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REFRACTOMETRY AS A TOOL IN DIABETIC STUDIES

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ABSTRACT : The refractive index as well as molar refraction, is the true index of purity of substance and plays a vital role in solution chemistry. A small addition of a foreign substance either in solid state of liquid form is going to effect the refractive index.

As such the variation of refractive indices in pure glucose solution as a function of concentration is studied in detail and this principle is extended to the study of the refractive indices of urine solution of diabetic patients. The refractive indices are measured by spectrometry and abbe refractometry. A detailed study of variation of refractive indices of urine samples containing different sugar concentrations, of patients of different age groups revealed that the increase in refractive index follows a linear scale and can be explained by the equation, $n=n_o\ [1+0.0025\log\ (a\ s)^{1/4}]\ [1+0.03\log 0.011C]$. These study provided an opportunity to project refractometry as an effective tool in diagnosing the diabetic level of a patient by making use of a simple calibration curve of increment in refractive index 'Δn', as a function of level of the disease.

KEYWORDS: Refractive index, spectrometer, abbe refractometer, Diabetes.

INTRODUCTION

Refraction is the bending of light as it passes between two transparent substances. Refractometers, instruments that measure the angle of refraction between air and aqueous solution, allow the rapid and inexpensive determination of solute concentrations in various fluids. Refractometers are especially useful for rapid screening tests in veterinary and medical laboratories because no chemical manipulations are required; merely the samples interaction with light produces a clinically useful reading. Refractometers are the standard instruments for determining protein concentration in plasma and body cavity fluids samples, and urine solute concentration. (Duncan *et al*., 1994 and Wolf *et al*., 1969).

The Refractometer has almost completely replaced gravimetric techniques, such as urinometry, for determination of urine specific gravity, due to the advantages of small sample size, speed of analysis, better accuracy, and relatively low cost (Wolf *et al*., 1969 and Dorizzi *et al*., 1987). Refractometers also have certain biological applications. Refractive index increments are necessary in determining the effect of certain drug compounds.

Ex: Valepotriates (Tittles *et al*., 1978). Refractive index may support tissue characterization for research and diagnostic purposes in cosmetic/pharmacy and medicine respectively (Knuettel *et al*., 2001). Also refractive index is a quantitative indicator of corneal hydration (Young et al., 2004). Refractometers also find its application in chemicals, agriculture, Food industry, Beverage industry and Petro chemicals. According to the paper report by 2005, 194 million people are currently affected with diabetes worldwide. India alone currently counts over 35 million and the counts may reach up to 73.5 million by 2025.

The present work provides an easy method in estimating the glucose concentration of diabetic urine samples, basing on corresponding changes in refractive indices with concentration by using refractometry as a technique.

MATERIALS AND METHODS

Preparation of the sample

The samples used in spectrometer and abbe refractometer are the diabetic urine samples. Urine samples of different diabetic patients of varying glucose concentration and age are collected from the local diagnostic laboratories and hospitals (Sathya Sai Diagnostic Laboratory, Srinivasa diagnostic Laboratories, Kotnis Praja Vydhyasala, Vijaya Durga Laboratories) in clean, dry, stoppered bottles. Samples are freshly used to calculate the refractive index.

METHODOLOGY

The Optics of Refraction:

Light travels more slowly on liquids and solids than it does in gases or in vacuum, and it changes its path depending on the medium. The change in speed causes the light to change direction as it passes between materials of different densities (Wolf, 1966). A physical characteristic of any material in its index of refraction(n), a constant related to the ratio of the speed of light in a vacuum to the speed of light in that medium. Snell's law of refraction defines the behavior of light as it passes between two media according to the angles made by the incident ray(e) and the refracted ray(e) and the indices of the refraction of the two materials:

$$n_i \sin é_i = n_r \sin é_r$$

For an incident ray Snell's law becomes the more familiar equation for n:

$$n = \frac{\sin é_i}{\sin é_r}$$

The value of n varies according to the wavelength of the incident light (Jeanne, 2001). The emission band of sodium, a yellow light, is commonly used. Multiple factors influence the n of an aqueous solution: the solute, its concentration, and the temperature of the solution (Naumann, 1964). Light passes through the sample and then through a series of prisms, producing a distinct line of demarcation on a reticule. Multiple prisms reduce dispersion of white light, allowing the use of room light for illumination (Pesalk, 2001).

In both techniques spectrometer and abbe refractometer are used to calculate the refractive indices of diabetic patients and correlate the glucose concentration in blood. The results drawn are compared with each other and finally inserted in a formula, which is used in estimating the glucose concentration of diabetic urine samples.

SPECTROMETER:

Before using the spectrometer for the experimental observation, the necessary optical and mechanical adjustments are to be made. The sample is taken in a hollow prism and the refractive indices are determined by the spectrometric methods using a research spectrometer (Andhra Scientific Company (ASCO), INDIA).

ABBEREFRACTOMETER:

The most common instrument used to measure the refractive index is the abbe refractometer. Urine samples are collected from different diabetic patients used for analysis.

Procedure: Diabetic urine samples of different age groups with varying degree of glucose concentration are collected in a clean and dry small bottles. A dropper may be used to transfer the fresh sample (urine sample) and applied on the surface that is between the two prisms of the abbe refractometer. The dropper should be plastic to minimize the possibility of scratching the prism surface. Scratching or marring the sample plate may cause blurring of the demarcation line for reading results. Wiping the platen with distilled water and a soft tissue is the cleaning method recommended. A light and dark band is observed through the eyepiece of the telescope. To obtain the optimum contrast between light and dark boundary, the instrument must be properly tilted towards the window or the light source. Increased sharpness of the boundary may be obtained by using a vertical, gold color fluorescent lamp. The observed band is adjusted with in the intersection of the cross wired exactly, by adjusting the screw provided. Read the appropriate scale through separate eyepiece at the point where the dividing line between bright and dark fields cross. A so ft cloth or tissue moistened with water is generally used to wipe the prism and dried thoroughly. The utilization of the instrument is very easy and the result can be obtained with in few minutes.

RESULTS AND DISCUSSIONS

The refractive indices of glucose solution at different concentrations have been measured by spectrometric and abbe refractometric methods and are presented in Table I. From the Table I an increase in refractive index of glucose with an increase in concentration is observed. It is quantified approximately, $n = n_0$ [1=0.0025m] where, 'm' is the mass of the glucose in 100ml of water. From the table the refractive indices at concentrations of 0.6grams, is reported to be 1.3290 and 1.330 by the experimental methods and 1.3290 by the formula.

Taking the clue from the above experiment the refractive indices of urine samples collected from different diabetic patients of different age groups and different gravity of the disease re collected and the refractive indices are determined. They are presented in Table II,III and IV.

Table II shows that there is an increase in refractive index values with the increase in refractive index values with the increase in urine glucose concentration in blood, the glucose passes in the urine(0.35-0.5% (trace), (0.5-1%(+), 1-1.5%(++), 1.5-2%(+++), greater than 2% (++++) the values obtained are quantified by using the formula, $n = n_0 (1+0.0025 \log_{10}s)$ where the values of s are 1,2,4,6,8 and 10 for normal, trace, +,++,+++,++++ states respectively. And 'n_0 'is the refractive index in fasting state and n is the refractive index in post–prandial state. Here refractive index is calculated based on the urine glucose concentration. For example the value obtained through Spectrometer for a patient of 60 years of age is 1.3340 and by abbe refractomerter is 1.334, which has close resemblance to the calculated value 1.3331.

Table III represent the refractive index values drawn by spectrometer. The data indicated that there is no marked variation in refractive indices of normal patient's urine sample in fasting and after break fast (post prandial). Slight variation in some samples may be due to other components present in urine. No change in refractive index may represent healthy condition of the particular patient. How ever quantification is possible based on the age and urine glucose concentration by the formula i.e. $n = n_0 [1+0.0025 \log_{10}(a\ s)^{1/4}]$. Where as the age ranging form 25-34,35-44,45-54,55-64,65 and above as equal to 0,2,4,2,6,8,10 and s is the urine glucose concentration. (S=1,2,3,6,8,10 for normal, trace, +, ++, +++,and ++++ states respectively. For example a patient having the age of 32 years has the refractive index 1.3338 drawn through Spectrometer. Where the calculated value is 1.3340, which is close to the Spectrometer value.

Table IV depicted increase in refractive index values of diabetic urine samples with the increased glucose concentration, obtained both by the Spectrometer and Abbe Refractometer. There is a possibility of quantifying the values to some extent by using the formula $n = n_0 [1=0.0025 \log (as)^{1/4}] [1+0.03\log 0.011C]$, where n_0 is the refractive index in free state has the values 2,4,6,8 and 10 for age of the patients in the range, 25-34,35-44,45-54,55-64 and above 65.s has the values of 1,2,4,6,8and 10 for normal, trace, +, ++, +++, ++++ types. C is the glucose concentration in mg%. Here the refractive index can be calculated by using any available data, either glucose concentration, age or urine glucose concentration. For example for patient having the glucose concentration of 233mg%, age of 68 years having the urine concentration of ++, show the refractive index drawn through spectrometer is 1.3420 and that of refractometer is 1.340, where both values show resemblance to the calculated value 1.3391 obtained by the above formula.

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TABLE I
REFRACTIVE INDEX OF GLUCOSE SOLUTION

Glucose Concentration g%	ABBEREFRACTOMETER Obtained	Calculated	SPECTROMETER Obtained	Calculated
	1.3270			
0.1	1.3270	1.3237	1.3296	1.3243
0.2	1.3270	1.3243	1.3300	1.3280
0.3	1.3280	1.3280	1.3300	1.3284
0.4	1.3280	1.3283	1.3304	1.3287
0.5	1.3280	1.3286	1.3304	1.3290
0.6	1.3290	1.3290	1.3304	1.3293
0.7	1.3300	1.3293	1.3308	1.3297
0.8	1.3300	1.3296	1.3326	1.3300
0.9	1.3300	1.3300	1.3334	1.3310
1.0	1.3320	1.3303	1.3320	1.3306
1.1	1.3320	1.3306	1.3320	1.3310
1.2	1.3320	1.3310	1.3320	1.3313
1.3	1.3330	1.3313	1.3330	1.3313
1.4	1.3340	1.3316	1.3340	1.3316
1.5	1.3340	1.3320	1.3350	1.3324
1.6	1.3350	1.3324	1.3360	1.3326
1.7	1.3360	1.3326	1.3360	1.3330
1.8	1.3360	1.3330	1.3370	1.3333
1.9	1.3370	1.3333	1.3370	1.3333
2.0	1.3370	1.3336	1.3370	1.3336

TABLE II
REFRACTIVE INDEX OF DIABETIC URINE SAMPLES DRAWN DURING FASTING AND POST PANDRIAL (SPECTROMETRIC METHODS)

S. No	Age	Urine Glucose Concentration	Fasting	Post Prandial	Calculated Value
1	32	Trace	1.3320	1.3326	1.3330
2	70	Trace	1.3352	1.3360	1.3362
3	25	Trace	1.3296	1.3300	1.3306

4	42	+	1.3330	1.3348	1.3351
5 35	+	1.3334 1.3358 1.3354			
6 60	++	1.3334 1.3412 1.3364			
7 56	++	1.3344 1.3360 1.3369			
8 53	++	1.3378 1.3390 1.3404			
9 40	+++	1.3434 1.3440 1.3464			
10	62	++++	1.3364	1.3434	1.3404

TABLE III
REFRACTIVE INDEX OF NORMAL PATIENTS URINE SAMPLE THROUGH SPECTROMETER

S.NO	Age	Fasting	Post Prandial	Calculated value
25-34				
1	25	1.3334	1.3334	1.3337
2	32	1.3338	1.3340	1.3340
35-44				
3	35	1.3304	1.3309	1.3309
1.3338 1.3343				
45-54				
5	48	1.3340	1.3346	1.3346
1.3358 1.3362				
1.3327 1.3377				
55-64				
8	60	1.3300	1.3308	1.3308
65>				
9	72	1.3434	1.3442	1.3442
10	81	1.3344	1.3352	1.3352

TABLE –IV
INCREASED REFRACTIVE INDEX OF DIABETIC URINE SAMPLES WITH INCREASED GLUCOSE CONCETRATION (THROUGH SPECTROMETER AND REFRACTOMETER)

S.No	Age	Glucose Concentration In blood mg%	Urine glucose	REFRACTOMETER		
				REFRACTOMETER obtained	SPECTROMETER Obtained	Calculated
1	34	110	nil	1.320	1.3214	1.3240
2	68	148	trace	1.320	1.3218	1.3308
3	60	150	trace	1.334	1.3340	1.3331
4	58	168	trace	1.333	1.3338	1.3330
5	67	189	+	1.337	1.3370	1.3354
6	31	200	+	1.338	1.3390	1.3358
7	40	210	+	1.339	1.3408	1.3368
8	68	233	++	1.340	1.3420	1.3391
9	45	240	++	1.342	1.3432	1.3394
10	43	257	+++	1.343	1.3464	1.3406
11	58	262	+++	1.346	1.3468	1.3411
12	72	272	+++	1.347	1.3470	1.3419
13	43	328	++++	1.347	1.3472	1.3448
14	55	440	++++	1.348	1.3484	1.3501

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