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Short Review on Spectral Methods for the Determination of Chromiun in Human Body Fluids and Tissues

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Abstract

Background: The data for the determination of chromium concentrations in biological samples are still contradictory and for some biological objects varied within orders of magnitude. They are not fully clarified the role and participation in the metabolism in healthy subjects and in various diseases and conditions. *Aim*: One reason for the outstanding problems in the study of the functions of the chromium in the human organism is a need for accurate, with low detection limits, and access to a biomedical laboratory methods for the analytical determination. That's why we set the aim to make a brief review of spectral methods for determination of chromium in human body fluids and tissues. *Methods, results*: Analytical methods which most commonly to apply at determining the chromium in biomaterials are the atomic spectral (AS) - Flame atomic absorption spectroscopy, Electrothermal atomic absorption spectrometry, Inductively coupled plasma optical emission spectrometry and Inductively coupled plasma mass spectrometry. They are accurate and have lower limits of detection, but not all biomedical laboratories have them. Of the molecular spectral, applicable methods with spectrophotometric indication for determination of chromium in biomaterials are the methods which used suitable chelating agents of chromium in micellar medium. They have limits of detection, comparable to AS-methods and are available to all biomedical laboratories. *Conclusions*: The lag in specifying the chromium concentrations in human bio-samples, related with clarifying the role of chromium in health and disease, can be overcome using both atomic spectral and spectrophotometric methods. Any study relating to the determination of chromium in human body fluids and tissues will contribute to the analytic of chromium and related the determining his biomedical research.

Keywords

Cr AS-methods Body Fluids, Cr AS-methods Tissues, Cr Photometric Body Fluids, Cr Photometric Tissues

1. Introduction

As one of the essential trace elements-biotics, chromium involved in the biochemical processes in the body. Its content in human body fluids and tissues varies from ng (ng / ml) in the blood serum to μg range (μg/g) in the hair [1]. Changes in chromium concentrations were observed in various diseases and conditions, but the involvement of chromium in the exchange is not yet fully elucidated [2, 3]

One of the reasons for delays in studying the function of chromium in the body is the need for accurate, accessible, with low detection limits of the analytical methods for its determination in biological samples. The low chromium concentrations - about three orders of magnitude lower than those of iron, copper and zinc, are a major cause of the more complicated and often inaccurate analytical determination.

In determining the concentration in nanomoles range, chemical methods - classical photometry and electrochemical methods require application of slow, laborious, multi-step methods for pre-concentration and separation, involving the use of large volume and mass of biological samples. The large volume or mass required for these methods, for example 10 or 20 ml of blood serum, making them unsuitable for analysis of most of the biological samples.

Instrumental methods of analysis - AAS (Atomic absorption spectrometry), NAA (Neutron activation analysis), Inductively Coupled plasma Optical Emission Spectrometry (ICP-OES) и Inductively Coupled plasma mass spectrometry (ICP-MS) have low limits of detection, but

require expensive and complex equipment. As with the chemical methods, and at the instrumental, the complicate and variable biological matrix requires the use of prior processing the samples in which the methods are becoming more labor intensive [1 – 6, 7, 8].

Methods for determining the appropriate metal chelating agents in micellar medium can compete with instrumental methods. They have low limits of detection, while photometric indication; these limits are combined with access for scientific and routine technique of execution. [9].

With regard to solving the analytical problems in the determination of chromium in biological samples, we set the aim to make a brief overview of spectral methods for determination of chromium in biological samples. Contemporary values for the distribution of the content of chromium in human body fluids and tissues are given in Table 1.

Table 1. *Chromium concentrations (C_{Cr}) in human body fluids and tissues.*

Samples		**Selected contemporary values in healthy people**		
Measuring units		***$C_{av.v.}$ ± SD**	**$C_{Cr(from-to)}$ or $C_{av.v}$**	**Reference**
Blood, ET-AAS	µg/l	0.22 ± 0.07	0.08–0.51	[1]
Blood, ET-AAS	µg/l		0.22–12.00	[4]
Blood, ET-AAS	µg/l	35.04 ± 26.02		[5]
Blood, ETAAS	µg/l	59.2 ± 2.94	51.92–63.50	[2]
Blood, ET-AAS	µg/l	6.19 ± 0.03		[6]
Blood, ETAAS	µg/l		0.5 - 5.1	[3]
Blood ISP-MS	µg/l	0.44 ± 0,27	0.01-1.2	[10]
Blood, ISP-OES	µg/l	0.23 ± 0,03	0.00-0.28	[11]
Serum, ISP-OES	µg/l	1,80±1,40		[7]
Serum, ISP-MS	µg/l		1.0	[8]
Serum, ET-AAS	µg/l	0.16±0.06	0.08-0.36	[12]
Serum, ET-AAS	µg/l	0.17 ± 0.05	0.06 – 0.43	[1]
Serum, ET-AAS	µg/l		12 – 60	[13]
Serum FAAS	µg/l	0.88 ± 0.2		[14]
Urine, ET-AAS	µg/l		0.30–0.90	[15]
Urine, ET-AAS	µg/l		0.24-1.8	[16]
Urine, FAAS	µg/l		6.10	[17]
Urine, ISP-OES	µg/l	9.00±7.00		[7]
Urine, FIA FAAS	µg/l	3.80 ± 0.20	0.04-50	[18]
Urine, ET-AAS	µg/l	9.20 ± 2.54	7.50–11.40	[2]
Urine, ET-AAS	µg/l	1.18 ± 0.23		[19]
Urine, ET-AAS	µg/l	0.32±0.20	0.08-0.90	[12]
Lung, ET-AAS	µg/g	0.117 ± 0.053	0.00161 – 0.40	[1]
Milk, ET-AAS	µg/l		16.0	[1]
Milk, ISP-OES	µg/kg		72	[20]
Human milk, ET-AAS	µg/l		from 0.69 ± 0.01 to 17.33 ± 0.59	[21]
Saliva, ET-AAS	µg/l		0.80 – 3.60	[1]
Skin, ETAAS	ng/g		21.0	[1]
Sweat, ETAAS	µg/l	2.0 ± 1.2		[1]
Nails, ISP-OES	µg/ml	2.37±0.64		[7]
Nails, ISP-OES	µg/g	1.47 ± 0.56		[22]
Nails, ET-AAS	µg/g		0.8–1.4	[15]
Nails, ET-AAS	µg/g	1.16±1.05		[16]
Erythrocytes, ET-AAS	µg/l		1.4–2.5	[15]
Erythrocytes, ET-AAS	µg/l		0.14-4.58, 1.54	[16]
Erythrocytes, ET-AAS	µg/l	3.21 ± 2.20		[23]
Plasma, FAAS	µg/dl	64.98±5.17		[24]
Liver, blood, FAAS	µg/ml		0.25-6.0	[25]
Liver, ET-AAS	ppm	2.49±0.89		[26]
Hair, FAAS	µg/g		0.20	[27]
Hair, FAAS	mg/g		1.0152±0.8844	[28]
Hair, FAAS	mg/g	3.97±3.44		[29]
Hair, ETAAS	µg/g	3.6 ± 0.45	3.05 – 4.2	[2]
Hair, ISP-OES	µg/ml	4.36±1.03		[7]
Hair, ISP-OES	µg/g	3.32 ± 0.22		[22]
Hair, ISP-OES	µg/g	3.32 ± 0.22		[22]
Hair, ISP-OES	µg/g	0.92 ±0.65		[11]
Hair, ET-AAS	µg/g	2.45 ± 1.3		[30]
Hair, ET-AAS	ng/g	4280 ± 278		[31]
Hair, ET-AAS	µg/g	1.1±0.3		[16]
Prostate, NAA-LLR	mg/kg	0.49 ± 0.07		[32]
Prostate, ISP-MS	mg/kg	0.53 ± 0.08		[32]
Tissues, ETAAS	µg/l		37.04	[5]

Samples		Selected contemporary values in healthy people		
Measuring units		**$^{*}C_{av.v.} \pm SD$**	**$C_{Cr(from-to)}$ or $C_{av.v}$**	**Reference**
Tissues, ET-AAS	µg/g	2.84±5.17		[5]
Bones, FAAS	µg/g	7.87±11.92		[33]
Urine, ET-AAS	µg/g		0.08	[34]
Hip joint tissues, ETAAS	µg/g		5.33 - 17.86	[35]

$^{*}C_{av.v.} \pm SD$ - Cr concentration average value ± standard deviation

Table 1 show that the analytical methods which most frequently used in the determination of chromium in biomaterials are spectral methods. It complements our rationale for the review of the literature and confirms our practical grounds the necessity of this review.

2. Atomic Spectrum (as) Analytical Methods for the Determination of Trace Chromium in Biomaterials

Traces of chromium in biomaterials are determined by atomic spectrum (AS) analytical methods by FAAS, ETAAS, ICP-OES and ICP-MS. The instrumental detection limits of chrome by FAAS, ETAAS, ICP-OES and ICP-MS are shown in Table 2.

Table 2. *Instrumental detection limits (ILOD) chromium by AS – methods.*

	FAAS	ETAAS	ICP-OES	ICP-MS	References
ILOD	0.01 ppm	0.1 ppb	0.01 ppm	0.01 ppb	[36]
	50-100 µg/l	0.1 µg/l	1–10 µg/l	0.1µg/l	[1, 37]

In more recent literature is considered that when determining the ILOD chromium by ICP-OES are comparable to those of ET-AAS, and not by FAAS - methods [37]. Emphasizes however, that ILOD in ICP-OES can be aggravated by several times to an order of magnitude in the presence of interfering complex matrices, such as in biological samples [37].

In determining chromium by FAAS require application of the procedures for pre-concentration and / or separation (Table 1 and 2). Thus, by FAAS, after using magnetic nanoparticles as the adsorbent in the solid phase extraction is determined chromium in environmental water and blood serum [14]. By FAAS with pre-concentration, usually by mineralization and / or separation are determined traces of chromium in plasma [24], in hair [27 – 29, 38], in blood [25], liver [25], urine [17, 18], bone [33]. FAAS is used in combination with flow injection analysis, FIA. With FAAS and FIA is determined chromium in urine [18].

In FAAS has a practical limitation, such as the volume of the test sample, which does not allow its use for the determination of chromium in all biological fluids.

ET-AAS is most used method for the determination of trace elements, including chromium in biological samples. Atomization, difference of interfering sources and techniques for their elimination and control in the determination of chromium is given in a general view of a number of specific articles / [1, 15, 16, 39, 40, 41, 42].

For the determination of chromium in ETAAS using preliminary concentration - mineralization and / or extraction mainly in isolated cases deproteinization of blood and serum. It is determined chromium in blood serum samples [1, 5, 12, 13, 43]; blood / [1 - 6, 30, 44]; urine [1, 2, 12, 15 - 17, 19, 34, 42, 45, 46]; milk [1, 21]; liver [26, 44]; hair [2, 30, 31]; bone tissues [4, 5]; nail [1, 15, 16]; erythrocytes [15, 16, 23]; saliva [1, 43]; lung [1]; skin [1]; sweat [1], hip joint tissues [35], marrow [44], kidney [44], and stomach [44]. Have been developed and direct ETAAS methods for the determination of chromium in blood [2]), urine [2, 34, 45], hair [31], blood serum [2].

Upon determination of the chromium and others metals in ICP-OES by pretreatment of the samples is used mainly mineralization [7, 22]. Chromium is determined in urine [7] blood [11], nail [7, 22], serum [7], urine [7], hair [7, 11, 22], breast [20], milk [20].

Among the advantages of the method: efficient excitation of the atoms and ions; high stability of discharge; wide linear dynamic range: 5-6 range; ICP-OES - method has some drawbacks: high risk of spectral interferences; direct determination of trace elements in OES, including chromium, amid high concentrations alkali and alkaline earth metals in biological samples is considered to be virtually impossible. Furthermore, the plasma is unstable in the presence of organic substances in biological samples or adding organic solvents [36].

In mass spectroscopy (ICP-MS), the pre-treatment generally requires dilution with nitric acid [32]. The limit of detection of the method in the determination of chromium in unfurnished conditions was 0.01 µg/l, but it does not occur if the total matrix concentration is 1.0 g/l. For comparison, in ETAAS influence matrix concentrations of 20 g /l [47]. So in determinations should be used in several ml samples - 2.5 - 5.0 ml [47] and practical limits of detection of chromium in the biomaterials are in the range 0.1 - 1.0 µg/l [48]. Limitations of the method when working with organic matrix depend on high concentrations of alkali and alkaline earth metals in biological samples.

In ICP-MS chromium is determined in the urine [8], blood serum [8, 10], whole blood [10], and prostate tissue [32].

3. Spectrophotometric Methods for the Determination of Trace Chromium in Biomaterials

Spectrophotometric methods are applied in the

determination of chromium in water analysis [49 - 53]. Another area of application is the analysis of soils, alloy steels, geological samples and industrial effluents [54-57]. A third area of application is the analysis of food and pharmaceutical additives [54, 58, 59]. In comparison to the above methods for the determination of chromium in the analytical sites, few are spectrophotometric method for determination of chromium in human body fluids and tissues. Chromium is determined by spectrophotometry in human blood serum [9, 60-65]. and human urine [9, 60].

The low concentrations of chromium in biological samples (Table 1) require for its determination to apply methods with low limits of detection. Furthermore, AS-methods suitable for the purpose and methods would be suitable chelating agents for chromium, in the micellar environment with spectrophotometric indication. With the help of these methods may be defined concentrations in nanogram range (ng/ml).

We have developed and implemented two methods of chelating agents for chromium in micellar medium and spectrophotometric indication in the determination of chromium in blood serum and urine [66, 67].

In comparison with conventional FAAS and photometric methods for determination of Cr in biological materials, new methods [66, 67] have the following advantages:

- Lower limit of detection, allowing the use of small-volume samples of blood serum and urine - 1.00 ml;
- High selectivity avoids extraction and stripping in the definition used in photometric and flame AAS methods;
- Good reproducibility in determining Cr. In determining the chromium concentration in the range 0.20 - 4.00 μmol/l in serum samples and urine: relative standard deviation, RSD = 3.24% and RSD = 1.81% on average in the same concentration range for serum and urine, respectively.
- Using the same analytical procedure - reagents, pretreatment of samples, analytical determination in the determination of Cr in blood serum and urine.
- Therefore, both methods can be applied for serial analysis in biomedical laboratories.

The reduced concentration of chromium in blood serum and urine in children with high blood pressure [66, 67] may be considered as a criterion for determining of functional and primary vascular changes in the cardiovascular system.

New methods for the determination of Cr in blood serum and urine are embedded in the Department of "Chemistry and Biochemistry" laboratory Molecular spectrophotometry, elemental and functional bioassay and the Department of Pediatrics of MU-Pleven.

Applications developed methods for solving medical-biological problems, not casuistry and real cases in medicine, and their implementation in those laboratories showed that methods for determining Cr in blood serum and urine in micellar environment with spectrophotometric indication have a place in modern clinical and toxicological studies.

4. Conclusions

The most ETAAS is used to determine the chromium in human body fluids and tissues. It is the fastest. In part of the ET-AAS-methods are used direct techniques for determining of chromium in bioassay.

In species with high concentrations of chromium, or those which may be taken larger in volume or mass samples such as hair, nails, urine, is applicable FAAS-method.

ICP-OES and ICP-MS are multi-element methods. In comparison to ET-AAS, the number of studies of chromium in bio samples with them is very measuring less.

AS - methods require expensive and operationally difficult technique with not available most clinical and biomedical laboratories.

Molecular spectral spectrophotometric method is applied in a number of areas to determine the chromium, whereupon the concentrations of chromium in the analytes are comparable to those in human body fluids and tissues. The definitions of chromium, however, in human biosamples in recent years are limited number and in limited numbers analytes. Contemporary applicable methods for the determination of chromium in the biomaterials, by spectrophotometric indication are methods that make use of suitable chelating agents for chromium in the micellar medium. In limit of detection, they are competitive on the AS-methods. And spectrophotometers have all medical and biological laboratories. Furthermore, the methods can be automated and use the available automatic analyzers in clinical laboratories.

We believe that the lag in specifying the chromium concentrations in human bio samples, related to clarifying the role of chromium in health disease can be overcome using both atomic spectral, so spectrophotometric methods. Any determination of chromium in human fluids and tissues, regardless of what method has been made, will contribute as in analytic of the chromium, and in related biomedical research its determination. The available for all medical laboratories spectrophotometric methods are one of the possibilities for participation of more researchers in resolving the analytical, biochemical, physiological, health problems associated with determining of chromium in human body fluids and tissues.

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