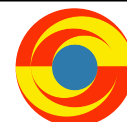
International Journal of Science and Qualitative Analysis
2015; 1(1): 6-10
Published online May 27, 2015 (http://www.sciencepublishinggroup.com/j/ijsqa)
doi: 10.11648/j.ijsqa.20150101.12



SciencePG
Science Publishing Group

The Impact of Enzymes in the Hepatic Function

Alma Daja[1, *], Entela Treska[2]

[1]Clinical and Biochemical Laboratory, Health Centre nr 2, Tirana, Albania
[2]University Hospital of Obstetrics and Gynaecology "Queen Geraldine", Tirana, Albania

Email address:
almadaja@live.com (A. Daja), entela.t@hotmail.com (E. Treska)

To cite this article:
Alma Daja, Entela Treska. The Impact of Enzymes in the Hepatic Function. *International Journal of Science and Qualitative Analysis*. Vol. 1, No. 1, 2015, pp. 6-10. doi: 10.11648/j.ijsqa.20150101.12

Abstract: The aim of this study is the application of Alkaline Phosphatase (ALP) and Gamma-glutamyl Transferase (GGT) activity, by using the corresponding kinetic methods, at the Clinical Chemistry Laboratories in our place. Enzymes are proteins synthesized in the cells of living organisms in order to play the role of bio-catalysts. Today are recognized about 2000 different enzymes. Some of those have been isolated in pure form of the chemical. We used serum as a material and we took into consideration 15 healthy patients, 15 patients who had viral hepatitis and 15 patients with idiopathic hyper-bilirubinemia. Solution where the substance is being analyzed, was treated with specific reagents according to the procedures such as incubation, heating, etc, until the color is formed. The absorbance of the coloring solution was measured using photometry at a wavelength of 405nm. As an analytical method for the determination of ALP, we chose 4-nitrophenylphosphate (4NPP) as a substrate, while as a buffer for the optimal pH adjustment of the reaction we used 2-amino-2 methyl -1-propanol (AMP). ALP was within permitted values from 100 to 290 U /l, whereas Gamma-glutamyl Transferase was at normal reference values of 5 to 51 U /l. Automated method was equal to the standard method, with a correlation coefficient of r = 0997. Accuracy within the series of measurements had a variation coefficient of only 1.25%. From the results taken from the laboratories, we had an increased precision, accuracy, sensitivity and reliability as a result of the technological process of electronic engineering of new modern techniques with monoclonal antibodies and immobilized enzymes.

Keywords: Clinical Chemistry, ALP Determination, GGT Assay Kit, Enzyme Classification, Hepatic Enzymes, Photometry

1. Aim of the Study

- The application of Alkaline Phosphatase and Gamma-glutamyl Transferase activity by using the corresponding kinetic methods, at the Clinical Chemistry Laboratories in our place.
- The study of the precision of both methods for the clinical chemistry requirements according to the International Federation of Clinical Chemistry (IFCC).
- The comparison of the above methods to the standard methods recommended by IFCC, to estimate the application possibilities in clinical chemistry practice.

2. Introduction

2.1. Introduction to Enzymes

Enzymes are proteins synthesized in the cells of living organisms in order to play the role of bio-catalysts. Enzymes accelerate substantially all those biochemical reactions that are thermodynamically possible. According to the definitions of Ostel, catalysts are substances that accelerate chemical reactions without interfering at the reaction products. Some typical examples of catalysts are well known in the field of organic chemistry. It is also well known the fact that some metals like nickel (Ni) and platinum (Pt), are used in very small quantities to accelerate chemical reactions and to create conditions of equilibrium as soon as possible [1,2]. Like these enzymatic catalysts, the cells also express the action in catalytic amounts, namely in concentrations lower than those of the substances participating in the reaction catalyzed by the enzyme. In the role of bio-catalysts, enzymes increase the speed of biochemical reactions without interfering in balance, determined by the law of mass action [3].

2.2. Classification of Enzymes

Today are recognized about 2000 different enzymes. Some of these enzymes have been isolated in the pure form of the chemical.

Different studies have shown that all the enzymes are divided into groups:

- Simple enzymes

- Composed enzymes

There have been many proposals on the classification of enzymes. Today stands the classification proposed by the Enzymes Comity (EC) at the International Federation of Clinical Chemistry (IFCC). This committee has taken into consideration 800 different enzymes [4,5]. After this review, is made the proposal that the classification of enzymes must be based on specificity. We have analyzed Alkaline Phosphatase (ALP) and Gamma-glutamyl Transferase (GGT), by using the corresponding work protocols [6].

2.3. Early Enzyme Discoveries

The existence of enzymes has been known for well over a century. Some of the earliest studies were performed in 1835 by the Swedish chemist Jon Jakob Berzelius who termed their chemical action catalytic. It was not until 1926, that the first enzyme was obtained in pure form, a feat accomplished by James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work was to make him earn the 1947 Nobel Prize. John H. Northrop and Wendell M. Stanley of the Rockefeller Institute for Medical Research shared the 1947 Nobel Prize with Sumner. They discovered a complex procedure for isolating pepsin. This precipitation technique devised by Northrop and Stanley has been used to crystallize several enzymes [7,8].

3. Material and Methods

We used serum as a material and we took into consideration 15 healthy patients, 15 patients who had viral hepatitis and 15 patients with idiopathic hyper-bilirubinemia. At a biological environment, which has a complex composition, it is quite appropriate to measure the speed of an enzymatic reaction and factors affecting that are as follows:

- Concentration of the substrate,
- The temperature of the environment where the enzymatic reaction will take place,
- pH of the environment,
- The presence of factors which may be enzymatic activators or inhibitors.

3.1. Clinical Chemistry Analyzers Technology

Clinical chemistry analyzers run assays on clinical samples such as blood serum, plasma, urine, and cerebrospinal fluid to detect the presence of a component relating to disease or drugs. Clinical chemistry analyzers are used in a variety of settings, including small clinics, research labs, and high-throughput hospital labs. They are also used at the point-of-care, such as in physicians' offices and patient bedsides [9].

Analytes (components) commonly include enzymes, substrates, electrolytes, specific proteins, drugs of abuse, and therapeutic drugs. The results give clinicians a feedback on toxicology and on renal, cardiac, and liver function [10].

How does a clinical chemistry analyzer work?

Analyzers are highly automated to maximize throughput, to improve user safety from bio-hazards, and to diminish the risk of cross-contamination. Samples are loaded into the machine and tests are programmed by the user. A probe measures an aliquot of sample and places it into a reaction vessel. Reagents are added from an on-board refrigerated supply. Incubation time is allowed, if required; then photometric or ion-selective electrode (ISE) testing determines the concentration of analyte. Results are displayed on screen or sent to a printer or computer [11,12].

3.2. Photometry in Clinical Chemistry

Photometry lies on the basis of some techniques used today for various definitions in clinical chemistry. To complete an examination we operate as follows:

- Solution where the substance is being analyzed, is treated with specific reagents with special procedure such as incubation, heating, etc, until the color is formed.
- Measurement at 1cm Cuvette with a wavelength that corresponds to the maximum of absorption. The absorbance of the coloring solution is measured using photometry.
- At the table we have a look at the value of Σ (λT) which belongs to the substance that will be analyzed. Then the only unknown subject remains the concentration C which is calculated by the formula (1).

$$C = F/\Sigma\ (\lambda T) \tag{1}$$

Absorbance that is calculated is the one that is caused by the molecules of the substance present in the solution [13,14]. While in the analytical practice, to perform the measurement of the absorbance at the area of a visible spectrum, we use special glass containers or plastic ones, called Cuvettes (d =1cm). Their absorption should not be considered. The rule of the measurements in automatic and semi-automatic photometers are expressed in figure 1:

Figure 1. *The steps of measurements in automatic/semi-automatic Photometers*

At the CH – 100 Photometer, the scheme of the colorimetric determination is as follows in figure 2:

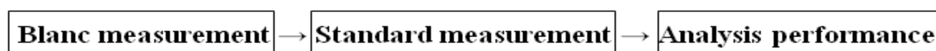


Figure 2. *Steps of measurements in CH-100 Photometer*

In clinical chemistry, each new analytical method is confronted with a method which is considered the "comparison method" or the "standard method" [15]. To confirm analytical Sclavo Diagnostics kit for Alkaline Phosphatase, we chose an optimized method from the German Sodality of Clinical Chemistry (GSCC). This optimized method is commercialized with O15-D_2 analytical kit.

3.3. Large Alkaline Phosphatase Opt Test Which Serves as a Standard Method

3.3.1. The Principle of the Method of Analytical Kit

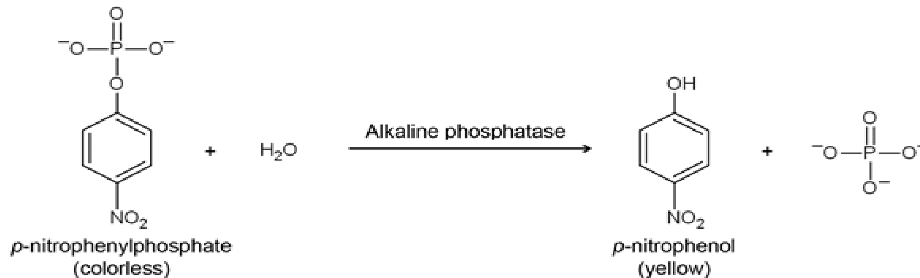


Figure 3. *Reaction characterized by alkaline phosphatase*

Final concentrations in the test, correspond to the standard concentrations recommended by the German Sodality of Clinical Chemistry (GSCC).

3.3.2. Reagents Used for the Method

- The buffer solution
- Substrate in the form of tablets

3.3.3. Preparation of Working Reagent

For each buffer solution, we dissolve a tablet of substrate. When the dissolution is completed, than the working reagent is ready to be used.

3.3.4. Measurement Conditions

- A wavelength of 405nm
- Temperature 37°C
- Serum or plasma used as a biological material
- Dilution limit of 550U / l

3.3.5. Analysis Performance

In a test tube we add 1 ml of work reagent. The tube is put in advance at the incubation at 37°C for 5 minutes. After that in the test tube we pass 0.2 ml blood serum or plasma. The solution is mixed well, and the absorbance is measured exactly after 1, 2 and 3 minutes.

ΔA is defined per minute and also the calculation is done according to the formula (2):

$$\text{Alkaline Phosphatase U/l} = \Delta A/\text{min} * 2757 \quad (2)$$

Where 2757 represents the factor for analytical kit produced by the firm of Dr. Bruno Lange (Germany).

3.4. Gamma-Glutamyl Transferase Determination Method

GGT enzyme is measured at a wave length of 405 nm. For the determination of GGT activity we use the analytical kit of Bioderice Company, Italy.

3.4.1. The Principle of the Method of Analytical Kit

GGT catalyses the transfer of the γ-glutamyl groups between donor and acceptor molecules. It is located in the cell membrane of all human cells and tissues and functions to transport amino acids into the cell [16].

Most commercial analytical methods for GGT make use of one of two synthetic substrates γ-L-glutamyl-p-nitroanilide and γ-L-glutamyl-3-carboxy-4-nitroanilide. The carboxy substrate is currently preferred because of a higher solubility and hydrolysis of the substrate does not occur spontaneously [17]. The older un-substituted substrate is more widely used. The reaction catalyzed by GGT is shown below:

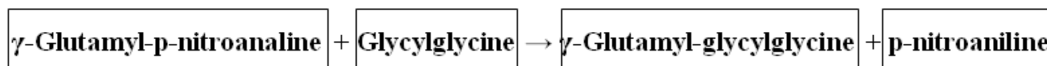


Figure 4. *Reaction catalyzed by Gamma-glutamyl Transferase*

Reaction can be monitored by following the increasing absorbance of the respective product at 405 nm. Glycylclycine is used as a buffer to maintain a pH of 8. Magnesium ions are added when p-nitroaniline is used to facilitate solubility. Although most of the enzyme originates from the liver, a small amount of GGT is released from the prostate, which may explain why man have slightly higher values than women [18].

3.4.2. Reagents Used for the Method

- reagent 1 (R1): glycylglycine 50mmol / l
- buffer: AMPD, pH = 8.1 100mmol / l
- reagent 2 (R2): γ-Glutamyl-p-nitroanaline 4mmol/l

3.4.3. Preparation of Working Reagent

Lyophilized substrate (R2 reagent) was dissolved in a buffer solution (R1 reagent). The solution formed, was

shaken to complete the digestion. Working reagent is stable 5 days at room temperature and 3 weeks in the refrigerator from 2-8°C.

3.4.4. Measurement Conditions

- A wavelength of 405 nm
- Incubation temperature 37°C
- Calibration of the apparatus against distilled water

3.4.5. Analysis Performance

In a test tube we add 1 ml working reagent. We refill the tube with 0.1 ml serum or plasma. The solution is stirred well. Environmental reaction is incubated at 37 degrees and thereafter absorbance is measured. Absorbance measurement is repeated after 2 and 3 minutes. Activity is calculated according to the formula (3):

$$GGT\ (U/l) = \Delta A/min * 1158 \tag{3}$$

Where 1158 is the factor of the analytical kit. The normal value of GGT according to the laboratory is 5-51 U /l.

3.5. Automation of Lysa 500 Plus Method, for Alkaline Phosphatase Determination

As an analytical method for the determination of ALP, we chose as a substrate 4-nitrophenylphosphate (4NPP) while as a buffer for the optimal pH adjustment of the reaction we used 2-amino-2 methyl -1-propanol (AMP). Under the optimal conditions, ALP that is present in biological materials, catalyzes this transformation reaction.

The parameters of the program are:

- Temperature 37°C
- The first wavelength 405nm
- The second wavelength 600nm
- Kinetic analytical method
- Positive slope
- The ratio biological material : working reagent is 1:50
- The volume of blood serum 4 µl
- Volume of reagent 200 µl
- Incubation time 60 seconds
- Absorbance Changes time 120 seconds
- Reagent limits 0 - 0.8
- Linearity limit 0-1000 U /l
- Sensitivity 0.37 ΔmA per U /l

4. Results and Discussion

From the results taken from the laboratories (table 1, 2, 3) we have an increased precision, accuracy, sensitivity and reliability as a result of the technological process of electronic engineering of new modern techniques with monoclonal antibodies and immobilized enzymes.

Table 1. *Hepatic parameter measurements of 15 healthy cases*

Type of analysis	Nr of cases	Mean value	Standard deviation
Total bilirubin	15	0.65	0.25
Direct bilirubin	15	0.27	0.016
SGOT	15	28	10
SGPT	15	19	9
GGT	15	19	9
ALP	15	156	50

Table 2. *Hepatic parameter measurements of 15 cases with idiopatic hyperbilirubinemie*

Type of analysis	Nr of cases	Mean value	Standard deviation	T-test	Probability P
Total bilirubin	15	1.7	0.4	$T_{exp} > T_{crit}$	$P < 0.05$
Direct bilirubin	15	0.35	0.15	$T_{exp} > T_{crit}$	$P < 0.05$
SGOT	15	26	9	$T_{exp} > T_{crit}$	$P > 0.05$
SGPT	15	21	11	$T_{exp} > T_{crit}$	$P > 0.05$
GGT	15	27	14	$T_{exp} > T_{crit}$	$P > 0.05$
ALP	15	97	52	$T_{exp} > T_{crit}$	$P > 0.05$

Table 3. *Hepatic parameter measurements of 15 viral hepatic cases*

Type of analysis	Nr of cases	Mean value	Standard deviation	T-test	Probability P
Total bilirubin	15	1.36	0.75	$T_{exp} > T_{crit}$	$P < 0.05$
Direct bilirubin	15	0.3	0.2	$T_{exp} > T_{crit}$	$P < 0.05$
SGOT	15	27	506	$T_{exp} > T_{crit}$	$P < 0.05$
SGPT	15	322	497	$T_{exp} > T_{crit}$	$P < 0.05$
GGT	15	206	237	$T_{exp} > T_{crit}$	$P < 0.05$
ALP	15	210	96	$T_{exp} < T_{crit}$	$P > 0.05$

Many determinations that in the past were made with radiometric method and chromatography, today are successfully made using photometry. Photometric techniques are gaining increasingly more important field in other sectors traditionally occupied by other methodologies. Today we perform enzymatic dosage at a standard temperature of 30°C or 37°C and rarely at 25°C.

When the reaction time is short, it is sufficient to pre-incubate the subject in a thermostat at a reaction mixture temperature recommended by the method. In modern photometers, this procedure is not necessary. Today photometers are designed to perform measurements in kinetic, allowing a more accurate control of the temperature. Requirements to measure the speed of the enzymatic reaction within a short time, or the starting speed determination of the reaction, are due to the fact that the more reaction is developed, the more different factors will interfere at the reaction velocity. Therefore it is necessary to perform kinetic enzymatic dosages within a short period of time. The same tests were performed using Lysa 500plus method.

With normal classical method, the analyst is obligated to repeat mechanically many times the same analytical link such as the photometric reading, incubation time etc, which leads to fatigue and reduced attention of the analyst. All this leads to an increased analytical inaccuracy associated not only with a progressive increase of random errors but also serious errors.

Some of these errors are:

- The transformations of analytical samples,
- Photometric readings,
- Calculation errors,
- Recording the measurement results etc

Secondly the increase of analysis number is followed by different problems of organizational and economic character. Today these difficulties are avoided increasingly, using automatic analysis system or simply said auto-analyzer, the benefits of which are:

- Automated kinetic method for the determination of ALP is possible using commercial kits for this determination,
- Automated method is equal to the standard method, with a correlation coefficient of r = 0997,
- Accuracy within the series of measurements has a variation coefficient of only 1.25%.

5. Conclusions

- Table 1, on the basis of the examination of 15 healthy subjects, we observed that ALP was within permitted values from 100 to 290 U /l, whereas Gamma-glutamyl Transferase was at normal reference values of 5 to 51 U /l.
- In Table 2, we made the examination of 15 subjects with idiopathic Hyperbilirubinemie suffered from *cholecyst* and *cholangitis*.
- In Table 3, we examined 15 subjects with mechanical icter, where we had a significant increase of the activity of alkaline phosphatase and Gamma-glutamyl Transferase, which was confirmed by the X-ray examination of the bile roads, that resulted to be blocked.

Blockage can be caused by:

- Infections
- Liver cancer
- Cancer metastases of the liver
- Liver cirrhosis (severe damage of the functional liver tissues).

References

[1] Bennett, T. P., and Frieden, E.: Modern Topics in Biochemistry, pg. 43-45, Macmillan, London (1969).

[2] Holum, J.: Elements of General and Biological Chemistry, 2nd ed., 377, Wiley, NY (1968).

[3] Martinek, R.: Practical Clinical Enzymology: J. Am. Med. Tech., 31, 162 (1969).

[4] Harrow, B., and Mazur, A.: Textbook of Biochemistry, 109, Saunders, Philadelphia (1958).

[5] Pfeiffer, J.: Enzymes, the Physics and Chemistry of Life, pg 171-173, Simon and Schuster, NY (1954)

[6] Tietz, N. W. Ed., Fundamentals of Clinical Chemistry (W. B. Saunders Co., Philadelphia, Pa., 1970).

[7] Stryer L, Berg JM, Tymoczko JL (2002).*Biochemistry* (5th ed.). San Francisco: W.H. Freeman. ISBN 0-7167-4955-6.

[8] Schomburg I, Chang A, Placzek S, Söhngen C, Rother M, Lang M et al. (Jan 2013). "BRENDA in 2013: integrated reactions, kinetic data, enzyme function data, improved disease classification: new options and contents in BRENDA". *Nucleic Acids Research* 41 (Database issue): D764–72. doi: 10.1093/ nar/gks 1049. PMID 23203881.

[9] Radzicka A, Wolfenden R (Jan 1995). "A proficient enzyme". *Science* 267 (5194): 90–931. doi:10.1126/science.7809611. PMID 7809611.

[10] Callahan BP, Miller BG (Dec 2007). "OMP decarboxylase--An enigma persists".*Bioorganic Chemistry* 35 (6): 465–9. doi: 10.1016/j.bioorg. 2007.07.004 PMID 17889251.

[11] Horton, R. Moran, L.A. Scrimgeour, G. Perry, M. Rawn, D. (2005) Principles of Biochemistry 4th Edition, Pearson Education (US)

[12] Becker, W. Kleinsmith, L.J. Hardin, J. Bertoni, G.P. (2008) The World of the Cell, Benjamin Cummings

[13] Anon. , Clini cal Me thods Manual, Bausch & Lomb , Inc., Rochester, N.Y. (1965)

[14] Johnston DE (1999). "Special considerations in interpreting liver function tests". *Am Fam Physician* 59 (8): 2223–30. PMID 10221307.

[15] McClatchey, Kenneth D. (2002). *Clinical laboratory medicine*. Lippincott Williams & Wilkins. pp. 288–. ISBN 978-0-683-30751-1. Retrieved 5 August 2011.

[16] Mengel, Mark B.; Schwiebert, L. Peter (2005). *Family medicine: ambulatory care & prevention*. McGraw-Hill Professional. pp. 268–. ISBN 978-0-07-142322-9. Retrieved5 August 2011.

[17] Kennedy I.W. “User evaluation of precision performance of clinical chemistry devices”, NCCLS, Vol 4, No 8, page 185-195, 1989

[18] Wachtel M. “Creation and verification of reference intervals laboratory medicine”, No 26, page 593-597, 1995.