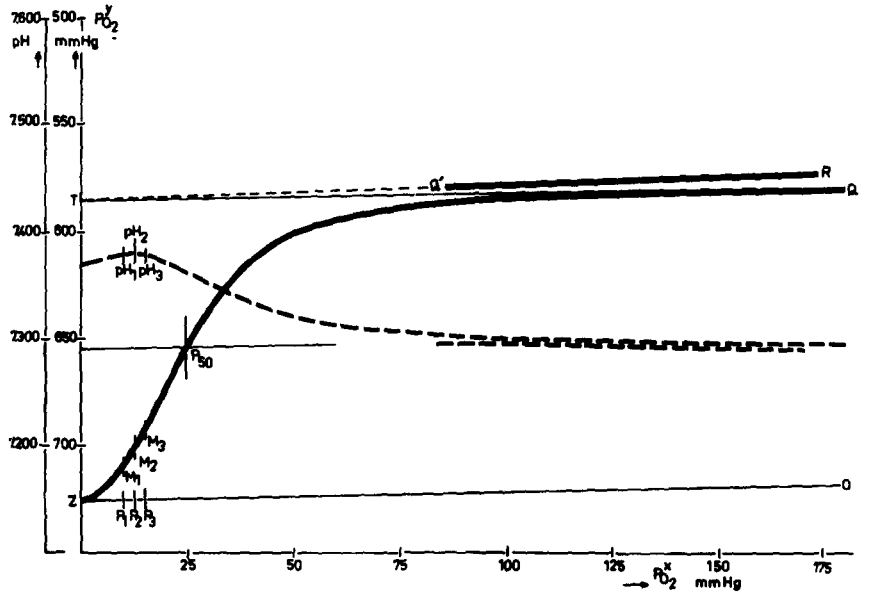

Fig. 1. An example of an oxyhemoglobin dissociation curve (O_2 content vs P_{O_2}) of a blood sample as generated by the Dissociation Curve Analyzer system. The thick solid line represents $P^y_{O_2}$ (P_{O_2} of the gasphase) versus $P^x_{O_2}$ (P_{O_2} of blood) and the thick broken like the change of blood pH. Detailed analysis is described in section 2.

fall in P_{O_2} in the gas phase, which is proportional to the oxygen content of the blood, is measured by a P_{O_2} electrode connected to the Y_1-axis. By reversing the signal of the Y_1 channel a plot of blood O_2 content versus blood P_{O_2} results. The change of the pH of the blood is recorded using the Y_2 channel.

An example of a curve, as obtained for blood samples, is presented in fig. 1. To increase the accuracy the curve is recorded in two portions, one halve (ZQ) going on to $P^x_{O_2}$ = 180 mmHg and the other halve ($Q'R$) proceeding on to 300–400 mmHg at a lower sensitivity of the recorder.

Before automatic computation the following manual procedure was used to estimate the parameters P_{50} and n_{Hill} from the curve:

1. Extrapolate the final branch $Q'R$ of the curve to $P^x_{O_2}$ = 0. This gives the intersection point T.

2. Draw a straight line TQ from point T as tangent to the end of the first branch (ZQ) of the curve.

3. Draw line ZO from point Z parallel to line TQ.

4. Draw vertical lines, dividing the curve into as many sections as desired; usually from $P^x_{O_2}$ = 10 mmHg to $P^x_{O_2}$ = 90 mmHg at intervals of 5 mmHg.

5. Calculate at the intersection points $M_1, M_2 \ldots M_N$ the corresponding hemoglobin saturation $S_{O_2} = S_1$, $S_2 \ldots S_N$ expressed as mole fraction; for example at $P^x_{O_2} = P_1$, the saturation S_1 equals to $P_1 M_1 / ZT$.

6. Read at $P^x_{O_2} = P_1, P_2 \ldots P_N$ the corresponding pH values: $pH_1, pH_2 \ldots pH_N$.

7. Correct these values of $P^x_{O_2}$ to pH 7.4 using the Bohr factor [7]

$$\frac{d \log P_{O_2}}{d\,pH} = -0.5. \qquad (1)$$

8. Calculate the constants K and n_{Hill} of the Hill equation, using the function

$$\log \frac{S}{1-S} = K + n_{Hill} \log P \qquad (2)$$

with the saturation values S between 0.30 and 0.70 and the corresponding P values, corrected to pH 7.4.

9. Taking $S = 0.50$, P_{50} is obtained as antilog $-K/n_{Hill}$.

3. Computational methods

The data of the $P^x_{O_2}$, $P^y_{O_2}$ and pH electrodes are

measured from $P^x_{O_2}$ equals zero until $P^x_{O_2}$ exceeds 350 mmHg.

Thereafter corrections are made with respect to the pH and $P^x_{O_2}$ values, assuming a linear drift of the asymmetry potential of the pH cell and of the sensitivity of the $P^x_{O_2}$ electrode. The following formules are used:

$$pH(i)_c = pH(i)_m + (7.384 - pH_I) - (i/N)(pH_{II} - pH_I)$$
$$i = 1, ..., N \quad (3)$$

wherein

$pH(i)_c$ = corrected pH value of measurement i;
$pH(i)_m$ = measured pH value of measurement i;
pH_I = gauging pH value of the NBS phosphate buffer "7.384" before the determination (as a rule $pH_I = 7.384$);
pH_{II} = gauging pH value of the NBS phosphate buffer "7.384" after the determination;
N = total number of measurements.

$$P^x_{O_2}(i)_c = P^x_{O_2}(i)_m \left[\frac{X_{cal}}{X_I} + \frac{i}{N}\left(\frac{X_{cal}}{X_{II}} - \frac{X_{cal}}{X_I}\right)\right]$$
$$i = 1, ..., N \quad (4)$$

wherein

$P^x_{O_2}(i)_c$ = corrected $P^x_{O_2}$ value of measurement i;
$P^x_{O_2}(i)_m$ = measured $P^x_{O_2}$ value of measurement i;
X_{cal} = calibration value of the $P^x_{O_2}$ electrode;
X_I = gauging value of the $P^x_{O_2}$ electrode before the determination (as a rule $X_I = X_{cal}$);
X_{II} = gauging value of the $P^x_{O_2}$ electrode after the determination.

Subsequently the $P^x_{O_2}(i)_c$ is recalculated to standard pH = 7.4 using the formule of Astrup et al. [7]

$$^{10}\log P^x_{O_2}(i)_{c,\,7.4} = {}^{10}\log P^x_{O_2}(i)_c - 0.5\,[7.4 - pH(i)_c]$$
$$i = 1, ..., N. \quad (5)$$

Whereas we assumed a linear drift of electrode sensitivity for $P^x_{O_2}$, we corrected for linear drift of the $P^y_{O_2}$ value itself, which is included in the correction for the physical dissolved oxygen hereafter. This is acceptable at small deviations, so that the usual differences of ca. 1% between the gauging values Y_I and Y_{II} of the $P^y_{O_2}$ electrode have not been considered.

To fix the origin of the curve a straight line

$$P^y_{O_2}(i)_m = A * P^x_{O_2}(i)_{c,\,7.4} + B \quad (6)$$

wherein $P^y_{O_2}(i)_m$ = measured $P^y_{O_2}$ value of measurement i, is calculated according to the least squares method through the first $P^y_{O_2}(i)_m$, $P^x_{O_2}(i)_{c,\,7.4}$ points, using the measurements up to $P^x_{O_2}(i)_{c,\,7.4} = 2.5$ mmHg or at least three points. All $P^y_{O_2}$ values are referred to this origin using the relationship

$$P^y_{O_2}(i)_c = P^y_{O_2}(i)_m - B \quad (7)$$

wherein $P^y_{O_2}(i)_c$ represents the corrected $P^y_{O_2}$ value.

Assuming blood is fully saturated at $P^x_{O_2}$ values above 250 mmHg, deviations from a horizontal course of the oxygen content line at $P^x_{O_2}$'s above 250 are due to physically dissolved oxygen and $P^y_{O_2}$ electrode drift, supposing both are linear. To estimate the amount of physically dissolved oxygen and electrode drift, the relationship

$$P^y_{O_2}(i)_c = C * P^x_{O_2}(i)_{c,\,7.4} + D \quad (8)$$

is derived by means of linear extrapolation from 250 mmHg $\leqslant P^x_{O_2}(i)_{c,\,7.4} \leqslant$ 350 mmHg (least squares method) to $P^x_{O_2}(i)_{c,\,7.4}$ equals zero.

Oxygen saturation fractions ($S_{O_2}(i)$) are then calculated by subtracting the curve deflection magnitudes, attributable to physically dissolved oxygen ($C * P^x_{O_2}(i)_{c,\,7.4}$), from $P^y_{O_2}(i)_c$ values, and dividing the remainder by the deflection produced by fully saturated hemoglobin (D). The following relationship is used

$$S_{O_2}(i) = [P^y_{O_2}(i)_c - C * P^x_{O_2}(i)_{c,\,7.4}]/D. \quad (9)$$

At this point all corrections being fulfilled, the coordinates of the Hill plot can be estimated using the equations

$$Y_{Hill}(i) = {}^{10}\log [S_{O_2}(i)/(1 - S_{O_2}(i)] \quad (10)$$

$$X_{Hill}(i) = {}^{10}\log P^x_{O_2}(i)_{c,\,7.4}. \quad (11)$$

According to our experience the equation of Hill proves to be valid between 0.30 and 0.70 oxygen saturation, and so in this range Y_{Hill} and X_{Hill} are related by a straight line

$$Y_{Hill} = n_{Hill} * X_{Hill} + K. \quad (12)$$

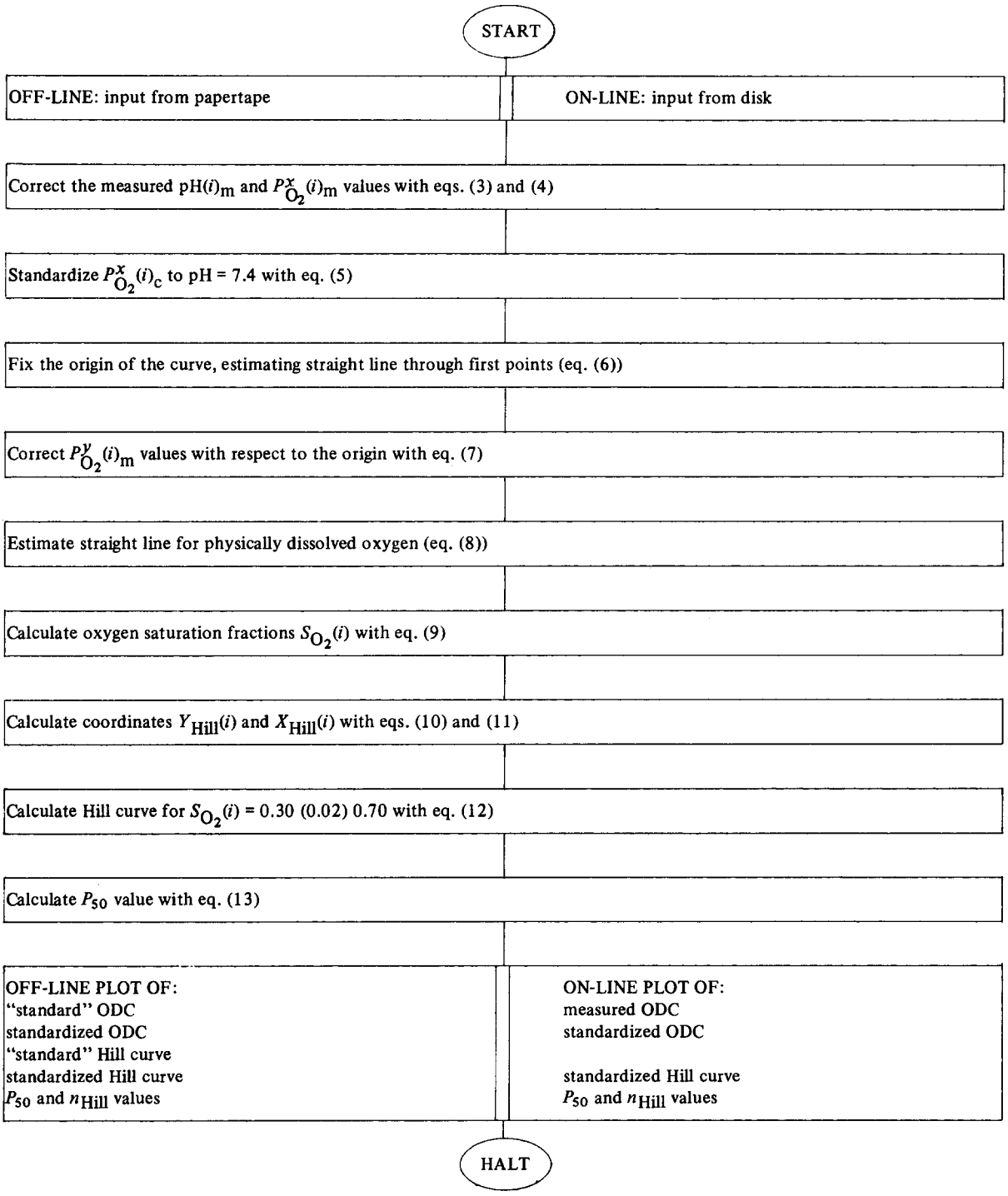

Fig. 2. Flowchart of the two computation methods: OFF-LINE and ON-LINE.

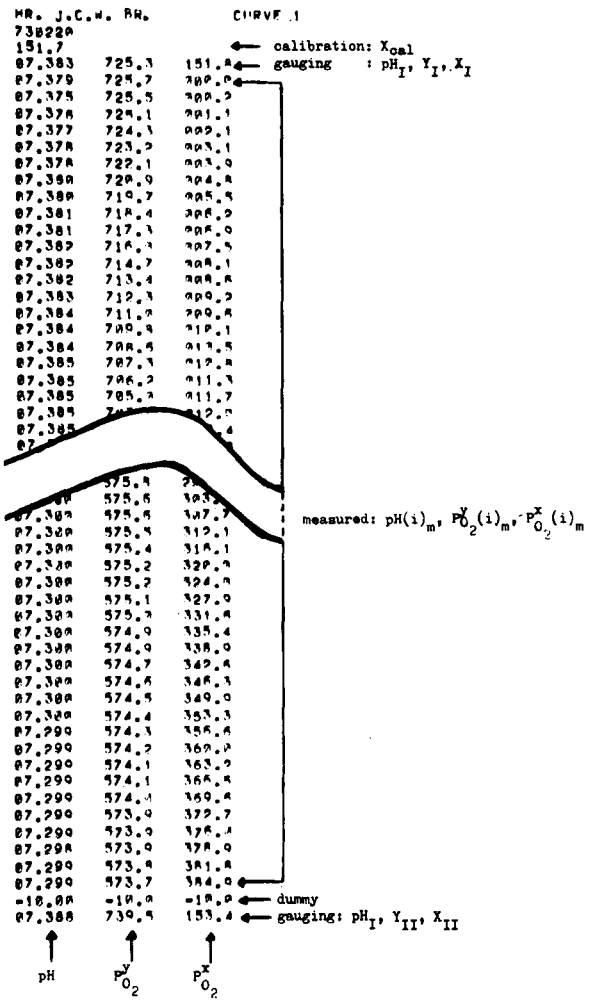

Fig. 3a. Example of input data, OFF-LINE collected for the computer, from the Dissociation Curve Analyzer system, generating the ODC of blood from a cardiac patient.

```
HR. J.C.W. BR          CURVE 1
738220
P50=    23.7358
LOGP50=     1.3754
   XCOR       SAT
   0.1944   0.1863
   3.8030   2.4308
   5.3749   4.0901
   7.3450   6.6567
   8.4212   8.4659
   9.4210  10.1362
  10.6089  12.7175
  11.4916  14.3192
  12.5842  16.8320
  13.1726  18.5800
  13.7507  20.2574
  14.7410  22.6315
  15.3288  24.2388
  16.1120  26.5460
  16.6802  28.0829
  17.4621  30.3901
  18.2226  32.6973
  18.6887  34.2361
  19.3702  36.4748
  20.1028  38.5709
  20.6859  40.1078
  21.4379  42.2040
  22.1624  44.3002
  22.8125  46.3981
  23.4607  48.4258
  24.1762  50.5219
  24.8880  52.4078
  25.5293  54.2940
  26.3309  56.1069
  27.2226  58.5512
  27.9522  60.2253
  28.5513  62.0419
  29.3974  64.2048
  30.4815  66.2233
  31.4254  68.2437
  32.3842  70.0531
  33.6184  72.2087
  34.7141  74.0144
  36.2644  76.3740
  37.5252  78.1057
  39.2378  80.0394
  41.2560  82.1770
  43.5287  84.0277
  46.3764  86.0787
  49.7213  88.2577
  53.3903  90.1516
  58.0994  92.0905
  63.9687  94.0077
  74.6856  96.2474
 146.1477  99.8194

FORMULE HILL PLOTT :   2.682 * X -3.689
```

Fig. 3b. Example of output data of the standardized ODC of a blood sample from a cardiac patient obtained by the OFF-LINE computation method.

This relationship is calculated by the least squares method using saturation values in this range in steps of 0.02, to obtain equal weight of each part of the Hill plot. The value of the slope equals n_{Hill}. Deriving the P_{50} value is now quite straight forward

$$P_{50} = 10^{-K/n_{Hill}}. \quad (13)$$

3.1. The OFF-LINE method

The BCD-data from the Dissociation Curve Analyzer System are sampled with intervals of 5 sec and collected on papertape by means of a teletype with papertape punch.

Patient information, date code, the calibration value of the $P^X_{O_2}$ electrode and the gauging values are

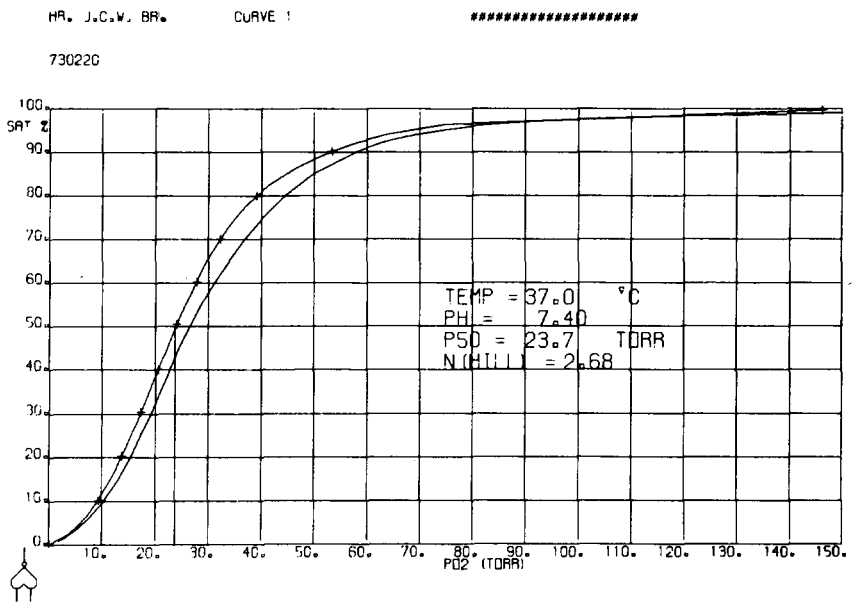

Fig. 3c. An OFF-LINE plotter output of the standardized ODC of blood from a cardiac patient indicated by crosses (+) and the "standard" ODC derived from data of Severinghaus [8].

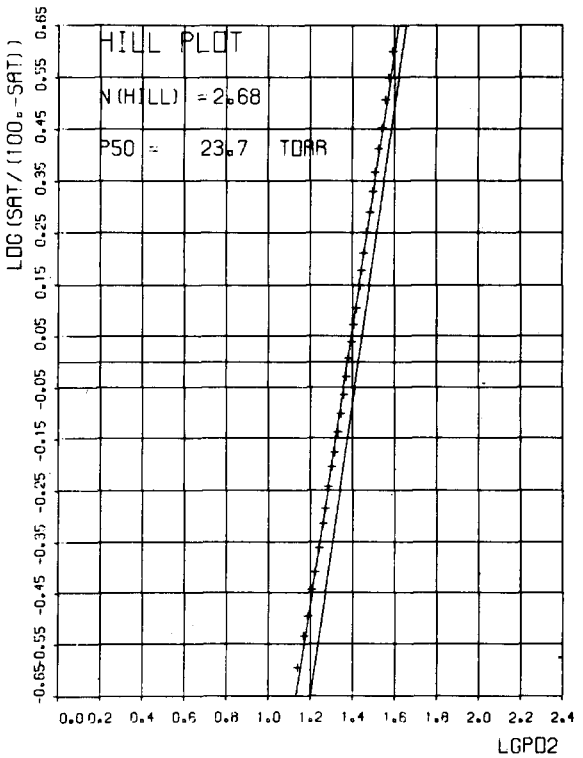

Fig. 3d. OFF-LINE Hill plots for the standardized ODC of blood from a cardiac patient and the "standard" ODC.

punched in the papertape by the operator of the measuring equipment; the terminating gauging values, which are preceded by a set of negative dummy values to secure a proper separation, are also punched by the operator.

So one complete papertape consists of respectively: patient-data (80 characters), date-code (10 characters), one calibration value (X_{cal}), three initial gauging values (pH_I, Y_I, X_I), measured data ($pH(i)_m$, $P^y_{O_2}(i)_m$, $P^x_{O_2}(i)_m$), dummy values and a set of three terminating gauging values (pH_{II}, Y_{II}, X_{II}).

The computation of the standardized oxyhemoglobin dissociation plot and Hill plot is straight forward. To compare the results also a "standard" oxyhemoglobin dissociation curve and Hill curve, derived from data of Severinghaus [8] are plotted.

3.1.1. Flowchart

A flowchart of the computer program is given in fig. 2.

3.1.2. A typical sample run

As a typical sample run the ODC of a blood sample from a cardiac patient is determined. The listing of the input-papertape for the computer is shown in fig. 3a, while the results of the computation are given in figs. 3b, 3c and 3d. Remarkable is a left-shift of the ODC with regard to the standard ODC, which is also typically for a smoker.

3.1.3. Hardware and software specifications

The BCD-data are decoded to ASC II-data, using a special purpose interface which was developed by our own. The program takes about 24K 18 bits-words of memory on a PDP-15 computer and is written in Fortran IV. A complete set of plots takes about 15 min, mainly by time consuming plotting.

3.2. The ON-LINE method

The three analog outputs of the measuring instruments are connected with the pH, $P^x_{O_2}$ and $P^y_{O_2}$ amplifiers in the laboratory. These amplifiers, generating a signal between 0.0 and 5.0 V, are connected with the analog input of an IBM-1800 process-computer by means of a cable with eight conductors of about 200 m length. The computer samples the signals every second; once in every minute the sampled data of the six 10-sec-intervals are smoothed and reduced to six numbers per channel, which are stored on a magnetic disk for further analysis. At the laboratory two switches, connected with the digital input of the computer, inform the computer which part of the measurement, i.e., gauging before or after the measurement, starting or stopping, is done. The computer reacts by switching on or off two check-lights in the laboratory. The identification is performed by printing the date and the starting and stopping time of the measurement. The number of measurements within a day (up to 5), the gauging values, times and date and smoothed data are kept in a part of the disk file.

3.2.1. Flowchart

The flow scheme (fig. 2) proceeds in nearly the same way as for the OFF-LINE method with the exception of the input and output data.

3.2.2. A typical sample run

An example of the plotter and printer output of the ODC of a blood sample from a normal individual is given in figs. 4a, 4b, 4c and 4d.

3.2.3. Hardware and software specifications

The $P^x_{O_2}$ and $P^y_{O_2}$ amplifiers have a gain of one. However, because of the high sensitivity of the method for fluctuations in the pH and the small changes of this signal in regard to its full scale, the pH amplifier has a gain of five. The digital information from the two switches has been transferred by LED's. Each change in the switches generates an interrupt on which the computer takes action.

The data-acquisition and data-reduction are performed with standard programs except for the smoothing routine.

The program takes about 2.800 16 bits-words memory under MPX.

The computation program takes about 10K words. A complete set of plots takes about 10 min, mainly consumed by plotting.

3.3. Comparison of the ON-LINE and the OFF-LINE method of aquisition and computation

We measured the ODC's of 5 different blood samples in one DCA-1. The three Digital-Acid-Base Analyzers PHM 72, used for the measurement of $P^x_{O_2}$,

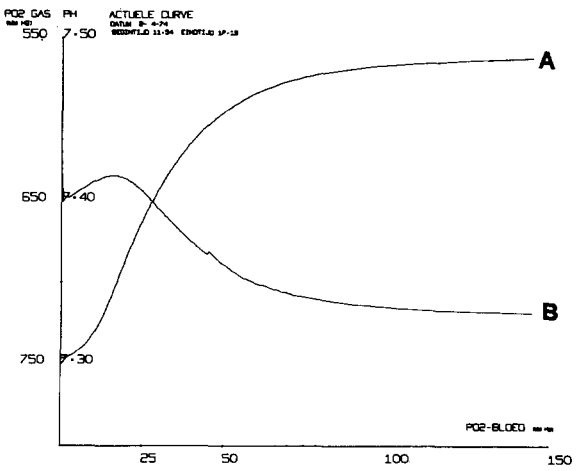

Fig. 4a. ON-LINE plotter output of the measured ODC of blood from a normal individual; curve A represents the uncorrected ODC (oxygen content versus P_{O_2}) and curve B the course of blood pH.

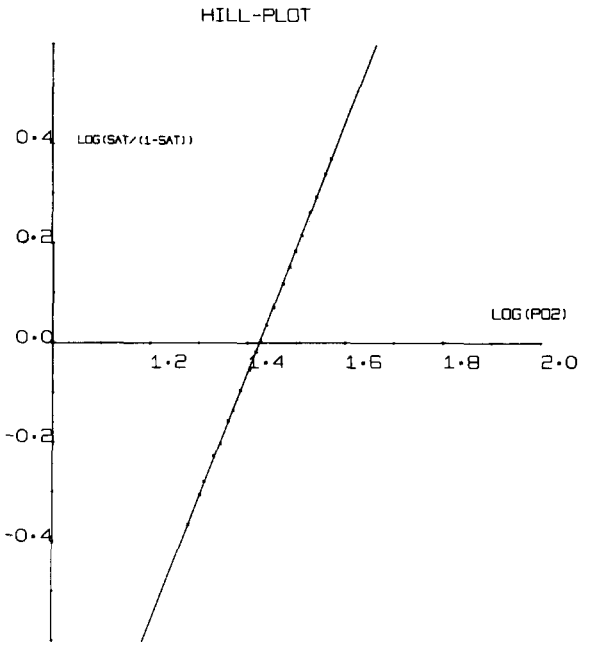

Fig. 4c. ON-LINE plotter output of the Hill curve for the standardized ODC of blood from a normal individual.

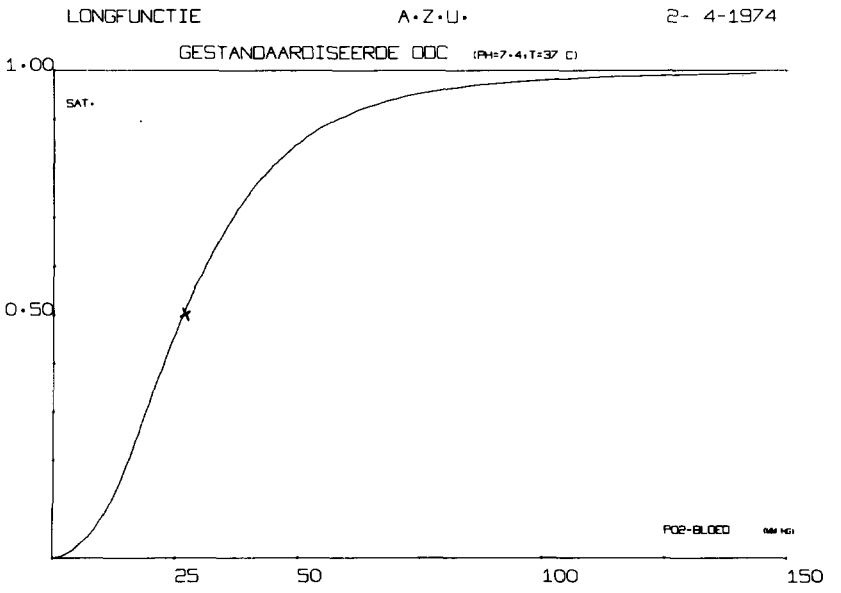

Fig. 4b. ON-LINE plotter output of the standardized (pH = 7.4) ODC of blood from a normal individual. Cross (X) indicates the normal value of P_{50} (= 27.0 mmHg).

$P^{y}_{O_2}$ and pH, generated their analog signals for the ON-LINE and their BCD outputs for the OFF-LINE method. So we could compare the two systems.

Table 1 shows the P_{50} and n_{Hill} values of the ODC's from five blood samples simultaneously obtained by the ON-LINE and OFF-LINE computation method. The mean differences of P_{50} (−0.2 mmHg) and n_{Hill} (−0.02) do not deviate significantly from zero and

```
RESULTS ODC MEASUREMENT ON  2- 4-1974 FROM 11.54 UNTILL 12.19 HR
 GAUGING VALUES

  PO2 BEFORE =149.6 MM HG, PO2 AFTER =151.5 MM HG

  PH BEFORE =7.383 PH AFTER =7.399

 TOTAL DIFFERENCE IN Y(=D) = 182 MM HG WITH A STARTING POINT OF Y= 746 MM HG
                 **************************************************
                 *                                                *
                 *     LOG(SAT/(1-SAT))= -3.620 + 2.545*LOG(PO2)  *
                 *                                                *
                 *     P50=26.43 MM HG                            *
                 *                                                *
                 **************************************************


THE POINTS WHICH DETERMINE THE HILL-PLOT ARE
SAT. WITH PO2 BLOOD
 0.300*   18.9
 0.330*   20.0
 0.343*   20.4
 0.371*   21.3
 0.384*   22.0
 0.409*   22.8
 0.421*   23.3
 0.445*   24.2
 0.468*   25.2
 0.490*   26.0
 0.501*   26.6
 0.520*   27.3
 0.541*   28.3
 0.568*   29.5
 0.587*   30.4
 0.604*   31.2
 0.622*   32.2
 0.647*   33.4
 0.663*   34.4
 0.686*   35.8
 0.701*   36.8
```

Fig. 4d. Output data of the standardized ODC of a blood sample from a normal individual obtained by the ON-LINE computation method.

are negligible in view of the standard deviation of the determination (standard deviation of the duplo of P_{50} = 0.21 mmHg and of n_{Hill} = 0.04).

Table 1
Comparison of P_{50} and n_{Hill} values of the ODC's from five blood samples obtained simultaneously by the ON-LINE and OFF-LINE computation method

P_{50} (mmHg)			n_{Hill}		
a on-line	b off-line	Δ (a–b)	c on-line	d off-line	Δ (c–d)
24.6	24.9	−0.3	2.43	2.43	0.00
23.3	23.3	0.0	2.59	2.60	−0.01
25.8	26.0	−0.2	2.39	2.41	−0.02
25.0	25.2	−0.2	2.51	2.53	−0.02
24.9	25.1	−0.2	2.47	2.49	−0.02
Mean difference		−0.2	Mean difference		−0.02

4. Mode of availability of the programs

The source listings of the programs and schemes of the interfaces are available on request from the biochemical laboratory of the departments of Cardiology and Cardiac Surgery, University Hospital, Utrecht, The Netherlands.

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