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To cite this version:

U. Möller, H. Köst, S. Schneider, H. Coufal. EVALUATION OF STAINED AND UNSTAINED ELECTROPHEROGRAMS BY PHOTOACOUSTIC SPECTROSCOPY. Journal de Physique Colloques, 1983, 44 (C6), pp.C6-121-C6-124. <10.1051/jphyscol:1983618>. <jpa-00223177>
EVALUATION OF STAINED AND UNSTAINED ELECTROPHEROGrams BY PHOTOACOUSTIC SPECTROSCOPY

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Résumé - Par spectroscopie photoacoustique tous les genres d'électrophérogrammes, même ceux de protéines colorées non teintes, peuvent être cartographiés avec une sensibilité et résolution excellentes et de plus, les groupes prosthétiques de ces protéines peuvent être identifiés.

Abstract - By photoacoustic spectroscopy all types of electropherograms, even unstained electropherograms of colored proteins can be mapped with excellent sensitivity and resolution and the prosthetic groups of these proteins identified.

INTRODUCTION

Conventional and laser densitometry of polyamide PAGE-IEF gels suffer from several severe drawbacks. Light scattering by the substrate and inhomogeneous or insufficient staining are the limiting factors. Photoacoustic spectroscopy and mapping should be able to overcome these problems and should therefore be of considerable interest for the evaluation of electropherograms.

EXPERIMENT

PAGE substrates were prepared either with carrier ampholytes Servalyt pH 3-10 or Servalyt pH 2-11 (Serva, Heidelberg, Germany) by a method described by Radola /1/. Substrate thickness is 0.175 mm, the thickness of the wet, protein containing polyacrylamide gel layer was 0.175 mm for the "maxigel" and 0.05 mm for the "minigel". For one experiment, a prefabricated gel, Serva Precote pH 3-10, was used. Drops of a protein marker solution (marker protein mix 9, Serva) were applied and subsequently focused. Upon completion of the focusing procedure the gels were either stained/destained or dried. During drying the gels shrink to five percent of their original thickness.

Conventional transmission densitograms of stained gels were recorded with a commercial laser densitometer LKB 2202 Ultrascan at a wavelength of 633 nm with a spatial resolution of 50 μm. PA mapping of stained and unstained ultra-thin gels was performed using an Ar⁺-laser tuned to different emitting lines or a tunable dye laser pumped by the Ar⁺-laser as excitation source. The laser beam is intensity modulated by an acousto-optic modulator, attenuated and focused on to the sample under study. The sample is contained in a PA cell described elsewhere in full detail /2/ as is the self supporting carbon film used as a reference sample /3/.

A comparison of both techniques, conventional transmission densitometry (Fig. 1a) and photoacoustic mapping (Fig. 1b), clearly shows that PA mapping gives at least the same resolution

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as the densitogram taken with a commercial laser scanner, however, with less background, even though a background correction had been carried out for the latter. Experiments on unstained electropherograms clearly show (Fig. 1c) that it is possible to locate chromoproteins on undyed PAGE substrates by PA-mapping. At the moment, this cannot be achieved with any other technique due to the low absorbance variation in the unstained sample.

![Densitograms of "minigels" prepared by applying a solution of 1 mg marker protein mix 9 (Serva Heidelberg) in 1 ml of Servalyte pH 3-10. Hollow arrows mark artifacts (denatured proteins) caused by the application of the solution in the center of the separation lane; for symbols used to identify proteins see Table 1.](image)

a) Transmission densitogram of "minigel" prepared by applying 1 μl of solution and subsequent staining of the electropherogram with Servablue G.
b) Photoacoustic densitogram of the same electropherogram.
c) Photoacoustic densitogram of unstained gel, 5 μl of solution applied. Only peaks due to chromoproteins (A,C,D,H) are observed.
To evaluate the potential of in situ photoacoustic spectroscopy, PA spectra of buffer solutions of pure chromoproteins and of the same proteins on a PAGE substrate were recorded. These PA spectra resemble closely conventional transmission spectra recorded with a normal spectrophotometer. The differences can be attributed to a wavelength dependence of the nonradiative quantum yield /4/. Spectra of phycocyanine are shown in Fig. 2 to exemplify this point. Taking these differences into account PA mapping at various probing wavelengths allows chromophore identification on unstained electropherograms.

![Photoacoustic spectrum of phycocyanine on gel in situ compared with PA spectrum of phycocyanine in solution and a conventional absorption spectrum](image)

Fig. 2 - Photoacoustic spectrum of phycocyanine on gel in situ (—) compared with PA spectrum of phycocyanine in solution (0.95 mg crude phycocyanine of Spirulina maxima/2 ml 0.01 Phosphate buffer pH 7, —) and a conventional absorption spectrum (......) of the same sample.

Since a large number of experimental parameters, like modulation frequency or thermal properties of sample or substrate, effect amplitude and phase of the photo acoustic signal quantitative measurements of the chromoprotein concentrations will be difficult. By a suitable standardization and calibration, however, this drawback of photoacoustic mapping could be overcome.

**CONCLUSION**

It was clearly demonstrated that photoacoustic spectroscopy is a valuable tool for the evaluation of the protein distribution on stained and unstained PAGE IEF substrates. The spatial resolution of the method is at least equal to that of commercial laser densitometers. Further improvements of resolution and sensitivity are envisaged; the application of multiplex imaging techniques /5,6/ should be particularly helpful. A mapping of the protein distribution at various wavelengths, the mapping of the chromoprotein distribution on gels enriched with only one type of chromoprotein and the recording of PA spectra of previously localized zones by a broad-band spectrometer can help to identify the chromophores of chromoproteins focused in unstained gels and to elucidate the nature of its prosthetic group.

**ACKNOWLEDGMENTS**

We are deeply indebted to Dr. E. Köst and Mrs. S. Nohel for providing and characterizing the electropherograms. We wish to thank Mr. K. Sieber of LKB (München, Fed. Rep. of Germany) for recording of densitograms, the members of the electronics shop of our institute for valuable help, Mrs. E. Kudler for skillful experimental assistance and Miss E. Benedikt for the preparation of figures.

Financial support by the "Deutsche Forschungsgemeinschaft" and the "Fonds der Chemischen Industrie" is gratefully acknowledged.
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