

## CHEMICAL COMPOSITION STUDY OF STANDARD CHICKEN SERUM

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**Abstract.** Sterile filtered chicken serum was purchased from “Sigma-Aldrich” and studied using X-ray photoelectron spectroscopy in order to reveal chemical composition and distribution of the predominant elements. The main peaks observed in survey spectra were of C 1s (76.94 %), N 1s (3.73 %) and O 1s (13.47 %) core levels. There have also been observed small amounts of P 2p (0.96 %), Cl 2p (1.79 %), and Na 1s (3.11 %). The study revealed that carbon spectrum contains four components with the corresponding chemical bonds: C–C, C–N/C–OH, C=N/N–C=C, and C=O. Nitrogen ions were present in three forms: imine, amine and positively charged nitrogen. Oxygen ions were bonded with carbon and nitrogen, and were connected with water molecules. The obtained spectroscopic data contains considerable variety of elements and is similar in part to the bovine serum albumin fraction V and human albumin.

**Keywords:** chicken serum, chemical composition, spectroscopy

**Introduction.** Serum is widely used for the purpose of monitoring and diagnosis support for most of poultry diseases also it is used in numerous diagnostic tests, as well as blood typing. Changes in protein profiles as well as levels in body fluids may be of diagnostic, prognostic, and therapeutic significance. Human serum and plasma proteins have already been mapped for clinical applications (Tissot *et al.*, 1991; Anderson and Anderson, 2002; Pieper *et al.*, 2003). In animal species, analysis and characterization of serum proteome are still hindered by incomplete genome information. A few studies have characterized the serum proteome for bovines (Wait *et al.*, 2002), rats (Gianazza *et al.*, 2002), horses (Miller *et al.*, 2004) and chickens (Huang *et al.*, 2006). If the spectroscopic studies of human or bovine serum can be partially found in the scientific literature, so far chemical composition research for chicken serum is absent. Such studies are relevant to the serum usability for technological needs. It is known that properties such as adsorption of proteins and formation of biofilms on solid surfaces plays an important role in various fields of practical application such as biomedical implantation, the food industry, water distribution systems, marine environments, etc. (Haras, 2005; Tengvall, 2003; Latour, 2008). The observed spectroscopic properties can be used to investigate serum interaction with a variety of materials in order to identify various biofilm formation possibilities. Research, such as protein adsorption monitoring is generally carried out using a quartz crystal microbalance, Fourier transform infrared spectroscopy, scanning electron microscopy, X-ray photoelectron spectroscopy (XPS), etc. (Kingshott and Hocker, 2006). The latter technique – XPS is a useful tool for studying the atomic composition and chemical bonds of proteins that have adsorbed substances up to a few nanometers into their surface (Briggs and Seah, 1990), so this method is frequently used to investigate albumin adsorption on various surfaces (for example, see (Martins *et al.*, 2003; Azioune *et al.*, 2005; Ithurbide *et al.*, 2007; Frateur *et al.*, 2007; Vanea and Simon, 2011; Gruian *et al.*, 2012)). However, it is most frequently the case that only

biocompatibility is determined through surface interactions with albumin, improved by the capacity of the material to adsorb proteins on its surface. The changes albumin or serum properties undergo after contact with a surface have not been studied in depth. This may be the result of insufficient XPS reference core level data.

The main aim of this research is to carry out an experimental study of XPS core level spectra and to reveal chemical composition of the predominant elements in uncontaminated chicken serum.

#### Materials and methods

Sterile filtered chicken serum was purchased from “Sigma-Aldrich”. A small amount of material was placed on a glass substrate and left to dry in ambient air for 24 hours in order to obtain a thin layer on the surface.

X-ray photoelectron spectra were acquired at room temperature by using an “ESCALAB MK II” spectrometer (VG Scientific, Great Britain). The photoelectrons were excited using a non-monochromatized MgK $\alpha$  radiation (1253.6 eV). The working pressure in the analysis chamber was  $5 \times 10^{-8}$  Torr during the spectrum analysis. The photoemission data has been collected and processed. After the Mg K $\alpha$  source satellites and background deduction multiple photoelectron spectra were separated into several peaks setting the peak position: binding energy, width (FWHM), relative area, and atomic concentration ratio. The accuracy of the relative intensities and binding energies of the measured lines were about 10% and 0.1 eV respectively. The random C 1s line with binding energy equal to 284.6 eV was used for correction of the charging effects. After background subtraction, a non-linear least squares curve fitting routine with a Gaussian/Lorentzian product function was used for the analysis of XPS spectra. The relative concentrations of chicken serum components were calculated using a standard quantification routine.

#### Results and discussion

A wide energy range surface scan was carried out in order to identify all the chemical elements in the chicken serum sample. The main peaks of highest intensity were observed at binding energies  $\sim 285$  eV,  $\sim 400$  eV, and  $\sim$

532 eV, which correspond to C 1s, N 1s, and O 1s bands respectively. There have also been observed small amounts of P 2p, Cl 2p, and Na 1s elements which are natural for the animal serum. The experimental results are

summarized in Table 1. The main spectroscopic components (carbon, nitrogen, and oxygen) are located similarly to the known data published in references (McArthur *et al.*, 2001; McArthur, 2006).

Table 1. Elemental ID and quantification in the standard chicken serum

Peak	Binding energy, eV	FWMH, eV	Relative area, a.u.	Atomic concentration, %	SF
P 2p	132.94	1.64	455.81	0.96	1.250
Cl 2p	199.83	1.32	1576.23	1.79	2.360
C 1s	284.77	1.97	28180.72	76.94	1.000
N 1s	399.48	1.38	2353.19	3.73	1.770
O 1s	531.99	3.10	13238.25	13.47	2.850
Na 1s	1072.30	3.76	7127.81	3.11	7.990

The detailed XPS spectrum of the carbon region is shown in Figure 1. The C 1s peak has been deconvoluted into four components. The first component, with a binding energy of 284.6 eV, corresponds to C–C bonds, the second component (285.7 eV) – to hydroxyl group C–OH and/or C–N binding, the third component (286.6 eV) – to C=N and/or N–C=C bonds (Kaciulis, 2012), and the fourth (287.8 eV) – to C=O bonds. The binding energy values of the C 1s peak components (~285 eV, ~287 eV and ~288 eV) obtained in our experiments are similar with the results reported for the bovine serum albumin absorbed on passivated chromium (Frteur *et al.*, 2007).

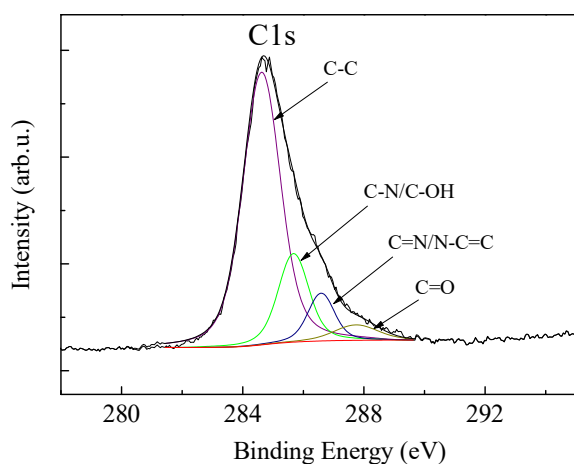


Fig.1: C 1s spectrum for BSA sample

The deconvolution of N 1s core level spectrum reveals three components, as shown in Fig. 2. Analysis of the deconvolution of experimental N 1s spectra based on known results (Martins *et al.*, 2003; Azioune *et al.*, 2005; Ithurbide *et al.*, 2007; Frateur *et al.*, 2007; Vanea and Simon, 2011; Rubio *et al.*, 2002; Kang *et al.*, 1990; Kang *et al.*, 1991; Hasik *et al.*, 2003; Liu *et al.*, 2004; Cruz-Silva *et al.*, 2008) allows us to conclude that fitting components with binding energies 399.3 eV and 400.3 eV are related to imine (-NH=) and amine (-NH-) groups, respectively, while a binding energy value of 402.2 eV is associated with positively charged nitrogen and can be attributed to the protonated nitrogen groups (-NH<sup>+</sup>-/NH<sub>2</sub><sup>+</sup>).

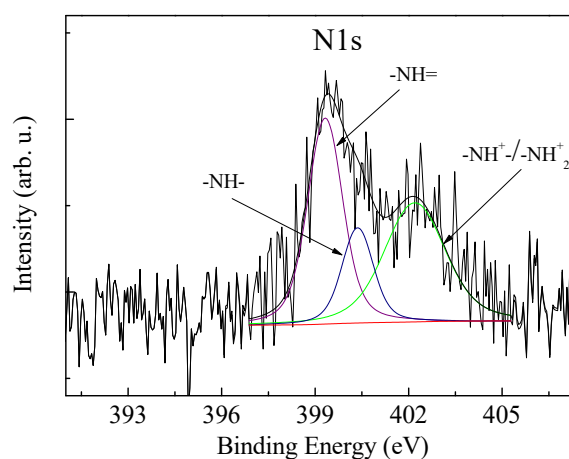


Fig. 2: N 1s spectrum for BSA sample

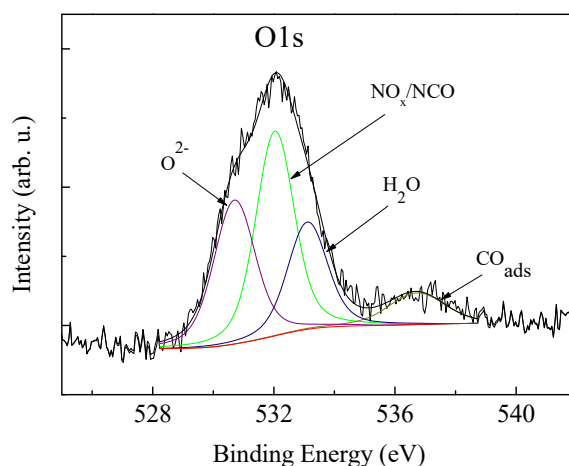


Fig. 3: A O 1s spectrum for BSA sample

Figure 3 shows the XPS spectrum of the oxygen region. The O 1s peak consists of three components with binding energies 530.7 eV, 532.0 eV, and 533.1 eV. The first and third components correspond to the oxygen O<sup>2-</sup> ions and oxygen ions in the water molecules, respectively (Dzhurinskii *et al.*, 1975; Hopfengärtner *et al.*, 1993; Lim and Atrens, 1990; Pilleux *et al.*, 1994; Becarria *et al.*, 1995; Gardner *et al.*, 1995). The third component, with a

binding energy of 532.0 eV, might be caused by the presence of NO<sub>x</sub> in the blood serum (Merritt *et al.*, 1983) and/or nitrogen-carbon-oxygen (NCO) bonds (Bui *et al.*, 1993).

### Conclusions

The standard chicken serum was studied by using X-ray photoelectron spectroscopy. The obtained spectroscopic data contains considerable variety of elements: carbon (76.94 %), nitrogen (3.73 %), oxygen (13.47 %), phosphorus (0.96 %), chlorine (1.79 %), and sodium (3.11 %). The main peaks of survey spectra were observed at binding energies ~ 285 eV, ~ 400 eV, and ~ 532 eV, which correspond to C 1s, N 1s, and O 1s bands respectively. The study revealed that carbon spectrum contains four types of chemical bonds: C–C, C–N/C–OH, C=N/N–C=C, and C=O. Nitrogen ions occur in three forms: imine (–NH=), amine (–NH–), and protonated nitrogen groups (–NH<sup>+</sup>–/–NH<sub>2</sub><sup>+</sup>). Oxygen ions were present as oxygen O<sup>2–</sup>, H<sub>2</sub>O, and, NO<sub>x</sub>/NCO.

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