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Short communication

Development of a simplified densitometer for the determination of aflatoxins by thin-layer chromatography

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Abstract

A simple, miniaturised and low power consuming (battery, fully semiconductor based) detector cell (SeBaDeC) was developed for the densitometric measurement of aflatoxins on TLC plates. A UV-light emitting diode (UV-LED) with a peak emission wavelength of 370 nm was used for fluorescence excitation, while a photo diode with a peak sensitivity of 440 nm in combination with a 418 nm cut-off filter was applied for detecting the fluorescence intensity. The resulting signal was further amplified by means of a commonly used operational amplifier integrated circuit (OA) and directly converted into a digital signal with a simple analogue-digital-converter (ADC). This signal was recorded at the serial (RS232) port of a portable PC and processed with a spreadsheet program. The software used for data recording is freeware and available in its source code, and the long lifetime of the UV-LED (up to 10 000 h) permits a maintenance free application of this device. This simplified device has shown to be able to detect concentrations of aflatoxins of 1 ng, thus offering a cheap and sensitive alternative to currently available TCL scanners.

Keywords: Densitometry; Aflatoxins

1. Introduction

The monitoring of aflatoxins (toxic metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*) in food at regulatory limits [1,2] depends directly on precise and reliable analytical methods for their determination [3]. Modern aflatoxin analysis is commonly based on high-performance liquid chromatography (HPLC), while thin layer chromatography (TLC) still offers the opportunity of a simple and robust technique [4,5].

TLC offers the advantage of separation of all aflatoxins, which can even further be isolated from interfering matrix residues by using immunoaffinity column clean-up prior to TLC [6].

When using TLC, the quantification of aflatoxins is commonly performed by comparison of sample spots with those from standards under UV light (semiquantitative approach) or by densitometric scanning of the developed TLC plates. In the first case the precision and reliability of the results depends directly on skilled and experienced personal.

The need for reliable, simple and objective determination methods for aflatoxins increased significantly, while in many parts of the world (especially in developing countries), TLC is the method of

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choice. Thus, a simple and reliable densitometer is highly desirable.

Several approaches have been made in the past to elaborate and validate means for the fluorescence determination on TLC plates with commercially available or simple prototype apparatus [7–14]. Impressive results by simple means have been made with so-called ''spotmeter'' prototypes [7,8]. These devices were dedicated to determine the fluorescence with a probe that was positioned over the aflatoxin spot. Spots as low as 1 ng were recorded. However, both devices measured the fluorescence transmission on the TLC plate, while the here-described approach allows the measurement of fluorescence reflection, thus permitting the utilisation of all types of TLC sheets and reducing possible influences of the substrate on the fluorescence signal [15].

Nowadays commercially available fluorescence densitometers have been developed to state-of-the-art products with excellent performance characteristics concerning precision, data resolution and flexibility. However, such instruments are rather bulky (due to the broad applicability for most TLC applications) and must be run directly off the mains.

The here described device for densitometric quantification has been developed with the intention to deliver a simplified, inexpensive and precise alternative to commercially available TLC densitometers.

2. Experimental

2.1. Chemicals

Gradient grade methanol, analytical grade methanol, analytical grade formic acid, hexane, paraffin and silicagel-60 TLC-plates (20×20 cm) were obtained from E. Merck (Germany). HPLC grade acetone and *tert*.-butylmethyl-ether were purchased from Scharlau (Germany). Aflatoxins (dry film) were purchased from Sigma–Aldrich (Italy).

2.2. Apparatus

Gas tight micro-litre syringes were obtained from Hamilton (USA). The TLC sample application device (Linomat), densitometer (Scanner 3) and the UV hand-lamp (366 nm) were purchased from CAMAG (Switzerland). The evaporation block was obtained from Pierce (USA).

The cut-off filter samples (KV 418) were provided as models from Schott (Germany)

The UV-light emitting diode (UV-LED Model: NSHU 590E) and the photodiode (Model: EP-440-3.6) were purchased from Roithner Lasertechnik (Austria), while all other semiconductors and passive electronic parts were purchased from Buerklin (Germany). Electrical circuits and mechanical constructions were produced and assembled as prototypes by the author.

Generic no-name 286 personal computer with parallel port and a portable PC (OmniBook 2000) Hewlett-Packard, USA was used.

2.3. Software

MS-DOS 6.21 (286 PC) or Windows95® (OmniBook 2000). The software for digitalisation and recording of analogue data was written by Francois Mocq [16]. The spreadsheet program used for data assessment was Microsoft-Excel (Microsoft Corporation). The software for circuit layout production (TARGET 2001! V8) was supplied from Ing.-Buero Friedrich, Eichenzell, Germany. The software for the determination of method performance characteristics (MVA) was obtained from Novia, Germany.

2.4. Extraction of samples and TLC

Test samples of paprika and pistachios were extracted and processed according to a recently developed method [17].

Mixed aflatoxin stock solutions in *n*-hexane–acetone–methanol (90+5+5 [v/v/v]) were applied on the TLC-plate with a sample application device (Linomat). The plates were pre-developed with methanol just over the application band to pre-concentrate the aflatoxins and subsequently developed with a mobile phase of *tert*.-butyl-methyl-ether/methanol/water (480+15+5 [v/v/v]).

The developed TLC-plates were first scanned with the commercially available Scanner (CAS) as a reference and subsequently scanned with the developed SeBaDeC. The chromatograms of the CAS

were integrated with the supplied CATS® software by CAMAG.

3. Results and discussion

A previously published circuit for the measurement of illumination [18] was modified slightly to study the applicability of the described detector principle. The fluorescence of the aflatoxins was measured after the cut-off of the excitation light (370 nm) using a filter (>418 nm) and transformed into an electrical signal. This signal was displayed on a digital multimeter. Less than 5 ng of aflatoxin B_1 yielded in definite signals.

Further improvements in the mechanical set-up and the electronic circuits in addition to a fully light and electrical field shielded detector cell should allow the detection of smaller amounts. For this reason, a discrete metal container (hosting the UV-LED, the photodiode, and the cut-off filter) was constructed to eliminate the above-described interference, while the wiring from the detector cell to the amplifier was made with shielded cables. In order to allow a reliable and easy data recording the amplified signal was converted with a simple analogue-digital-converter (ADC) in combination with an easy understandable software [16]. This set-up allowed direct data recording to the parallel port of a PC. The circuit layout of the final version is shown in Fig. 1 and the schematic drawing in Fig. 2.

However, the way of signal recording and processing from the detector is not limited to the approach described above. Many kinds of data logging, data storage or processing systems are nowadays widely used in miniaturised devices of daily use (electronic thermometers, mobile phones). Nevertheless such advanced approaches were not considered in this work. The actual aim of this work was the evaluation of the measuring principle with a miniaturised and simplified apparatus.

Thin-layer chromatograms with aflatoxin B_1 concentrations ranging from 1 to 9 ng absolute per spot were developed. Aflatoxin B_1 was chosen to be the single analyte to demonstrate the performance, since it is the predominant aflatoxin found in contaminated food products and is also explicitly regulated as a single contaminant [2].

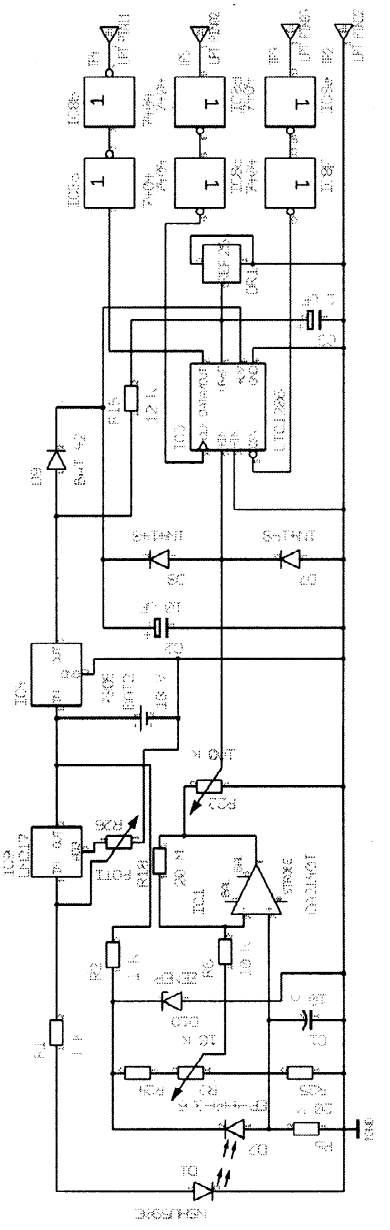

Fig. 1. Layout of the analogue amplifier and analogue-digital-converter. The output signal at pin 6 of the previously discussed analogue amplifier IC 1 (circuit I) is converted by the IC 3 to digital signals. The diode pair D7/D8 is implemented to protect IC 3 from voltages above 5 V by short-circuiting voltages above 5 V and below 0 V (during calibration with R23). The IC8 (4704) works as a buffer, thus protecting IC 3 from external influences.

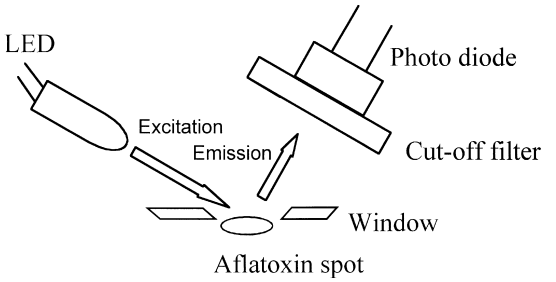

Fig. 2. Schematic drawings of the SeBaDeC.

For comparison aflatoxin chromatograms were first scanned with the CAS and subsequently re-scanned with the SeBaDeC. The scan with the SeBaDeC was performed in an angle of 90° to the development of the TLC-plate in order to allow the simultaneous determination of all aflatoxin B_1 spots in one scan. The correlation coefficient, the limit of detection (LOD) and limit of quantification (LOQ) were calculated from the 95% confidence interval with a method validation software and were found for CAS to be 0.9998, 0.4, and 0.5 ng, respectively, and those for SeBaDeC to be 0.9961, 1.5, and 2.2. ng, respectively.

Further calibrations with all four aflatoxins were made after improving the movement of the detector cell over the TLC-plate. This was achieved by a simple threaded bold support that pushed the detector along the plate when the bold was revolved manually. The results of calibration are listed in Table 1.

For further characterisation of the detector the long-term drift of the signal was investigated. Therefore, the detector cell was positioned over an aflatoxin free spot of the TLC-plate and the signal was recorded for 50 min. The drift was found to be 1.8% over the measured time range. This indicates that during a scan time of approximately 3 to 5 min no measurable drift should occur.

As aflatoxins are subject to UV-light degradation [19] the radiation during the fluorescence measurement might effect results significantly. Signal fading rates of 50% within 3 min were reported with spotmeters [5] and limited the maximum radiation exposure of spots during measurement to 10 s. However, the power ratings of the UV-LED are as low as 750 μW at a single small bandwidth of 370 nm. In contrary to this, previously described UV-light sources were based on fluorescent gas tubes or mercury tubes with significantly higher power ratings of several Watt. This led to the assumption that the described fade should be significantly lower for the SeBaDeC. For confirmation the detector cell was positioned over an aflatoxin B_1 spot and the signal was recorded over a time range of 45 min and additionally for 10 min over a blank position. The signal fade was calculated to be less than 1.5% within a time frame of 1 min. This time was assumed to be the maximum exposure time during several measurements.

Finally, fortified test samples of paprika powder and pistachios were analysed by TLC and the aflatoxin B_1 content was measured with both densitometers, the SeBaDeC and the CAS. As shown in Table 2 the data obtained in this comparison are very similar which confirms that the here proposed SeBaDeC is capable to determine aflatoxin spots already with a sufficient precision, while Fig. 3

Table 1
Calibration parameters of aflatoxins B_1, B_2, G_1 and G_2 derived from the 95% confidence interval of the calibration curve[a]

Analyte→	AfB_1	AfB_2	AfG_1	AfG_2
Correlation (r=)	0.9983	0.9954	0.9944	0.9504
LOD [ng]	1.2	1.7	1.7	4.8
LOQ [ng]	1.9	2.8	2.5	7.1
RSD [%] (method)	2.8	3.8	5.0	15.7

[a] The correlation coefficient, LOD and LOQ (SeBaDeC) were calculated from the 95% confidence interval with a method validation software (MVA). The calibration parameters for the aflatoxins B_1, B_2 and G_1 were satisfactory, while the values for G_2 were unexpectedly high due to deviations of the obtained signals. However, repeated experiments indicated that aflatoxin G_2 calibration data is conceivably lower in the range of the other aflatoxins.

Table 2
Results of the analysis of fortified paprika powder with aflatoxins B_1 and G_2

Aflatoxin added [ng/g]	Aflatoxin B_1 found		Aflatoxin G_2 found	
	CAS	SeBaDeC	CAS	SeBaDeC
Blank	0.0	0.0	0.0	0.0
1	0.9	0.9	0.9	0.9
2	1.5	1.6	1.5	1.7
3	2.8	2.5	2.7	3.0
4	3.3	3.0	3.0	3.3

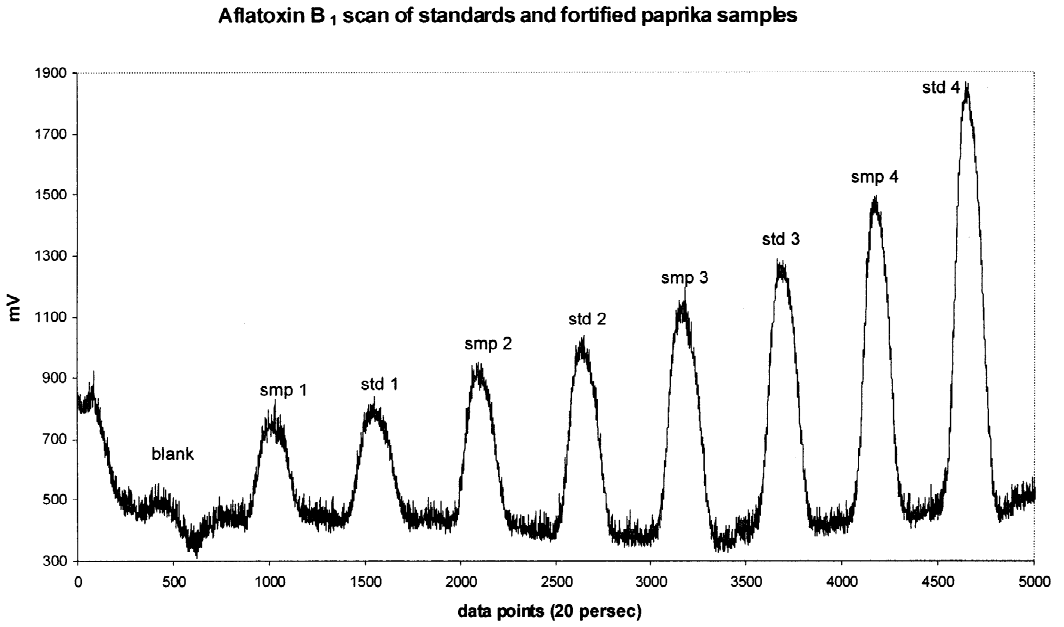

Fig. 3. Horizontal scan of a TLC plate. Peaks [smp 1] to [smp 4] are fortified and analysed paprika samples in the range of 1 to 4 ng/g aflatoxin B_1. Peaks [std 1] to [std 4] are the corresponding standards of aflatoxin B_1.

shows the corresponding densitogram of AfB1 in paprika powder.

4. Conclusion

The proposed miniaturised device (solid stated detector cell) was found to be an inexpensive and promising alternative to the currently used equipment for aflatoxin quantification. Due to the low power consumption of the light source this SeBaDeC principle offers the operation by means of batteries. Furthermore the lifetime of the used UV-LED is superior compared to mercury lamps or gas tubes. Signal processing and data recording were based on commercially available and inexpensive electronic components. The performance data demonstrated, that the SeBaDeC is suitable for the determination of aflatoxins at European regulatory limits of 2 ng/g aflatoxin B_1 (respectively 4 ng/g total aflatoxins) in combination with adequate TLC methods. However, at the current state of development, further improvements of the prototype in design of the cell and electronic circuits are conceivable.

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