ABSTRACT

Dynamics of Blood Coagulation
by
Jie Gao

In this paper we present a new method to analyze whole blood coagulation dynamics. Using a PC-286 computer equipped with a DASH-16 A/D board.

The row data from a SONOCLOT analyzer is amplified and digitized on the DASH-16 A/D board and stored in the PC-286 hard disk. The data is filtered through a digital Butterworth filter. The filtered data is then fit to mathematical relationship by the least square method. The coefficients which define the mathematical relationships are used to identify blood specimens with normal coagulation dynamics as distinguished from specimens with abnormal dynamics.

The digital filter and curve-fitting routine are implemented on MATLAB software.

DYNAMICS OF BLOOD COAGULATION

by
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Dynamics of Blood Coagulation

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This thesis is dedicated to
my wife Dong-Yun for her encouragement and support

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CHAPTER 1

INTRODUCTION OF WHOLE BLOOD
RECALCIFICATION TIME

Blood coagulation is not only a necessary defense to prevent blood loss following injury, but it now appears that activation of clotting systems is an integral part of the host's immune response. Abnormal regulation appears to be a contributory factor in numerous disorders including coronary disease, allergies and inflammatory responses. In addition, clot deposition around cancer cells may aid the progressive growth of malignant tumors[1].

In the United States the great majority of deaths are due to diseases in which the blood clots too rapidly. These hypercoagulable states include patients with heart attacks, cancer and diabetes.

On the other extreme are those diseases in which blood takes too long to clot and bleeding becomes a life threatening possibility. The most common hypocoagulable state could be hemophilia.

The majority of healthy individuals would have a normal coagulation status, one between these extreme conditions.

Unfortunately, the traditional laboratory tests used to monitor clinical coagulation are insensitive to the majority of hypercoagulable states. These tests are insensitive for two main reasons. The first is that the tests are performed on blood plasma which contains no blood cells. It is well known that the cellular elements such as platelets and monocytes may make very significant contributions to accelerated clotting. Second, the reagents added for performing these tests, the prothrombin time and the partial thromboplastin time are procoagulants themselves.

In other words these reagents reduce the clotting times of plasma from about 6 minutes to less than 15 seconds and 50 seconds respectively. Therefore these clotting accelerants are added in such quantities that they mask the lower concentrations that may be present in the plasma.

Therefore, whole blood coagulation is much more sensitive to procoagulants since no procoagulants are added and all the cells are present. So, with an appropriate instrument capable of detecting the earliest formation of a blood clot (early fibrin formation) the whole blood recalcification time should be sensitive to change in blood clotting. (The word "recalcification" is used because blood is collected in the anticoagulant sodium citrate which complexes calcium ions to prevent clotting. When added to instrument excess calcium ions are added to initiate clotting.)

Figure 1 is a Blood Coagulation Spectrum that gives the Recalcification Times. From this Spectrum, the time from 0-10 minutes spans five ranges of blood coagulation. Range A, from 0-2.5 minutes represents thrombophilia, range B from 2.5-4.6 minutes represents clinically-relevant hypercoagulability, range C from 4.6-7.5 minutes represents normal blood coagulation, range D from 7.5-9.5 minutes representatives clinically-relevant hypocoagulability, E from 9.5--- minutes represents hemophilia[2].

THE COAGULATION SPECTRUM

Recalcification Times(Minutes)

A=THROMBOPHILIA

B=CLINICALLY-RELEVANT
   HYPERCOAGULABILITY

C=NORMAL

D=CLINICALLY-RELEVANT
   HYPOCOAGULABILITY

E=HEMOPHILIA

Time(min)
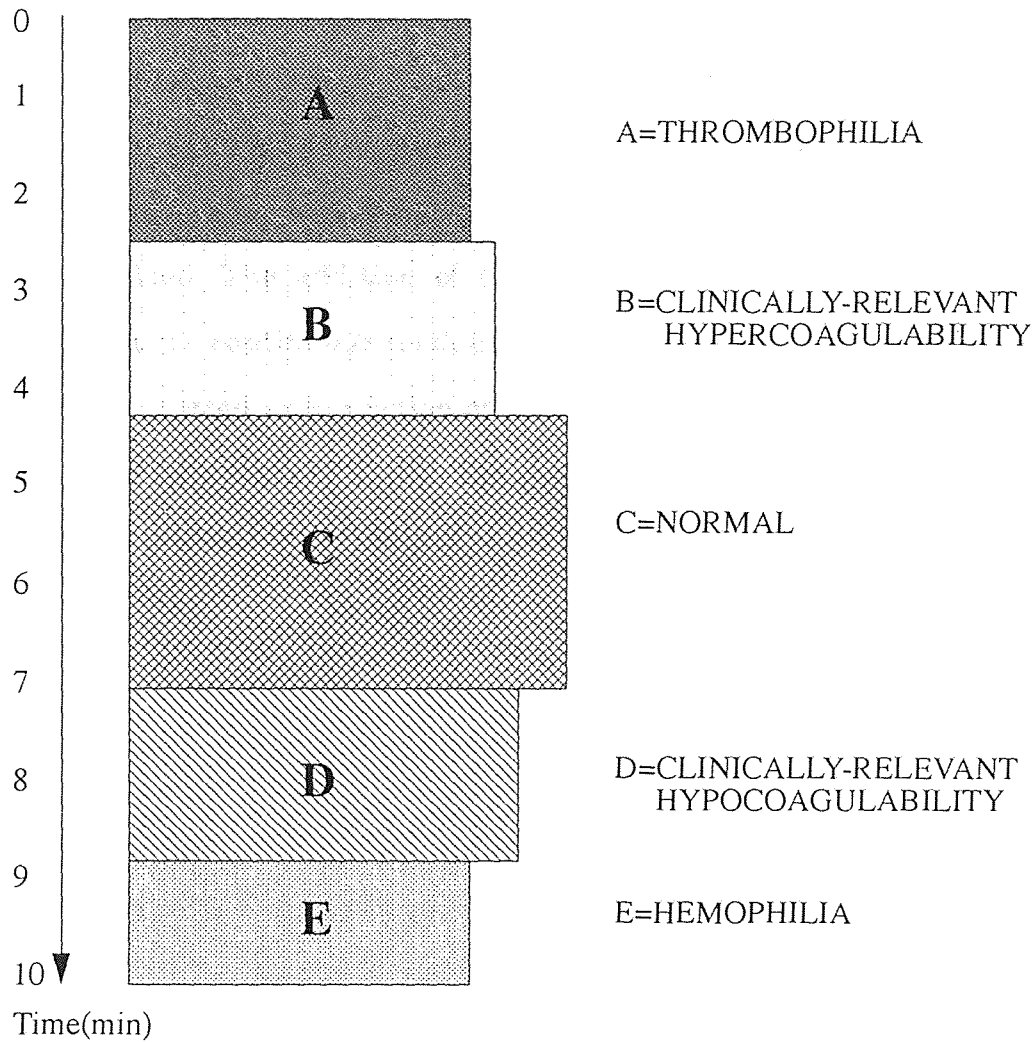
Figure 1 Blood Coagulation Spectrum

This information together with utilization of a sensitive instrument, the Sonoclot Coagulation Analyzer(SONOCLOT) (Sienco Inc., Morrison Co.) allows us to obtain clinically useful data.

For the test, the peripheral venous blood was drawn into 5 ml siliconized glass tubes containing 0.5 ml of buffered citrate. Citrated whole blood (1.0ml) was added to 12x75mm plastic tubes. The bloods were incubated at 37^0C for 10 minutes, after which 40 μl of 0.1M $CaCl_2$ was added to 300 μl of the incubated blood. The addition of the $CaCl_2$ initiates the process of clotting. A reproducible continuous readout of changes in impedance (viscous drag) in the clot was plotted as a function of time. Clot impedance started to increase only with the formation of fibrin. The recalcification time is defined as the time in minutes necessary for the clot to produce an impedance rise of 1 inch from the baseline[3].

CHAPTER 2

THE SONOCLOT COAGULATION ANALYZER

Sonoclot Coagulation Analyzer (Model DP-154) is a rate-reaction instrument for recording a continuous time-based signature of the entire clotting process from initial fibrin-fibril formation, through fibrin cross-linking, to full clot development, contraction and lysis. It is capable of analyzing samples of whole blood or citrated and recalcified whole blood, or even plasma[4].

2.1 Principles of Operation

An axially vibrating probe is immersed to a controlled depth in a small sample of fluid. The viscous drag (viscous impedance) imposed upon the probe by the fluid is sensed by the transducer. The electronic circuitry quantifies the drag as a change in electrical output. The signal is transmitted to a time-base strip chart recorder which provides a signature of the entire clot information, clot contraction and clot lysis process. For this project a digital signal processing system, to be described, was connected in paralleled with the strip chart recorder.

2.2 Capabilities

The Sonoclot Coagulation Analyzer is well suited to a wide range of clinical diagnostic applications and research studies.

a. Characterizing the coagulable state for screening, in surgery and in therapy.

b. Monitoring anticoagulant levels, both functional and by assay.

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c. Clearly identifying and quantifying platelet function in whole blood and in plasma.

d. Identifying and characterizing disfibrinogens.

e. Measuring fibrinolytic activity.

f. Measuring the viscosity of blood, plasma and serum.

2.3 Specifications

Samples: whole blood, blood plasma

Sample Volumes: 0.4ml in plastic cuvettes, 0.4ml in glass cuvettes, 0.05ml in micro-cuvettes.

Heater Block: Temperature control-factory set at $37^{o}C \pm 0.2^{o}C$.

Output: 0-20 millivolts, universal binding posts, 3/4 spacing continuous read-out into strip chart recorder

Recommended recorder: 0-10millvolts, potentiometer

Stirring: automatic 5 second timed, magnetic

Sensor: Transducer sealed in sensing head

2.4 Sonoclot Analyzer Application Areas

Cardiovascular Surgery: determine restoration of clot and platelet function after the administration of protamine. Discriminate hemostatic vs. surgical bleeders.

Thrombotic Potential: estrogen related, orthopedic surgery, neurosurgery, plasma and blood recalcification rate, heparin monitoring; coumadin monitoring; disfibrinogens identification; blood compatibility of bioengineering materials; viscosity measurements: blood, plasma, serum[4].

CHAPTER 3

DIGITAL SIGNAL PROCESSING

The Sonoclot Coagulation Analyzer is connected to a strip chart recorder and data is extracted manually from the tracing. For this project, the Analyzer is also interfaced to a PC-286 or PC-386 computer equipped with A/D conversion capability; the data is processed by digital filtering and curve-fitting software and the result printed out.

The following is a flow chart of the data processing procedure

```
┌─────────────────────────┐
│    Sonoclot Analyzer    │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│        Amplifier        │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│    DAS-16 A/D Board     │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│     DAS-16 Streamer     │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│       Binary Code       │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│       Unpack Code       │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│   Change to Matlab File │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│      ASCII File.m       │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│  Enter Matlab Software  │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│  Loading File & Plotting│
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│   Using Digital Filter  │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│      Curve Fitting      │
└─────────────────────────┘
             ↓
┌─────────────────────────┐
│   Printing The Results  │
└─────────────────────────┘
```
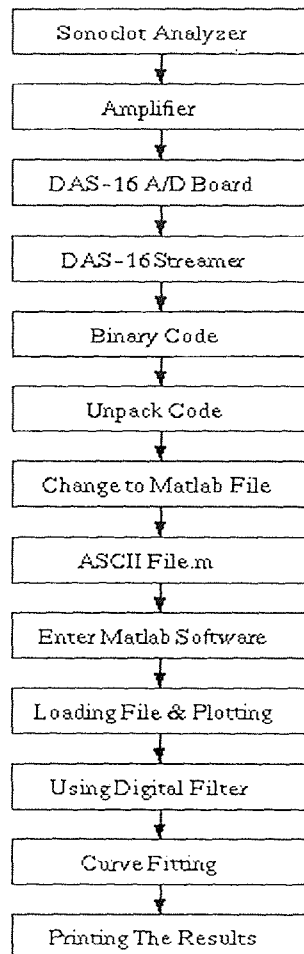
3.1 DAS-16 A/D Board

Metra Byte's DASH-16 is a multifunction high speed analog/digital I/O expansion board for the IBM Personal Computer. It is a full length board that installs internally in an expansion slot of an IBM P.C. or P.C./X.T. and turns the computer into a fast high precision data acquisition and signal analysis instrument. The board is of multilayer construction with integral ground plane to minimize noise and crosstalk at high frequencies[5].
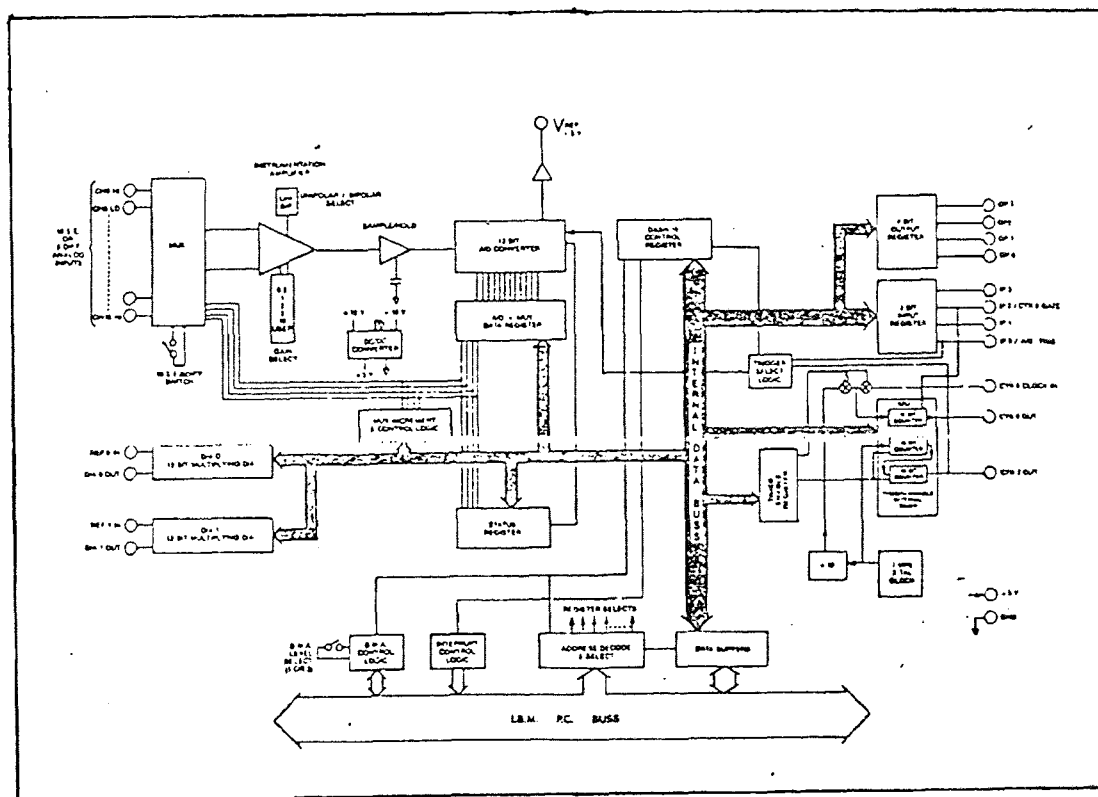

Figure 2 DAS-16 A/D Board Block Diagram

3.1.1 DASH-16 Channel Configuration Switch

DASH-16 uses an industry standard(AD574A) 12 bit successive approximation converter with a 25 microsecond conversion time. The channel input configuration is switch selectable on the board, providing a choice between 16 single end channels or 8 differential channels with 90db common mode rejection and ±10v common mode range.

16 [switch diagram] 8   SLIDE SWICH SET TO 16 SINGLE
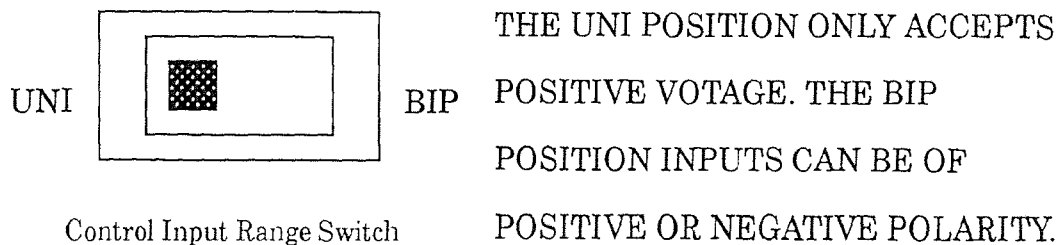   ENDED ANALOG INPUT CHANNELS
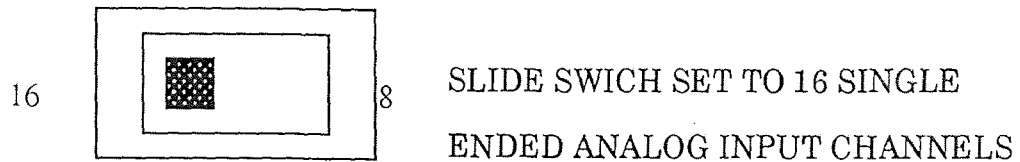
Channel Configuration Switch

The arrival of faster versions of the AD574A converter will allow an upgrade of the speed in D.M.A.(Drive Memory Addressing) mode by plug in replacement. The A/D may be triggered in three ways, by software command, by internal programmable interval timer or by direct external trigger to the A/D. For this project an internal programmable interval timer is used.

3.1.2 DASH-16 Unipolar and Bipolar Switch

High input impedance ranges of +1v,+2v,+5v&+10v unipolar and ±0.5v, ±1v, ±2.5v, ±5v & ±10v bipolar are switch selectable.

UNI [switch diagram] BIP   THE UNI POSITION ONLY ACCEPTS
                            POSITIVE VOTAGE. THE BIP
                            POSITION INPUTS CAN BE OF
Control Input Range Switch POSITIVE OR NEGATIVE POLARITY.

The BIP position is used in this project. Other ranges may be realized with a single user installed resistor. All inputs are multiplexed through a low drift, fast setting instrumentation amplifier/sample-hold combination and the channel input configuration is switch selectable to operate as either 16 single ended or 8 differential channels.

3.1.3 DASH-16 Interval Timer

A 3 channel programmable interval timer (intel 8253) provides trigger pulses for the A/D at any rate from 250kHz to 1 pulse/hr.
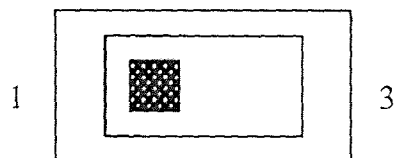
3.1.4 DASH-16 Gain Switch
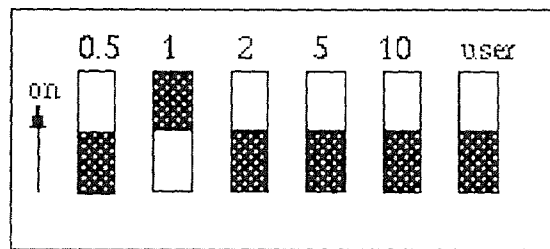
Gain Switch

The gain switch on the DASH-16 configures the entire board for the gain selected. Only one slider of this DIP switch should be in the ON position.

3.1.5 DASH-16 DMA Level

DMA Selects Switch

USING THIS SWITCH SELECTS DMA LEVEL 1 OR 3.

This slide switch selects the operating DMA level. On a floppy disk computer set it to level 3. A hard disk equipped computer select level 1, although operation of IBM XT machines is also possible on level 3.

3.1.6 Accessories (Screw Terminal Connector Board)

All I/O is brought to miniature screw terminal connectors which accept 12-22 gauge wire. A small breadboard area with 15 volt power is available for amplifiers, filters and other user required interface circuits. This offers the user easy access to all I/O lines on the DASH-16 board.

3.1.7 Connector Pin Assignments

All I/O is through a standard 37 pin D type male connector that projects through the rear panel of the computer.

The DASH-16 has been designed to provide high data rate throughout using the D.M.A. (Direct Memory Access) capabilities of the IBM P.C. at rates above 10,000 samples per second. Real time triggering of the A/D plus D.M.A. assures perfect synchronism in sampling, unaffected by other computer operations. These capabilities are essential to applications such as signal analysis, Fast Fourier Transform, vibration and transient analysis where high data rates must be sustained for short intervals of time.

3.3 Amplification of the SONOCLOT Analyzer Output Signal

The IC op-amp is a solid-state device capable of sensing and amplifying DC and AC input signals[11].

$$V_{out} = \left(\frac{R_f}{R_1} + 1 \right) V_{in} \tag{3.1}$$
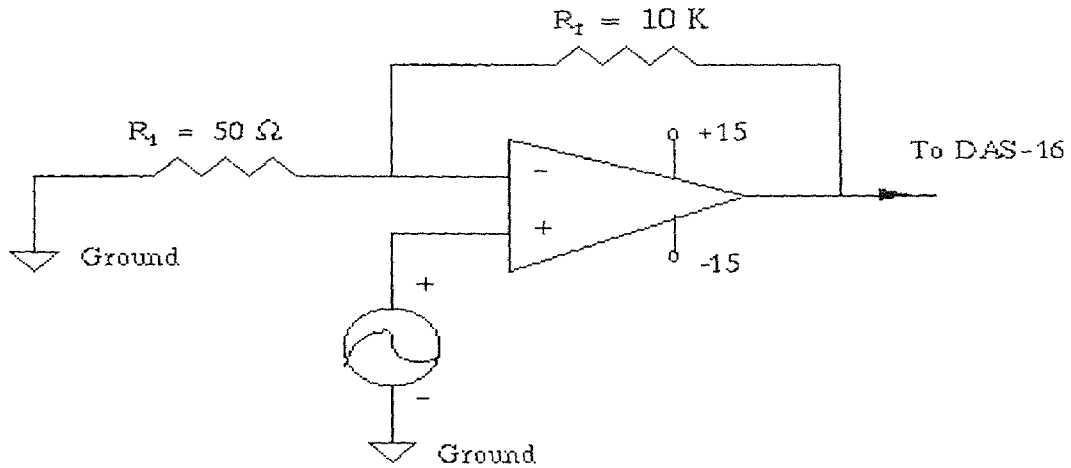
Figure 3 OP-AMP Circuit

This single-stage amplifier with a gain of 10k/50=200 is sufficient to interface the output from the SONOCLOT to the DASH-16 A/D board.

3.4 The Capture of Blood Coagulation Data and Used Streamer

MetraByte's stream-16 data streamer is a high speed data acquisition software routine for use with the MetraByte DASH-16 A/D expansion board. The stream-16 allows analog input data from a DASH-16 to be directly stored on hard disk at sample rates of up to 60,000 samples per second. The stream-16 utilizes the DMA capability of the DASH-16 to collect analog data and stream it out to a data file on a disk.

3.4.1 Data Streamer System Requirements

The System requirements for the DASH-16 data streamer are:

a. IBM PC-XT/AT or equivalent

b. A Standard disk driver

c. MetraByte DASH-16 A/D expansion board

d. Hard disk

3.4.2 Data Streamer Diskette Contents

The Data streamer diskette contains the following executable files:

a. STREAMER.EXE Data streaming utility

b. DSINSTAL.EXE Data streamer installation utility

c. MKFILE.EXE File creation utility

d. UNPACK.EXE Data file translation utility

3.4.3 Data File Preparation

To keep up with the high data rates of the DASH-16, data files for the Data Streamer must meet certain requirements:

a. Data files must reside in the root directory of the disk. The data streamer will not stream data to files contained in sub-directories.

b. Data files must be created before running the data streamer. The data streamer will not create data files.

c. Data files must be contiguous. They must reside on sequential sectors on the disk. The streamer will verify this before attempting to stream to a file.

d. MKFILE.EXE is a utility that creates data files, and reserves space on the disk.

The MKFILE.EXE utility can be used to create Data streamer data files in the root directory.

Example: MKFILE c:\bbl68.dat 1

This example creates a file called bbl68.dat in the root directory of disk c with a file size of 1k. If MKFILE cannot find enough space to create a file of the size, it will report an error[5].

3.4.4 Using the Streamer

To run the data streamer, type "streamer" at c: prompt and the STREAM.BAT file will load and execute the data streamer using the entered configuration parameters.

The data streamer display shows all the data acquisition parameters. Use the cursor keys to move from field to field[6].

The Data Streamer Display

```
┌─────── KEITHLEY .. MetraByte .. DAS 16 .. Streamer ───────┐
│                                                           │
│   ┌──────────── Data .. File .. Information ───────────┐   │
│   │ Drive _____                            │   │
│   │ Data File _____    Log File _____   │   │
│   │ Data File Size _____     Number of Sample ____ │   │
│   │        Total Sample Time _____                 │   │
│   └───────────────────────────────────────────────────┘   │
│                                                           │
│   ──────────── DAS 16 .. Card .. Set up ────────────       │
│   ┌───────────────────────────────────────────────────┐   │
│   │ Sample Rate(kHz)_____  First Chan _____ Last Chan ____ │
│   │ Trigger Star _____   Clock Source ____       │   │
│   │ Base Address(HEX) _____ DMA Level ____          │   │
│   │ ADC Mode _____     Single Ended.Clock Speed _____ │
│   └───────────────────────────────────────────────────┘   │
│                                                           │
└───────────────────────────────────────────────────────────┘
```

a. File Name: Type the drive, file name and extension of the data file to receive streamed data. If the data file is acceptable for streaming, the data streamer will display the file size, number of samples, and the total sample time.

b. Sample Rate: If using internal triggering, type in the sample rate in kilohertz. The total sample time will be recalculated at the new sample rate.

c. Scan Limit: Type in the high and low channels to be included in the scan. The complete scan sequence is displayed under the scan limits.

d. Sample Triggering Mode: The sample triggering mode determines which trigger source will be used. Internal triggering is derived from the on-board timer. External triggering is supplied via the digital I/O part.

e. Start Triggering Mode: The start triggering mode determines which triggering source will start the series of samples. Type E for external, I for internal triggering.

f. Collecting data: To begin sampling data, press the F1 key.

g. Exiting: To exit from the data streamer program type F10.

3.4.5 Convert Binary Code into ASCII Code

The data files written by the data streamer are binary files. It is converted by the streamer into ASCII format. The UNPACK.EXE utility accomplishes this. The resulting file may be typed or examined with a text editor.

Example: UNPACK c:\bbl68.dat, c:\matlab/bbl68.m,0-512/B/DASH16

This example unpacks samples 0 through 512 from the data streamer data file: "c:\bbl68.dat" to the ASCII file "c:\matlab\bbl68.m". It interprets the data as bipolar.

3.5 Using PC-Matlab Process Capture Data and Plot It

3.5.1 Matlab Software

Matlab is a high-performance interactive software package for scientific and engineering numerical computation. Matlab integrates numerical analysis, matrix computation, signal processing, and graphics in an easy-to-use environment where problems and solutions are expressed just as they are written mathematically without traditional programming. Matlab is used for research and to solve practical engineering and mathematical problems. Typical uses include general purpose numeric computation, algorithm prototyping, and solving the special purpose problems with matrix formulations that arise in disciplines such as automatic control theory, statistics, and digital signal processing[7].

1. Matlab System Requirements:

There are three versions of Matlab for IBM and compatible personal computers: PC-Matlab, At-Matlab, and 386-Matlab.

2. At-Matlab requires:

a. IBM PC/AT or compatible 80286 or 80386- based MS-DOS computer.

b. 2M bytes or more of memory, of which at least 1M byte must be extended memory.

c. MS-DOS or PC-DOS version 3.1 or higher

d. An 8087, 80287 or 80387 numeric coprocessor chip.

e. A hard disk with at least 1.5M bytes of free space.

f. A 360k byte $5\frac{1}{2}$ inch floppy disk drive.

Matlab also has four optional toolboxes available that provide additional application-specific capabilities.

1. The Control System Toolbox which contains more than fifty additional commands for control engineering and systems theory, with an emphasis on state-space techniques.

2. The System Identification Toolbox which adds more than forty commands for parametric modelling and system identification

3. The Robust-Control Toolbox that adds thirty-five commands for robust control system design.

4. The Signal Processing Toolbox with fifty commands for one-dimensional and two-dimensional digital signal processing. It includes functions for the design and analysis of digital filters and for power spectrum estimation (FFT analysis). The Signal Processing Toolbox is bundled along with the PC-Matlab system and is installed automatically during Matlab installation. Much of Matlab's power is derived from its extensive set of functions. Matlab has a large number of functions, well over 200. The general categories of mathematical functions available in Matlab include:

Elementary mathematical; special functions; elementary matrix; special matrices; matrix decompositions and factorizations; data analysis polynomial; differential equation solution; nonlinear equation and optimization, numerical integration; signal processing.

Figure 4 is a plot of raw data that was digitized by the DASH-16 A/D board and processed by the DASH-16 streamer file converter into an ASCII format. The software command to accomplish this is:

UNPACK c:/bbl68.dat,c:/matlab/bbl68.m,0-512/B/DAS16

Where bbl68.dat is the binary data file, bbl68.m is the ASCII data file which is stored in MATLAB software. The file is plotted using command:

load bbl68.m

plot(bbl68)

The resulting plot reveals a noise component that must be filtered out.
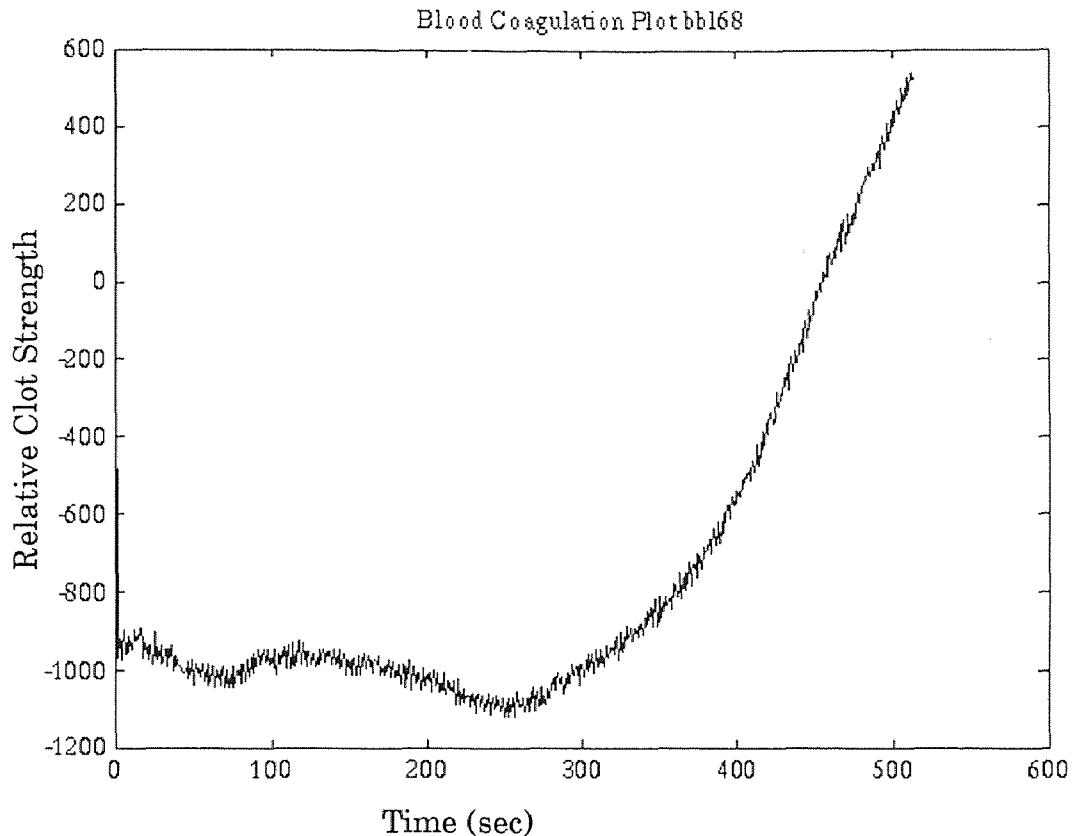
Figure 4 Primary Digitized Data Plot

3.5.2 Filter Selecting and Signal Processing

Matlab has a collection of filter functions immediately useful for signal processing in the Signal Processing Toolbox. The Signal Processing Toolbox is a collection of algorithms, expressed in M-files, that implements a large variety of modem signal processing techniques. The function in the Signal Processing ToolBox for digital filter design can be grouped into four categories:

IIR(Infinite Impulse Response) filter design using analog prototypes

Direct IIR filter design

Direct FIR(Finite Impulse Response) filter design

Inverse filter design

Filters are most often used to enhance signals by removing unwanted components from them. The most common filter of this type is the frequency selective filter, designed from frequency response specifications, that allows only certain desired frequency bands to pass through[8].

The z-transform of the difference equation for the digital filter yields the frequency domain or transform function model description:

$$y(z) = \frac{b(1) + b(2)z^{-1} + \ldots + b(nb+1)z^{-nb}}{1 + a(2)z^{-1} + \ldots + a(na+1)z^{-na}} x(z) \qquad (3.2)$$

A variety of names are used to describe filters with either of na=0 or nb=0. When nb=0, the filter is sometimes called IIR (infinite impulse response), all-pole, recursive, or auto regressive(AR). When na=0, the filter may be called FIR (finite impulse response), all-zero, nonrecursive, or moving average(MA). If both na and nb are greater than zero, the filter may be called IIR, pole-zero, or recursive, or autoregressive moving average(ARMA).

The output from the SONOCLOT was sampled at a 1 Hz frequency. There is some noise in the raw recorded data plot as shown in Figure 4. A Butterworth IIR filter was selected to filter out the noise.

Butterworth filters are defined by the property that the magnitude response is maximally flat in the passband. The property of the Butterworth filter is that the approximation is monotonic in the passband and the stopband. In the low-pass case, the first 2n-1 derivatives of the sequined magnitude response are zero at w=0.

In Matlab Signal Processing Tools box, [b,a]=butter(n,Wn) designs a n order low-pass digital Butterworth filter with cutoff frequency Wn and returns the filter coefficients in length n+1 row vectors b and a. Wn, the cutoff frequency must be a number between 0 and 1, where 1.0 corresponds to half the sample frequency.

For example, we design a fifth order Butterworth filter we set [b,a]=butter(5,0.2/0.5), at here n=5, Wn=0.4 Hz, so in this Butterworth filter the cutoff frequency is 0.4 Hz.

In Matlab, the command FILTER filters data using a digital filter. The filter realization is the transposed direct form II structure, which can handle both FIR and IIR filters. Access to initial and final conditions is available.

The block diagram for the transposed direct form II realization of filter is
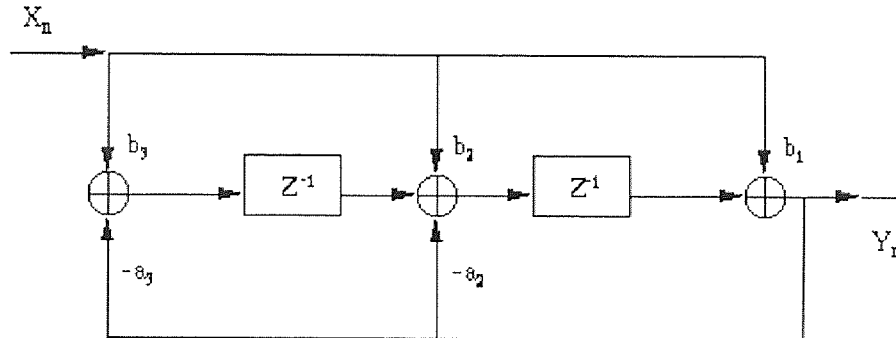

Figure 5 Digital Filter Direct II Structure

y=filter(b,a,x) filters the data in vector x with the filter described by coefficient vectors **a** and **b** to create filtered data vector. The operation performed by the filter is described in the time domain by the difference equation:

$$y(n) = b(1)x(n) + b(2)x(n-1) + \ldots + b(nb+1)x(n-nb)$$

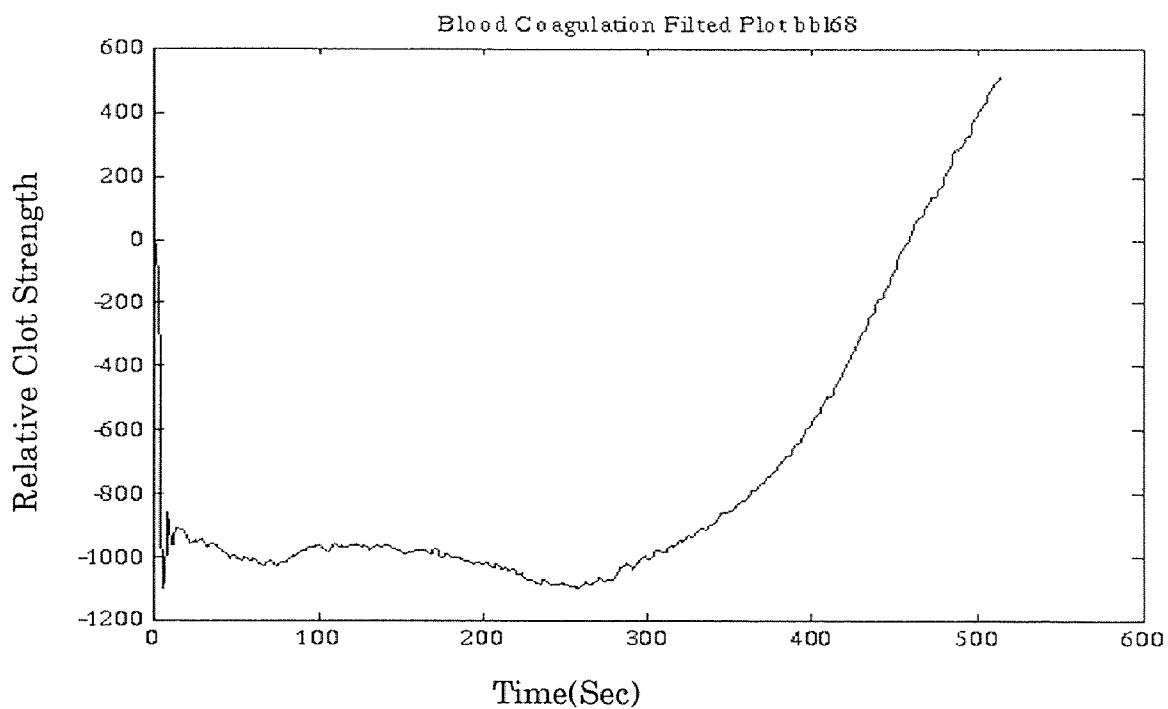$$- a(2)y(n-1) - \ldots - a(na+1)y(n-nb) \qquad (3.3)$$

An equivalent representation is the z-transform of frequency-domain description:

$$y(z) = \frac{b(1) + b(2)z^{-1} + \ldots + b(nb+1)z^{-nb}}{1 + a(2)z^{-1} + \ldots + a(na+1)z^{-na}} x(z) \qquad (3.4)$$

Figure 8(a) and (b) are representative tracings of data before and after filtering. For these curves, the unites of **y** axis are meaningless, the numbers represent relative clot strength.
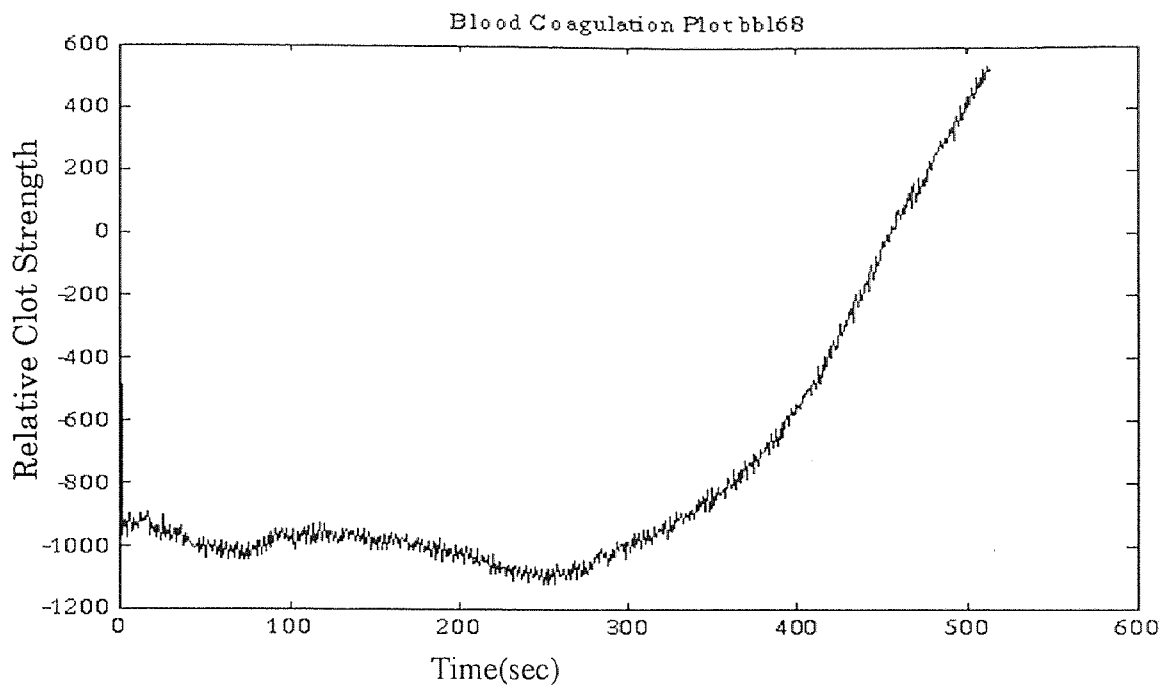
(a) Before Filtering

(b) After Filtering With Low Pass Butterworth Filter of
Order Five and Cutoff Frequency 0.4 hz.

Figure 6 Representative Data Recorded From the Sonoclot
Analyzer (a), and After Filtering (b)

CHAPTER 4

CURVE FITTING

4.1 Curve Fitting

The digitized blood coagulation data is subjected to digital signal technical processing.The Matlab software package is used to fit the measured data to a polynomial equation whose parameters might to used to characterize the kinetics of blood coagulation.

In general, a polynomial fit to data in vectors x and y is a function p(x) of the form

$$p(x) = c_1 x^n + c_2 x^{n-1} + \ldots + c_{d-1} x + c_d \tag{4.1}$$

The degree of this polynomial is n and the number of coefficients is d=n+1. The coefficients c_1, c_2, ... c_d are determined by solving a system of simultaneous linear equations, **Ac=y**, where the columns of A are successive powers of the x vector, also called the Vandermonde matrix. The solution to the equations **Ac=y** is obtained using the Matlab "matrix division" operator, **c=A/y.**

The function polyfit.m in the Matlab toolbox automates this procedure, it uses given data in a vector **X**, **Polyfit** command, finds a polynomial p such that p(x) fits the data in a vector y in a least square sense. **Polyfit(x,y,n)** returns the coefficients, in descending powers of x, of the n-th order polynomial that fits vector y to x.

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Example: load bbl68.m

[b,a]=butter(5,0.2/0.5);

g=filter(b,a,bbl68);

p=[1,2,3,4];

x=0:512;

y=g';

c=polyfit(x,y,3);

fit=polyval(c,x);

plot(x,fit,x,y,'.')

Using these Matlab commands we obtain the polynomial equation that best fits the measured data(Figure 7).

The precision of the MATLAB software is 4 significant figures so that a coefficient smaller than 0.0001 would be represented as 0.0000. In order to obtain coefficients smaller than 0.0001, it was necessary to solve the polynomial equation by hand.

Figure 7 Measured Curve of Sample bbl68 Fitted to a
3rd Order Polynomial
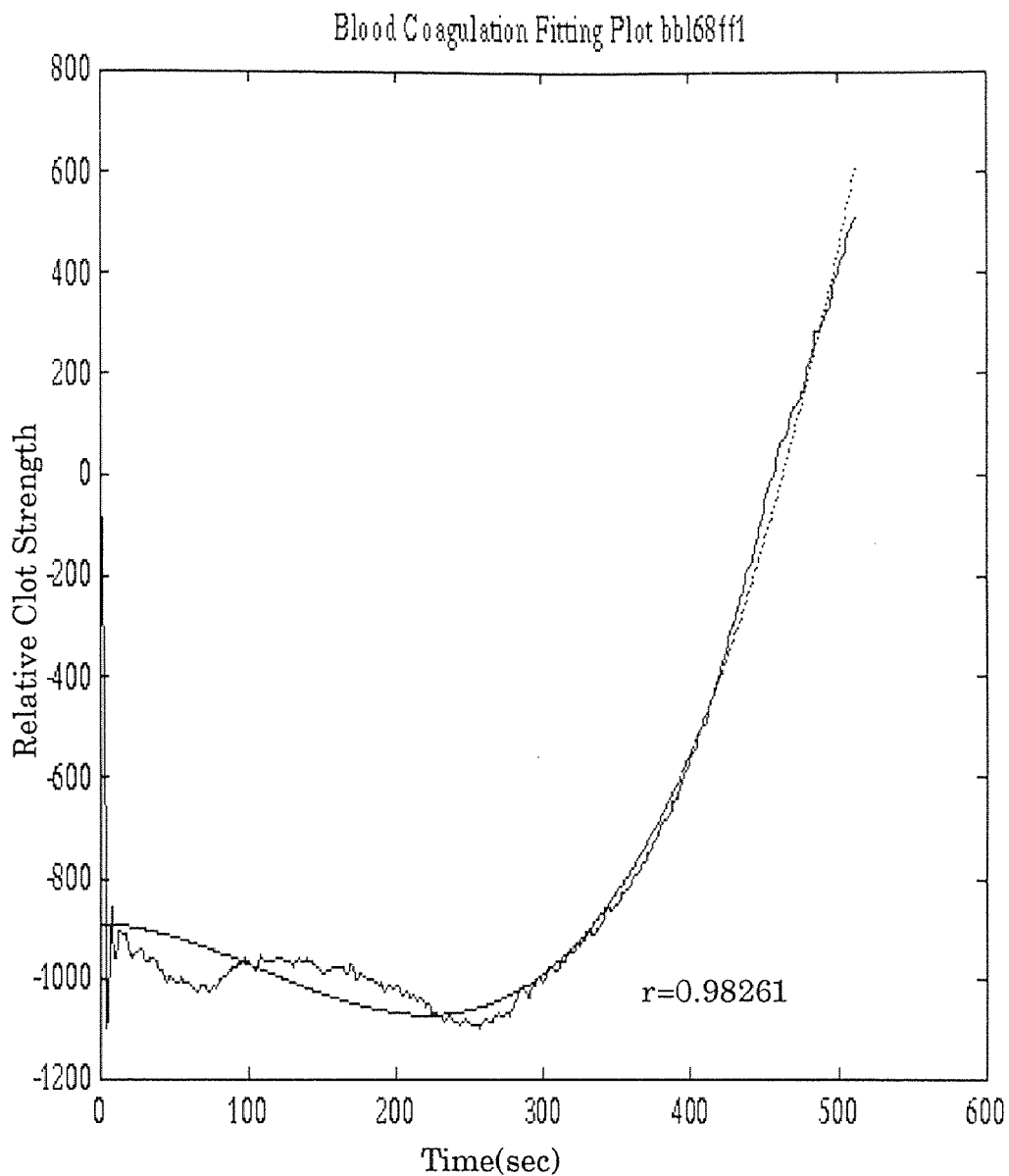
$$p\,(x) \;=\; 0.0000316x^3 - 0.0102x^2 - 0.1072x - 888.0834 \qquad (4.2)$$

Figure 9 is the digitized and filtered data obtained from blood sample

bbl68 and fitted to a 3rd order polynomial with coefficients b_0=-888.0834,

b_1=-0.1072, b_2=-0.0102, b_3=0.0000316

The correlation coefficient is a measure of the strength of the association between the measured data and the best fit polynomial.

The correlation coefficient r is:

$$r = \frac{n\Sigma xy - (\Sigma x)(\Sigma y)}{\sqrt{[n(\Sigma x^2) - (\Sigma x)^2][n(\Sigma y^2) - (\Sigma y)^2]}} \qquad (4.3)$$

where n is the number of paired observations[10].

The correlation coefficient relates that the pattern of behavior of the two set of variables is in that one can be used effectively to predict the other. Perfect agreement between two variables will result in a coefficient of 1.00. A coefficient of say, 0.92 would reveal that the correlation is very strong, while 0.21 would show it to be very weak[12].

In Matlab, the command Corrcoef(x,y) returns a matrix of correlation coefficients calculated from an input x matrix with variable y matrix.

With the MATLAB command 'corrcoef(y,fit) we obtain a correlation coefficient r_1=0.98261 for the 3rd order polynomial (Equation 4.2) that best fits the measured data of sample bbl68 illustrated in Figure 7.

With the command p=[1,2,3,4,5] (see p24), the data can be fit to a 4th order polynomial.
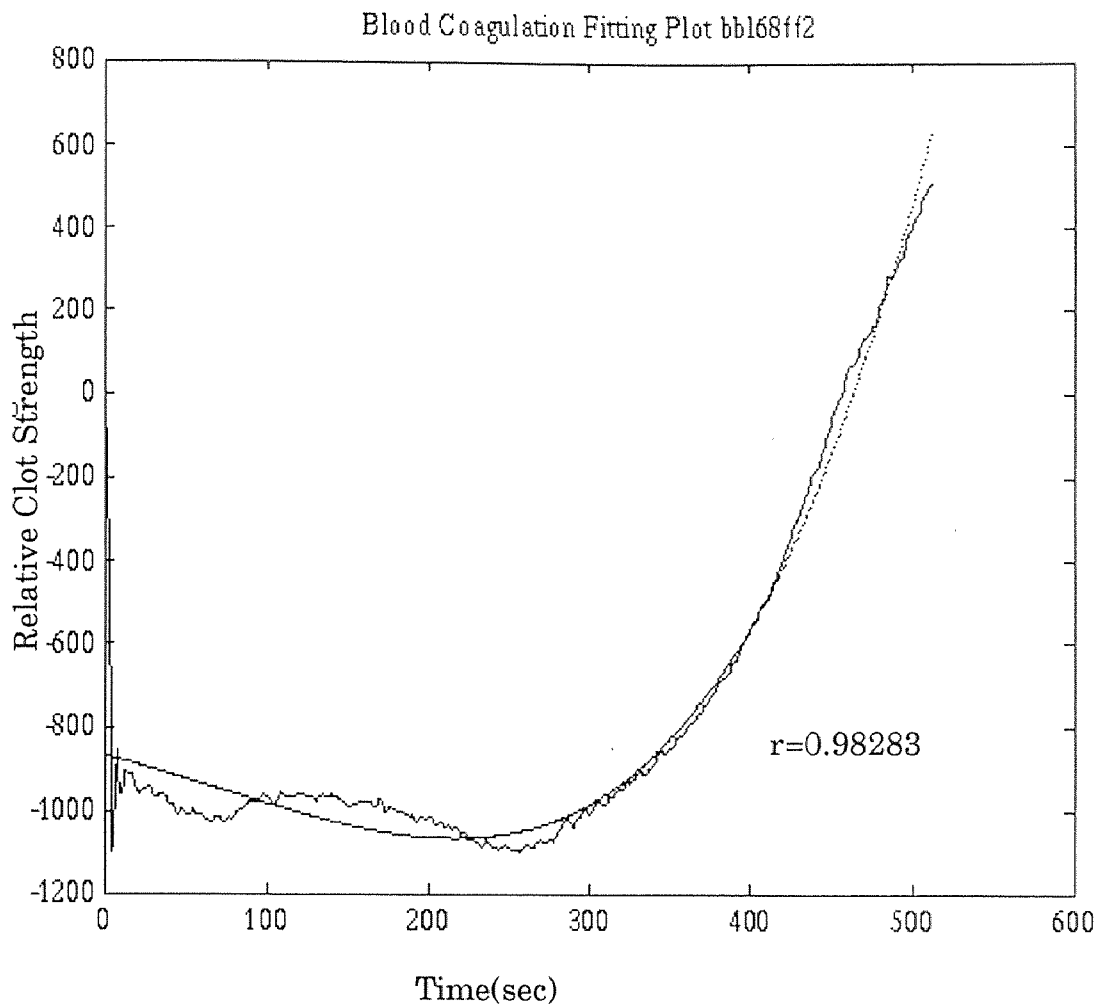
$$p(x) = 2.4122 \times 10^{-8}x^4 + 6.4072 \times 10^{-6}x^3 - 0.0019x^2 - 1.0494x - 864.1731$$

$$(4.4)$$

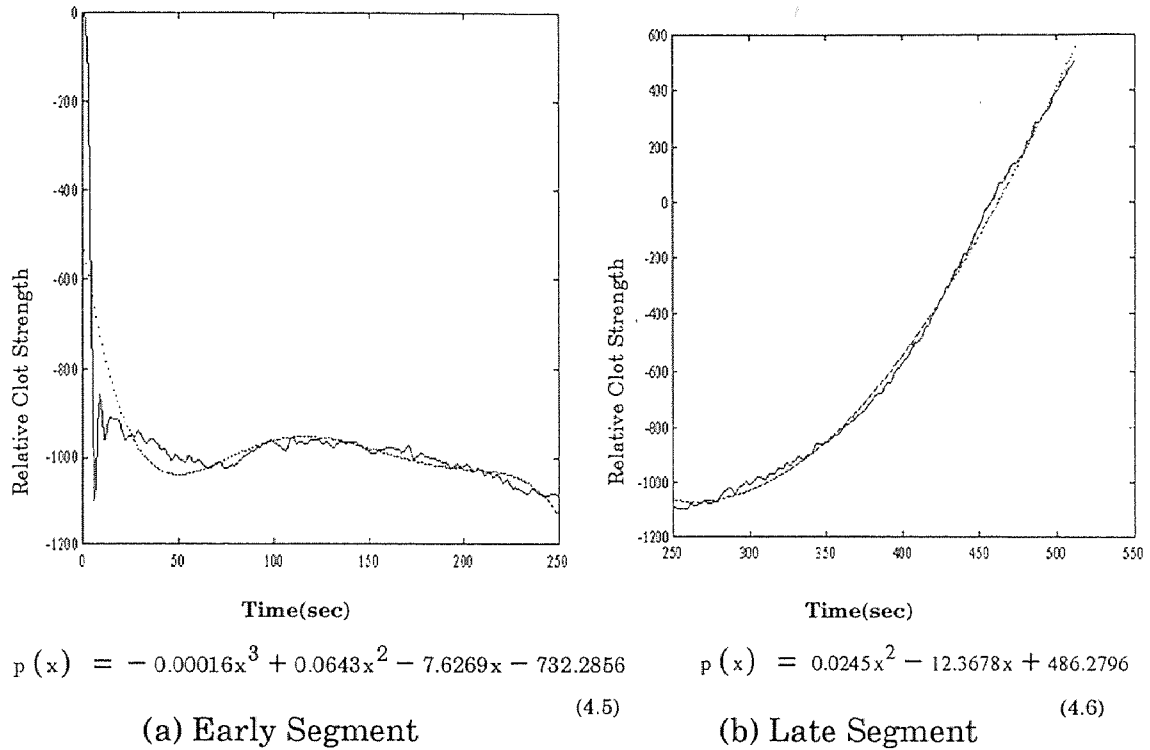Figure 8 Measured Curve of Sample bbl68 Fitted to a
4th Order Polynomial

$$p\left(x\right) = 2.4122 \times 10^{-8}x^4 + 6.4072 \times 10^{-6}x^3 - 0.0019x^2 - 1.0494x - 864.1731 \qquad (4.4)$$

The same measured curve of sample bbl68 illustrated in Figure 7 is redrawn in Figure 8 and compared to the 4th order polynomial Equation 4.4. The correlation coefficient of Equation 4.4 is calculated to be $b_2=0.98283$ which is just slightly improved over the $r_1=0.98261$ for the 3rd order polynomial Equation 4.2

We hypothesize that the relevant characteristics of the dynamics of blood clot formation are contained in the latter part of the curve, from sample time 250-512(t=4.16-8.53 min) in figures 9 (a) and (b). Consequently a third curve-fitting algorithm was evaluated in which the measured data was divided into two segments an 'early' segment from the sample time 0-250(t=0-4.16 min) and a 'late' segment from sample time 250-512(t=4.16-8.53 min).

$$p(x) = -0.00016x^3 + 0.0643x^2 - 7.6269x - 732.2856$$

(4.5)

(a) Early Segment

$$p(x) = 0.0245x^2 - 12.3678x + 486.2796$$

(4.6)

(b) Late Segment

Figure 9 Cutting Fitting Curve

In Figure 9, the data of blood sample bbl68 is segmented into an early segment from sample time 0-250(t=0-4.16 min) and fitted to the 3rd order polynomial with a correlation coefficient r=0.7082, and a late segment from sample time 250-512(t=4.16-8.53 min), fitted to the 2nd order polynomial with a correlation coefficient r=0.9987. This correlation coefficient is the highest, compare to the correlation coefficient for the early curve using 4th and 5th order polynomials.

4.2 Sensitivity Analysis

Figure 10 and 11 are qualitative illustrations demonstrating the sensitivity of the shape of the fourth order polynomial curve to each of the five parameters in the polynomial. In Figure 10, curve A is constructed using the mean values of b_0 to b_4, rounded out, from the curve-fitting data for the normal blood sample, Table 1 (p34). For curve B, C, D and E the absolute values of coefficients b_4, b_3, b_2, and b_1 respectively, were increased by 10% from the values in curve A. It appears that a 10% change in the absolute value of b_3 (curve c) has the largest effect on the shape of curve A.

Figure 11 is similarly constructed from the curve fitting data from abnormal blood, Table 2 (p35). The parameters in curve A are the rounded out mean value of b_0 to b_4 from Table 2, and in curves B, C, D and E the absolute values of b_4, b_3, b_2 and b_1 respectively, are increased by 10% from their values in curve A. Clearly the coefficients b_3 (curve C) and b_2 (Curve D) have the most profound effect on the shape of curve A.

It should be noted that coefficients b_1-b_4 have opposite signs in the normal blood data as compared to the abnormal blood data.
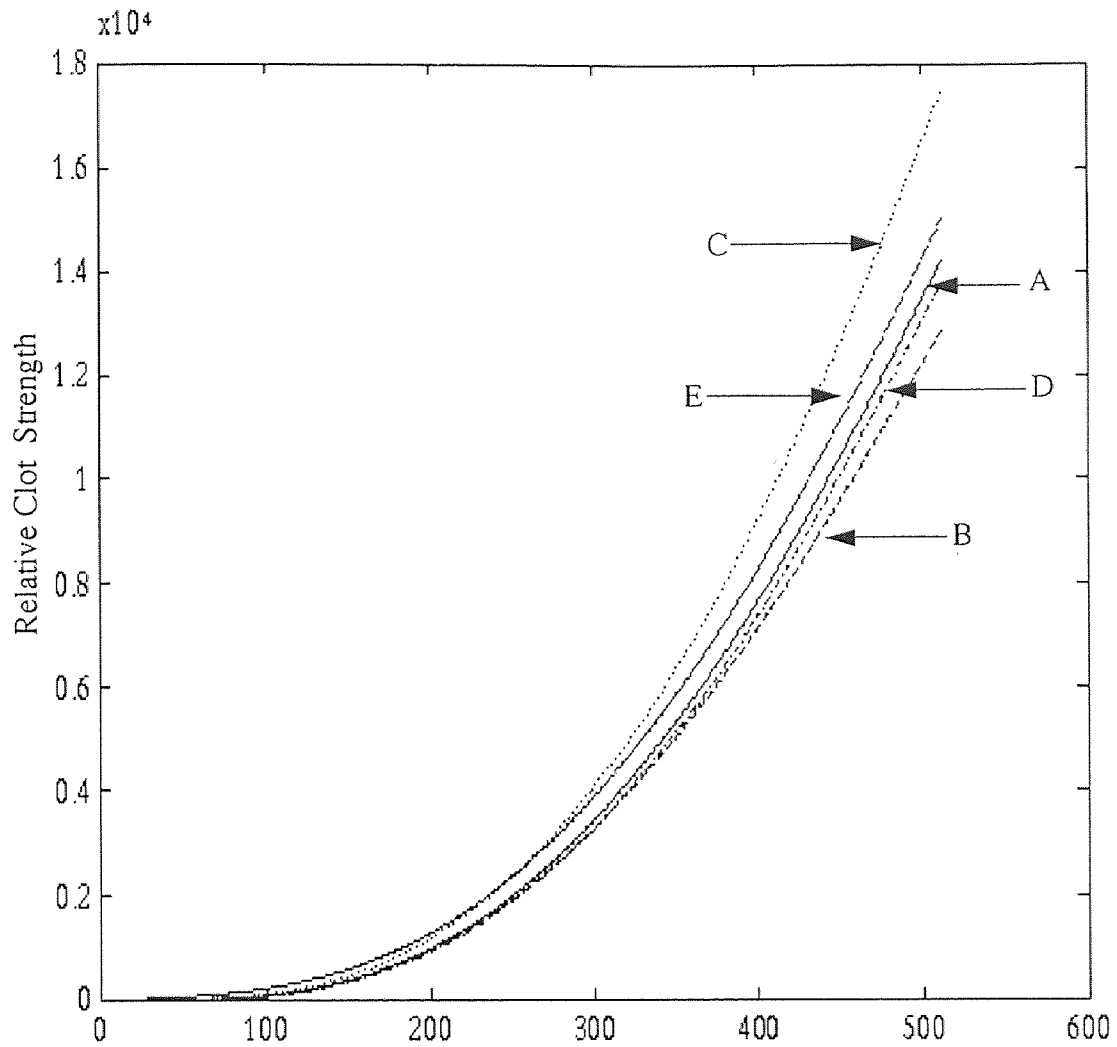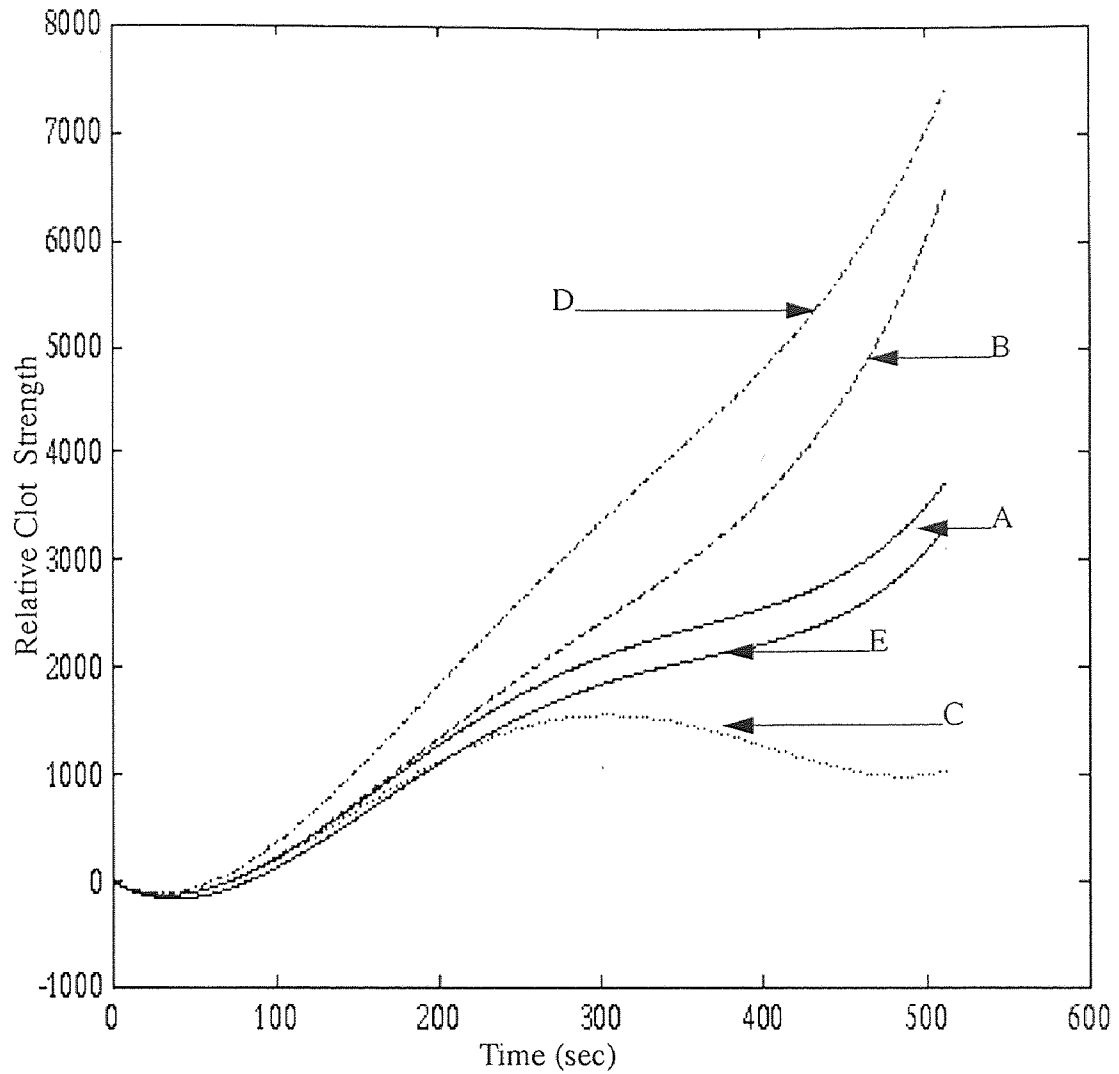
Figure 10 Parameter Sensitivity Analysis From
Normal Blood Data

$$p\,(x)\ =\ b_4 x^4 + b_3 x^3 + b_2 x^2 + b_1 x + b_0 \tag{4.5}$$

NORMAL	b_4	b_3	b_2	b_1	b_0
A	-2.0×10^{-7}	2.4×10^{-4}	-0.017	0.4	-1.02×10^{-3}
B	-2.2×10^{-7}	2.4×10^{-4}	-0.017	0.4	-1.02×10^{-3}
C	-2.0×10^{-7}	2.64×10^{-4}	-0.017	0.4	-1.02×10^{-3}
D	-2.0×10^{-7}	2.4×10^{-4}	-0.0187	0.4	-1.02×10^{-3}
E	-2.0×10^{-7}	2.4×10^{-4}	-0.017	0.44	-1.02×10^{-3}

Figure 11 Parameter Sensitivity Analysis From
Abnormal Blood Data

$$p(x) = b_4 x^4 + b_3 x^3 + b_2 x^2 + b_1 x + b_0 \qquad (4.6)$$

ABNORMAL	b_4	b_3	b_2	b_1	b_0
A	4.0×10^{-7}	-4.2×10^{-4}	0.14	-8.0	0.012
B	4.4×10^{-7}	-4.2×10^{-4}	0.14	-8.0	0.012
C	4.0×10^{-7}	-4.62×10^{-4}	0.14	-8.0	0.012
D	4.0×10^{-7}	-4.2×10^{-4}	0.154	-8.0	0.012
E	4.0×10^{-7}	-4.2×10^{-4}	0.14	-8.8	0.012

CHAPTER 5

RESULTS AND CONCLUSION

The data from five normal blood samples(bbl88, bbl89, bbl90, bbl93, bbl94) and five abnormal blood samples(bbl81, bbl82, bbl83, bbl85, bbl86) are summarized in Table 1 to 2. The abnormal samples were obtained from patients with known clotting disorders, or were rendered artificially abnormal by the addition of a procoagulant or anticoagulant.

The table list the polynomial coefficients that best fit the measured data. The zeroth order coefficient, i.e. the coefficient at time t=0, is meaningless since it represents the output from the SONOCLOT analyzer at the instant it is turned on, when numerous action, in addition to the viscosity of the blood sample, affect the reading. Thus it is only necessary to examine the coefficients with subscripts greater than zero which are the ones affected only by the mechanical properties of the blood sample. Table 1 and 2 lists the polynomial coefficients and the correlation coefficient for 2nd and 4th order polynomials for the normal and abnormal samples.

In Table 1 the measured blood coagulation dynamic curves are fit to a 2nd order polynomial with coefficients b_0, b_1, and b_2. Comparing the meaningful coefficients b_1 and b_2 for the normal and abnormal blood samples, it is noteworthy that the algebraic signs of the two coefficients differ for the normal and abnormal samples. Thus the best-fit polynomials are distinctively different equations for normal and abnormal blood. The student t test applied to coefficients b_1 and b_2 yield P values smaller than 0.0001 which is interpreted as a negligible probability that the observed differences are chance observations.

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The correlation coefficients between the 2nd order polynomial and the measured data ranges between about 0.93 and 0.98 for the normal blood, and a somewhat improved 0.96 and 0.98 for the abnormal blood.

Table 2 summarizes the results in which the reassured blood coagulation dynamic curves are fit to a 4th order polynomial with coefficients b_0 to b_4. Here again, each of the meaningful coefficients b_1 to b_4 differ in algebraic sign for the normal and abnormal bloods, yielding statistically significant P values. The correlation coefficients for the 4th order fit are, as expected, closer to unity than for the 2nd order fit.

It must be emphasized that for this preliminary project the samples of abnormal blood that were selected were grossly abnormal. A visual glance at the shape of the blood coagulation dynamic curves produced by the SONO-CLOT instrument would clearly identify that the curves differed from those of normal blood. With the instrumentation and computer algorithms in place, it will now be necessary to determine whether the curve-fitting method can detect and quantitate subtle differences in shapes of the curves that might not be evident upon visual inspection. This will require a large-scale collection of blood samples from patients with expected blood coagulation disorders and careful authentication of these disorders.

**Table 1**   2nd Order Polynomial Coefficients

	NORMAL			
	b_2	b_1	b_0	r
bbl88	0.0093	-3.4185	-0.0433	0.9345
bbl89	0.0132	-4.1307	0.0100	0.9744
bbl90	0.0085	-1.8439	0.0352	0.9656
bbl93	0.0126	-3.2620	0.0256	0.9838
bbl94	0.0099	-3.3003	0.0335	0.9569
Mean	0.0107	-3.1911	0.0122	
SD	0.00186	0.7438	0.02915	
	ABNORMAL			
bbl81	-0.0036	6.0544	0.0159	0.9770
bbl82	-0.0076	8.7600	-0.0196	0.9839
bbl83	-0.0108	12.4590	-0.0457	0.9807
bbl85	-0.0120	11.0889	-0.0448	0.9637
bbl86	-0.0126	12.6521	-0.0387	0.9740
Mean	-0.00932	10.2029	-0.02658	
SD	0.003341	2.4966	0.02323	
P (t test)	0.0001	0.0001		

Table 2 4th Order Polynomial Coefficients

	NORMAL					
	b_4	b_3	b_2	b_1	b_0	r
bbl88	-5.1988×10^{-7}	4.12×10^{-4}	-0.0150	0.2739	0.0031	0.9524
bbl89	-1.2024×10^{-7}	1.00×10^{-4}	-0.0143	0.0283	-0.0161	0.9781
bbl90	-1.2562×10^{-7}	1.00×10^{-4}	-0.0132	0.6273	-0.0090	0.9752
bbl93	-0.8091×10^{-7}	1.00×10^{-4}	-0.0253	0.8300	0.0403	0.9866
bbl94	-1.1982×10^{-7}	4.80×10^{-4}	-0.0180	0.1060	-0.0234	0.9653
Mean	-1.9329×10^{-7}	2.384×10^{-4}	-0.01716	0.3731	-0.00102	
SD	1.6407×10^{-7}	1.7086×10^{-4}	0.004370	0.3077	0.02243	
	ABNORMAL					
bbl81	1.3344×10^{-7}	-2.0×10^{-4}	0.0863	-6.5250	0.0216	0.9911
bbl82	1.4026×10^{-7}	-5.0×10^{-4}	0.0783	-2.3720	-0.0052	0.9921
bbl83	4.4050×10^{-7}	-2.0×10^{-4}	0.1706	-9.0309	-0.0131	0.9936
bbl85	6.1926×10^{-7}	-6.0×10^{-4}	0.1758	-10.9338	0.046	0.9894
bbl86	5.9937×10^{-7}	-6.0×10^{-4}	0.1832	-11.1303	0.0108	0.9920
Mean	3.8657×10^{-7}	-4.2×10^{-4}	0.1388	-7.9984	0.01202	
SD	2.1311×10^{-7}	1.8330×10^{-4}	0.0464	3.2655	0.02086	
P(t test)	0.0026	0.0008	0.0002	0.0009		

APPENDIX

Clotting Profile Plot of Blood bbl81ff1

Time (sec)
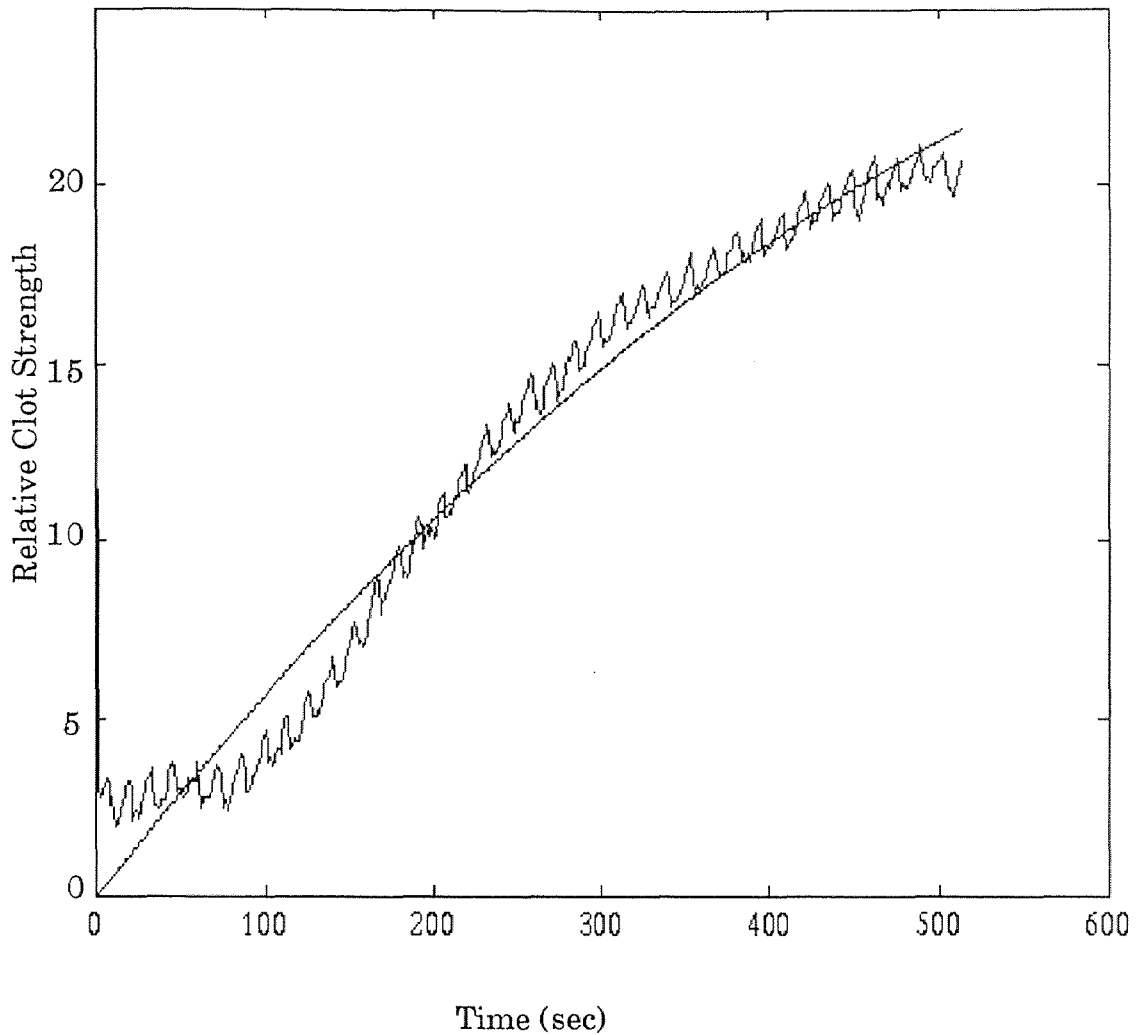
Fitting Equation: $p(x) = -0.0036x^2 + 6.0544x + 0.0159$

Correlation Coefficient: r=0.9770

Data File: bbl81   Sample Rate: 1Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min
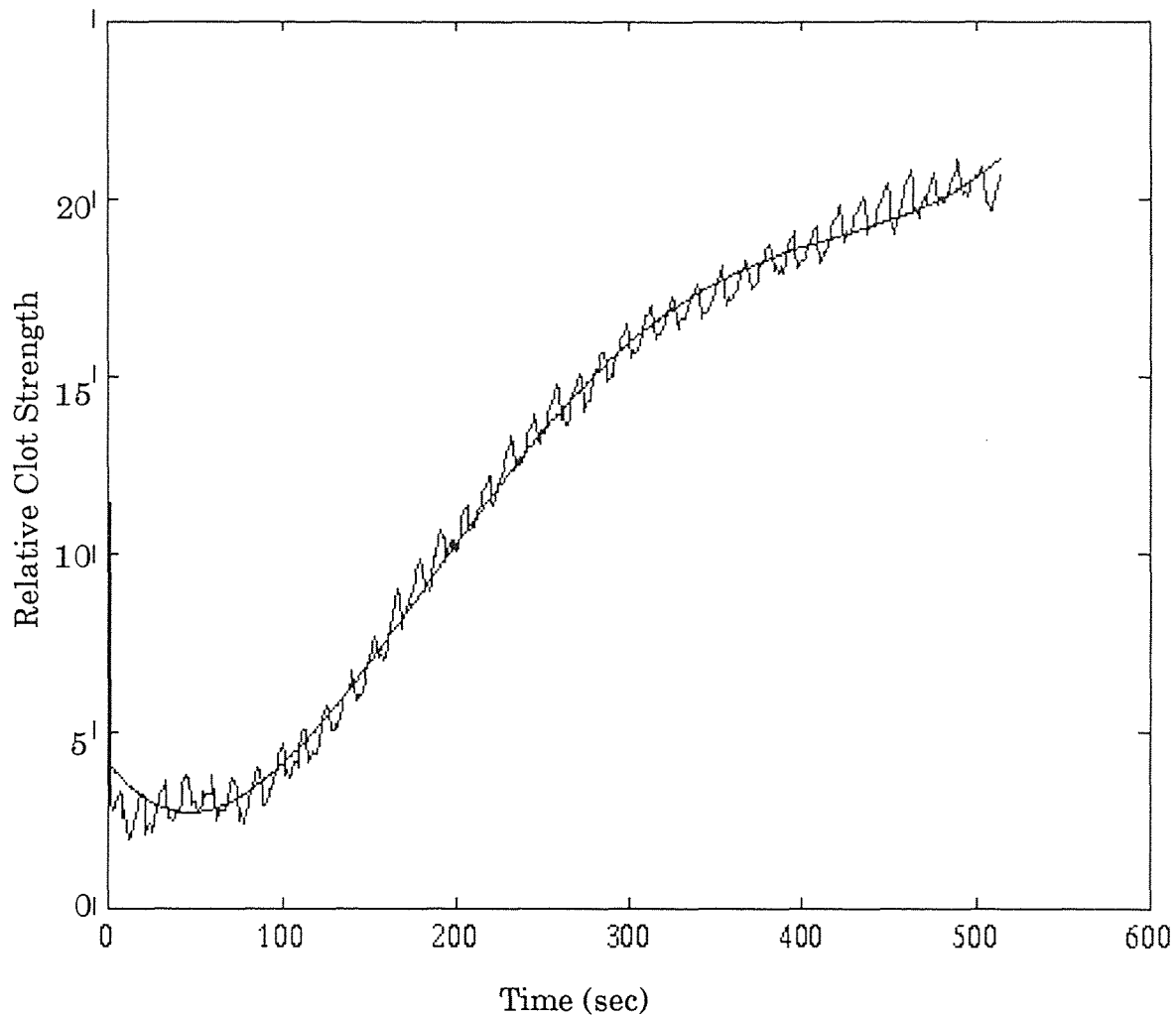
Channel Switch: 16   Gain Switch: 10
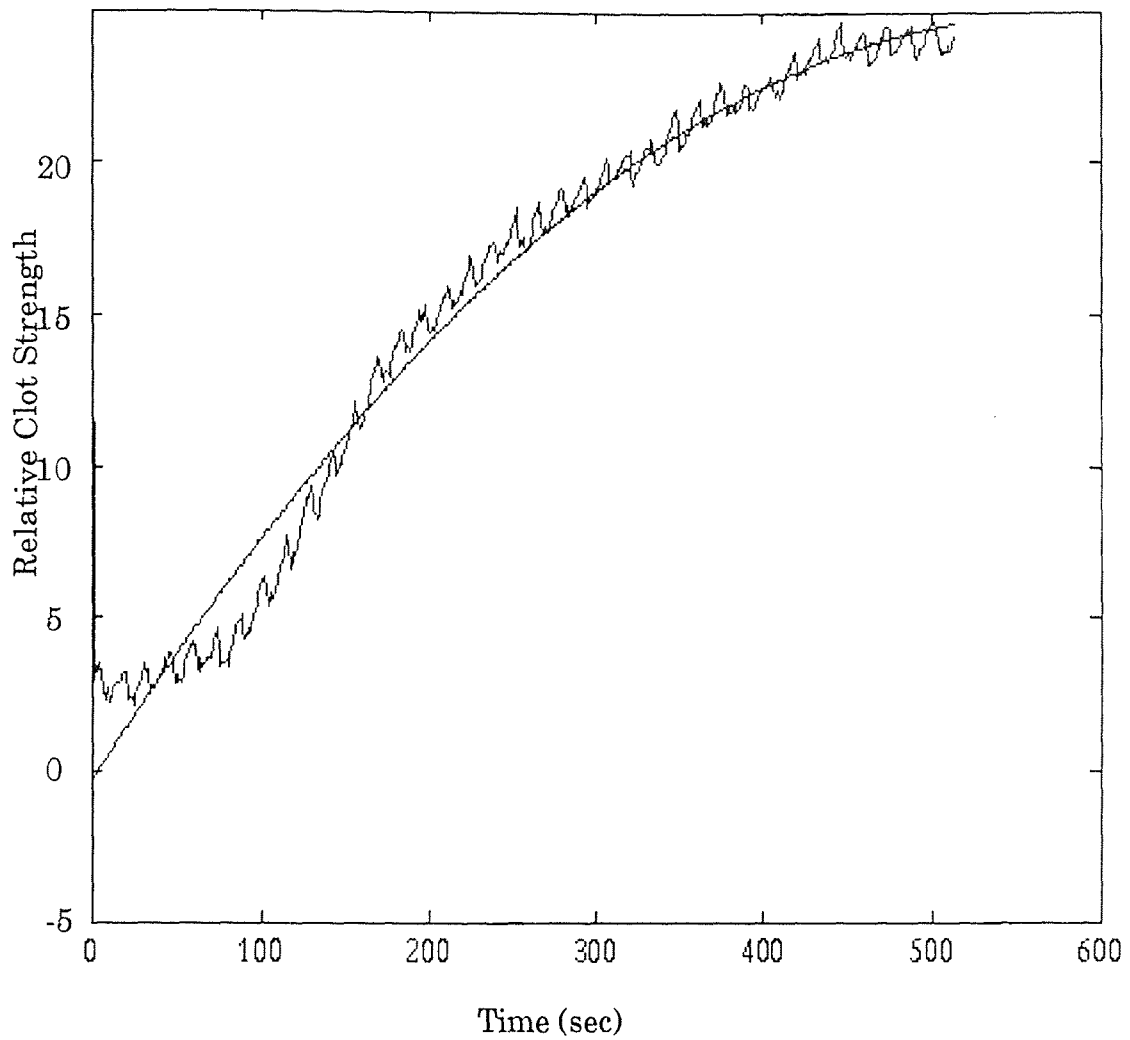
Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl81ff2

Fitting Equation:

$$p(x) = 1.3344 \times 10^{-7}x^4 - 0.0002x^3 + 0.0863x^2 - 6.525x + 0.0216$$

Correlation Coefficient: r=0.9911

Data File: bbl81	Sample Rate: 1 Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl82ff1

Fitting Equation: $p(x) = -0.0076x^2 + 8.760x - 0.0196$

Correlation Coefficient: r=0.9839

Data File: bbl82	Sample Rate: 1 Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl82ff2

Time (sec)
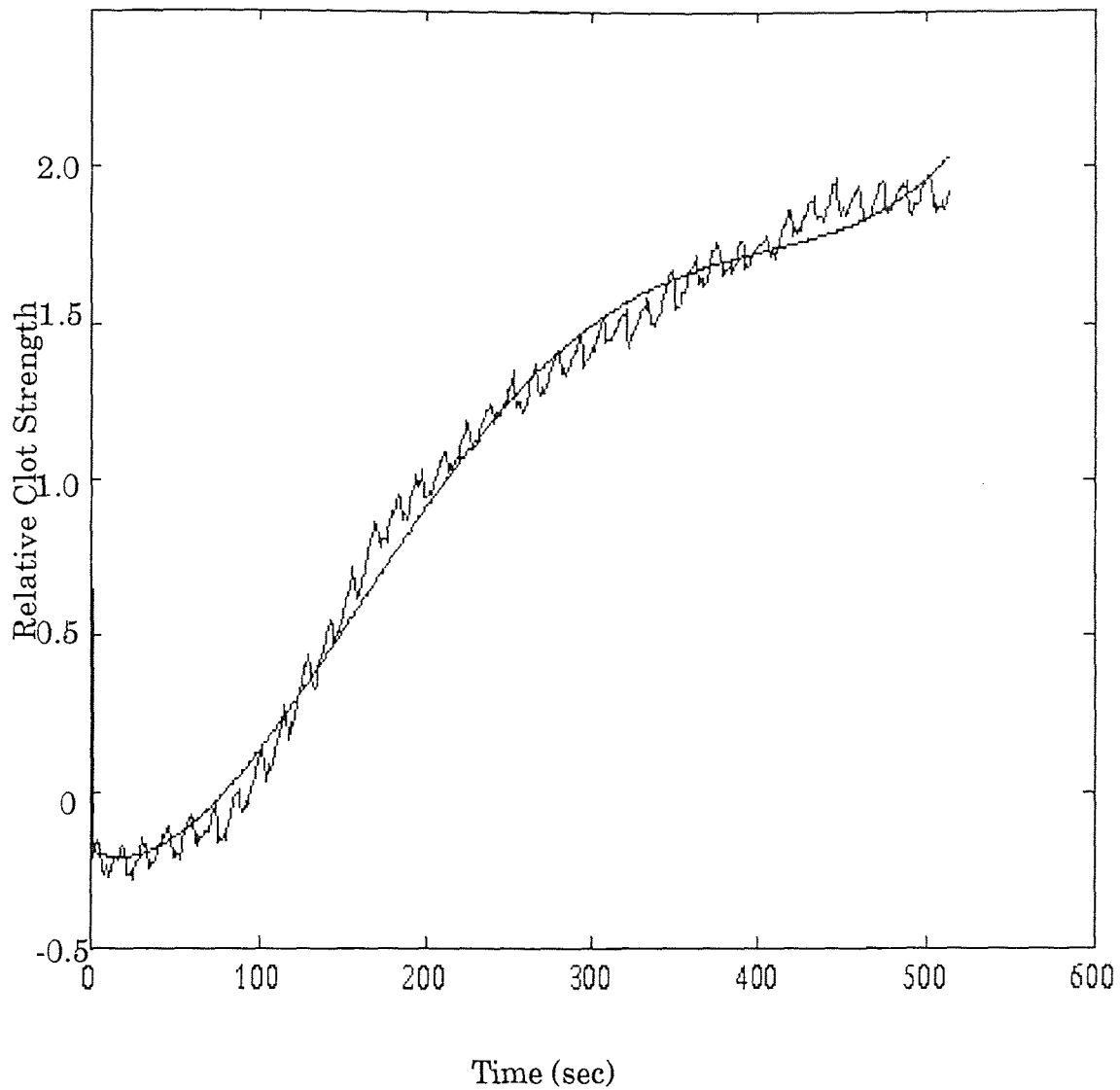
Fitting Equation:

$$p(x) = 1.4026 \times 10^{-7} x^4 - 0.0002 x^3 + 0.0783 x^2 - 2.372 x - 0.0052$$

Correlation Coefficient: r=0.9921

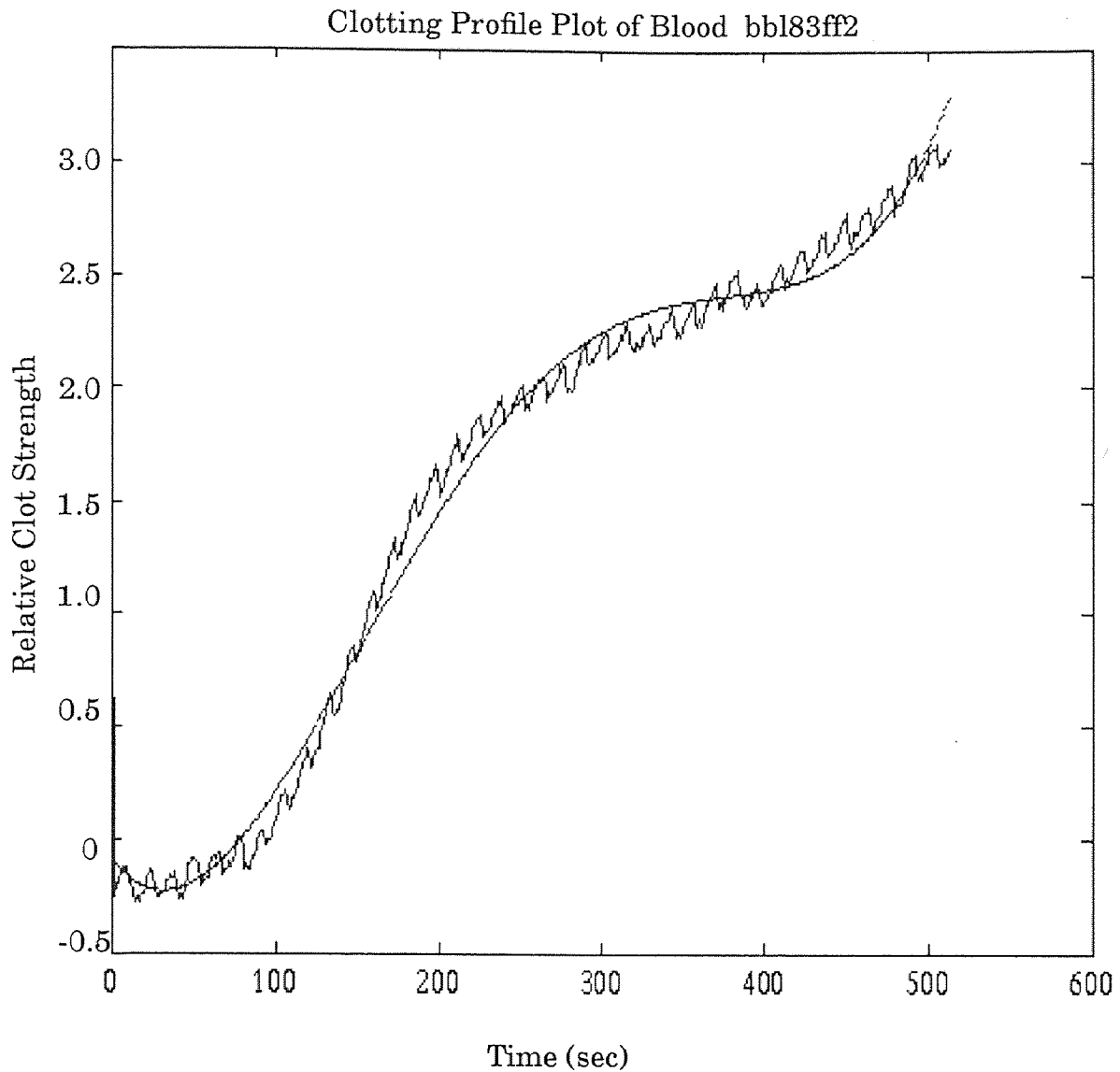|	
Data File: bbl82	Sample Rate: 1 Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl83ff1
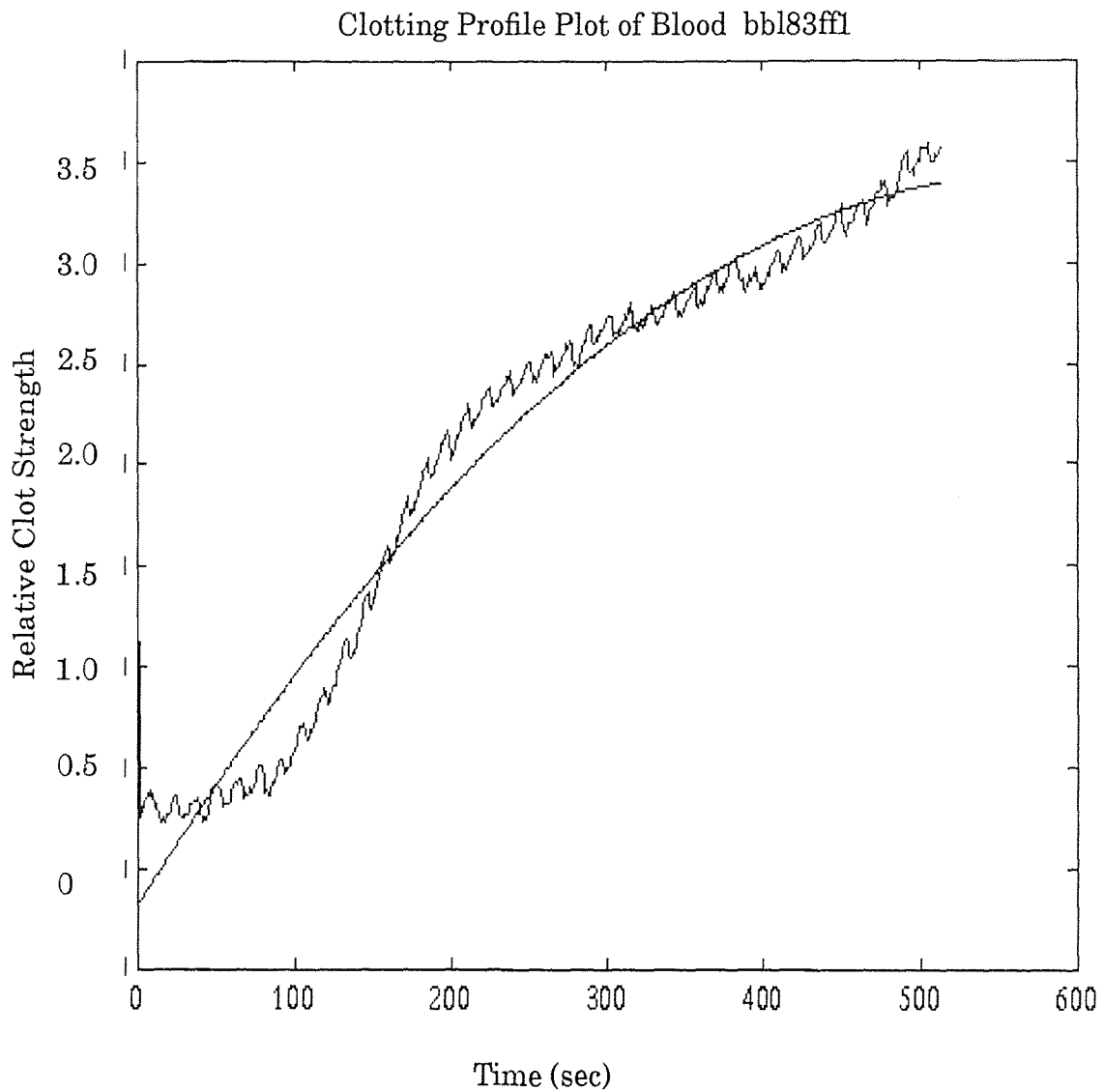
Fitting Equation: $p(x) = -0.0108x^2 + 12.4590x - 0.00457$

Correlation Coefficient: r=0.9807

Data File: bbl83	Sample Rate: 1Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan:2

Clotting Profile Plot of Blood bbl83ff2

Fitting Equation:

$$p(x) = 4.405 \times 10^{-7}x^4 - 0.0005x^3 + 0.1706x^2 - 9.0309x - 0.0131$$

Correlation Coefficient: r=0.9936

Data File: bbl83   Sample Rate: 1 Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min
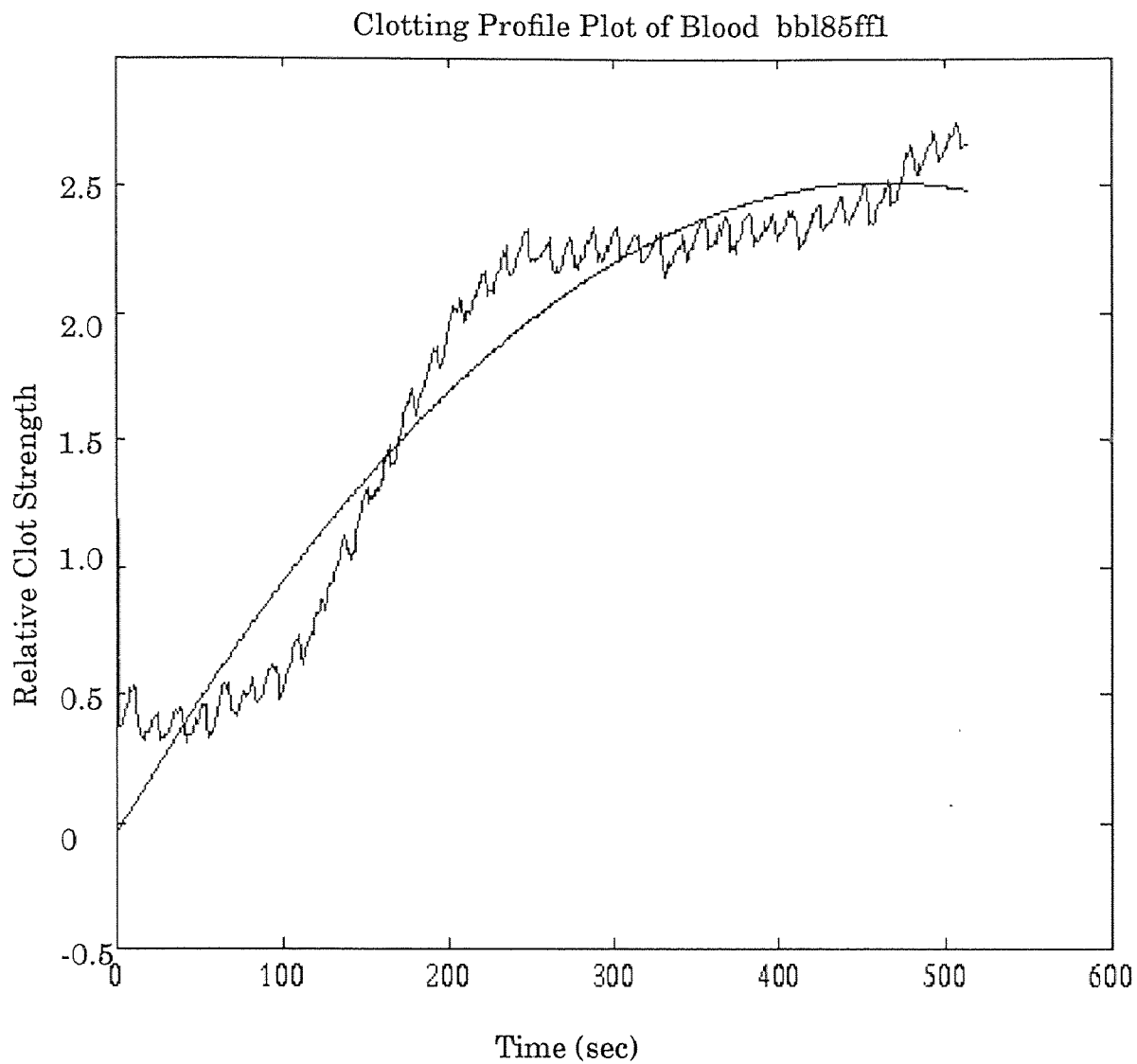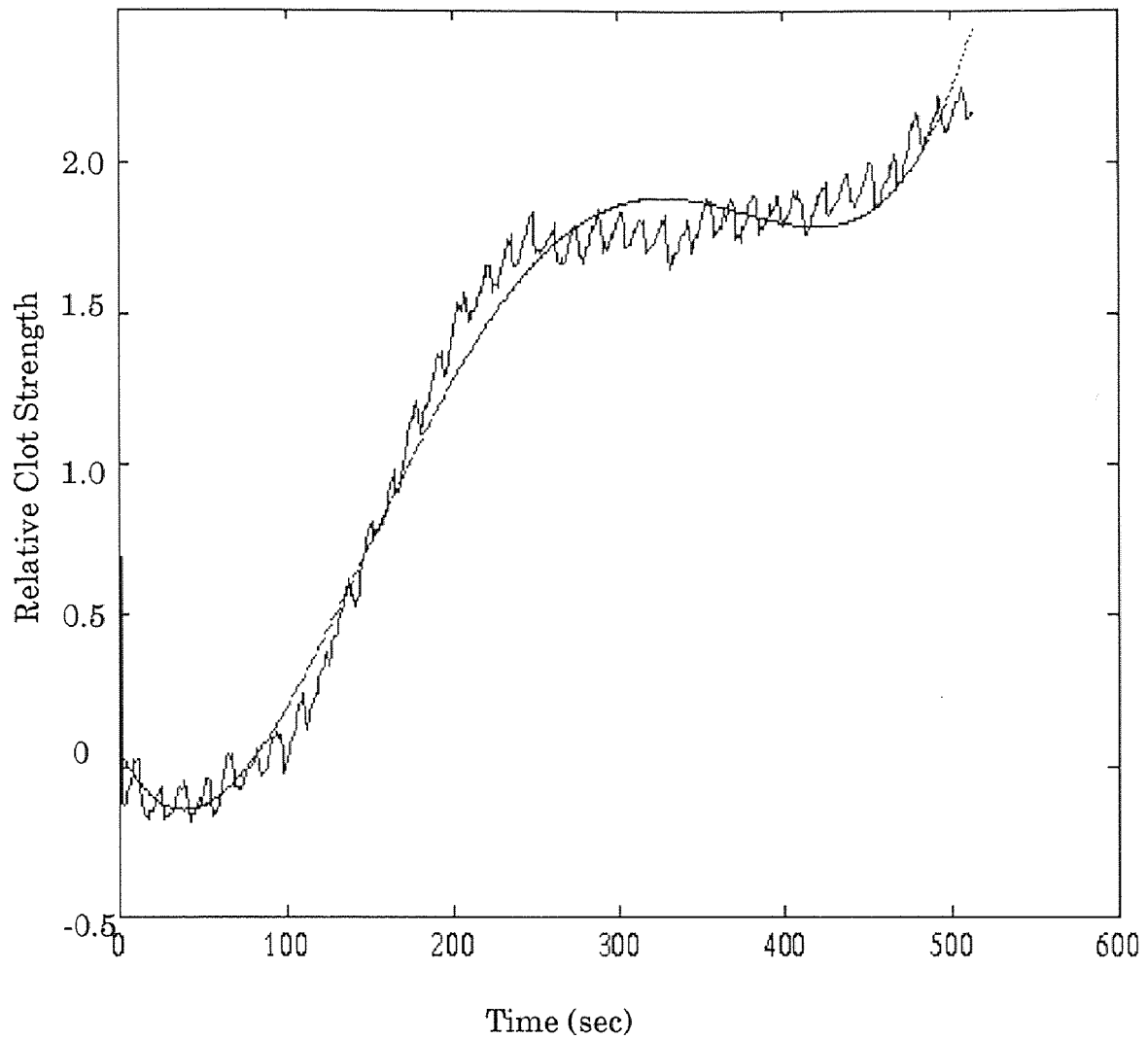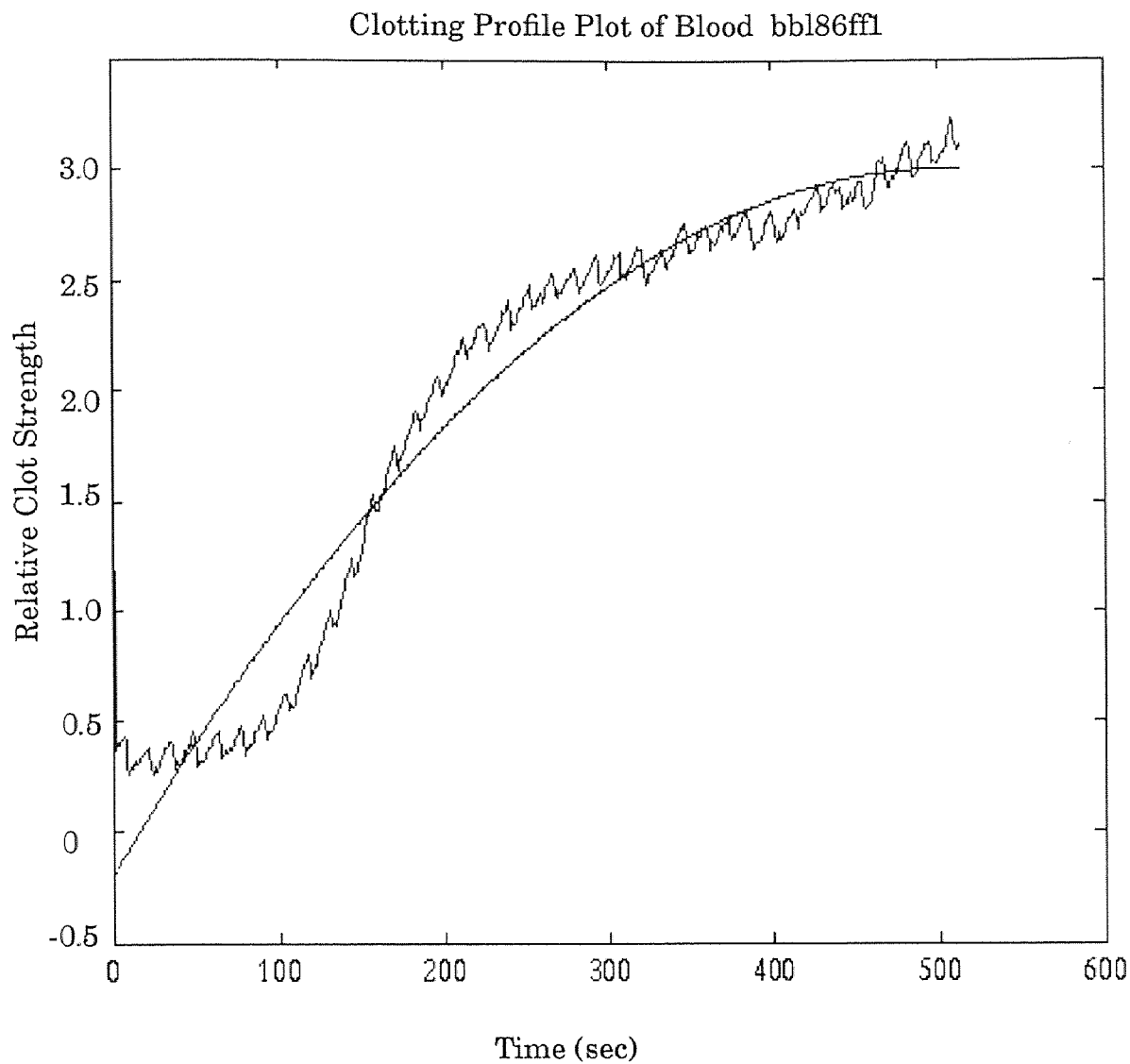
Channel Switch: 16   Gain Switch: 10

Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl85ff1

Fitting Equation: $p(x) = -0.0120x^2 + 11.0889x - 0.0448$

Correlation Coefficient: r=0.9637

Data File: bbl85	Sample Rate: 1 Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl85ff2

Fitting Equation:

$$p(x) = 6.1926 \times 10^{-7}x^4 - 0.0006x^3 + 0.1758x^2 - 10.9338x + 0.046$$

Correlation Coefficient: r=0.9894

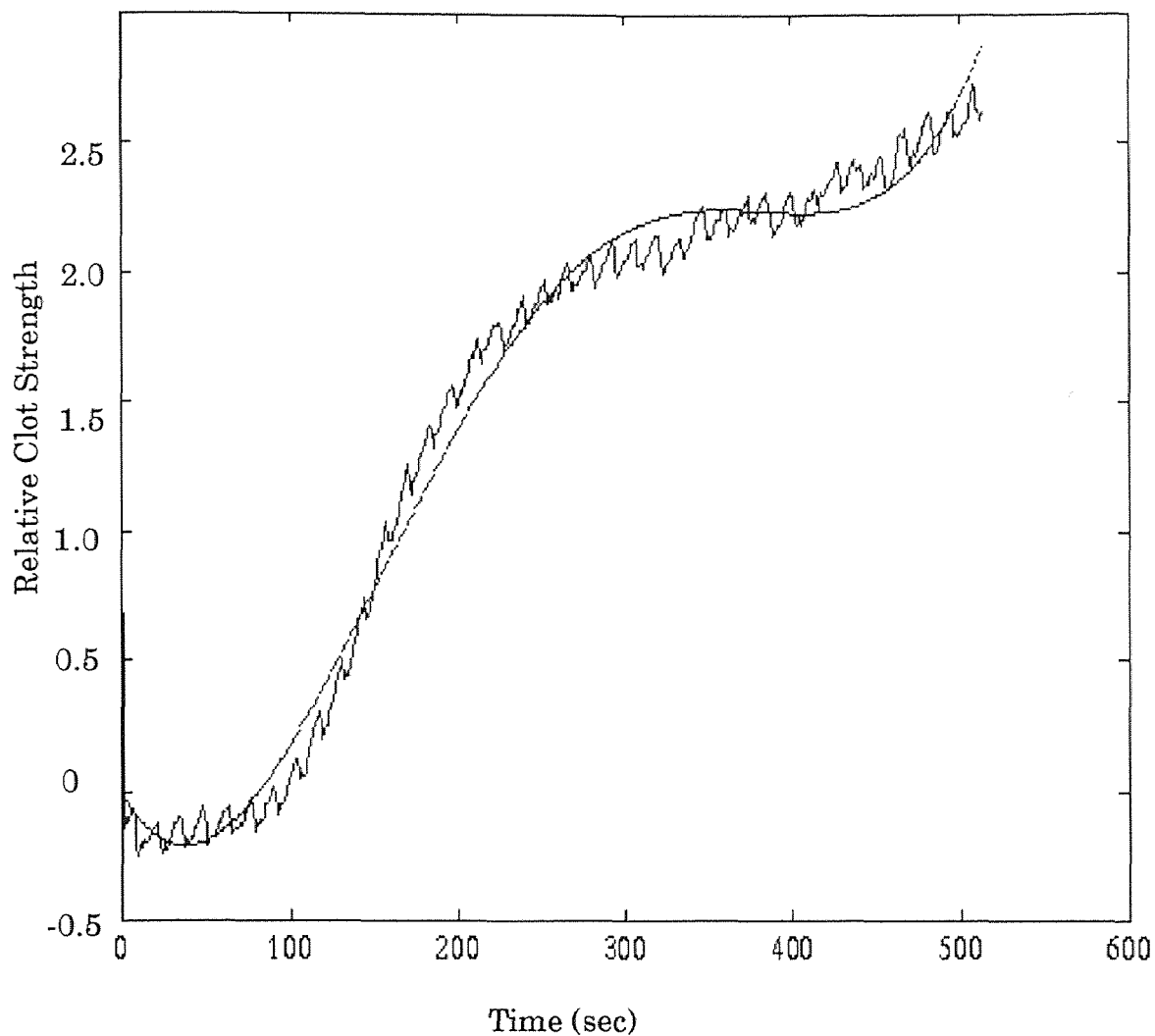|	
Data File: bbl85	Sample Rate: 1 Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl86ff1

Fitting Equation: $p(x) = -0.0126x^2 + 12.6521x - 0.0387$

Correlation Coefficient: r=0.9740

Data File: bbl86	Sample Rate: 1Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl86ff2

Time (sec)

Fitting Equation:

$$p(x) = 5.9937 \times 10^{-7}x^4 - 0.0006x^3 + 0.1832x^2 - 11.1303x + 0.0108$$

Correlation Coefficient: r=0.9920

Data File: bbl86   Sample Rate: 1Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min

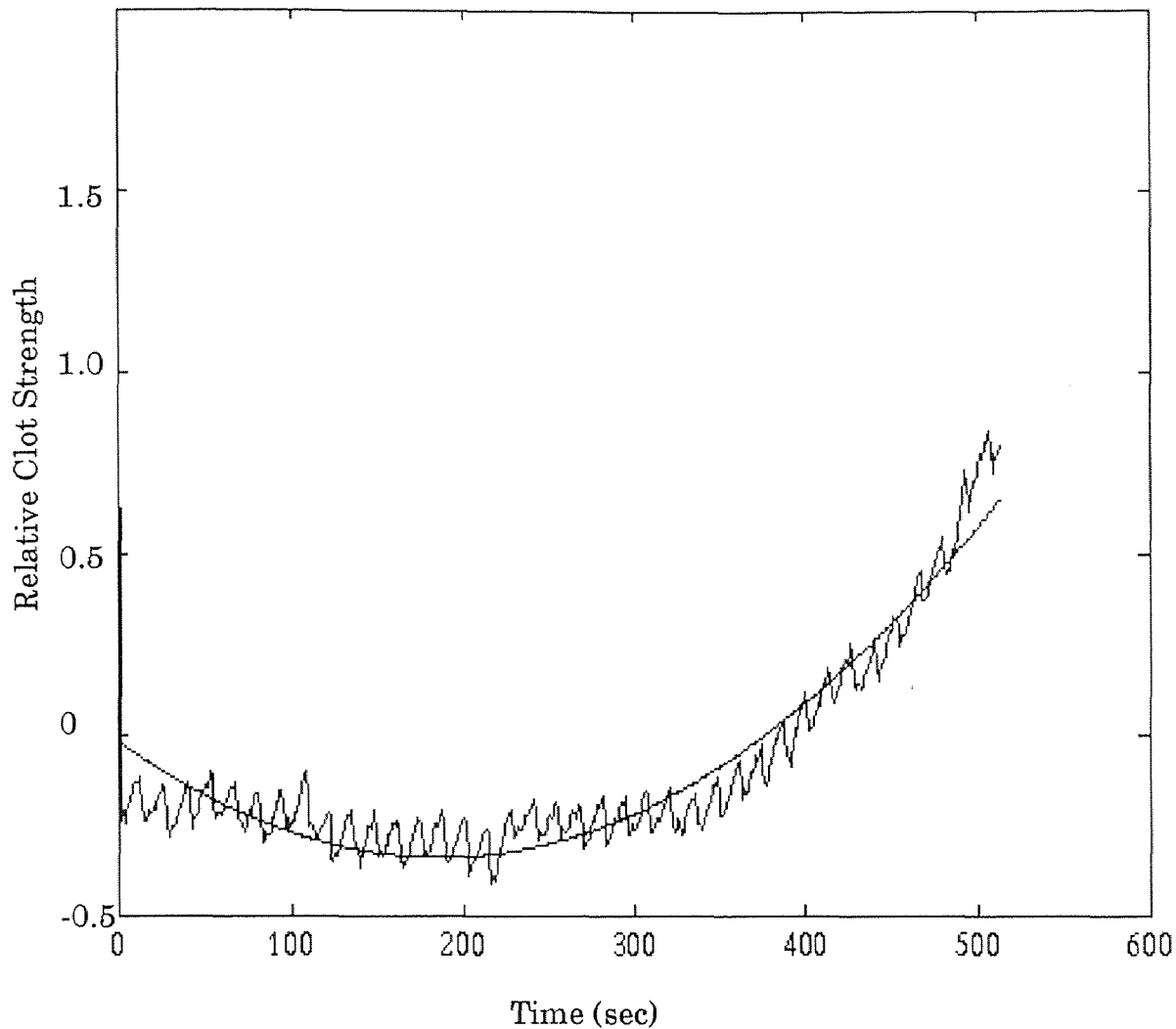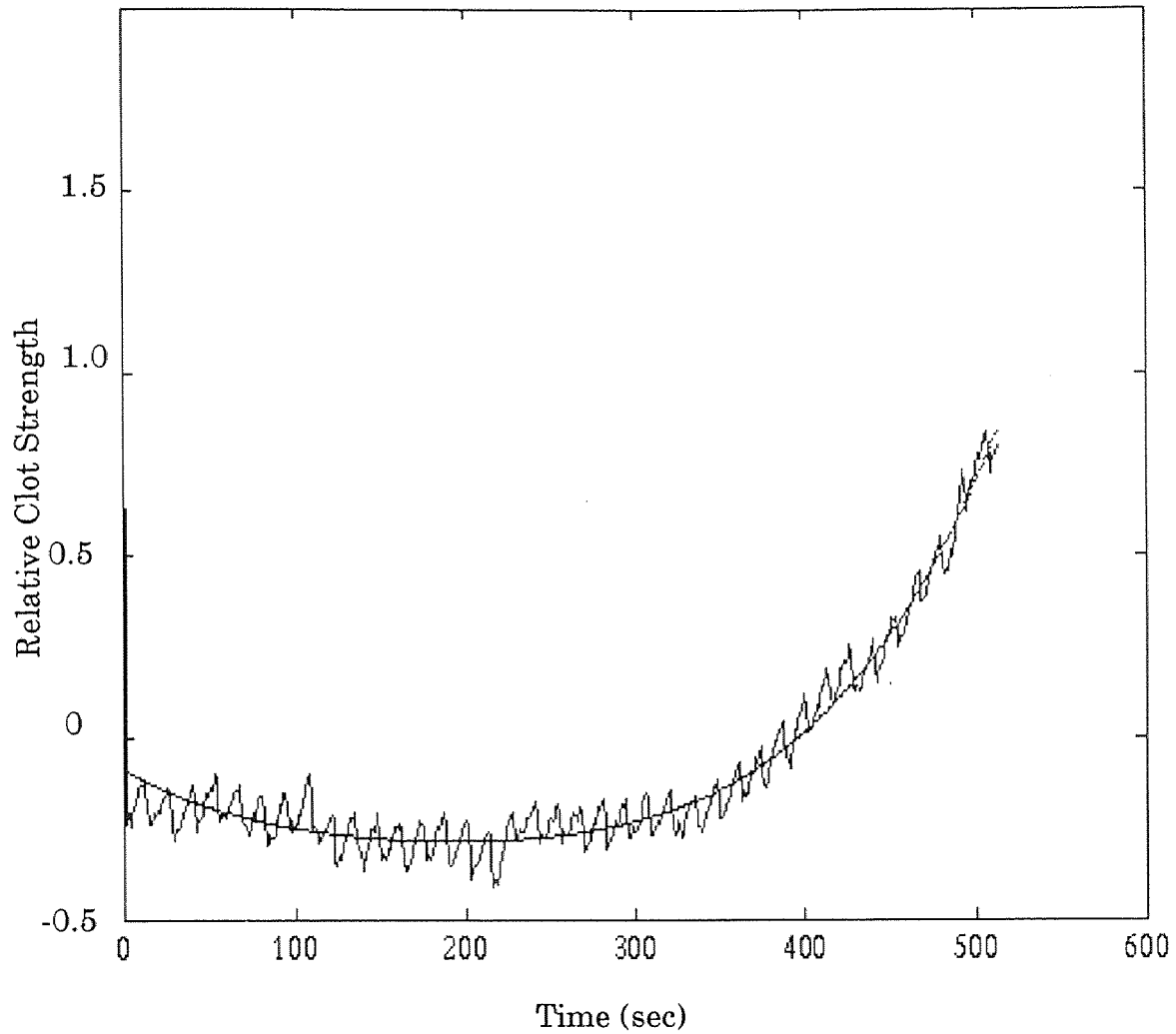Channel Switch: 16   Gain Switch: 10

Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl88ff1

Fitting Equation: $p(x) = 0.0093x^2 - 3.4185x - 0.0433$

Correlation Coefficient: r=0.9345

Data File: bbl88   Sample Rate: 1Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min
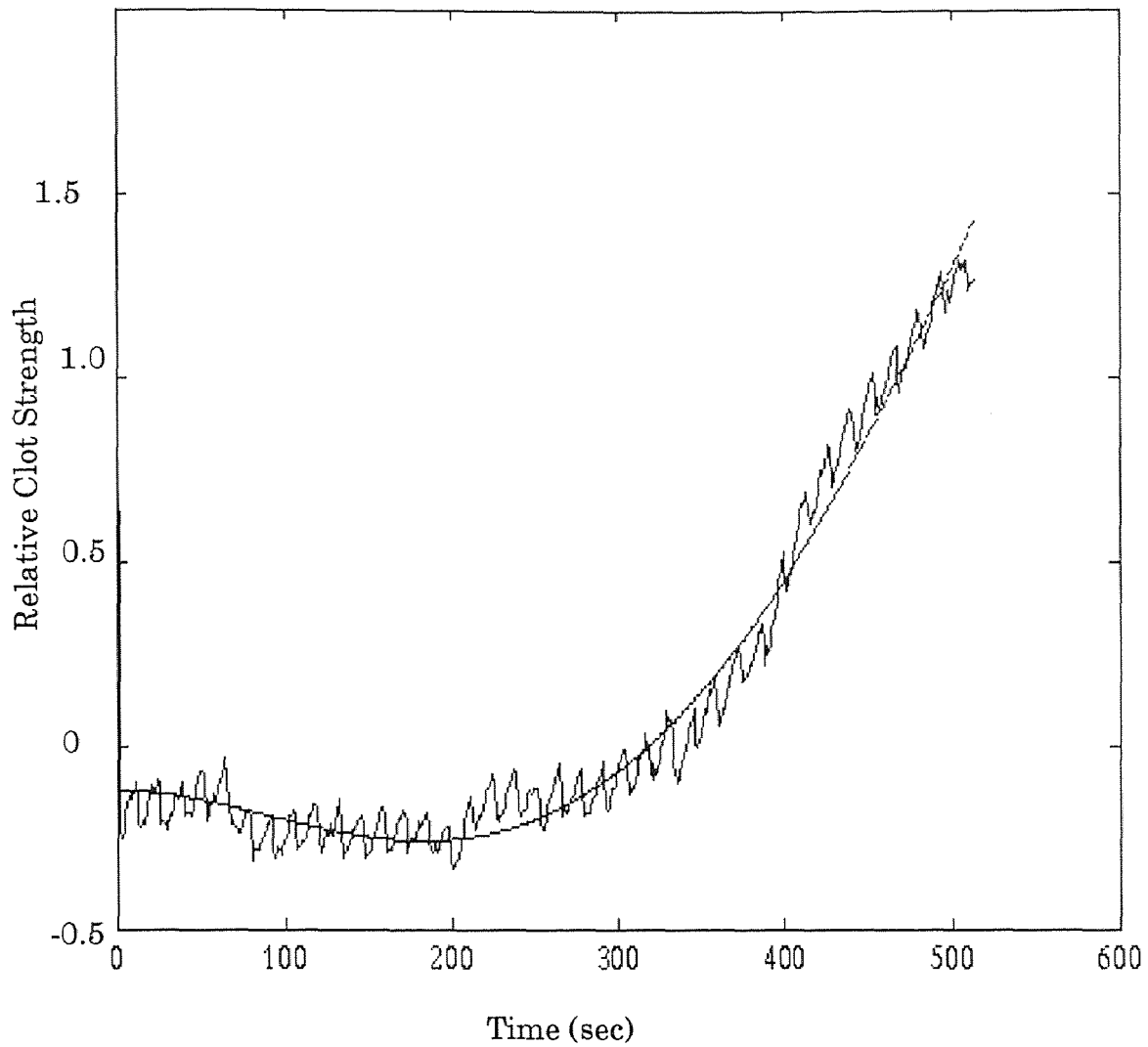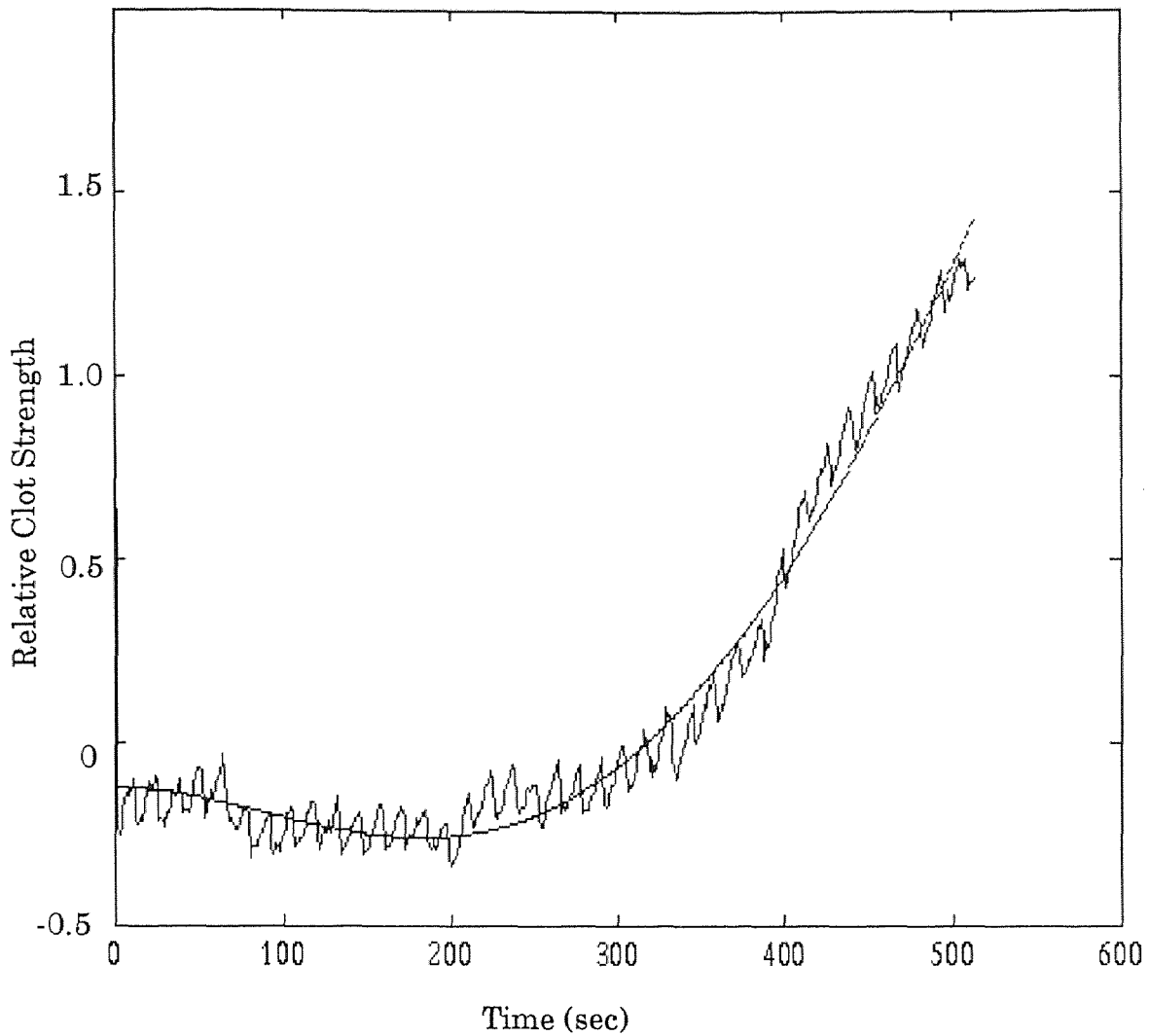
Channel Switch: 16   Gain Switch: 10

Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl88ff2

Fitting Equation:

$$p(x) = -5.1988 \times 10^{-7}x^4 + 0.000412x^3 - 0.015x^2 + 0.27394x + 0.0031$$

Correlation Coefficient: r=0.9524

Data File: bbl88	Sample Rate: 1Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl89ffl

Fitting Equation: $p(x) = 0.0132x^2 - 4.1307x + 0.0100$

Correlation Coefficient: r=0.9744

Data File: bbl89	Sample Rate: 1Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl89ff2

Fitting Equation:

$$p(x) = -1.2024 \times 10^{-7}x^4 + 0.0001x^3 - 0.0143x^2 + 0.0283x - 0.0161$$

Correlation Coefficient: r=0.9781

Data File: bbl89   Sample Rate: 1Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min
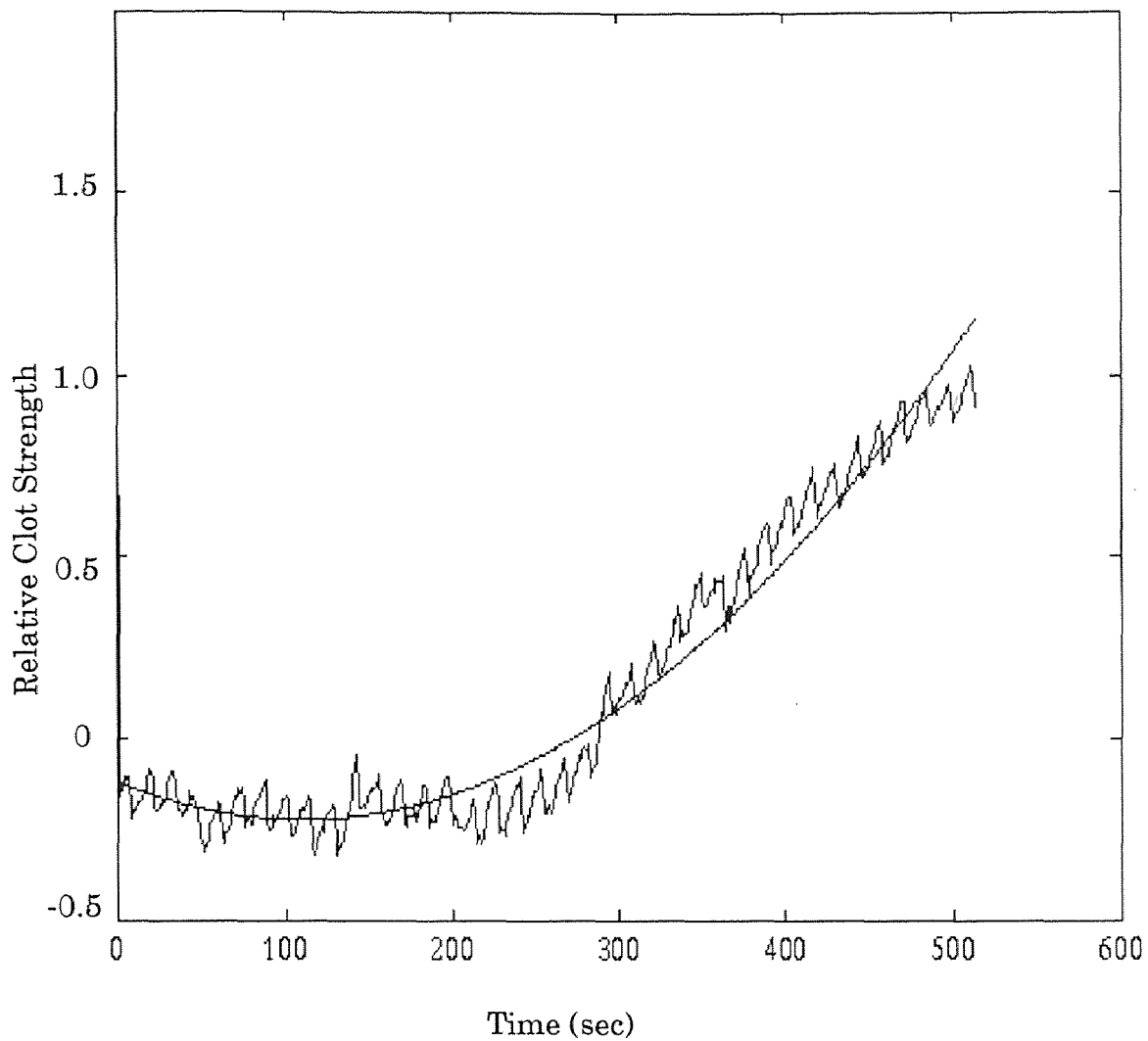
Channel Switch: 16   Gain Switch: 10

Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl90ff1

Time (sec)

Fitting Equation: $p(x) = 0.0085x^2 - 1.8439x + 0.0352$

Correlation Coefficient: r=0.9656

Data File: bbl90   Sample Rate: 1 Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min
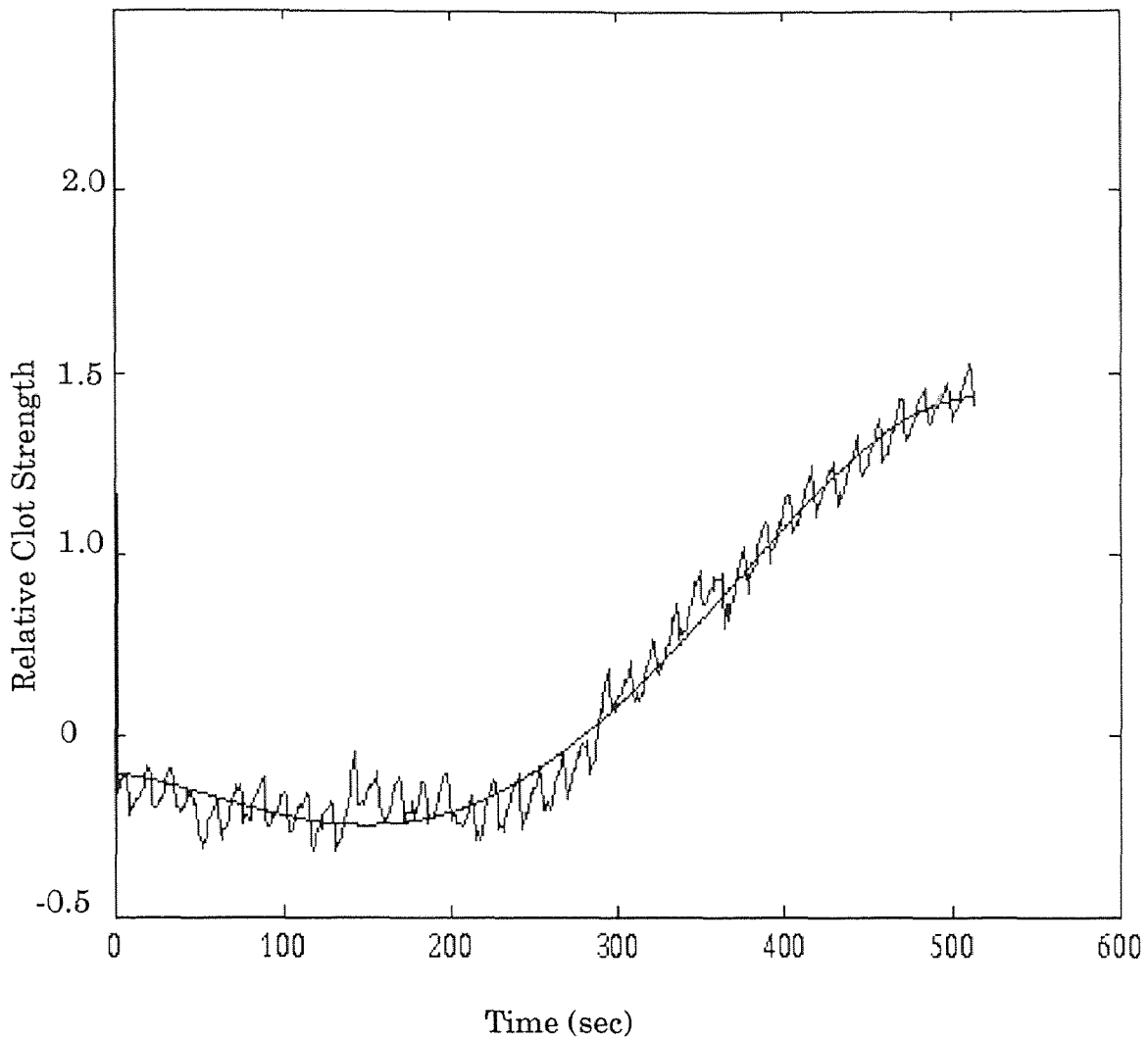
Channel Switch: 16   Gain Switch: 10

Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl90ff2

Fitting Equation:

$$p(x) = -1.2562 \times 10^{-7} x^4 + 0.0001 x^3 - 0.0132 x^2 - 0.6273 x - 0.0090$$

Correlation Coefficient: r=0.9752

Data File: bbl90   Sample Rate: 1Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min
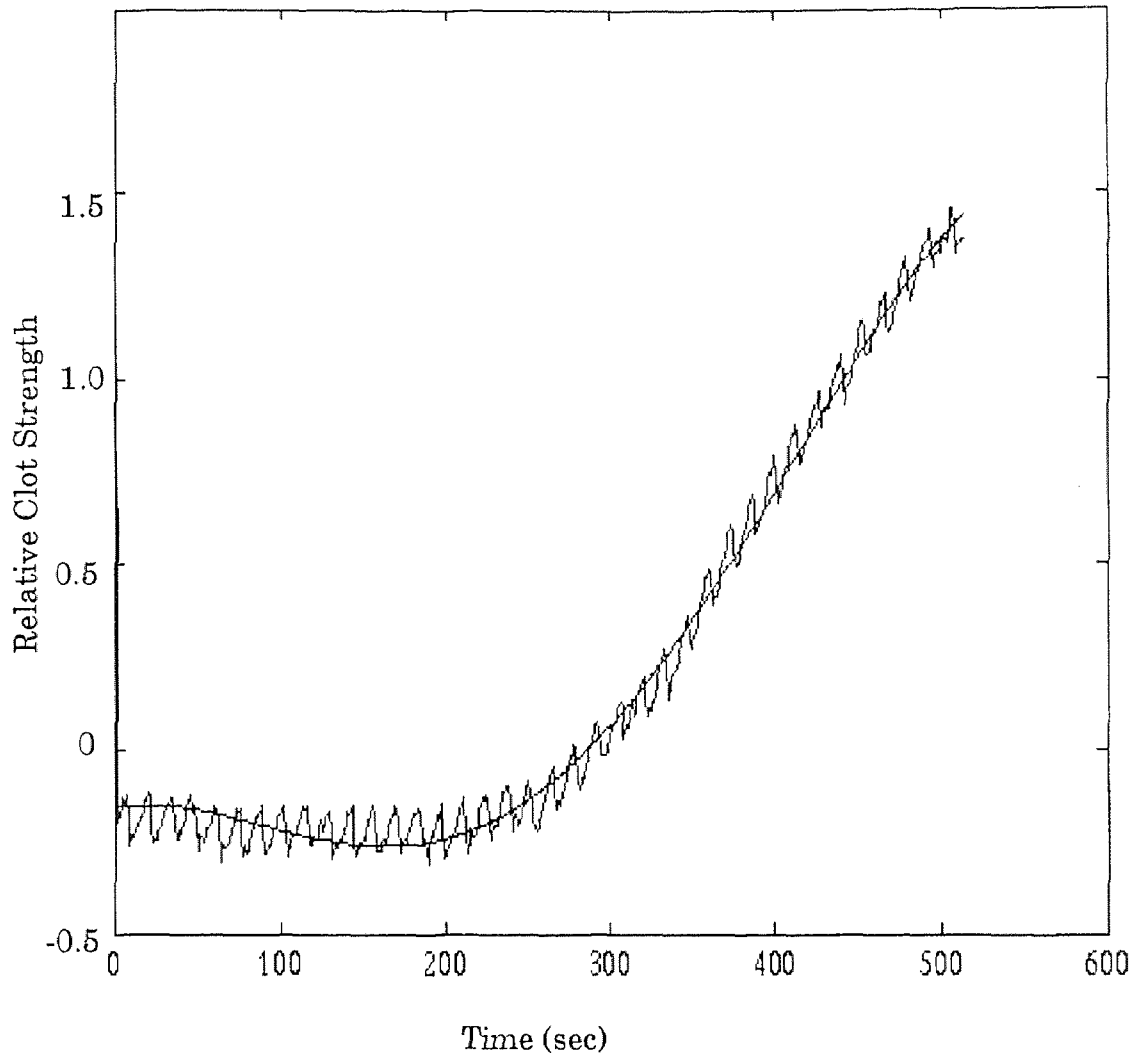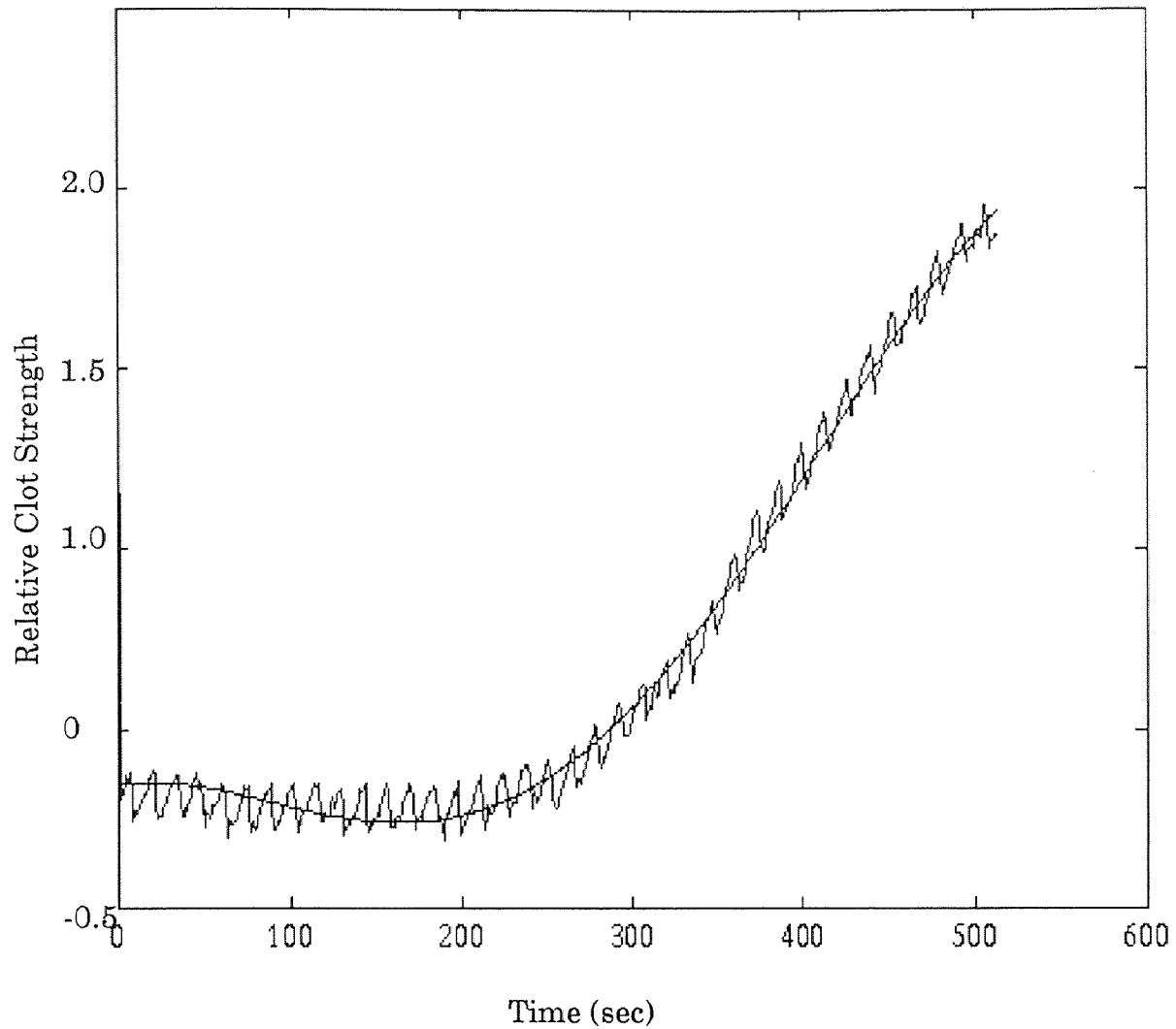
Channel Switch: 16   Gain Switch: 10

Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl93ffl

Time (sec)

Fitting Equation: $p(x) = 0.0126x^2 - 3.262x + 0.0256$

Correlation Coefficient: r=0.9838

Data File: bbl93	Sample Rate: 1 Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl93ff2

Fitting Equation:

$$p(x) = -0.8091x \times 10^{-7}x^4 + 0.0001x^3 - 0.0253x^2 + 0.830x + 0.0403$$

Correlation Coefficient: r=0.9866

Data File: bbl93	Sample Rate: 1Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2

Clotting Profile Plot of Blood bbl94ff1

Time (sec)

Fitting Equation: $p(x) = 0.0099x^2 - 3.3003x + 0.0335$

Correlation Coefficient: r=0.9569

Data File: bbl94   Sample Rate: 1Hz

Data File Size: 1024   Number of Sample: 512

Base Address: 300(HEX)   Total Sample Time: 8.53 min

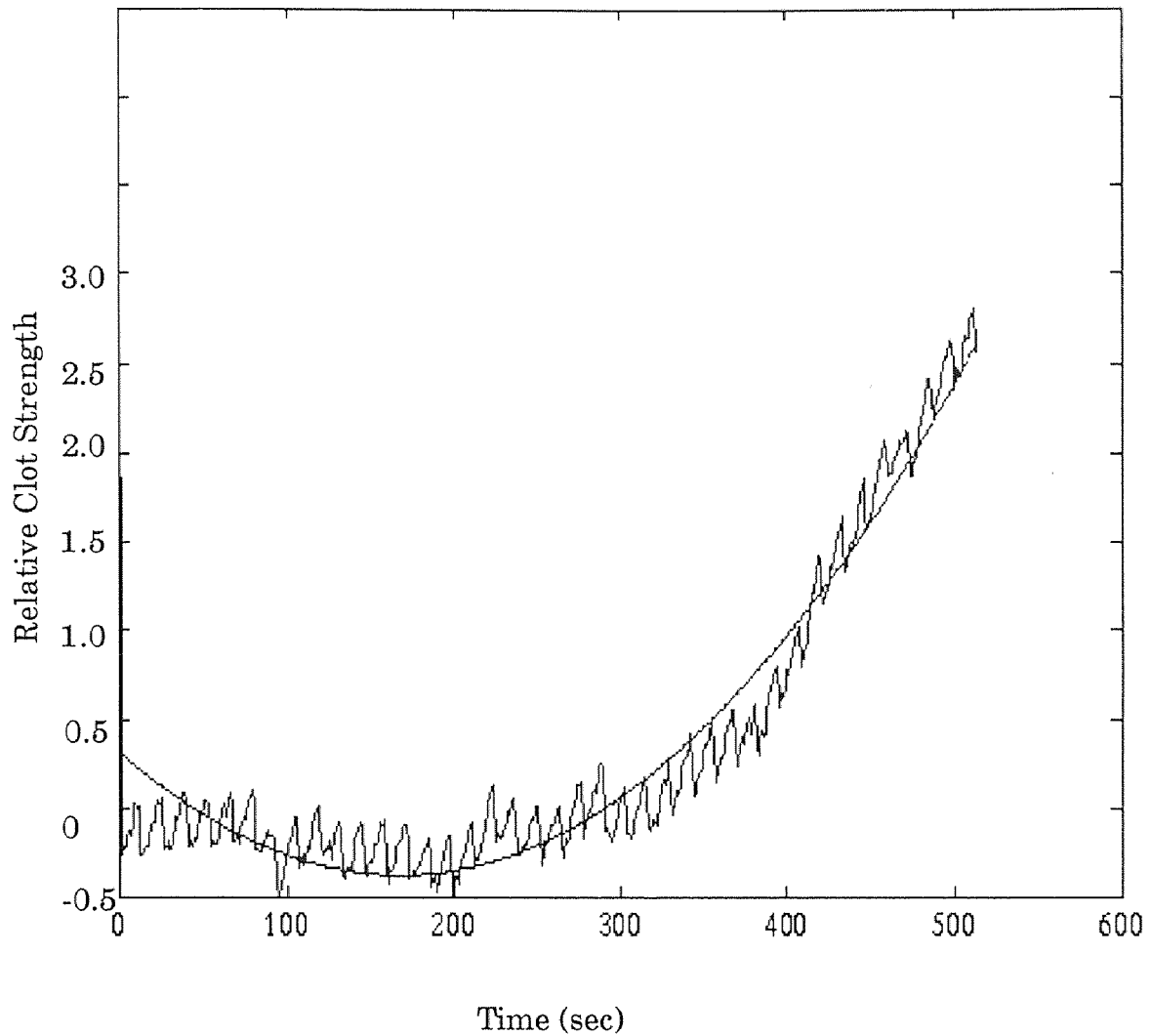Channel Switch: 16   Gain Switch: 10
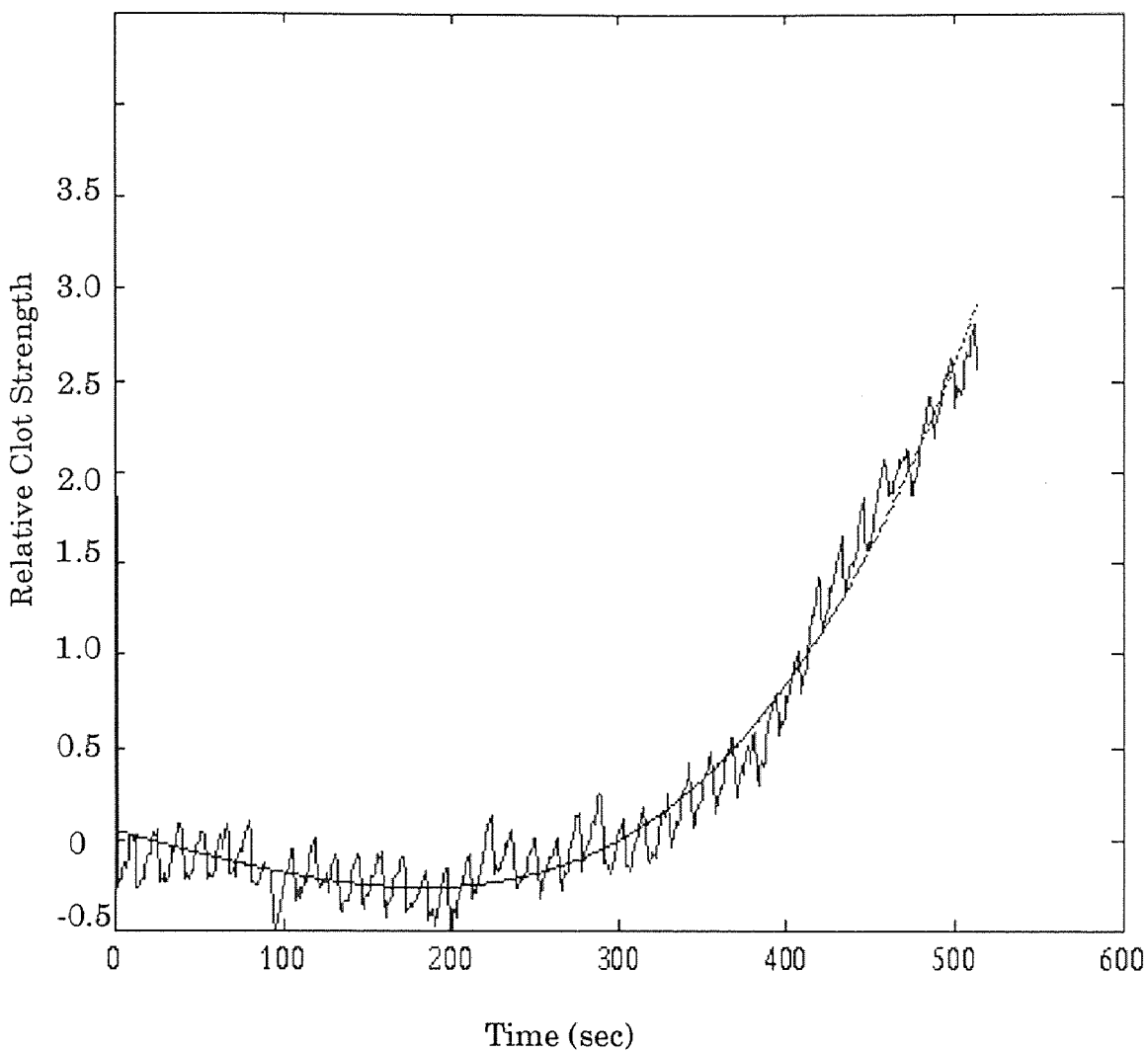
Range Switch: BIP   DMA Switch: 1

First Chan: 2   Last Chan: 2

Clotting Profile Plot of Blood bbl94ff2

Fitting Equation:

$$p(x) = -1.1982 \times 10^{-7}x^4 + 0.00048x^3 - 0.018x^2 + 0.10603x - 0.0234$$

Correlation Coefficient: r=0.9653

Data File: bbl94	Sample Rate: 1Hz
Data File Size: 1024	Number of Sample: 512
Base Address: 300(HEX)	Total Sample Time: 8.53 min
Channel Switch: 16	Gain Switch: 10
Range Switch: BIP	DMA Switch: 1
First Chan: 2	Last Chan: 2
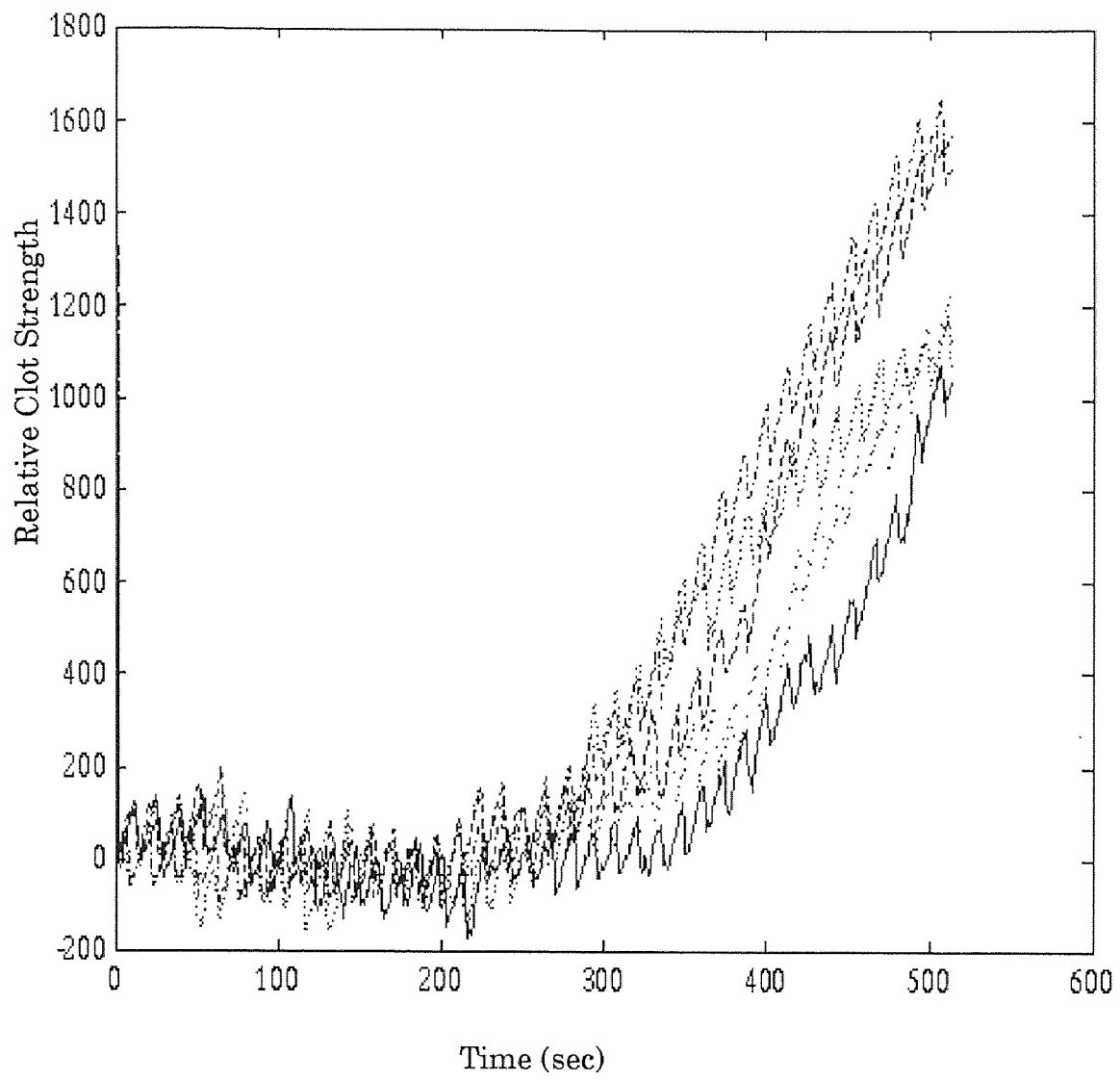
Raw Data of Normal Whole Blood Coagulation

Blood Sample: bbl88, bbl89, bbl90, bbl93, bbl94
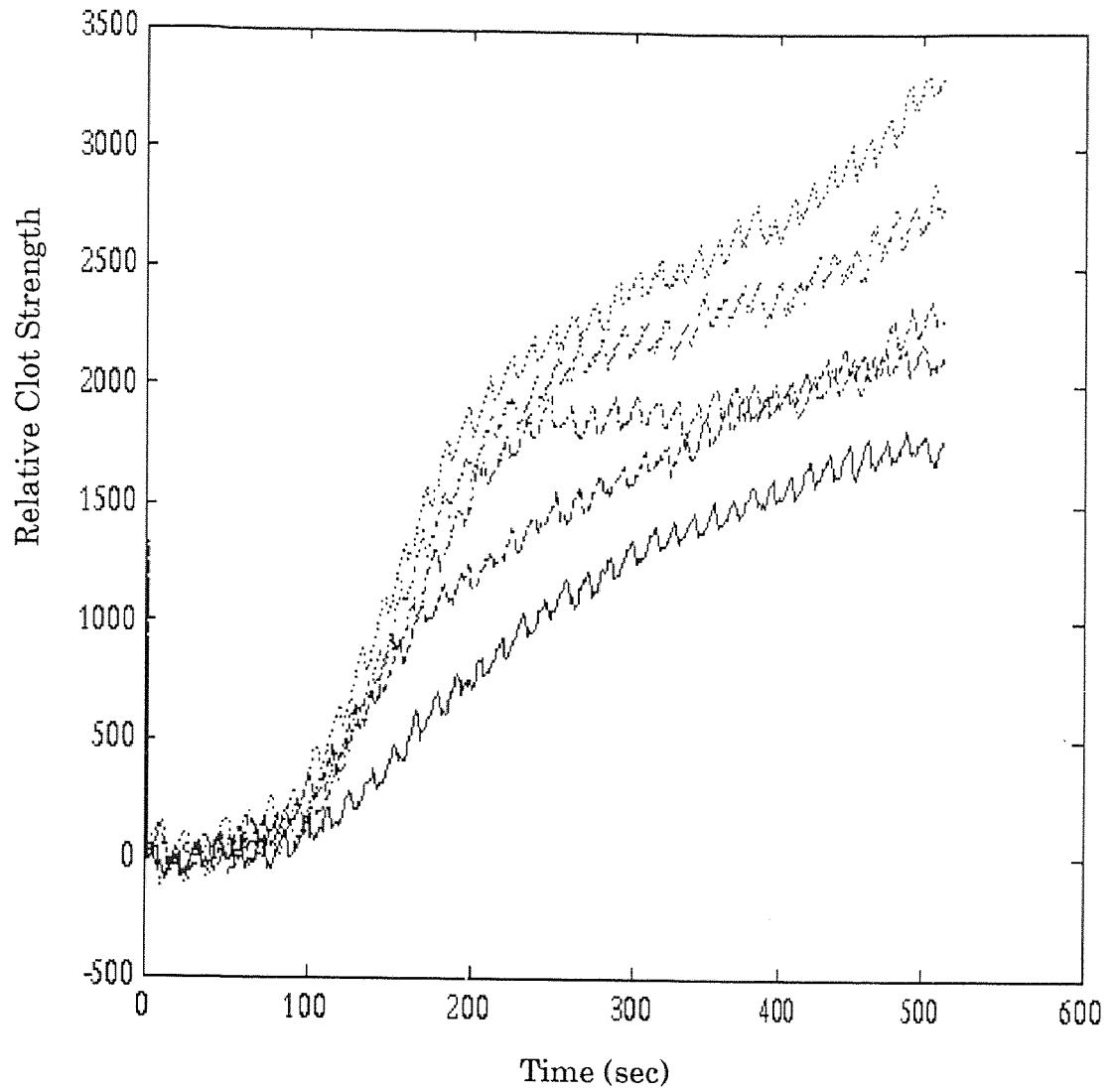
Raw Data of Abnormal Whole Blood Coagulation

Blood Sample: bbl81, bbl82, bbl83, bbl85, bbl86

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