Cobalt (Co) and chromium (Cr) ions, which can be potentially released from total hip replacements (THR) manufactured from CoCr alloy, are classified by IARC as potentially carcinogenic and carcinogenic, respectively, to humans. Their formation and systemic distribution thus demand special attention. The aim of the present work is to develop an accurate and reliable method for the determination of Co and Cr concentration down to µg/L (ppb) level in human serum that will also provide information about the species of the Cr ions present. For that purpose we used an electrochemical method of adsorptive stripping voltammetry (AdSV). AdSV is the most successful method for electrochemical trace analysis with reported limits of detection down to 10^{-10} M range. The sensitivity of AdSV method is based on the preconcentration, i.e., accumulation, of dissolved metal ions from solution at mercury electrode. Metal ions are complexed by the addition of an appropriate complexing agent, which is selective for that particular metal. The metal complex formed is then adsorbed at a mercury electrode resulting in 100-1000-fold increased concentration of metals ions at the electrode compared to the concentration initially present in the solution. The collected metal complex is then stripped, i.e., reduced, with the electrochemical reaction occurring at the characteristic reduction potential, where a peak appears. The height of the peak is proportional to the concentration of the analyte, i.e., metal. The quantification is carried out by the standard addition method.

Methods:
We have further optimized the original methods developed for water solutions to achieve the lower detection limit and higher sensitivity needed for measurements of Co and Cr in human serum. Measurements were performed using an EG&G Instruments PAR Electrochemical Trace Analyzer Model 394 with a cell Model 303A and a stirrer Model 305. The system contained a mercury working electrode, an Ag/AgCl reference electrode and a Pt auxiliary electrode. All the chemicals used were of suprapure quality. Strict precautions were taken to prevent metal contamination during sample collection and processing. 0.5 ml of serum was acid digested by the combination of sulfuric acid, nitric acid and hydrogen peroxide in a 10 ml Kjeldhal flask. The digested sample was then dissolved in an appropriate buffer. For Cr determination 0.1 M ammonia buffer of pH 9.2±0.2 was used with the addition of diethylene triamine pentaacetic acid (DTPA). The concentrations of Co and Cr were measured in four healthy persons with no history of metal implants. The second blood tube was taken for measurements. For each person at least two measurements were made.

Results:
The AdSV method developed involved the formation and interfacial accumulation of the metal complex onto the working mercury electrode and subsequent measurements of the reduction peak of the accumulated complex. The adsorption potential for Co, where the adsorption of metal complex with DMG proceeds, was at −0.8 V, and adsorption time was 60s. The complex was then stripped in the negative direction from −1.1 V to −1.3 V, where the reduction peak occurred at −1.20 V. The adsorption potential for Cr, where the accumulation of metal complex with DTPA proceeds, was at −1.2 V, and adsorption time was 20s. The metal complex was then stripped in the negative direction from −1.15 V to −1.45 V, where its reduction peak occurs at −1.35 V. The optimum parameters of adsorption potential and time and concentration of complexing agent, we can measure concentrations down to 0.05 µg/L level in human serum.

The quantification was performed by the standard addition method using standards prepared daily from the stock solution. Figure shows an example for the measurement of Co in digested human serum dissolved in DMG containing ammonia buffer. The concentration of Co in the sample was determined after two standard additions of 0.3 and 0.6 µg/L of Co. The mean concentrations of Co and Cr measured in healthy persons is as follows:

Co: 0.11 ± 0.03 µg/L (range 0.07 – 0.17), and
Cr: 0.25 ± 0.04 µg/L (range 0.23 – 0.31)

The results agree well with reference data. We have tested the method on reference serum of human origin (Seronorm, Norway) and by comparing the results with ETAAS and ICP-MS. With ETAAS method satisfactory agreement was obtained (ETAAS: 0.27 µg/L Co and AdSV 0.21 µg/L Co; ETAAS: 0.54 µg/L Cr and AdSV 0.41 µg/L Cr). ICP-MS method was not able to measure cobalt concentrations lower than 0.5 µg/L.

Discussion:
The concern with using M/M THRs is related to the possibility of increased magnitude of metal wear and long-term local and systemic effects. Part of this concern stems from the difficulty in measuring accurately the amount of released ions. There are many difficulties in obtaining uncontaminated samples, and the number of research centers capable of testing samples at the ppb level are few in number and expensive to run. With this work we hoped to have contributed to the development and application of electrochemical techniques in the biomaterials field. The electroanalytical procedures described herein based on the use of stripping voltammetry technique represents an interesting alternative to the traditional methods for trace metal analysis due to the low cost of the equipment required (up to 20,000 USD), the short measuring time (10min) and low detection limit (down to 0.05 µg/L). We will use this method for measurements of cobalt and chromium in sera of patients with THRs. The method has a further advantage of being a species-sensitive method not just an element-sensitive method, which gives us a starting point for speciation of chromium in serum. It is well known that compounds of Cr(VI) are 10- to 100-times more toxic than compounds of Cr(III). The possibility of differentiation among various oxidation states of chromium in serum has not yet been offered by any method.

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