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Short communication

Quantitative thin-layer chromatographic identification of dental base alloys¹

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Abstract

In the corrosively aggressive medium of the oral cavity, the use of identical dental alloys requires identification of the existing metal construction. One of the methods allowing this identification is quantitative thin-layer chromatography with anodic sampling. Using a 4.5-V battery and suitable electrolytes, seven dental alloys for fixed and removable dentures based on cobalt were analysed. Chromatograms of alloy samples were developed with a mixture of acetone and 2 M HCl. Scanning of the thin-layer chromatographic spots produced chromatographic curves and the area under the curve was proportional to the content of cobalt in the alloy studied. Regression analysis showed a very high coefficient of correlation ($r=0.999$) between the area of the spot and the proportion of cobalt. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Metal as the main part of the denture is incorporated daily into biological medium. The choice of an alloy depends on its composition and properties which have to satisfy both the functional duration of the appliances and friction in the oral cavity, as well as avoid galvanism or sensitisation or allergy [1].

As the oral cavity is an aggressive corrosive medium, it is recommended that in every following prosthodontic therapy, a familiar dental alloy is used, i.e. one of the same or at least similar composition as the existing dentures already in the oral cavity of the patient. As there is no law in Croatia obliging the

therapist to file information on the type of material used, it has to be identified in a different way. Furthermore, knowledge of the alloy composition is essential for evaluating undesired micrographic and macrographic alloy changes that may well result from inadequate or negligent laboratory treatment [2,3].

There are two main groups of methods for identification of the composition of dental alloys based on sampling. The first one comprises laboratory analyses with specially prepared samples of certain dimensions and shapes. This group includes X-ray emission spectroscopy (XES), proton induced X-ray emission (PIXE) spectroscopy, atomic absorption spectroscopy (AAS), glow discharge optical emission spectroscopy (GDOES), etc. The second group of the methods comprises analyses of samples taken from the patient's mouth.

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Using X-ray emission spectroscopy, Kosovel et al. [4] estimated that dental alloys did not, after melting, reveal any significant changes in the ratio of the main components detected. The greater presence of zinc on the sample surface after melting may be attributed to the fact that this element migrated to the surface in all samples where it was an additional component. Živko-Babić et al. [5] have proved, through PIXE spectroscopy, the diffusion of certain metals from the crown inside the natural tooth, where gold and copper showed greater mobility in comparison to silver and palladium. It is very important for the dentist to identify metallic dental materials already incorporated in patient's mouth. The analysis of samples taken from the mouth are: abrasive stripping voltammetry, 'splitter test' with energy dispersive X-ray (EDX) analysis and anodic sampling with thin-layer chromatography (TLC). Stripping voltammetry is based on an abrasive transfer of alloy traces onto a solid graphite electrode surface and a subsequent electrochemical oxidation of the deposit [6,7]. The amount transferred is very small, i.e. ranging from 10^{-6} to 10^{-8} . Different metallic phases may be oxidized at a different potential to the characteristic peaks in the current-voltage curve.

Such a voltammogram can be used for a fingerprint recognition of a certain alloy. The counter electrode is made of platinum, and the saturated calomel electrode is the reference. Scholz et al. [6] advocated the use of this method for analysing amalgams (filling material for restoring the posterior teeth), while Živko-Babić et al. [7], using the same method, analysed the composition of dental alloys (for casting prosthodontic appliances), with a different metal as the main component in them. The

'splitter test' together with EDX analysis is a unique method enabling fast identification of the metal components in the mouth [8]. Some metal powder is ground off the crown cemented in the mouth with a rotating carborundum stone, and spread on a round, self-adhesive, graphite foil, which then becomes conductive. The metal powder spread in this way is identified with the EDX analysis, with high precision, up to 1% of the mass proportion of a certain metal. Suzuki [9] used X-ray fluorescence spectroscopy to detect the allergen metals in intra-oral metal restorations. The dental literature mentions the use of chromatography in the analysis of enzymes in the saliva [10], while gas chromatography helps evaluate low-molecular-mass substances in polymers, e.i. polymethylmethacrylic (PMMA), which is used for removable dentures [11,12].

To the best of our knowledge, data on use of anodic sampling with TLC for the identification of the dental alloy composition is not described in the literature. Therefore the possibility of identifying a certain component or a majority of the components in an alloy of unknown composition, as well as the intra-oral identification on an incorporated prosthetic reconstruction, has been a real challenge.

2. Experimental

2.1. Material

The chromatographic test was conducted on seven dental base alloys for fixed and removable dentures. Each alloy contains cobalt as the main component in different mass proportions. The composition of the tested alloys is shown in Table 1.

Table 1
Composition of dental base alloys according to the manufacturer (weight%)

No.	Samples	Co	Cr	Mo	Si	Fe	Mn	W	C	V
1	Dentalit C	61	31.5	5.5	0.5	1			0.1	1
2	Sheralit F	64–66	28–29	5–6			0.3		0.4	
3	Wironium	62.5	29.5	5	1	0.5	0.5		0.2	
4	Biosil F	64.8	28.5	5.3	+		+		+	
5	Remanium CD	65	28	4.5	1.6	+	+			
6	Keralloy	66.5	20	6	1			6.0	+	
7	Wirobond	61	26	6	1	0.5		5	0.005	

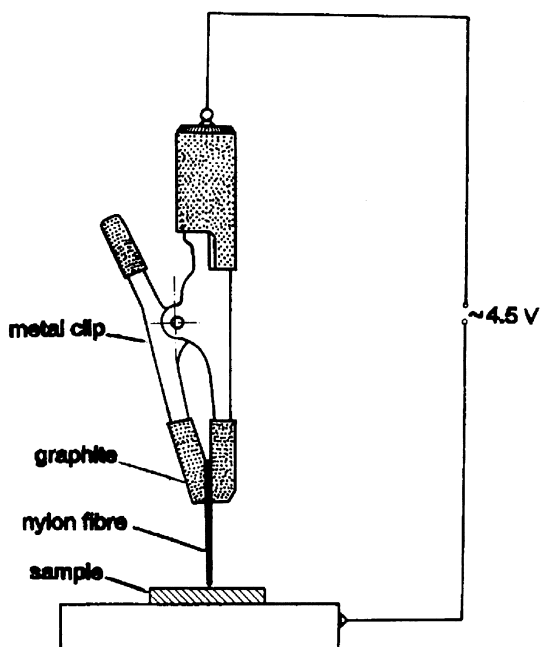


Fig. 1. Scheme of the apparatus for microanodic sampling.

2.2. Equipment

The scheme of the apparatus for anodic sampling is shown in Fig. 1. It consists of a metal clip coated with pure graphite which holds a nylon fibre tip (2×20 mm). The traces obtained of alloy were applied on the chromatographic plates (200×200 mm) with a coat of microcrystalline cellulose, thickness 0.1 mm, without an indicator (E. Merck, Darmstadt, Germany). The plates were placed in the chromatographic vessel (300×80×300 mm) for developing the chromatograms. A CAMAG-TURNER fluorometer 111 (Muttensz, Switzerland) was used for measuring the intensity of the cobalt spots on the chromatogram.

2.3. Chemicals

The sampling was performed in glycerol–water–HCl (conc.) solution, in a 10:10:1 volume ratio. Chromatograms were developed by the solution of acetone–HCl (conc.)–water, in a 86:8:6 volume ratio. Visualization of cobalt spots was carried out by

spraying the chromatograms with the alizarin S ethanolic solution (5 g/l).

2.4. Sampling principle

The principle of anodic sampling lies in the anodic dissolution of the tested material by a special minielectrode. The electrode consists of a metal handle covered with graphite, inside of which is a nylon fibre wetted by a certain electrolyte. A 4.5 V-battery serves as a source of direct current. The minielectrode is connected to the negative battery pole through the metal conductor, while the other conductor is connected to the positive battery pole (Fig. 1). During the sampling, the electrode with the negative pole is connected to the area where the sample needs to be tested (Fig. 2), while the other electrode is attached to another metal in contact with the sample or to a part of the denture in the oral cavity (Fig. 3). A new nylon fibre was used for each

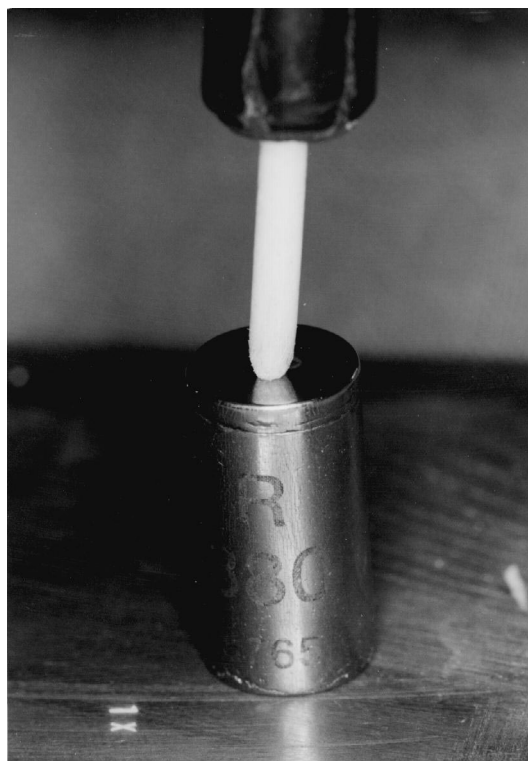


Fig. 2. Alloy sampling of reference material.



Fig. 3. Alloy sampling in the oral cavity of patient.

sampling at room temperature (ca. 23°C), previously wetted by the electrolyte. By closing the electric circuit, the dissolution of anodic sampling occurs and the newly formed ions are transferred from the sample to the lower part of the wetted cluster of nylon fibre. During the dissolution of the sample, a reaction occurs where the dissolved metals in ionic form are transferred to the nylon fibre of the electrode. The sampling ends after 20 min. The following reactions occur during the anodic sampling [13,14]:



The nylon fibre is attached to the absorbing layer of the chromatographic plate immediately after the sampling, 20 mm from the lower end of the plate, and is kept there for 10 s. The microcrystalline cellulose is then the stationary phase, and the developer, the mobile one. Through the capillary forces, a part of the electrolyte is transferred from the nylon fibre to the dry chromatographic layer, where the total sample mass obtained by anodic sampling is transferred as well. The chromatographic plate is dried with hot air, cooled and placed in a chromatographic vessel filled with a developer. The chromatographic process is then initiated with the developer and the migration distance is 120 mm. The chromatographic plate is dried with hot air. The

dried chromatograms are sprayed with ethanolic solution of alizarin to visualize the spots, which are then dried until all the traces of hydrochloric acid, absorbed onto the chromatographic layer, have been removed. Chromatographic plates with absorbed hydrochloric acid were caused by solution ammonium spray.

By spectrophotometric scanning of the spots containing cobalt with the CAMAG–TURNER device, a curve showing a change in the concentration of cobalt in the mentioned stains is obtained. The areas under the chromatographic curves are defined by the Monte Carlo method [15]. The regression curve is obtained by the least-squares method, the closest to the dependence of the relative surfaces and the cobalt mass, i.e. its proportion in the tested alloys [16,17].

3. Results

The chromatographic curves obtained by spectrophotometric scanning of the cobalt spots for all seven samples are shown in Fig. 4. The areas under the chromatographic curves determined by the Monte Carlo method are shown in Table 2. The regression line obtained from the relationship between the area and the mass of cobalt spots was $y=0.966+0.13x$. The standard error of estimate was 1.073. A high coefficient of correlation ($r=0.999$) between the areas under the curve and the proportion of cobalt in the tested alloys was obtained. The results show that the measured mass percentages of cobalt vary in relation to the declared values, 0.0% for Dentalit C and Remanium CD, 0.8% for Wironium and Keralloy, 1.2% for Biosil F, 1.6% for Wirobond and 2.9% for Sheralit F.

4. Discussion and conclusion

Metal constructions or alloy components may provoke tissue reactions, and the use of alloys of different composition (degrees of precious metals) may cause oral galvanism. All dental alloys are named after the main component which is present in the largest proportion. As cobalt is the base of the tested alloys, we tried to specifically prove and confirm its proportion, while simultaneously testing

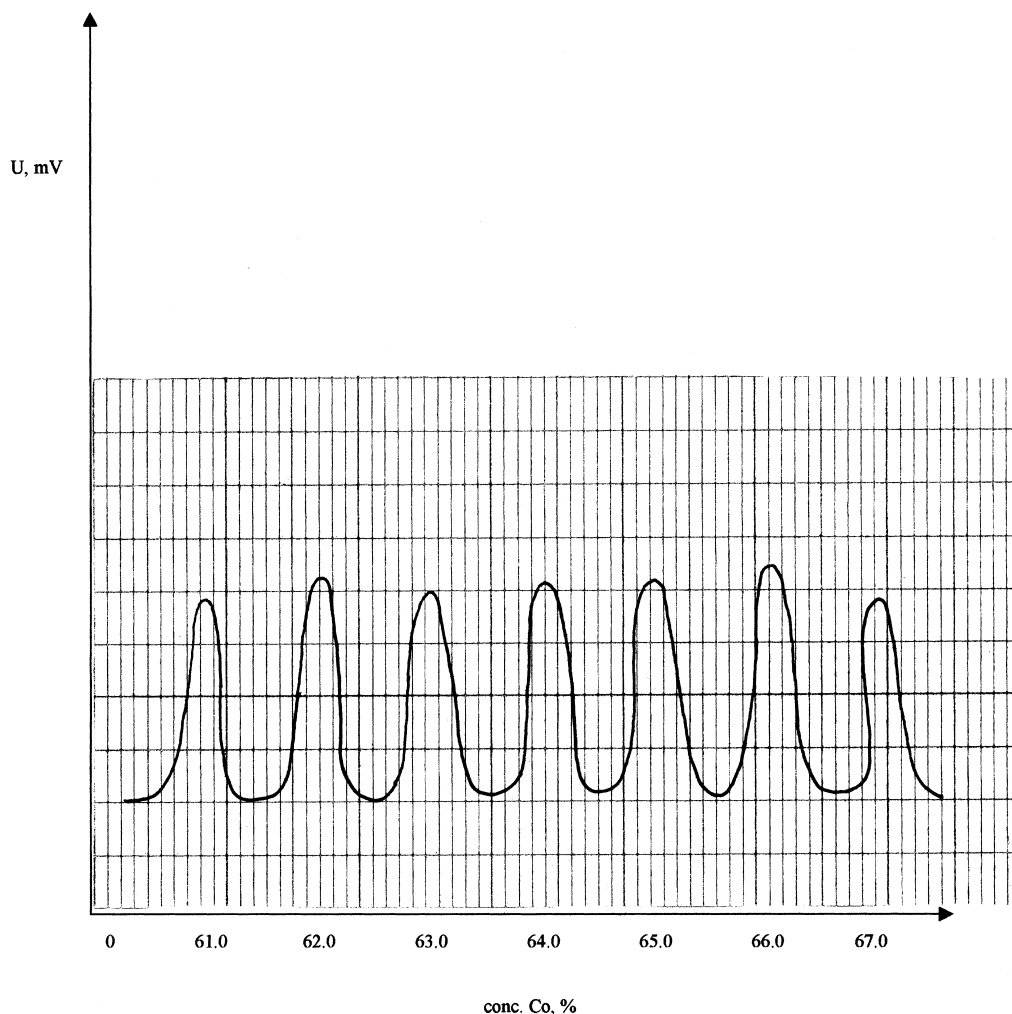


Fig. 4. Chromatographic cobalt curves obtained by scanning the spots.

Table 2
Relationship between the area under the chromatographic curves and cobalt in the spots

Samples	A/cm^2	Co (weight%)
Dentalit C	9.0	61.0
Sheralit F	10.2	67.0
Wironium	9.5	63.0
Biosil F	9.7	64.0
Remanium CD	9.8	65.0
Keralloy	9.8	65.0
Wirobond	8.8	60.0

the listed sampling methods. The identification of the proportion of the main metal in base alloys, which are cobalt or nickel, is sufficient to suppose which other metals and nonmetals are present, i.e. which alloy composition is to be used in the next prosthetic therapy. The proportion of the other elements varies more or less depending on the purpose (fixed or removable denture), since it is known that the alloy composition is determined by its properties in use. However, if allergic reactions occur, the identification of exact elements is needed. Nickel has proved to be an allergen provoking an allergy in 10% more

women than in men [18]. Because of this, methods that permit the determination of the composition of an oral sample are needed.

TLC in combination with anodic sampling has proved to be appropriate in terms of the time of the sampling, the size of the sample, as well as no limit restrictions regarding the element's mass. A very small sample, not larger than 10^{-6} g, may be identified by this method. Furthermore, the result of such an analysis may be used as a fingerprint, as well as for quantitative analysis. In the first case, the chromatogram resulting from an unknown sample is compared with the chromatogram resulting from 'reference materials'. In the second case, the so-called calibration curve of the main elements of dental alloys is obtained through the analysis of reference materials, which then helps to determine the proportion of a certain element in an unknown sample. We have limited this pilot study to the research of Co-based reference alloys. In order to identify other metals that are present in the alloys (Cr, Mo, Mn, Fe), electrolytes that produce soluble salts with the listed elements in the ionic form should be chosen, on condition that all ions obtained have a positive charge. The voltage of the direct current has to be much higher than that needed to transform the metal into the corresponding ion. In our case, it has been shown that the direct current voltage ($U=4.5$ V) is high enough to get ions by dissolving the alloys during anodic sampling. There is a possibility that, if the direct current voltage is not high enough, the dissolution of certain elements from the alloy occurs, which leads to false results. Conversely, the choice of electrolytes is important due also to the possibility of the electrolyte elements influencing the chromatographic process. The method proved to be limited in cases of alloys containing silver, since the insoluble AgCl occurs in the reaction of HCl solution with silver, and also in the case of silicon and carbon testing similar to the situation in the EDX analysis. Interferences are possible if the alloy contains elements with the same R_F values (e.g. Al and Cr). The sampling itself is equally fast as in stripping voltammetry. There is no need for grinding as in the 'splitter test' and it is reproducible. Stripping voltammetry is only a qualitative method and its reproducibility is limited in comparison to the other chemical analysis. The comprehensive interpretation

of the voltammograms in reference [6] was still missing because the peak position of a certain metal can vary considerably when this metal is an alloy component. Nickel was the only constituent giving an oxidation signal in the accessible potential range in Wiron 77. In Wiron S and Wirron 88 the electrochemical oxidation of nickel was inhibited [6]. The 'splitter test' with EDX analysis is, according to Wirz et al. [8] the most sensitive method, whose implementation is simple and fast and provides qualitative and quantitative data. The 'splitter test' shows insufficiencies in comparison with anodic sampling, since the crown in the mouth has to be ground, although insignificantly. The EDX analysis of these samples showed deviations between -2.5% to $+2.5\%$ in measurement of the unknown alloy, which corresponds to the results obtained by us. It is evident that a large proportion of a certain element results in lower deviations in the measurements. However, the EDX analysis cannot identify nonmetals such as silicon and carbon frequently found in dental alloys, while the use of X-ray emission spectroscopy is limited in the case of carbon and magnesium [19].

By summarizing all the advantages and deficiencies of the method enabling the analysis of orally taken samples, the anodic sampling with TLC seems to be the most suitable method. The results obtained in this test of reference alloys identify not only the composition (main component), but also the proportion of a particular element, in this case cobalt, with relatively high accuracy, and represent a pioneering move in the field of prosthodontics and its dental alloys. In our future work we will try to extend the use of this method to clinical sampling, where the answers are of much greater importance.

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