

ISSN 1726-5479

SENSORS & TRANSDUCERS

9^{vol. 144}
/12



IEEE 1451

TEDS Sensors, IEEE 1451 Standards

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Issue 9
September 2012

www.sensorsportal.com

ISSN 1726-5479

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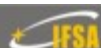
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International Frequency Sensor Association (IFSA) Publishing

Digital Sensors and Sensor Systems: Practical Design

Sergey Y. Yurish



Formats: printable pdf (Acrobat) and print (hardcover), 419 pages

ISBN: 978-84-616-0652-8,
e-ISBN: 978-84-615-6957-1

The goal of this book is to help the practitioners achieve the best metrological and technical performances of digital sensors and sensor systems at low cost, and significantly to reduce time-to-market. It should be also useful for students, lectures and professors to provide a solid background of the novel concepts and design approach.

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Conference Announcement



Topic E2: Transportation & Mobility

The Euromat conference series, organised by the Federation of European Materials Societies (FEMS), is one of the largest events of its kind in Europe, covering the full width of materials science and technology. We would like to direct your attention to the following Symposia which are focussing specifically on transport applications:

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PSoC Based Blood Coagulation Instrument for the Analysis of PT & APTT

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Received: 22 July 2012 /Accepted: 21 September 2012 /Published: 28 September 2012

Abstract: Internal bleeding and internal clotting is the a major cause of death in patients with chronic Hepatitis, Carcinoma, Hemophilia, patients undergoing anticoagulant therapy and Diabetic patients where the natural Blood clotting mechanism that coagulates and clots the bleeding injury doesn't work properly. Blood coagulation analyzer is the best tool in diagnosing these patients towards deciding the course of treatment. Though there are several clotting factors which are involved in blood clotting mechanism, Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) are the major diagnostic tools in deciding the course of therapy and dosage. In this paper the design and development of Coagulation Analyzer using PSoC (Programmable System-on-Chip) CY8C28433 is presented. The designed Analytic Instrument has shown comparatively better results of Prothrombin Time and Activated Partial Thromboplastin Time with standard Instruments. The error on comparison is less than 2 % as agreed by international standards. The designed instrument is useful in diagnosing of internal bleeding and clotting by interpreting the results AT comparatively lower cost.

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Keywords: Haemophilia, Warfarin, PT, APTT.

1. Introduction

Blood is a liquid connective tissue that acts as the main transporting system of the body. It transports nutrients, respiratory gases, metabolic wastes and other substances from one part of the body to

another. The main components of blood are Erythrocytes or Red Blood Corpuscles (RBC), Leucocytes or White Blood Corpuscles (WBC), Thrombocytes or Blood Platelets and Blood plasma. Blood possesses two remarkable properties. The blood remains in a fluid state throughout our life, but when it is shed, it loses its fluidity in a few seconds. Both these properties are essential for the life. The property of blood i.e., losing its fluidity and setting into a semisolid jelly when shed, is called Clotting or Coagulation of Blood [1-3]. This property is due to the natural phenomenon carried over by the property of plasma proteins and platelets. On further keeping, the clot retracts to a smaller volume and presses out a clear straw coloured fluid called the serum which will not clot anymore.

2. Coagulation of Blood

When there's bleeding due to injury blood is shed, the platelets disintegrate and liberate thromboplastin. It is also derived from damaged tissues and the plasma. It initiates the clotting process. Platelets are necessary for clot reaction to occur. Therefore failure of a clot reaction is an indication that the number of platelets in the blood might be lower. The classic theory of blood coagulation as proposed by Paul Morawitz explains the blood clotting mechanism as follows. Thromboplastin converts prothrombin into thrombin with the help of calcium ions. Ionic calcium greatly helps in the formation of active thromboplastin by acting as a cofactor in the coagulation process. Prothrombin is a plasma protein and it is present in normal plasma [4, 5]. It is manufactured in the liver. Vitamin K is required by the liver for normal formation of prothrombin. During clotting, prothrombin is converted to thrombin by thromboplastin. Thrombin is an active enzyme which converts soluble protein fibrinogen into the insoluble protein fibrin [6, 7]. Thus platelets play a critical role in the influencing the cascaded coagulation process. Finally, fibrin stabilizes the platelet-rich thrombus called blood clot. Fibrin is the fine threads which finally form the frame work of the clot entrapping blood cells, platelets and plasma. The fibrin threads adhere to damaged surfaces of blood vessels, therefore the blood clot becomes adherent to any vascular opening thereby preventing blood loss [8, 9]. The normal coagulation time is about 5 to 8 minutes. The fluidity of the blood vessels depends on intact blood platelets, intact blood vessels and the presence of anticoagulant such as heparin and antithrombin. The developed Instrument works under the principle of Opto Mechanics and the reaction of the sample with reagent is Turbo-Densitometry. Opto Mechanics is the principle used to detect blood coagulation by measurement of the transmitted light intensity [10].

3. System Block Diagram

The system block diagram is shown in Figs. 1 and 2 which depicts the design of the developed instrument. The block 1 represents sample block, which consists of a Light source, Hyper RED LED (KL33HHC) with water transparent lens emitting 670 nm wavelength incubation chambers and Sample cup holder. Below the sample cup holder a dc motor with magnetic rotation mechanism is fixed to mix the blood sample with the reagent. This magnetic agitator is controlled by the micro controller. The measurement block also has a temperature control unit to maintain the block at 37 °C. The temperature is controlled by PSoC using wire wound resistor for heating and temperature sensor. Based on the temperature sensor output the PSoC switches ON or OFF the heater. Block2 represents the photo sensor, Photodiode SI336-8BQ. The optical sensor S1336-8BQ (Si photodiode - HAMAHATSU PHOTONICS) is used to detect the amount of transmitted light from the solutions. These Si photodiode also has sensitivity in the UV to near IR range. Active area of photodiode is 5.8 mm - 5.8 mm and photosensitivity of the diode is 0.12 (A/W). It has excellent linearity with respect to incident light, low noise, wide spectral response range and long life [11]. Block3 represents the temperature sensor LM35DZ which is used to monitor the temperature of the Measurement Block (Fig. 2). Block 4 is PSoC Chip CY8C28433-24PVXI from cypress semiconductors which includes two programmable gain amplifiers (PGA) one for Photodiode and the other for Temperature sensor

LM35DZ, 14-bit ADC, Multiplexer and processing unit all built inside a single chip. PSoC controller, which integrates all the above components, becomes the dominant system architecture. A single PSoC device can integrate several peripheral functions with a microcontroller saving customers design time, board space and power consumption. The output signals from the photo diode and the temperature sensor are given to the I/O Pins of PSoC for signal amplification. Further these amplified signals are selected by Internal Multiplexer of PSoC for Analog to Digital conversion which is another internal block of PSoC. The PSoC CY8C28433 is built-in with 12 Digital blocks, 6 regular and 4 limited Analog blocks, one I²C, 2 Decimators, up to 24 Digital I/O, up to 24 Analog inputs, 2 analog outputs 1k RAM and 16k programmable memory [12]. Block 5 represents the keypad, Block 6 indicates LCD, Block 7 specifies RS232 which provides connectivity to a PC. An LCD is connected to PSoC displays the menus and the results.

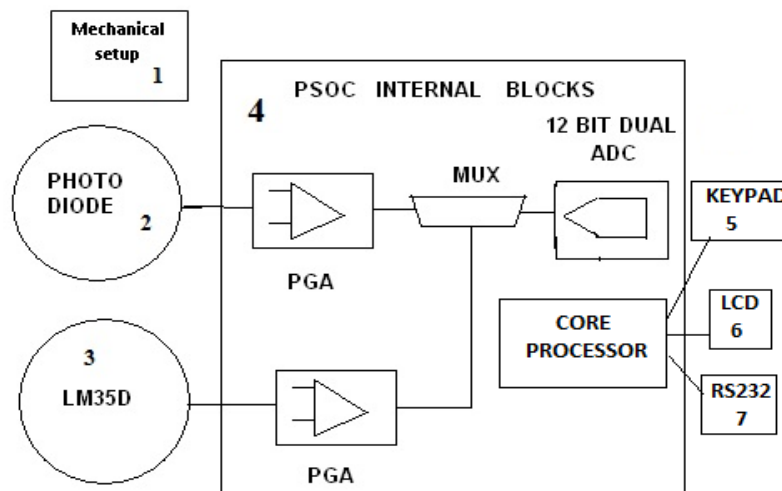


Fig. 1. System Block Block diagram.

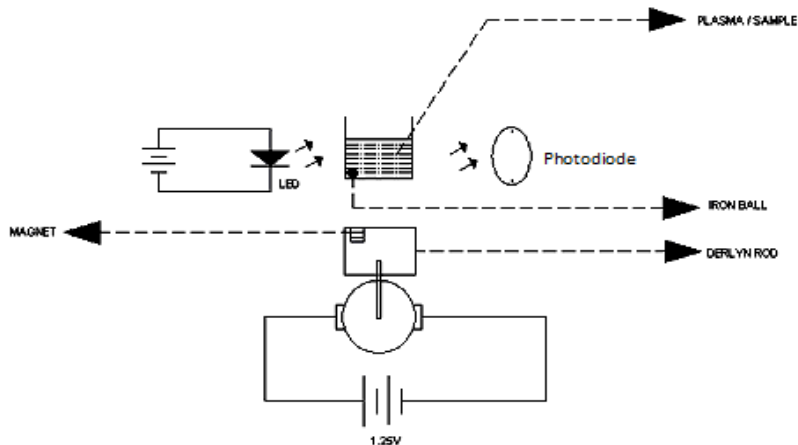


Fig. 2 Measurement Block.

4. Materials and Methods

PT reagent kit, Liquiplastin consists of PT reagent 5ml. APTT kit, Liquiceilin-E [13] consists of 5ml of APTT reagent and 5ml of activator Calcium chloride solution (25 mmol/l).

4.1. Sample Preparation

The Venous blood from the patient is collected using 2 ml syringe. About 1.8ml of the collected blood is dispensed into a test tube containing 200 micro-liter of 3.2 % tri-sodium citrate (0.11 mol/l) [14]and mixed well. The mixture is of ratio 9:1. It is then centrifuged for 15-20 minutes. The clear plasma formed as supernatant is then separated and transferred to another fresh test tube [15].

4.2. Measurement of PT

For performing Prothrombin Test, a cuvette with an iron ball is placed in a sample block. Now the sample of 50 micro- liter Plasma is taken in a cuvette. It should be incubated at 37 °C for 2 minutes by keeping into the incubation block. After that 100 micro litre of liquiplastin (clotting Reagent) is pipetted into the cuvette forcibly. As soon as the reagent is added the intensity decreases which is measured by photodiode and there by the controller starts the motor, rotating the magnet which enables the mixing of sample and reagent by rotating the iron ball inside the sample cup, simultaneously measuring the time in seconds using the timer. When the clot is formed the motor is stopped by the controller as sensed by the drastic change in intensity of light due to the increased turbidity of the sample density. The time in seconds between starting and stopping of the motor gives a measure of Prothrombin time. A mixture of plasma separated from 5-10 normal patients pooled and PT time is measured for this Fresh Normal pooled Plasma (FNPP) , the test is repeated for 5-6 times and is averaged. This average is MNPT (Mean Normal PT for reagent) and is used to calculate Karl Pearson’s Coefficient; R. R is calculated using the formula.

$$R = \frac{\text{Patient PT}}{\text{MNPT for Reagent}} \quad (1)$$

It is recommended by the WHO that MNPT should be established for each lot of PT reagents by each laboratory, since PT results are dependent on the combination of reagent lot, instrument and technique followed at each laboratory. International normalized ratio INR is calculated using R and International sensitivity Index (ISI) value that is provided by the manufacture of the reagent

$$\text{INR} = (R)^{\text{ISI}} \quad (2)$$

The INR calculation avoids the confusion of establishing the normal values as different laboratory has different normal ranges. INR index is accepted as international standard.

4.3. Measurement of APTT

In APTT 50 micro-liter Plasma is added with 50 micro liter of liquicelin-E into a sample cup containing iron ball for mixing and incubated for 2minutes. It’s then placed in the sample holder and then about 50 micro-liter of calcium chloride (25 mmol/l) solution is added. Now the motor is started and the timer in microcontroller measure time in seconds until the clot is formed which is indicated by the drastic change in the photodiode output. This measurement in seconds gives the APTT for the sample. There is no international convention like INR for APTT like PTT. Karl Pearson’s Coefficient R is calculated using the formula:

$$R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{MNAPTT of Reagent (in seconds)}} \quad (3)$$

Normal values using LIQUICELIN-E reagent are between 22-34 seconds. Between manual and Turbo densitometric instrument results a variation of 1-2 seconds may be expected. For photo optical instruments, it is recommended that each laboratory must establish normal range of their own.

5. Software

Development of software for the present system involves the following modules configuring analog and digital blocks as peripherals inside PSoC, initialization of LCD, starting ADC, reading 14 bit data signals, measurement and maintenance of temperature at 37 °C, measurement and monitoring of Changes in Photodiode output, starting the motor when the reagent is mixed with sample, stopping the motor when clot is formed, recording the clot time in seconds from the timer, calculating and displaying the results on LCD. The Flow chart for performing the above is given in the flowchart (Fig. 3).

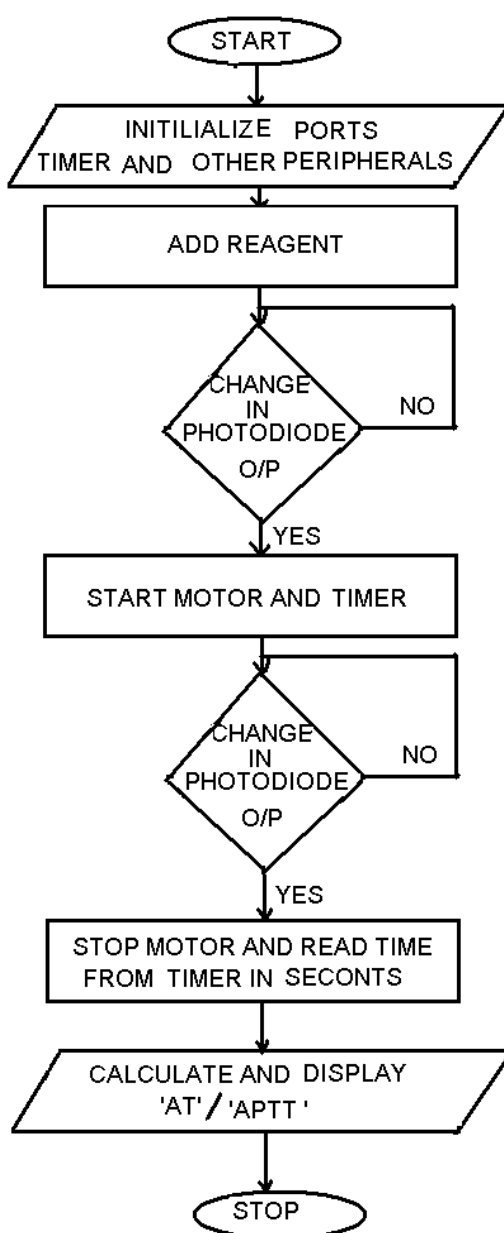


Fig. 3. Flow chart.

6. Results and Discussion

The Table 1 shows the Prothrombin time measured for 20 Patients by using the developed instrument. The Values are compared with the two other Standard Instruments URIT-160 and COAG 120. It can be seen that the values are very close to the values obtained with Standard Instruments. The Normal Value for PT is 10 to 14 seconds. It is observed that the Patients having PT above these values are suffered from internal bleeding due to diseases like Diabetes, Cancer or chronic hepatitis. MNPT is estimated to be 13.5 for the developed Instrument. ISI for the particular lot of the reagent is given as 1.6 (Reagent Manufacture's data). These values are used to calculate R and INR. APTT and calculated R values are measured for 10 samples and compared with the values of the standard instruments as shown in Table 2.

Table 1. Prothrombin Test results compared with standard instruments.

Sample	PT Sec			R			INR		
	Developed	URIT 160	COAG 120	Developed	URIT 160	COAG 120	Developed	URIT 160	COAG 120
Plasmatrol	10.2	10.9	10.6	1	1.069	1.039	1	1.113	1.063
Patient 1	13.2	12.1	12.8	1.294	1.186	1.255	1.51	1.314	1.438
Patient 2	45.3	41.2	43.8	4.441	4.039	4.294	10.863	9.333	10.294
Patient 3	13.1	12.9	13.3	1.284	1.265	1.304	1.492	1.457	1.529
Patient 4	7.5	7.5	6.9	0.735	0.735	0.676	0.611	0.611	0.534
Patient 5	13.2	13.8	14.3	1.294	1.353	1.402	1.51	1.622	1.717
Patient 6	87.4	87	92	8.569	8.529	9.02	31.095	30.863	33.754
Patient 7	12.6	13.2	12.9	1.235	1.294	1.265	1.402	1.51	1.457
Patient 8	10.8	11.3	11.2	1.059	1.108	1.098	1.096	1.178	1.161
Patient 9	54.8	55.1	54.7	5.373	5.402	5.363	14.735	14.862	14.691
Patient 10	14.5	15.3	15.6	1.422	1.5	1.529	1.756	1.913	1.973
Patient 11	13.4	13.6	14.2	1.314	1.333	1.392	1.548	1.584	1.698
Patient 12	14.2	13.5	14	1.392	1.324	1.373	1.698	1.567	1.661
Patient 13	12.6	13.7	14.1	1.235	1.343	1.382	1.402	1.603	1.678
Patient 14	32.2	30	33.2	3.157	2.941	3.255	6.293	5.618	6.608
Patient 15	28.3	30.7	31.2	2.775	3.01	3.059	5.119	5.831	5.983
Patient 16	13	14.5	13.2	1.275	1.422	1.294	1.475	1.756	1.51
Patient 17	10.8	12.6	10.6	1.059	1.235	1.039	1.096	1.402	1.063
Patient 18	11.4	11.7	12.9	1.118	1.147	1.265	1.195	1.245	1.457
Patient 19	15.5	14.1	14.9	1.52	1.382	1.461	1.954	1.678	1.834

Table 2. Test results of PT and APTT of PLASMATROL with Developed Instrument.

PT Sec	R	INR	APTT Sec	R
13.8	1.022	1.035	34.1	1.033
14.0	1.037	1.06	33.8	1.024
13.1	0.97	0.952	33.1	1.003
12.9	0.956	0.931	33.0	1
13.5	1	1	32.9	0.997
13.8	1.022	1.035	34.5	1.045
13.8	1.022	1.035	32.8	0.994
14.1	1.044	1.071	34.0	1.03
13.2	0.978	0.965	33.4	1.012
13.9	1.03	1.048	33.5	1.015

7. Validation of Developed Instrument

The developed Instrument is validated using Quality control for its known values and ranges. PLASMATROL H-I normal plasma control is used to check the PT and APTT values on the developed Instrument. It was observed that the values are very close to the target and well within the establish range. Table 3 shows the target and ranges for APTT and PT for plasmatrol control Lot no.309106. Table 4 shows the PT and APTT values obtained with Developed Instrument best agrees with the Quality control targets assuring the validation of the developed instrument.

Table 3. PLASMATROL H-I normal control data.

LOT No	PT		APTT	
	Mean Sec	Range Sec	Mean Sec	Range Sec
309106	13.5	10.5-16.5	33.0	31-47

Table 4. Activated Partial Thromboplastin Time in Seconds by Using the developed Instrument and Standard Instruments.

Sample	Developed	URIT 160	COAG120	R		
				Developed	URIT160	COAG120
Patient 1	32.8	32.3	31.1	0.959	0.944	0.909
Patient 2	33.1	33	32.6	0.968	0.965	0.953
Patient 3	71.8	70.9	70	2.099	2.073	2.047
Patient 4	28.9	29.1	28.5	0.845	0.851	0.833
Patient 5	35.6	34.8	35.1	1.041	1.018	1.026
Patient 6	31.5	31.5	31	0.921	0.921	0.906
Patient 7	29.6	28.9	29.2	0.865	0.845	0.854
Patient 8	25.4	26	26.8	0.743	0.76	0.784
Patient 9	30.1	30.7	31.3	0.88	0.898	0.915
Plasmatrol	37.5	36.7	38.9	1.096	1.073	1.137

7.1. Precision and Accuracy

The test results of Quality control PLASMATROL as shown in Table 4 explain a good precision and accuracy of the developed instrument. The S.D is 0.41 for PT (Sec) and 0.57 for APTT (Sec). The % error between observed values and the Target value as per table 4 is less than 1 %.

7.2. Statistical Study- PT

The statistical studies prove that the results obtained by using the developed instrument highly correlate with the results obtained using the standard Instruments. The Plotted Regression lines between PT results of developed and standard instruments in Fig 4 (a) and 4(b) approximates the line of Equality which confirms that, the developed Instrument is best fit with the standard Instruments. It is arrived by calculating the Karl Pearson's Coefficient of R and INR which shows the good agreement between the developed Instrument and the Standard Instruments. Fig. 4(c) and 4(d) shows linear regression analysis of R and Figs. 4(e) and 4(f) of that of INR as compared with standard instruments. The correlation between the Developed instrument and the standard ones were observed to be of 99 %.

Table 4 gives APTT measured for 10 Patients by using the designed Instrument. The values are compared with that of Standard Instruments. Values of corresponding Karl's pearson Coefficient R are calculated The Normal values of APTT vary from 25-35 seconds. The coefficient R is calculated.

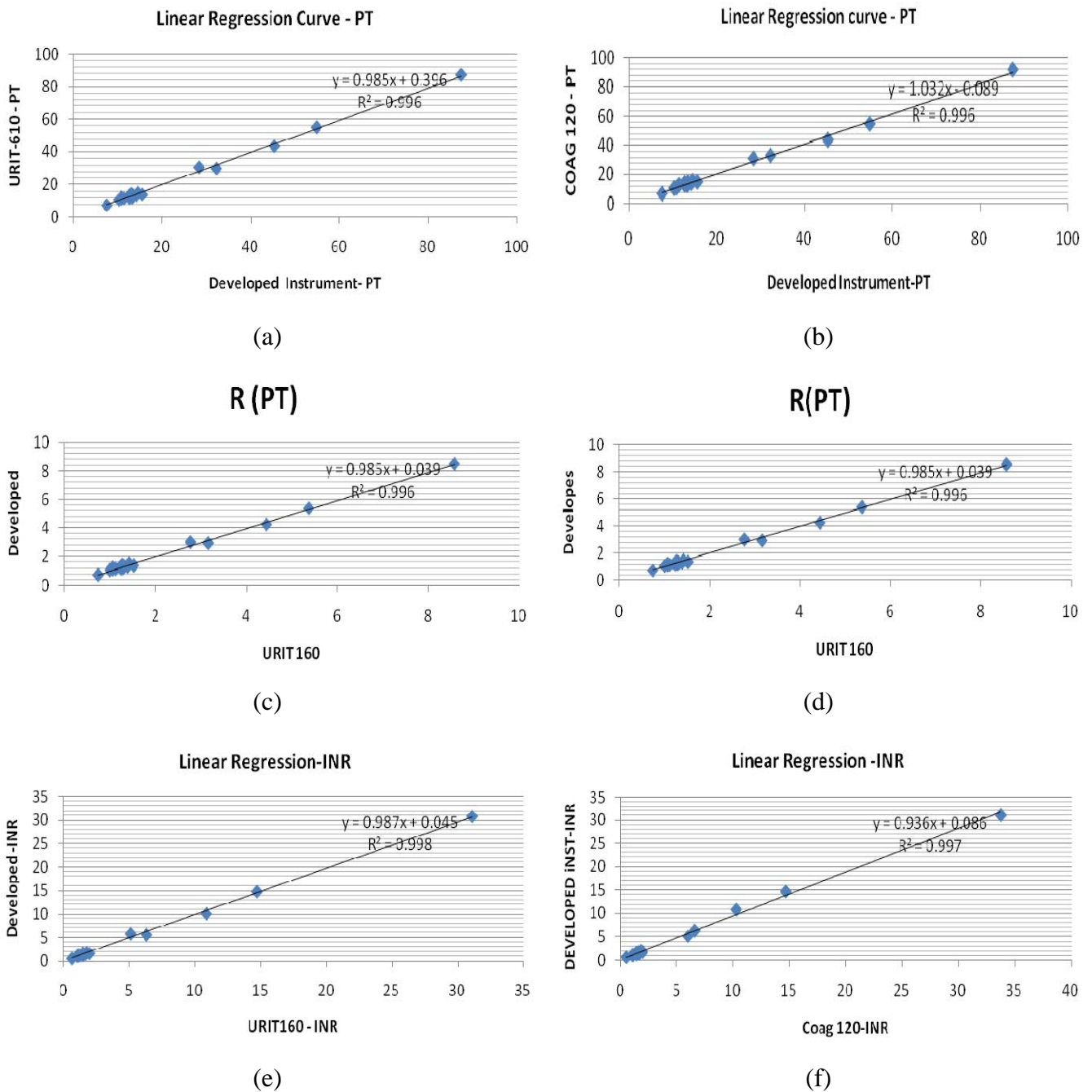


Fig. 4. Linear Regression PT and INR curves.

7.3. Statistical Study- APTT

The linear regression study of compared values between the developed Instrument with the standard instrument shows a good correlation of 99 %. Figs. 5(a) and 5(b) shows the regression line for APTT and 5(c) and 5(d) that of R as compared with standard instruments. MNAPTT is estimated to be 34.2 which is used to calculate R, Karl's pearson Coefficient. It's observed that the slope of linear regression is close to 1.

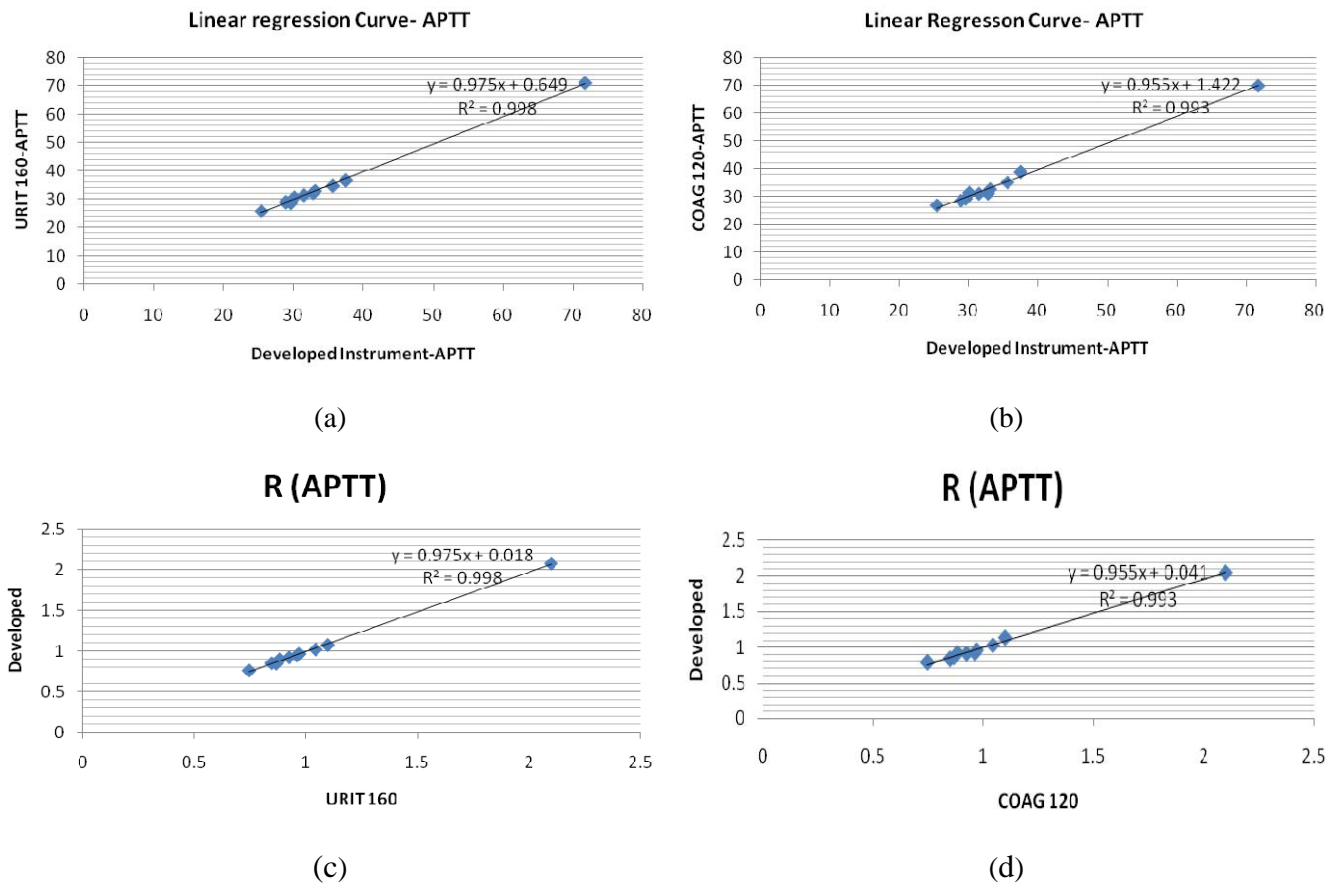


Fig. 5. Linear Regression APPT curve.

7.4. Interpretation on Results

Interpretation of INR results may give a clear picture of the patient’s condition. INR level above 5 indicates that there is a high chance of bleeding, In cases where the INR is 5 or less there is a high chance of having a clot [16]. Normal range for a healthy person is 0.9–2.0. For people on warfarin (anticoagulant) therapy INR falls between 2.5–5.0 [17]. Warfarin is effectively used in cases of arterial fibrillations [18]. INR arrived from PT measurement provides an excellent monitoring of warfarin dosage [10]. From the tables 3 patients 2 and 6, showing higher INR were reported to be under anticoagulant therapy against blood clot disease conditions like Clot in cardiac vessels, Thrombosis, Thromboembolism or deep vein thrombosis (DVT). Patients 14 and 15 were reported with internal bleeding due to liver dysfunction. An estimation of R and INR enables the physician in deciding the anticoagulant dosage and further course of therapy.

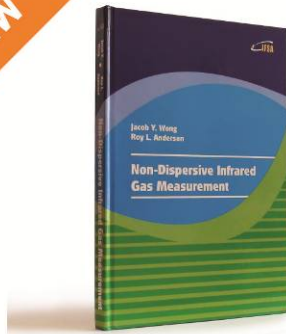
8. Conclusion

The Instrument for Blood coagulation measurement is designed and developed which plays a vital role in bio medical Instrumentation. The value of PT and APTT measurements are compared with the standard instruments URIT-160 and COAG-120. It is found that the values are well suited with that of above said Standard Instruments. Moreover the system is easy to operate and does not require any skilled persons. This blood clotting time machine is low cost, portable and user friendly diagnostic tool for physicians. The present instrument developed can perform tests PT and APTT. However the software can be extended for performing the other Blood clotting factor assays.

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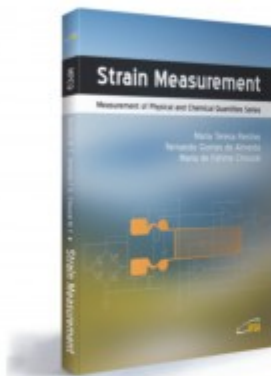
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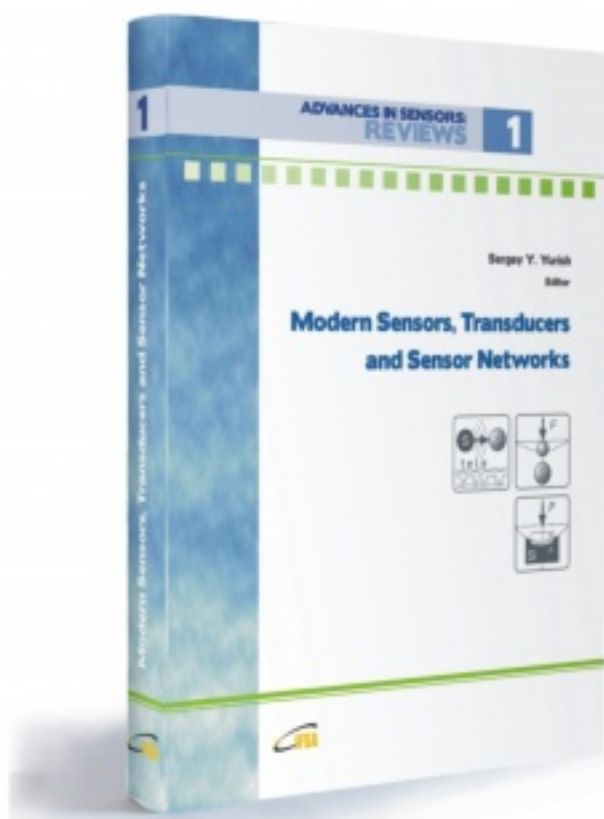
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