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LASER INDUCED BREAKDOWN SPECTROMETRY FOR QUANTITATIVE ANALYSIS OF Cd IN ARTIFICIAL SWEAT LEACHATE OF JEWELLERY

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A new method has been developed for the determination of cadmium in artificial sweat leachate using laser induced breakdown spectrometry (LIBS). Small volume of liquid samples could be applied onto the solid carrier, dried under the IR lamp, and subsequently analysed by the LIBS device. For calibration, two series of standard solutions of artificial sweat were prepared: alkaline (pH 8) and acidic (pH 5.5). The known volumes of the Cd standard (with concentration of 1 ± 0.001 g l^{-1}) were added into the samples of artificial sweat; calibration standards prepared ranging from 0-40 mg l^{-1} for acidic and 0-30 mg l^{-1} for alkaline sweat. To optimize the LIBS measurements, the regression parameters (R^2, AIC, MEP)

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were the criteria of choice. For our purposes, three cadmium lines at wavelengths 214.44 nm, 226.50 nm, and 228.80 nm were monitored. In model solutions, the method developed has provided the limits of detection (LODs) of 0.08 mg l^{-1} *for acidic and of 0.06 mg* l^{-1} *for alkaline sweat.*

The optimized method could then be used for the determination of Cd in artificial sweat leachates of jewellery samples, when the values obtained were in good agreement with the results from the reference analysis by ICP-OES. The proposed method is fast, simple, and requiring only minimal volume of samples. For analysis of leachate of jewellery, fabrics or products of daily consumption, this approach employing LIBS can be an interesting alternative to commonly used techniques, such as ICP OES or AAS.

Introduction

Quantitative analysis of toxic elements in leachates of simulated sweat may be crucial to distinguish between harmful and harmless objects potentially coming into contact with human skin. Such types of analyses can involve an idea about the transport of harmful and toxic components (e.g. Cd, Co, Ni, Pb and others) into the human sweat and about their effects in direct contact with the body. Similar analyses can concern not only jewellery, but also toys, coins, textile, and other items of everyday consumption [1-3]. The elements observed can cause various allergies and irritation or dermatitis. In several publications, similar experiment was conducted with the opposite purpose — to demonstrate how much sweat is harmful for metallic materials and what damage may cause. A view from the other side, when human sweat can damage the metal parts of tools and devices, was also described [4]. For analogous experiments, a simulated human sweat is being used. The experiments with leachate should be performed in unambiguously defined solutions that are usually controlled by the national or international regulations [5-8].

For analysis of artificial sweat, the most frequently used techniques are ICP-OES, ICP-MS, and AAS [9-12]. Not so common method is e.g. potentiodynamic polarization applicable to determine the degree of damage of tools caused by sweat [13].

Advantages of these approaches to the solution analysis are low detection limits and speed of analysis. The disadvantage may then be the investment into the equipment and rather high operational / running costs.

Laser Induced Breakdown Spectrometry (LIBS) offers advantages for analysis of sweat leachates and similar liquid samples. Many commercially available spectrometers for LIBS are not suitable for direct analysis of such liquids. Solutions can be applied on the surface of a solid substrate, e.g. paper, metal or plastic carrier and, after drying, carriers can be analysed directly by LIBS as described in several publications [14,15], but the analysis of sweat samples by this technique has not yet been described. Proposed procedures for sample preparation minimise the matrix effect of samples and improve the limits of detections. Other advantage is a reduction of the sample volume for analysis; the respective measurements being fast and simple. In our opinion, LIBS is an interesting alternative to the analytical methods mentioned above and employing ICP-MS or AAS.

In the method proposed herein, LIBS was used for determination of Cd in two types of artificial sweat applied onto solid carrier. For leaching of jewellery two types of solutions were used differing in conditions simulating the pH of human sweat. When using commercially available spectrometers, the samples of solutions cannot be analysed directly, but a small volume (several tens of microliters) is applied onto the solid carrier that is dried and then analysed by LIBS. After drying, the samples may be stored for some time in closed bags. Similar procedure for this type of analysis is not used often; nevertheless, the resulting speed, ease of use, ability to analyse very small volumes, and the possibility to store samples seem to find some application.

Experimental

Reagents and Standards

First, two different types of artificial human sweat were prepared according to Decree of the Ministry of Health on hygiene requirements for toys and products for children up to 3 years (No. 84/2001 Coll.). Acid sweat was prepared from 0.5 g $C_6H_9N_3O_2{\cdot}HCl{\cdot}H_2O$ (L-histidine monohydrochloride; Sigma-Aldrich, purity 98 %), 5 g NaCl + 2.2 g $NaH_2PO_4\ {\cdot}2H_2O$ (both p.a. and purchased from Lach-Ner,). Acidity was adjusted with the aid of 0.1 mol l^{-1} NaOH (Penta, p.a.) to pH 5.5 and made up to a total volume of 1 l. Alkaline sweat was prepared from 0.5 g $C_6H_9O_2N_3{\cdot}HCl{\cdot}H_2O$ (as above), 5 g NaCl + 5 g $Na_2HPO_4{\cdot}12\ H_2O$ (both as above) and made alkaline with pH 8 by adding the appropriate amount of 0.1 mol l^{-1} NaOH (as above) in the total volume of 1 l. Subsequently, two series of calibration standards of Cd were prepared in the concentration range of 0-40 mg l^{-1} (acid sweat) and 0-30 mg l^{-1} (alkaline sweat). Each standard was applied in a volume of 20 µl onto the solid paper carrier. Carriers were dried under IR-lamp and analysed by LIBS. Carriers for liquid samples were prepared from an ordinary plain paper using a metal die with a diameter of 17 mm.

Samples

Samples of jewellery were provided by Czech Environmental Inspectorate (CEI). The respective specimens came from three inspections of suppliers who had served on internet shops or stores with a low cost jewellery from China. Leachates of samples were prepared based on the area of jewellery (size in cm^2), calculated and for leachates as a two-fold amount of the artificial sweat used (in ml). Area sizes and volumes of solutions are shown in Table I. Samples were plunged into the solution in plastic container and stored at 30 °C for 168 hours according to the EN 1811 and No. 84/2001 Coll.

Afterwards, the samples were removed and the leachates prepared for measurements by LIBS, similarly as the calibration standards. Leachates were analysed by ICP-OES directly.

Table I Area size of jewellery and volume of artificial sweat used

Sample	Area size, cm^2	Sweat volume, ml	Type of sweat
9-A	9	18	Alkaline
7-A	6.25	12.5	Alkaline
2-B	9	18	Acid
Bangle	15	30	Alkaline
Ring	3	6	Acid

Laser Induced Breakdown Spectrometry (LIBS)

Instrumentation

In this work, a commercially available compact spectrometer for LIBS (model “LEA S500”; Solar TII Ltd., Belarus) was used. The system consists of a dual pulse Q-switched Nd: YAG laser operating at 1064 nm. The laser emits two co-linear pulses with duration of about 12 ns and with energy per pulse varying typically between 80 and 150 mJ at a maximum repetition rate of 20 Hz; the inter-pulse delay setting being from 0 to 20 ms. The spectrometer is equipped with Czerny–Turner spectrograph offering a focal length of 500 mm and grating of 1800 lines mm^{-1}, when providing a linear reciprocal dispersion of 1 nm mm^{-1}. The wavelength range of the spectrograph is from 170 to 800 nm, while particular measurements are carried out in 30 nm wide spectral regions. Spectra are recorded with the aid of a back-thinned and front-illuminated CCD camera (2048×14 pixels) having a fixed integration time (gate width) of 1 ms; the optical spectral resolution being better than 0.03 nm for the whole spectral range. Samples are placed inside the ablation chamber on motorized sliding table and if necessary, the ablation chamber can be flushed with an inert gas (He, Ar) or even evacuated.

Optimization of LIBS Parameters

On LEA S500 spectrometer, spectra were recorded in a 30 nm-wide spectral window. For this reason, more Cd-lines were sought so that they could be monitored in one spectral window. The most suitable region was evaluated to be from 205 to 235 nm. This area contained strong Cd-lines lying at wavelengths of 214.44 nm, 226.50 nm, and 228.80 nm (see Fig. 1). Subsequently, measuring conditions were optimized for the artificial sweat with the Cd concentration of 10 mg l^{-1}. Optimisation of the respective parameters for LIBS was being done with respect to the given SBR (signal-to-background ratio). The value of each optimised parameter providing the highest value of SBR was then selected as an optimum. The parameters with the greater effect on the analytical signal were optimised first: focal spot diameter and the energy of laser pulse. Spot size was varied from 200 to 500 μm in the step increments of 100 μm. The small width of the spot was advantageous because of feasibility to analyse the sample area at more analytical points that do not overlap. The effect of the laser pulse energy was tested in the range from 80 to 120 mJ.

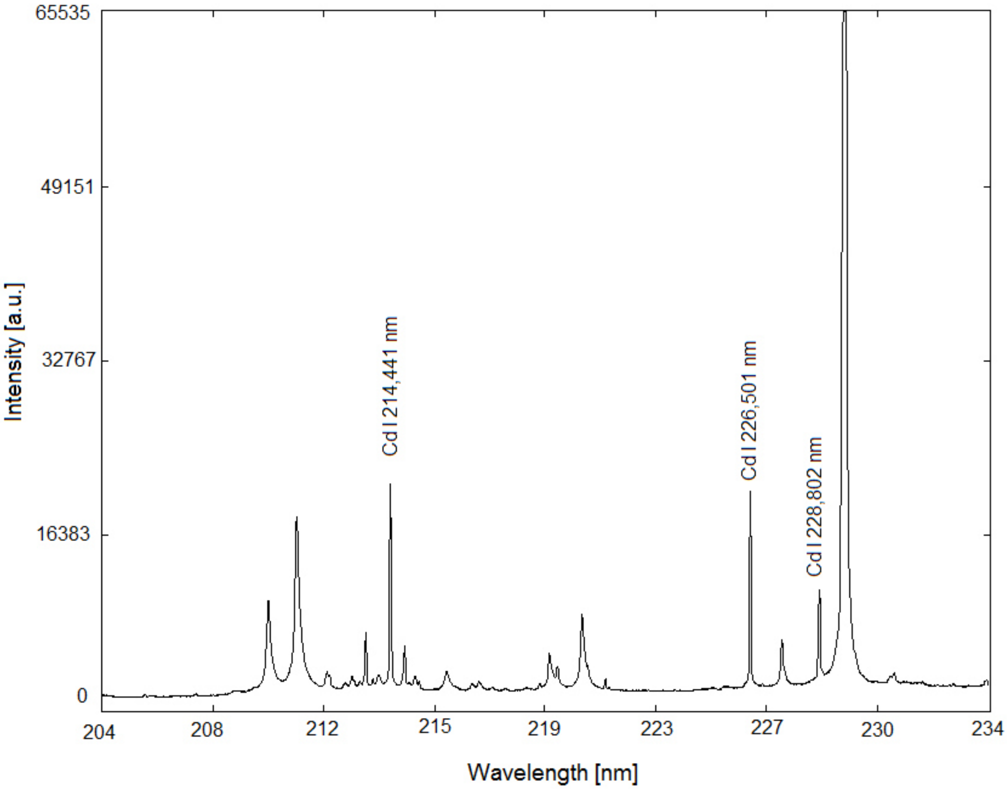

Fig. 1 Spectrum of Cd lines used for analytical purpose (Cd concentration 40 mg l^{-1} in artificial acidic sweat)

Subsequently, the width of the entrance slit, interpulse delay, the number of analytical points, and the number of pulses per point were optimised, too. The width of the entrance slit was changed from 10 to 30 µm, which was important for good spectral resolution and the ability to separate close wavelengths. It determined the amount of radiation reaching the detector. Furthermore, the interpulse delay was a parameter used in the double pulse (DP) mode of LIBS and indicating the time when the plasma had admitted the second laser pulse. The respective delay has been optimized in an interval from 0 to 10 µs; a number of analytical points (4-25) being selected to cover the maximal area of the carrier and to prevent replicate ablation at the same site. A number of pulses per one analytical point (1-3) was chosen so as to avoid breakdown of the carrier material. Experimental parameters were adjusted to obtain maximal intensity of the monitored lines and good reproducibility of the measurement. During each measurement, the paper carrier was dried under IR-lamp and placed in between two plastic plates with opening. These plates were encumbered by a steel block on the sliding table in the ablation chamber.

ICP-OES Analysis

ICP-OES technique was employed as a reference method for validation of the results obtained with LIBS. An ICP-OES spectrometer Integra XL (GBC, Scientific Equipment, Braeside, Australia) was equipped with a standard conical nebulizer (with sample flow rate of 2 ml min^{-1}) and cyclonic spray chamber (both glass Expansion, West Melbourne, Australia). Two spectral lines (228.80 and 226.50 nm) were selected; the results of both lines being averaged. The working conditions for ICP-OES analysis, when a calibration up to 1 mg l^{-1} Cd has been used, are summarized in Table II.

Table II Conditions set up for analysis by ICP-OES

Sample flow rate	1.5 ml l^{-1}	Photomultiplier voltage	600 V
Plasma power	1000 W	Viewing height	6.5 mm
Plasma gas	10 l min^{-1}	Number of replicates	3
Auxiliary gas	0.6 l min^{-1}	On peak integration time	1 s
Sample gas	0.65 l min^{-1}	Off peak background correction	Two points

Results and Discussion

Optimisation of LIBS Parameters

Based on our previous experiences with optimization of LIBS parameters, the focal spot diameter was expected to be a parameter with the strongest effect on the shape of the calibration curve and hence, it was optimised first. The highest value of SBR was obtained for a focal spot diameter of 200 µm. This value was used as fixed for further optimisation.

The second most important parameter was the energy of laser beam; its evaluation being performed based on the SBR graph depicted in Fig. 2. The highest value of SBR for this parameter corresponded to an energy of 110 mJ. For this set of conditions, we could obtain the spectrum without saturation and with sufficiently high intensity of all analytical lines monitored. Other parameters for optimisation were the width of entrance slit and interpulse delay. Regarding the former, the highest SBR value obtained was for 25 µm. The best value of the latter for our compact LIBS instrument was ascertained to be 7 µs, as documented by the graph of SBR in Fig. 3.

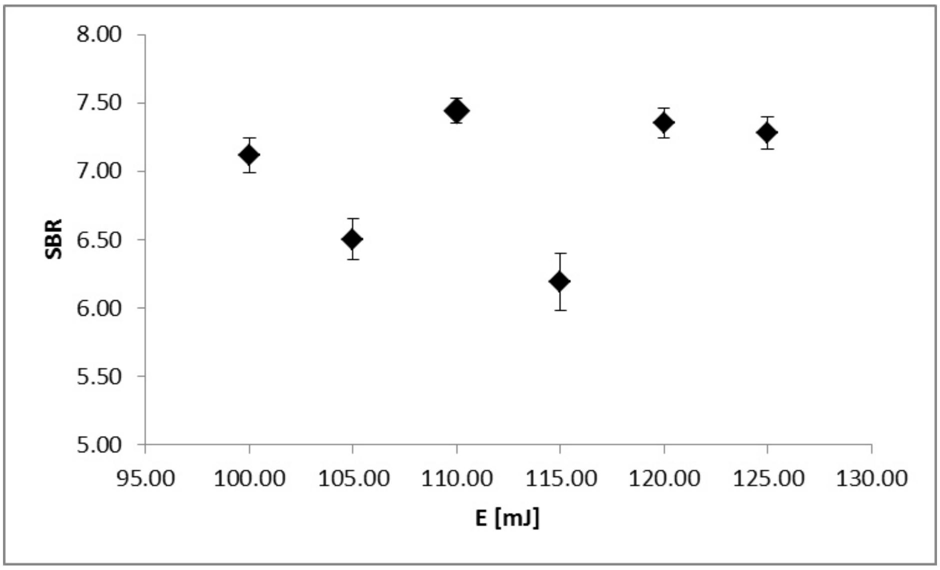

Fig. 2 Optimisation of the laser energy based on the highest value of SBR (signal-to-background ratio). Optimised for Cd concentration of 10 mg l^{-1} at wavelength of 214.441 nm

Another parameter, the number of pulses for analytical point was limited by the material acting as solid carrier. In our experiments, we had used an ordinary plain paper, and more than one pulse per analytical point already caused a rupture and destruction of carrier. The number of analytical points was then limited by dimensions of the ablation chamber and by the size of analysed samples. The focal spot diameter was set as the smallest possible; therefore, it was possible to use 9 analytical points for one analysis.

Thus, the final conditions for LIBS measurements were set as follows: (i) the focal spot diameter, 200 µm; (ii) energy of laser beam, 110 mJ; (iii) width of entrance slit, 25 µm; (iv) interpulse delay 7 µs, (v) one pulse per analytical point, and (vi) 9 analytical points for one analysis.

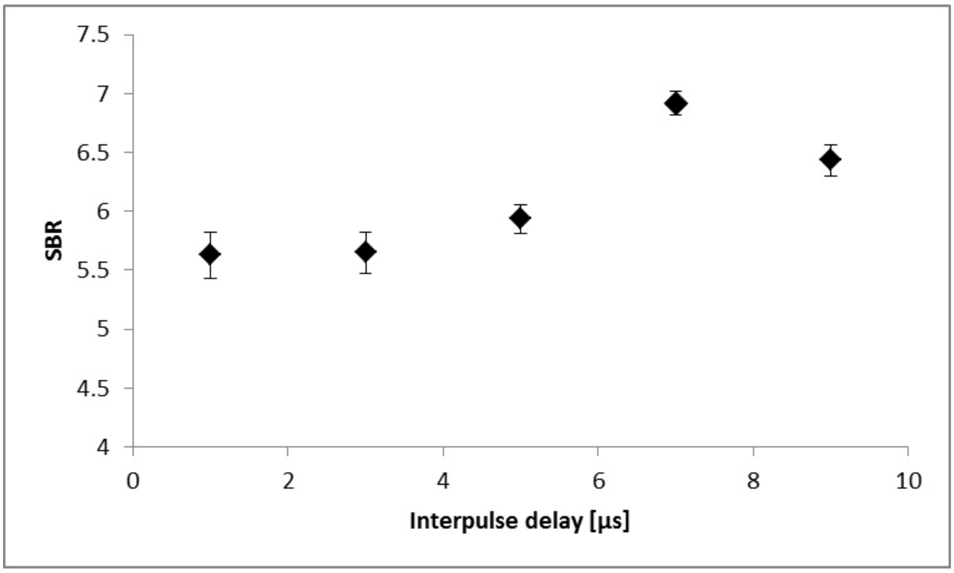

Fig. 3 Optimisation of interpulse delay based on the highest value of SBR (signal-to-background ratio). Optimised for Cd concentration of 10 mg l^{-1} at wavelength 214.441 nm

Under optimised conditions, we measured the calibration standards for Cd within the concentration ranges of 0-30 mg l^{-1} and 0-40 mg $^{-1}$ for alkaline and acidic sweat, respectively; the respective calibrations being shown in Figs 4 and 5. Statistical parameters (coefficient of determination R^2, regression equation and the value of Akaike Information Criterion – *AIC*; Mean Quadratic Error of Prediction – *MEP*; and the result from Fisher–Snedecor test – *p* value) are then listed in Table III. As seen, statistical parameters monitored for each calibration curve are not very different. For evaluation of the concentration of Cd in sweat leachates of jewellery, we choose the analytical lines with wavelengths of 214.44 and 226.501 nm, providing almost identical results, which could also be confirmed by statistical parameters AIC and MEP. Analytical line for Cd at 228.80 nm was not so sensitive, which might be affected by the highest peak in the spectrum. Slope of the calibration curve in this case was a half compared to the other lines being evaluated as insensitive again for our purposes. To assess the quality regressions, the *p* values were calculated for each model. In all cases, the calculated value was lower than that for a significance level of $\alpha = 0.05$, and the corresponding models could be specified as suitable.

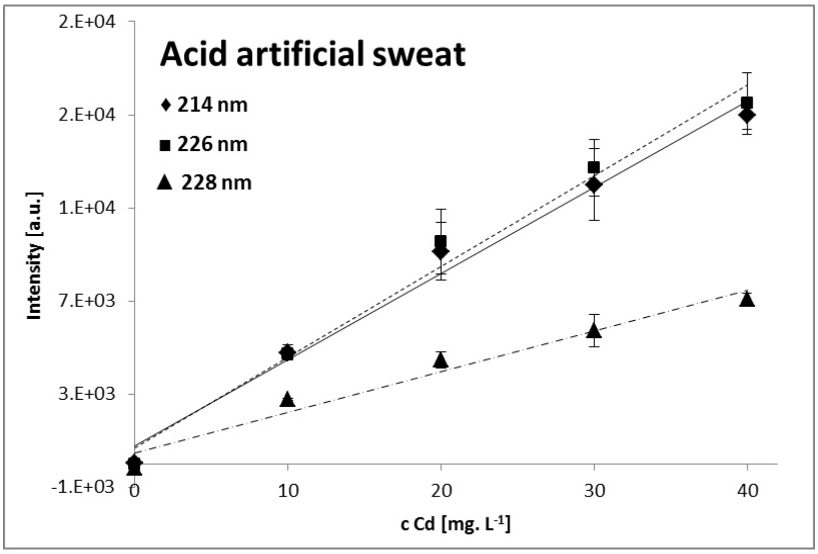

Fig. 4 Calibration curves of artificial acidic sweat measured under optimal conditions at wavelengths of 214.441, 226.501, and 228.802 nm

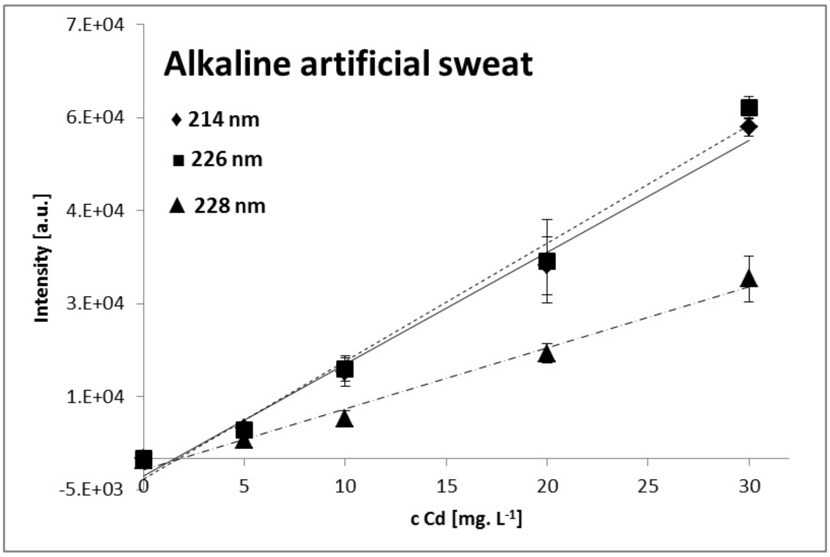

Fig. 5 Calibration curves of artificial alkaline sweat measured under optimal conditions at wavelengths of 214.441, 226.501, and 228.802

The limit of detection (*LOD*) was determined according to the 3σ/*s* criterion, where σ is standard deviation of intensity calculated from 36 replicates measured for the lowest calibration standard (i.e. blank) under optimal conditions; *s* being the slope of the calibration curve. The *LOD* estimated for the the conmtent of Cd was 0.08 mg l^{-1} for acidic and 0.06 mg l^{-1} for alkaline artificial sweats, respectively.

Table III Statistical parameters calculated for each calibration model of the artificial sweat

Sweat type	Wavelength	β_1	*Intr*	R^2	*MEP*	*AIC*	*p value*
Acid	214 nm	370	767	0.9862	2.32×10^6	70.3	6.8×10^{-4}
	226 nm	389	710	0.9851	1.81×10^6	69.3	7.3×10^{-4}
	228 nm	174	469	0.9641	9.35×10^5	65.6	2.9×10^{-3}
Alkaline	214 nm	1 802	–2 729	0.9892	1.91×10^7	80.1	4.8×10^{-4}
	226 nm	1 901	–3 204	0.9854	2.92×10^7	82.2	7.5×10^{-4}
	228 nm	983	–1 730	0.9873	6.11×10^6	74.9	6.1×10^{-4}

β_1 – slope of the curve, *Intr* – intercept of the curve, R^2 – coefficient of determination, *MEP* – Mean Quadratic Error of Prediction, *AIC* – Akaike Information Criterion, *p value* – result from Fisher–Snedecor test

Verification of the Proposed Method Employing LIBS

In order to verify functioning of the developed LIBS method, five samples of inexpensive jewellery were measured. Three samples of this selection were leached in the artificial alkaline sweat and the remaining two leached in artificial acidic sweat. The resulting sweat leachates were then analysed by LIBS as a dried drop on the solid paper carrier. In the case of ICP-OES measurements, leachates could be analysed directly.

Table IV Results of LIBS and ICP OES analysis of unknown jewellery samples

Sample	LIBS µg cm^{-2} week^{-1}	ICP OES µg cm^{-2} week^{-1}	Recovery %	Sweat type
9-A	193 ± 10	200 ± 6.2	96.5	Alkaline
7-A	299 ± 20	277 ± 8.2	108.0	Alkaline
2-B	733 ± 53	733 ± 8.7	100.0	Acid
Bangle	351 ± 16	339 ± 2.5	103.0	Alkaline
Ring	7766 ± 312	7400 ± 27	104.0	Acid

The results for both methods are shown in Table IV, where the respective values are the arithmetic mean and standard deviations calculated from three (ICP OES) and four (LIBS) replicate measurements of particular samples; the proper LIBS analysis having been performed under optimized conditions as described above. As seen, the recovery rate values were within the range of 96-108 %, which confirms good agreement between the two methods (Note: The results are recalculated for the content of Cd in the unified area of samples and expressed per week.)

Conclusion

A method for the determination of Cd in samples of artificial sweat leachates from jewellery has been proposed based on the use of commercially available compact LIBS instrument. The method developed can be characterised as rapid, simple, requiring minimal amount of samples and providing sufficiently low limits of detection, when the value of 0.08 mg l^{-1} Cd for acidic and 0.06 mg l^{-1} Cd for alkaline artificial sweat could be determined. The corresponding *LODs* recalculated for the content of Cd in the given sample volume(s) were 0.0016 µg.20 μl^{-1} for acidic and 0.0012 µg.20 μl^{-1} for alkaline artificial sweat, respectively. In our opinion, such *LODs* are low enough to allow one to use LIBS directly in practical analysis of real samples.

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