Iraqi J. Laser, Part B, Vol. 7, pp. 15- 19 (2008)

IRAQI JOURNAL OF LASER

Primary Diagnosis of Abnormal Urine via Auto Fluorescence Process

Rana R. Al-Ani

Institute of Laser for Postgraduate Studies, University of Baghdad, Baghdad, Iraq

(Received 20 June 2008; accepted 15 October 2008)

Abstract: The main objective of this study is to determine the suitable excitation wavelengths for urine components reaching to select the suitable lasers to execute the auto fluorescence due to their high intensities. The auto fluorescence was measured at 305, 325 and 350 nm excitation wavelengths for eleven urine samples which were also analyzed by conventional methods (chemical and microscopic examination). Data manipulation using Matlab package programming language showed that urine sample with normal chemical and biological components have emission peaks which are different from the infected urine samples. Despite the complexity of the composition of urine, fluorescence maxima can be observed. Most likely, the peaks observed do not result from a single fluorescent urinary metabolite, but depend on differences in urine component that is changed according the clinical situation of person.

Introduction

Urine is a multi-component mixture consisting mainly of organic compounds in a solution of mineral salts (Valcarcel *et al.,* 1987). Some of components are natural fluorescent and urine can be described graphically by means of either fluorescence topogram (excitation/emission matrix) (Wolfbeis *et al.,* 1985; Leiner *et al.,* 1987) or by their synchronous fluorescence spectra (SFS). Fluorescence technique is capable of instantaneous analysis, it is objective and no sample preparation is required (Grubina and Zenkevich, 1997).

As the urine is a metabolic product in the human and in case of a number of diseases which lead to metabolism disorder (such as diabetes, nephritis, hepatitis, etc.) some additional organic components may appear in it such as protein, bilirubin, etc. (Guminetsky *et al.,* 1999).

The biochemical analysis of urine until has based mainly on qualitative and quantitative determination of individual components considered to be markers of an assumed disorder. To isolate an individual compound among many others in the complex mixture of urine requires additional slow and costly processes-isolation, purification and chemical modification. All these bring complication and artifacts (Nikolajsen *et al.,* 2001).

Urine analysis is valued as quick screening method for the presence of lower urinary tract infections (Wigton *et al.,* 1999*;* Tanagho *et al.,* 2004), and include urine sediment analysis (i.e examining for cells, microorganisms, casts and crystals (Holmes and Wong 1999).

The main advantages of fluorimetry as a quantitative analytical techniques is its high sensitivity and, to a lesser extent, its selectivity (LIoyd, 1971; Katarine *et al.,* 2003).

Computerization of spectral measuring and filing the results enables to give a likely diagnosis or a deviation from standard. This method can also serve a doctor-clinician either to confirm or to exclude a concrete diagnosis (Danckwortt and Eisenbrand 1964; Chan and Paul 1995).

The present work is concentrated on the selection of appropriate excitation wavelengths for urine components (normal, abnormal).

Similar lasers wavelengths could be used in stead of them to get clearer and accurate auto fluorescence emission.

Materials and Methods

Urine samples

Urine specimens were collected from not fasting and healthy human (men and women) from ages 27 to 39 years old. Urine was chemically examined by commercial test strips for urobilinogen, glucose, bilirobin, ketones, specific gravity, blood, pH, proteins, nitrite and leukocytes. Microscopic examination was carried out to determine the presence of: white blood cells (WBCs), red blood cells (RBCs), casts epithelial cells and crystal. All values were measured at High Power Field (HPF).

Apparatus

Fluorescence spectra were measured using a spectrofluorometer model SL 174 fluorescence spectrophotometer connected to a PC, fitted with 1 cm cells and a xenon-arc source. The detector sensitivity was set at 915 V, and the excitation and emission slits were adjusted to provide a 20 nm spectral band-pass. The scan speed was 400 nm/min.

Auto fluorescence Measurements

Eleven urine samples were used in the experiment; 3.5 ml of urine sample without centrifugation were poured in quartz cuvette (10 mm).

The fluorescence spectra were measured at three excitation wavelengths (305, 325, and 350 nm) at room temperature. The data were plotted using Microsoft Excel and Matlab package programming language.

Results

A total of 11 persons were included in the present study. The age of persons ranged from 27 to 39 years.

Table (1-a,b) illustrates the results of chemical and microscopic examination of 11 urine samples. All the examined samples have normal values for urobilinogen, glucose, bilirobin, ketones, nitrite and casts.

The blood were measured ++50, +++250 and +10 for samples 8, 9 and 10 in their respective order. Trace amount of protein for samples 3- 10 were observed. Leukocytes values were +25 for samples 4, 5 and 6 and it was +++ 500 for samples 8, 9 and 10.

The values of W.B.C. were ranges from 1 to 2 /HPF for sample 3, from 2 to 3 /HPF for samples 5 and 6, from 3 to 7 /HPF for sample 8, from 3 to 4 /HPF for sample 9, from 15 to 20 /HPF for sample 10 and from 0 to 1 /HPF for sample 11.

Table (1-a) Results of chemical examination of urine samples

S	Chemical examination									
	Uro	**Glu**	**Bil**	**Ket**	**S.G**	**Bl**	**PH**	**Pro**	**Nit**	**Leuk**
1	0.1	Nil	Nil	Nil	1.030	Nil	6	Nil	Nil	Nil
2	0.1	Nil	Nil	Nil	1.030	Nil	6	Nil	Nil	Nil
3	0.1	Nil	Nil	Nil	1.025	Nil	6	Trace	Nil	Nil
4	0.1	Nil	Nil	Nil	1.025	Nil	6	Trace	Nil	+25
5	0.1	Nil	Nil	Nil	1.030	Nil	6	Trace	Nil	+25
6	0.1	Nil	Nil	Nil	1.030	Nil	6	Trace	Nil	+25
7	0.1	Nil	Nil	Nil	1.015	Nil	9	Nil	Nil	Nil
8	0.1	Nil	Nil	Nil	1.030	++50	6	Trace	Nil	+++500
9	0.1	Nil	Nil	Nil	1.030	+++250	6	Trace	Nil	+++500
10	0.1	Nil	Nil	Nil	1.015	+10	7	Trace	Nil	+++500
11	0.1	Nil	Nil	Nil	1.030	Nil	6	Nil	Nil	Nil

Table (1-b): Results of microscopic examination of urine samples

S	Microscopic examination							
	App	Rea	W.B	R.B	Ca	Epi	Cr	Others
1	Clear	Acidic	Nil	Nil	Nil	Nil	+	Nil
2	Clear	Acidic	Nil	Nil	Nil	Nil	+	Nil
3	Turbid	Acidic	1-2	Nil	Nil	+++	Nil	Monilia few
4	Clear	Acidic	Nil	Nil	Nil	Nil	Nil	Nil
5	Turbid	Acidic	2-3	Nil	Nil	+	Nil	Nil
6	Turbid	Acidic	2-3	Nil	Nil	Trace	+	Nil
7	Clear	Alkaline	Nil	Nil	Nil	Nil	++	Nil
8	Turbid	Acidic	3-7	0-1	Nil	++	++	Monilia few
9	Turbid	Acidic	3-4	Nil	Nil	++	Nil	Nil
10	Turbid	Alkaline	15-20	2-3	Nil	+	Nil	Nil
11	Clear	Acidic	0-1	Nil	Nil	Trace	Nil	Nil

The values of R.B.C. cells were ranges from (0 to 1)/HPF in sample 8 and in sample 10 the value ranges from (2 to 3)/HPF. Trace amount of epithelial cells in HPF for samples 6 and 11 is observed. Sample 3 value is (+++) while samples 5 and 10 values are (+) and samples 8 and 9 values are (++). Found of crystals in HPF for samples 1, 2 and 6 give + but the samples 7 and 8 give ++ were observed.

Primarily study were done using 25 excitation wavelengths starting from 295 nm wavelength and ending with 420 nm wavelength with 5 nm intervals between each excitation wavelength, according to the experimental data that will obtained for all of these experiment wavelengths only three excitation wavelengths (305, 325, and 350 nm) were included in this study. No observable changes were observed with other wavelengths.

To examine the differences between the fluorescence spectra of urine samples, three excitation wavelengths (305, 325, and 350 nm) were selected for all the eleven urine samples. At 305 nm excitation urine samples showed high fluorescence emission intensity peak, at 405 and 410 nm for samples 1 and 2 while low fluorescence emission intensity peak appears at 405 in sample 8, 415 nm in samples 5 and 11 and 420 nm for samples 4 and 10. No clear peaks appeared for samples 6, 7 and 3. Another peak appears at 625 nm for sample 9 (Fig 1).

At 325 nm excitation, urine samples showed high fluorescence emission intensity peaks at 410 nm for sample 2 while low fluorescence emission intensity peak appeared at 405 nm for sample 1, 415 nm for samples 4, 7 and 10 and 425 nm for sample 11. No clear peaks appeared for samples 3, 5, 6 and 8. Another peak appears at 665 nm for sample 9 (Fig. 2).

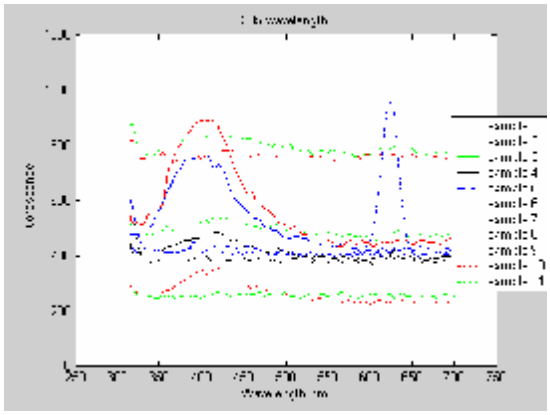

Fig. 1: Emission spectrum of urine samples at 305 nm excitation wavelength

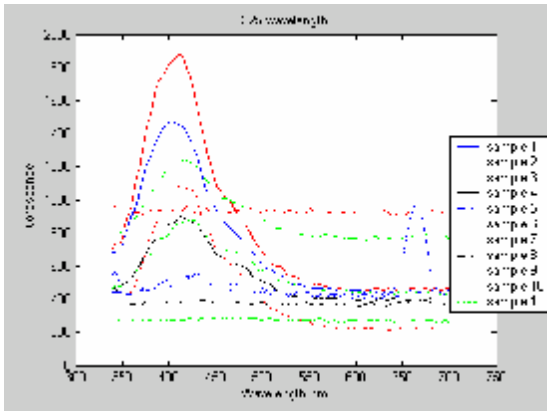

Fig. 2: Emission spectrum of urine samples at 325 nm excitation wavelength

At 350 nm excitation, urine samples showed high fluorescence emission intensity peaks at 415 nm and 420 nm for samples 2 and 7 in the other side at 415 nm for samples 1 and 10, 420 nm for sample 4, 425 nm for sample 11 and 430 nm for sample 5 shows low fluorescence emission intensity peaks. No clear peaks appeared for samples 3, 6 and 8. Another peak appeared at 700 nm for sample 9 (Fig 3).

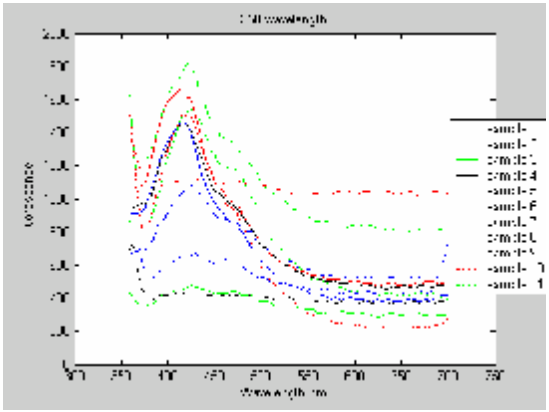

Fig. 3: Emission spectrum of urine samples at 350 nm excitation wavelength

Discussion

Although studies for urine fluorescence in human are limited, there are few studies which attempted to find the fluorescent components that contribute the emission spectra at certain excitation wavelengths.

To explore the differences between the fluorescence spectra of urine samples and urine with clinical examination, Figures (1, 2 and 3) is clear that, the variation in spectra profile for all urine samples depend on differences in urine components that is changed according the clinical situation of person.

The changes in the behavior of the fluorescence spectra that were observed at the 305, 325 and 350 nm wavelengths can be explained in term of presences of certain concentration of fluorophores that appear in the urine of individuals suffering from certain clinical disorders and these change behavior are clear in Figures (1, 2 and 3), and this fact in a good agreement is corresponding to the data were published by Leiner *et al.,* (1987) which stated that changes in the clinical manifestation of patients lead to changes in concentration of some fluorophores in urine. These fluorophores are Indol derivative, the excitation wavelength in range of 300-320 nm give emission spectrum of a wide range fluorophores like 5-Hydroxyindole-3-acetate, Xanthine, 4-pyridoxic acid and 3-Hydroxyanthranilic acid that have emission spectrum at the ranges of 345-435.

The selected wavelengths (305, 325, and 350 nm) are in good agreement with the results obtained from table (2), that illustrates the excitation-emission spectrum for fluorophores human urine sample. Anwer *et al.,* (2009) observed that at 290 nm excitation wavelength the normal urine samples showed very low intensity of fluorescence emission compared with the bacteriuria samples. While 305, 325 and 350 nm excitation wavelengths, these regions exhibit the highest fluorescence intensity in the present work.

Daubener *et al.,* (1999) found the components of urine samples are different in the proteins, blood, crystals, bilirubin and epithelial cells. This components can contribute certain emission spectra with different intensities at (305, 325, and 350 nm) excitation wavelength. Data from Leiner *et al*. (1984) except for tryptophan and melatonin for which data were taken from Kusnir *et al.,* (2005).

Table (2) List of fluorophores in human urine which can be excited at 290 nm

Fluorophores	Excitation/emission maxima (nm)
5-Hydroxyindole-3-acetate	300/345-355
5-Hydroxytryptophan	295/340
Indol-3-acetate	290-360
Indoxyl sulphate	290/380
Melatonin	290/330
Skatol-5-sulphate	290/380
Skatol-6-sulphate	290/360,370
Tryptophan	280/350

Conclusions

It could be concluded from the obtained results that the auto fluorescence process is an effective method to distinguish between the healthy and abnormal urine. The results of selected excitation wavelengths indicated that the N2 laser and XeCl laser are the suitable excitation source of the auto fluorescence.

References

Anwer A.G., Perinchery M.S., Ewa M.G., and Subramanyam V. (2009). Distinctive autofluorescence of urine samples from individuals with bacteriuria compared with normals. Clinica Chimica Acta **401**, 73- 75.

Chan S.C. and Paul E.S. (1995). The demographic and morbidity patterns of patients seen in an outpatients department in a Malaysian general hospital Fam Physician **7**, 3- 10.

Danckwortt P.W. and Eisenbrand J. (1964). Luminescenz-Analyse im filtrierten ultraviolettem licht. Akad. Verlags-Ges., Leipzig, pp. 162.

Daubener W., Hucke C., Seidel k., Hadding U. and Mackenzie C.R. (1999). Interleukin-1 Inhibits gamma interferon induced bacteriostasis in human uroepithelial cells. Infect. Immun. **67**, 5615- 5620.

Grubina L.A. and Zenkevich E.I. (1997). Express fluorescent method for the quantitative analysis of human organism porphyrins upon oncological diseases and ionizing radiation influence, 5th Int. Conf. on Methods and Applications of Fluorescence Spectroscopy, Berlin, September, pp. 21-24.

Guminetsky S.G., Gaika O.R., Kokoschuk G.I., Grigorishin P.M. and Kirsh N.L. (1999). Investigation of absorption spectra of the main human urines organic components in absence of proteins. Spie **3904,** 579- 589.

Holmes H. and Wong E. (1999). Urinary tract infections in adults. Am Acad Fam Phys. **59**, 5

Katarine D., Jaroslav K. and Ludmila P. (2003). Diagnostic monitoring of urine by means of synchronous fluorescence spectrum. J. Biochem Biophys Methods **55**, 111-119.

Kusnir J. and Leskova L. (2005). Concentration matrices solution for fluorescence definition of urine. Analytical letters **38,** 1559-1567.

Leiner M.J.P. (1984). Ph.D thesis, University of Graz.

Leiner M.J.P., Hubmann M.R. and Wolfbeis O.S. (1987). The total fluorescence of human urine. Anal. Chim. Acta **198,** 13- 23.

LIoyd J.B.F. (1971). Synchronized excitation of fluorescence emission spectra. Nature Phys Sci. **231**, 64- 65.

Nikolajsen R.P.H., Hansen A.M. and Bro R. (2001). Attempt to separate the fluorescence spectra of adrenaline and noradrenaline using chemometrics. Luminiscence, **16**, 91- 101.

Tanagho E.A., Aninch M.C. and Smith's J.W. (2004). General urology. 15th ed. Lange Medical Books, N.Y.

Valcarcel M., Gomez Hens A., Rubio S., Petidier A. (1987). Direct quantification of copoporphyrins and uroporphyrins in urine by derivative synchronous fluorescence spectroscopy. Clin. Chem. **33,** 1826- 1831.

Wigton R.S., Longnecker J.C. and Bryan T.J. (1999). Variation by specialty in the treatment of urinary tract infection in women. J. Gen Intern Med. **14,** 491- 494.

Wolfbeis O.S. and Schulman S.G. (1985). "The fluorescence of organic natural products, molecular luminescence spectroscopy: Methods and Applications", Wiley, N.Y, pp. 167- 370.

.

التشخيص الاولي للادرار غير الطبيعي بطريقة الفلورة الذاتية

رنا رياض العاني

معهد الليزر للدراسات العليا ، جامعة بغداد ، بغداد ، العراق

الخلاصة

الهدف الرئيسي من هذه الدراسة هو تحديد الاطوال الموجية اللازمة لتهيج مركبات الادرار وصولاً الى اختيار الليزرات المناسبة المستخدمة في الاثارة بسبب شدتها العالية للحصول على الفلورة الذاتية. تم قياس الفلورة الذاتية عند الاطوال الموجية (305، 325 و 350 نانومتر) المهيجة لاحدى عشر عينة ادرار فضلا عن ذلك تحليلها بواسطة الطرق التقليدية (الكيميائية وبالمجهر الضوئي). أظهرت معالجة البيانات بأستخدام لغة برمجة الحزمة البرمجية (Matlab) بأن عينات الادرار ذات المكونات الكيميائية والبايولوجية الاعتيادية تمتلك قمم طيفية (نقاط عظمى) تختلف عن عينات الادرار المصابة. بالرغم من الطبيعة المعقدة لمكونات الادرار يمكن ملاحظة قمة التفلور بوضوح ومع ذلك فأن القمم الملاحظة هي غير ناتجة عن مكون أيضي واحد لكن يعتمد على عدة مكونات والتي تتغير وفقاً لحالة الشخص السريرية .