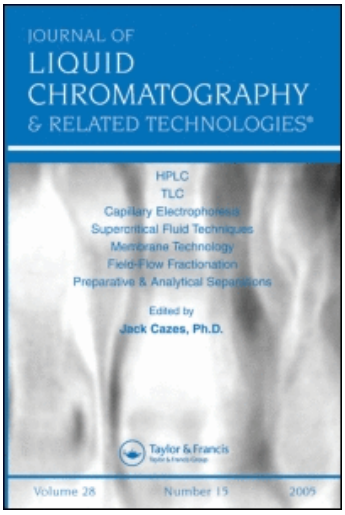

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High-Speed Video Densitometry: Principle and Applications

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HIGH-SPEED VIDEO DENSITOMETRY: PRINCIPLE AND APPLICATIONS

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In the past few years, TV-type multichannel detectors /Vidicon-, Plumbicon- and Orthicon tubes etc./ found application in several fields of analytical chemistry /1/. Recently, video-technique was introduced to the densitometric evaluation of thin-layer chromatograms as well /2/; the term 'video densitometry' is used in this context.

TV-type multichanel detectors reveal several properties unusual to commercially available densitometers. The main difference originates from the working principle: the scanning is carried out electronically rather than by mechanical movement of the specimen and/or light source. The scanning is very fast and its geometry is different from those used in traditional densitometers. These new

* Presented at the First Symposium on Advances of TLC and HPLC, May 14-15, 1982, Szeged, Hungary.

features called for novel technical solutions in instrumental design; on the other hand they allowed to adapt densitometric evaluation to new application fields. The present report deals with theoretical and practical aspects of using TV-type detectors in densitometry, with special regard to novel features and new possibilities of application.

General aspects

The working principle of TV-type detectors can be shortly described as follows: the image of the specimen /chromatographic spot/ is projected to a target-plate which is a two-dimensional array of unit detectors continuously scanned by an electron beam. The unit detectors behave like capacitors whose charge is proportional to incident light intensity and that are periodically discharged by the electron beam /Fig. 1/. Optical magnification /d/w using the terms of Fig. 1/ can be selected in a wide range according to the sizes of the chromatographic specimen. At 'usual' magnification /when a 200X200 mm chromatoplate fills the field of vision of the camera/, d/w is about 0.05 and several hundreds of unit detectors take part in the measurement of a single chromatographic spot or band.

For the purposes of mathematical treatment, the scanning scheme of video-densitometry can be considered as a stepwise two-dimensional scanning. The signal dis-

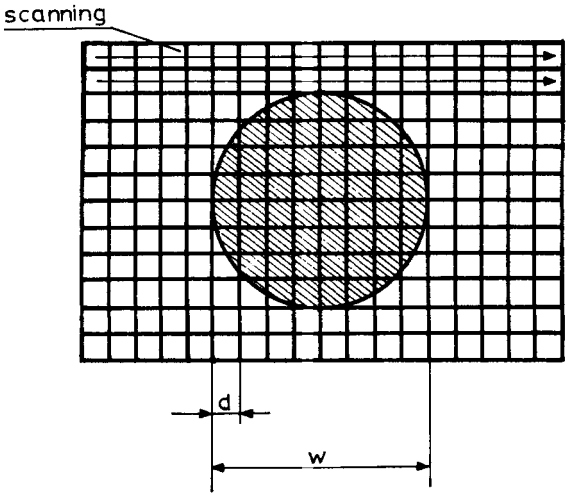

FIGURE 1

tortion of the spot density integral will thus contain two terms: the first one - common to all scanning geometries- originates from finite detector size /3/, the second one is a result of the stepwise nature of the scanning. According to our results /4/, in the case of an 'average chromatographic spot' /w = 5 mm, peak height = 0.5 absorbance/ the over-all signal distortion is below 1% of the spot density integral, and in the 'useful range' of quantitating a given component, it is nearly independent from spot-size data, indicating that the new scanning geometry is not a source of non-linearity.

Spectral sensitivity of Vidicon-type detectors allows quantitative determination in the visible range, extending the range to UV would need special detector de-

sign /quartz face plate and camera optics/. Detection limit for amino acids stained with ninhydrin lies in the nanomolar range /2-5 nanomoles depending on layer quality and spot shape/, as measured with a commercially available Vidicon tube /NOR-1" 2255 Heiman/. A further characteristic feature of Vidicone-type detectors as compared to conventional ones is the variance of detector response along the field of vision. This fenomenon originates partly from inhomogenities of the target plate and partly from that of illumination, and can be corrected for by shading correctors as used in video-techniques. Finally, it should be noted that the output signal of the TV-camera /the so called video-signal/ is too fast to be integrated by an ordinary integrator like those used in liquid and gas chromatography. On the other hand, combined with a high-speed integrator and data processing, TV-type detectors can drastically reduce measurement time as compared to conventional densitometers.

Application

Although the ide of building a Vidicon based densitometer dates back to the late sixties /5/, the first commercially available video densitometer /6/ /Telechrom OE 976, Chinoin-Budapest/ appeared only in 1976. The schematic diagram of this instrument is shown in Fig. 2. The instrument works according to a quasi double-beam

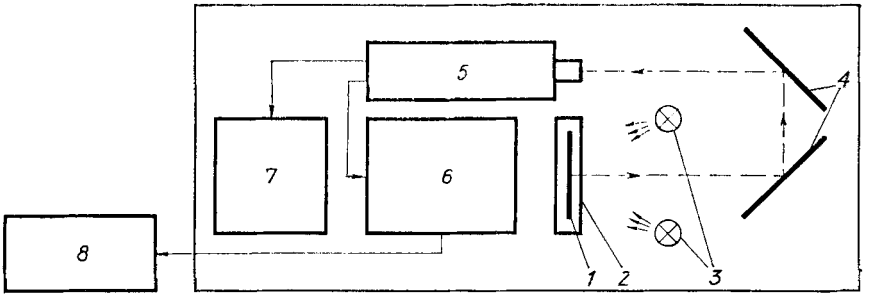

FIGURE 2

principle: the background intensity is sampled in each line of the scanning.

The new version of the instrument /Fig. 3/ can be used both in reflectance and in transmittance mode and

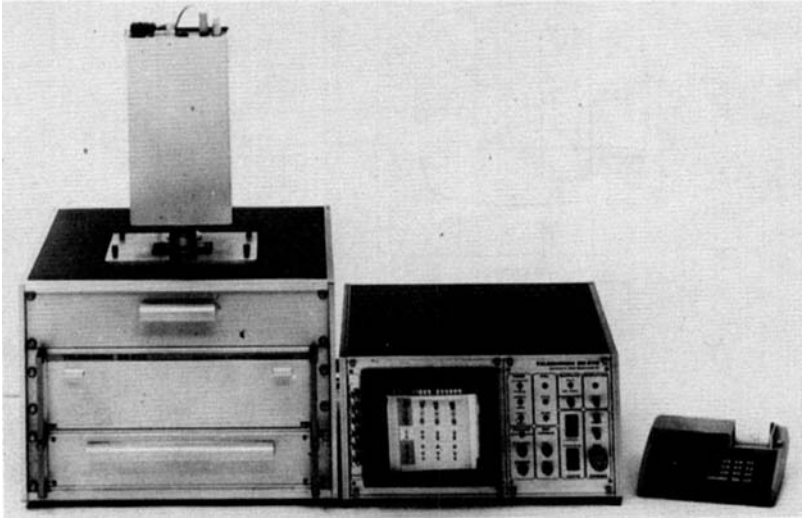

FIGURE 3

is equipped with UV light-source for fluorescence measurements. The control unit of the instrument fulfills two funktions. In contains the high speed integrator working according to a digital procedure /6/ that can be regarded as a three-dimensional extension of the area-measurement used in image analysis. The integrator is adapted to perform two integrations concomittantly, one of which is the component in question, the other one can be the total /coloured/ material of the sample or an internal standard. Another function of the control unit is the selection of the area of measurement. This is carried out by the operator with the aid of the monitor. Fig. 4 shows a schematic picture of the moni-

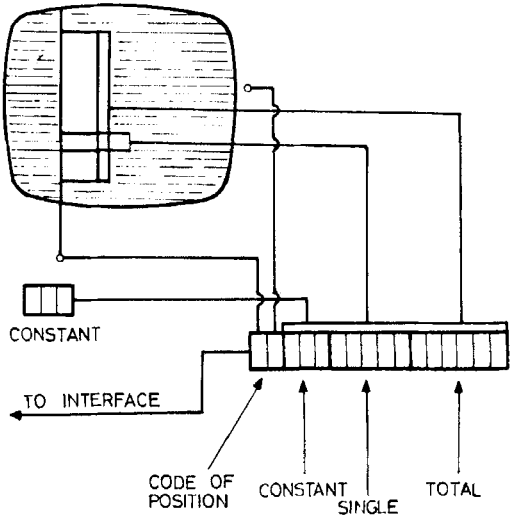

FIGURE 4

tor during measurement, indicating the flow-chart of the data-transfer.

The measurement is carried out either in manual or in automatic mode according to a pre-set program. Geometrically uniform chromatograms can be evaluated automatically, with small readjustments of the measurement program. The measured data are displayed on the monitor and also transferred to an on-line connected programmable calculator /Hewlett-Packard HP 97/. The calculator can be programmed to carry out the simpler calculations directly connected with the measurement, such as calibration curve fitting /reading from curve/, the use of internal and external standards, calculation and concentration per cents, etc. /7/. It is interesting to note that a densitogram is not required for the measurement. However, it can be displayed on the monitor and also on an optional recorder. As can be seen, the Telechrom video densitometer is adapted to large scale routine work and is especially suited to the direct determination of concentration per cents and component ratios. These properties made it possible to apply TLC methods in new fields of application as shortly summarized below. It should be mentioned finally, that, in addition to the hardware approach described above, the video signal may be processed also by software means to yield quantitative information. The fully computerized way is especially attractive since it may include all phases of signal processing /such as shading correction, spot selection and

integration, data processing etc./, with a substantial decrease in operator time.

Determination of essential amino acids in plant proteins

The concentration of the nutritionally limiting amino acid /usually lysine, tryptophan or methionine/ is a direct index of the nutritional quality and is used as a coarse ranking indicator in plant breeding programmes. Ion-exchange TLC on chromatosheets precoated with strong cation exchange resin /8/ makes it possible to separate each common /protein bound/ amino acid with a one-dimensional run. A few /ul of plant hydrolysate is chromatographed according to this technique /using different sodium citrate buffers/, the chromatograms stained with ninhydrin are subsequently used for quantitation by videodensitometry. The concentration is expressed in per cent of the total amino acid content of the sample which is determined from the same chromatogram. Fig. 5 shows the determination of lysine in wheat. The spot density integral of lysine /L/ and that of the total amino acid content /T/ are linearily related to the sample size. However, their ration /the percentage of lysine in the total amino acid content/ is independent of the sample size within a broad range. Consequently, accurate weighing and sample application are not necessary provided that the amount of sample per spot is within this range. All this renders sample preparation simple and productive which is one of

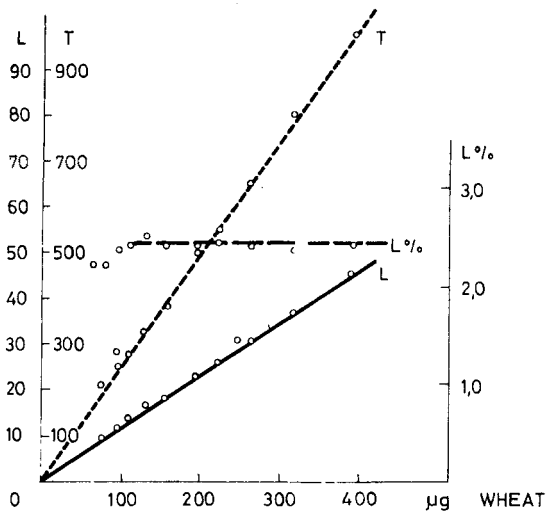

FIGURE 5

the ultimate reasons why the method can be applied economically in large scale screening programs. The video densitometric method showed good correlation with the amino acid analyzer, a comparison is shown in Fig. 6. The reproducibility of the method is characterized by a coefficient of variation of about 3-4%, including chromatography and hydrolysis /reproducibility of the densitometric measurement is below 1% c.v./.

The method has been applied in screening for lysine /2/ in cereals /barley /9/, wheat /10/, maize /13/, rye and sorgum /9/ / by different laboratories. The daily output was usually 300-800 analyses. Finally, a practical result should be mentioned: at the laboratory of the International Atomic Energy Agency /FAO/IAEA Seiberdorf,

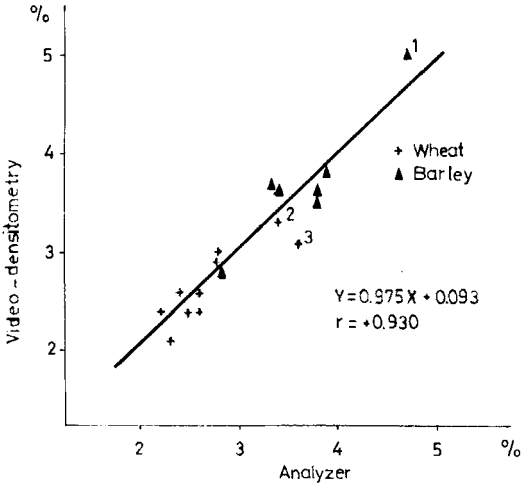

FIGURE 6

Austria/ about 16 000 wheat lines were analyzed in 1977 and a mutant was selected which contained about 30% more lysine than the control variety /9/.

The same system /ion-exchange TLC and video densitometry/ was used to determine biogenic amines in feed meals /16/ and diaminopimelic acid /17/.

Free amino acids in blood and in tissue samples

Amino acid metabolism disorders, such as phenylketonuria /phenylalaninaemia/, histidinaemia, lysinaemia, ornithinaemia, tyrosinaemia are manifested by an elevated level of one /or more/ of the above amino acids in the blood, curability of these diseases depends primarily on an early

diagnosis. Ion-exchange TLC in a pH = 4.2 ; Na^+ = 0.4 M sodium citrate buffer /14/ makes it possible to separate all of the above amino acids in one dimension, using 50-100 µl of blood dried on filter paper. Video-densitometry provides an adequately rapid quantitative answer and helps to identify even the mild forms of the above disorders, extending thereby significantly the scope of the screening.

A similar system was used to detect individual amino acid transforming enzymes in micro amounts of tissue samples /12/. Aliquots /a few µl/ were withdrawn from an enzyme reaction mixture and spotted directly to the chromatographic plate. The developped and stained chromatograms were analyzed by video-densitometry, the enzyme activity was calculated automatically from the slope of the progress curve. The sensitivity of this method offers new possibilities for clinical diagnostics.

Analysis of drugs

TLC technique is routinely used in many fields of the pharmaceutical industry. At the Chinoin Pharmaceutical Works, Budapest, TLC combined with video densitometry is used in the content uniformity test of multicomponent drugs /Pernovin, Amidazophen, Efedrin, ascorbic acid, Drotaverin etc./ /15/. Determination of D-Penicillamine in blood samples /11/ can be mentioned as an example of clinical application.

Although examples quoted in the present report - reflecting the author's scope of interest - con-

centrate mainly on amino acid analysis, we have to emphasize that video densitometry may be employed in most cases when general conditions of densitometric quantitation are fulfilled. TLC and other flat bed chromatographic techniques provided simple and efficient means for the separation of a wide range of substances and are ideally suited for large scale routine work. However, these techniques are now becoming neglected in many fields of chemical analysis for lack of an adequately productive quantitative method. We feel, that video densitometry may offer a viable alternative to solve a number of problems /such as the analysis of pesticides and food additives, screening for alkaloid producing plant lines etc./ where both the resolving power of a chromatographic technique and an adequately fast quantitative answer are equally important.

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