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Features of determining relationships in aqueous biological solutions between biophysical constants and environmental parameters using optical methods

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Abstract. The relevance of early diagnostics of chronic kidney disease (CKD) is due to its high prevalence and asymptomatic course of the disease in the early stage. There is a need to develop new approaches to the diagnosis of early-stage kidney damage that are accessible, non-invasive and reliable, with an emphasis on the use of optical methods. A new method of rapid diagnosis based on refractometric measurement of the refractive index of urine has been proposed. New relationships between the refractive index, osmolality O_s , and density ρ have been established. Based on the values of O_s and ρ , doctors determine the presence of kidney disease or damage at an early stage (the patient is then referred for clinical examination). The proposed method provides high accuracy (the refractive index measurement error of ± 0.00005 in a wide temperature range of 288–303 K made it possible to determine O_s and ρ based on these measurements with an error of 2 mOsm/l and 0.00015 g/cm³, which is sufficient to determine kidney damage at an early stage. This makes it competitive when compared to traditional methods of rapid kidney function testing (urinalysis, test strips, and mobile density meters). The results obtained for the diagnosis of kidney function were clinically confirmed during the examination of patients. This confirms the possibility of using refractometry for rapid diagnosis of kidney condition to detect damage at an early stage.

Keywords: kidney, early-stage damage, refractometry, osmolarity, density, urine, rapid diagnostics

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Материалы конференции

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Особенности определения связей в водных биологических растворах между биофизическими константами и параметрами среды с использованием оптических методов

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Аннотация. Актуальность ранней диагностики хронической болезни почек (ХБП) обусловлена их высокой распространенностью и бессимптомным течением заболевания на начальной стадии. Обоснована необходимость разработки новых подходов к диагностике определения поражения почек на ранней стадии, ориентированных на доступность, неинвазивность и достоверность, с акцентом на использование оптических методов. Предложен новый метод экспресс-диагностики, основанный на рефрактометрическом измерении показателя преломления мочи. Установлены новые зависимости между показателем преломления, осмолярностью O_s и плотностью ρ . По значениям O_s и ρ врачи определяют наличие заболеваний почек или их поражение на ранней стадии (далее пациент направляется на клиническое обследование). Предложенный метод обеспечивает высокую точность (погрешность измерения показателя преломления ± 0.00005 в широком диапазоне изменения температур 288–303 К позволила обеспечить определение на основе этих измерений O_s и ρ с погрешностью 2 мОсм/л и 0,00015 г/см³, что достаточно для определения поражения почек на ранней стадии. Это делает его конкурентоспособным при сравнении с традиционными способами экспресс-контроля состояния почек (урометрия, тест-полоски и мобильный измеритель плотности). Полученные результаты по диагностике состояния почек получили клиническое подтверждение при обследовании пациентов. Это подтверждает возможность применения рефрактометрии для экспресс-диагностики состояния почек для выявления их поражения на ранней стадии.

Ключевые слова: почки, оптическое излучение, рефрактометрия, осмолярность, плотность, моча, экспресс-диагностика, ранняя стадия

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Introduction

Chronic kidney disease (CKD) is a serious medical and social problem of mankind [1, 2]. According to the World Health Organization, the prevalence of CKD in the adult population reaches 16 percent or more [3–5]. Kidney disease is characterized by a long asymptomatic course and late diagnosis [3–5]. In most cases, pathology is detected only at stages 3–4, when therapeutic treatment options are significantly limited and treatment requires significant material costs [5–7]. This situation is due to the lack of reliable, accessible and easy-to-use methods of express diagnostics, unlike other areas of personalized medicine [8, 9].

Modern methods of express diagnostics of kidney condition used in clinical practice have a number of significant limitations. Urometry, despite its prevalence, is characterized by a significant measurement error (± 0.005 g/ml) and a pronounced dependence of the results on ambient temperature [10, 11]. In addition, visual assessment of the position of the liquid meniscus in the urometer introduces a significant subjective component into the measurement process. Test strips, although convenient to use, have limited accuracy due to color interpretation of results and high sensitivity to the presence of protein, glucose, and drugs in urine [12]. Another important disadvantage is their limited shelf life and demanding environmental conditions. The use of mobile density meters is unsatisfactory in terms of measurement accuracy ρ (should be ± 0.0001 g/ml or less) or in terms of operating costs and the price of the device itself for a large number of users (small medical institutions, polyclinics and personal use).

All this makes the development of new approaches to screening renal pathology at an early stage an extremely urgent task. Therefore, the aim of the work is to develop a method for express control of kidney condition on the basis of optical measurements and techniques for detection of kidney lesions at an early stage based on the results of measurements.

Materials and methods

In modern clinical medicine, data on the state of urine density are mainly used for rapid diagnosis of renal status [10–12]. There are no big problems to measure urine density in a stationary laboratory using an ultrasonic density meter with an error of 0.2%. There are also various models of spectrophotometers for the study of urine samples. It should be noted that the determination of urine osmolarity O_s is of particular importance in clinical diagnosis of renal diseases [3–6]. This parameter serves as an important indicator of the functional state of the kidneys and water-electrolyte balance of the organism [3–6]. However, the existing methods of determining this parameter, in particular osmometry, require the use of complex laboratory equipment, considerable time expenditure and special conditions of analysis, which limits their use even in routine clinical practice [2–6, 12].

The new control method we propose makes it possible to reduce the problem of determining O_s in clinical conditions and to determine O_s in express mode (currently unachievable). In addition, it is proposed to determine the density ρ_u of urine based on the measured refractive index n_u . We propose using the results of a single measurement of n_u in a urine sample to determine the values of O_s and ρ_u , taking into account the temperature T , with an error margin that will allow for the early detection of kidney disease. This approach has made it possible not only to obtain representative quantitative characteristics of the studied indicators, but also to conduct an interparametric comparison with a high degree of reliability.

Urine samples were used as research objects. Samples were collected from patients in various physiological states, including normohydration, dehydration, and conditions accompanied by water-salt imbalance. This variety of conditions allowed us to cover a wide range of osmolality, density, and refractive index values, which, in turn, ensured high data variability and the ability to identify consistent patterns. More than 150 people were examined. Measurements were taken using certified equipment, which minimized the influence of external factors and increased the reliability of the results. Taken together, this made it possible to establish relationships between the parameters studied and draw reasonable conclusions about the nature of their connection in the context of changes in the physiological state of the body.

In order to establish these dependencies, simultaneous studies on certified equipment of the change in n_u , O_s and ρ_u from the change in temperature T were carried out as follows. A urine sample was collected from the patient. Then from this sample, three urine samples were placed in a density meter, osmometer and refractometer. Synchronized measurements were made of the change in these quantities from changes in temperature T , which will be the base parameter in the construction of $O_s(n_u)$ and $\rho_u(n_u)$ relationships in the future. The temperature was varied in the range from 288 to 303 K, which corresponds to the standards of urine sample diagnostics [1–6].

The refractive index was measured using a mobile refractometer SNEL-105, which operates on autonomous power (measurement error ± 0.00005). This device can be used for rapid testing without any problems. This was a distinctive feature of the experimental study (the equipment used for rapid testing at the sampling site would not change). The density of the

urine samples was determined using a DSA 5000 M ultrasonic densitometer (a laboratory device with an error of 0.00001 g/ml). Osmolarity was measured using an OSKR-1M osmometer with a cryostat (measurement error of 1 mOsm/l in the measurement range from 500 to 2000 mOsm/l). This range includes O_s values that correspond to the onset of early-stage kidney disease (581–732 mOsm/l).

Results and discussion

The paper cites one example of experimental testing of our proposed method for rapid diagnosis of kidney condition. From a sample of studies involving 150 patients, 10 urine samples were selected from patients of different ages and genders who did not complain of kidney problems. The selection criterion was slight excess weight (no more than 15% deviation from the normal height-weight ratio). This was a recommendation from doctors at the First Saint Petersburg State Medical University (Saint Petersburg). Figs. 1 and 2 show examples of the dependence of n_u and ρ_u on temperature T for sample 1 (patient 1).

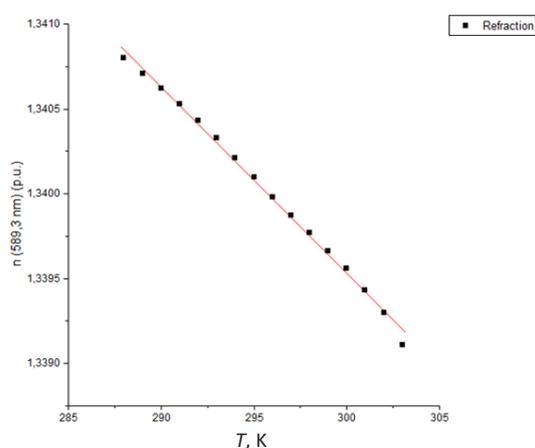


Fig. 1. Dependence of n_u on T

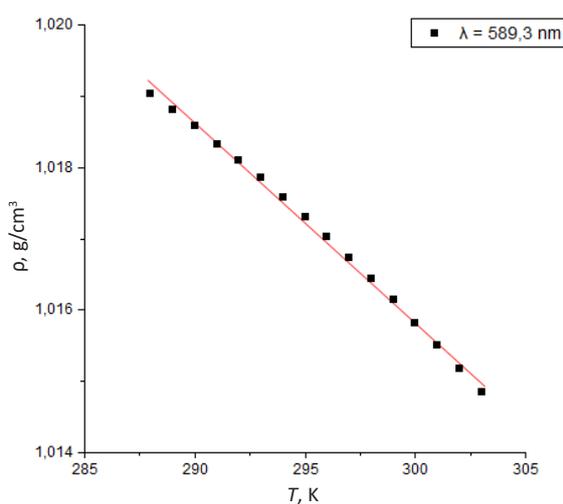


Fig. 2. Dependence of ρ_u on T

Using the least squares method, linear approximation models describing the established dependences were obtained from the experimental data:

$$\rho = 2.4322n - 2.2444,$$

$$O_s = 33.128\rho - 33.107.$$

These dependences are represented in Figs. 3 and 4. The validity of the obtained approximations was confirmed by statistical processing of the results: correlation coefficients were calculated and the significance of regression dependencies was evaluated. To increase the reliability and reproducibility of the experimental data, each measurement was repeated three times, after which the obtained values were averaged. This approach ensured the stability of the results to random deviations and allowed us to form reasonable conclusions about the nature of relationships between osmolarity, density and refractive index of aqueous biological urine solutions. Similarly, using the experimental data (Fig. 3, 4), a linear relationship between osmolarity and density of the solution under study was established: $O_s = 33.128\rho - 33.107$. The results obtained confirm that osmolarity can be indirectly estimated by measuring the density as well as the refractive index, which significantly simplifies the analysis procedure and makes it more accessible in conditions of limited laboratory resources.

For the remaining 10 urine samples, the same studies were performed. Analytical relationship between $\rho_u(n_u)$ obtained through the results shows that average value for the coefficient a equals 2.4343 and the average value for the coefficient b equals 2.2479. The general formula for

describing the relationship is: $\rho_u = an_u + b$. The deviation of coefficient a from the average value for a group of 10 samples is ± 0.0215 , which is about 1% of the average value. The deviation of coefficient b from the average value for a group of 10 samples is ± 0.0127 , which is less than 1% of the average value.

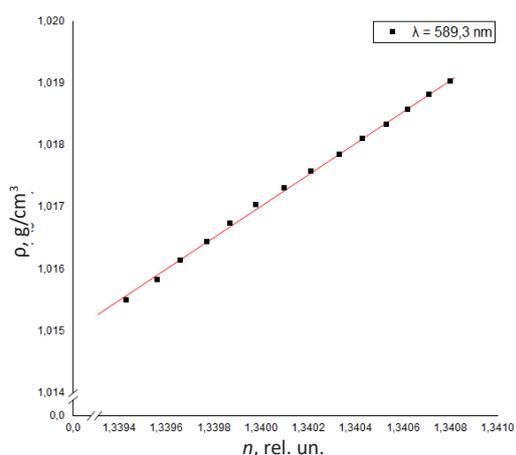


Fig. 3. Density dependence on refractive index

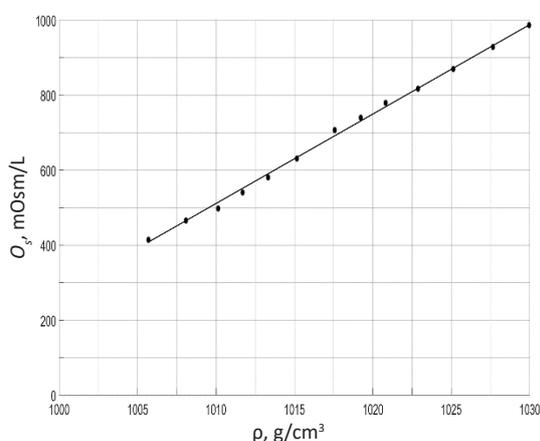


Fig. 4. Osmolarity dependence on density

Similar tests were conducted on a sample of 13 patients who did not show signs of early kidney disease during rapid diagnosis.

To ensure the implementation of the rapid diagnosis method for early kidney disease based on the measurement of the refractive index using a mobile refractometer SNEL-105 or two devices (a refractometer and a mobile densitometer), or a mobile densitometer (density meter), average dependencies for patient groups are required (with suspected early-stage kidney disease or no such suspicion).

Fig. 5 shows examples of the final dependences of density change on refractive index and osmolality on density, averaged over samples from the two groups of patients considered. Analysis of the results obtained shows that measurements of the refractive index ± 0.00005 in a wide range of temperatures T to 288–303 K allow for an error of 2 mOsm/L and 0.00015 g/cm^3 when determining O_s and ρ based on these measurements, which is sufficient for detecting kidney damage at an early stage. The dependences (Fig. 5) show areas of both early-stage kidney disease and the absence of disease.

This level of accuracy is entirely satisfactory for doctors. The early stage of kidney disease in terms of osmolality is in the range of 581–732 mOsm/L, and in terms of density, 1.0141–1.0152 g/cm^3 . The relative errors are less than 1%, which fully meets the requirements for rapid diagnosis.

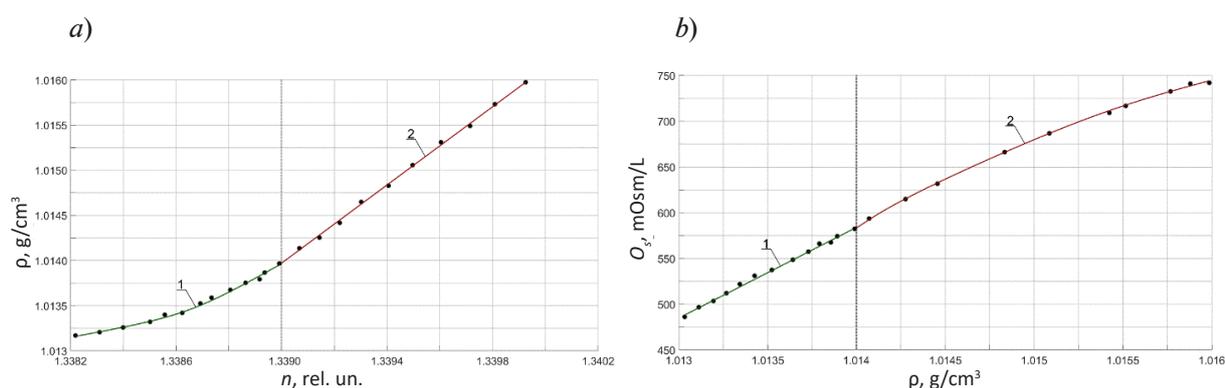


Fig. 5. Density dependence on refractive index (for two groups of patients). Graph 1 corresponds to patients without kidney damage, graph 2 corresponds to patients with early stage kidney disease (a). Osmolarity dependence on density (for two groups of patients). Graph 1 corresponds to patients without kidney damage, graph 2 corresponds to patients with early stage kidney disease (b)



Conclusion

Studies conducted on samples from more than 150 patients and the results obtained confirmed the validity of constructing dependencies $\rho_u(n_u)$ and $O_s(n_u)$ based on the established correlation between three physicochemical parameters of aqueous urine solutions. These dependencies can be used for rapid diagnosis of kidney disease at an early stage and to monitor the effect of drugs during treatment, as the measurement process is simple to implement and painless (it can be performed as many times per day as necessary), unlike invasive methods such as blood sampling.

Based on the results of studies using the newly developed methodology, the following should be noted. More than 150 people who felt well and did not complain of kidney problems were examined. Twelve people were found to have suspected early-stage kidney disease. All twelve people underwent a full clinical examination at the First Pavlov State Medical University of St. Petersburg using certified equipment. Nine people who were not suspected of having early-stage kidney disease and the authors of the article also underwent the examination. The examination consisted of laboratory blood and urine tests, as well as diagnosis of kidney condition using certified equipment.

In 11 out of 12 patients with suspected early-stage kidney disease, doctors confirmed the diagnosis based on the results of the examinations; in one patient, the diagnosis was not confirmed. In 13 patients from the group where no early-stage kidney disease was suspected, doctors confirmed the absence of kidney disease based on the results of the examination.

According to express diagnostics, the refractive index measurements we offer can be compared with density measurements using the VIP-2MR mobile densitometer (measurement error 0.0001 g/cm³). The measured value of ρ is also used to diagnose kidney disease at an early stage (confirmed by a clinical examination – diagnosis). Unlike a mobile refractometer, this device is almost twice as expensive and its maintenance during measurements is at least five times more expensive than when using a refractometer. Therefore, it is more expedient to use two refractometers with broader functional capabilities than one mobile densitometer. This is especially true in conditions that require high-speed and repeated analysis, which is desirable in order to understand the effectiveness of drugs during treatment.

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