United States Patent [19]
Lindstedt et al.

[11] 3,746,869
[45] July 17, 1973

[54] METHOD OF PHOTOMETRICALLY PLOTTING LIGHT SCATTERING OBJECTS

[75] Inventors: **Sven Lindstedt**, Goteborg; **Laszlo Treiber**, Vastra Frolunda, both of Sweden; **Peter Stollnberger**, Munich; **Walter Tausch**, Aalen, both of Germany

[73] Assignee: **Carl Zeiss-Stiftung**, Wuerttemberg, Germany

[22] Filed: **Sept. 28, 1971**

[21] Appl. No.: **184,416**

[30] **Foreign Application Priority Data**
Sept. 30, 1970 Germany .... P 20 47 952.1

[52] **U.S. Cl.** .... **240/218**, 250/219 FR, 250/227, 356/73, 356/201, 356/209
[51] **Int. Cl.** **G01n 21/26**
[58] **Field of Search** .... 250/218, 219 FR, 250/219 DF, 227, 83.3, 219 WE, 219 DQ, 219 DR; 356/201, 202, 203, 73, 205, 206, 180, 195, 212; 350/96 R, 96 B

[56] **References Cited**

UNITED STATES PATENTS

2,632,855	3/1953	Bendz	250/219 FR
3,567,328	3/1971	Riley	250/218
3,566,083	2/1971	McMillan	250/227
3,504,978	4/1970	Shibata	250/218
3,504,983	4/1970	Richmond	250/219 FR
2,718,597	9/1955	Heigl	250/218
2,834,247	5/1958	Pickels	250/219 FR
2,930,898	3/1960	Nuttall	250/218
3,618,061	11/1971	Livers	250/218
3,320,428	5/1967	Wagstaffe	250/218

Primary Examiner—James W. Lawrence
Assistant Examiner—D. C. Nelms
Attorney—Sandoe, Hopgood & Calimafde

[57] ABSTRACT

The invention contemplates an improved method and apparatus for photometrically plotting light scattered by a light-scattering object, as in the plotting of thin-layer chromatograms. Light is irradiated upon one surface of the object, and both the diffusely reflected light and the directly reflected light are simulatenously evaluated, in such manner (a) that base-line variations are minimized and (b) that light-scattering signal attributable to the observed substance is maximized and more readily identified.

18 Claims, 7 Drawing Figures

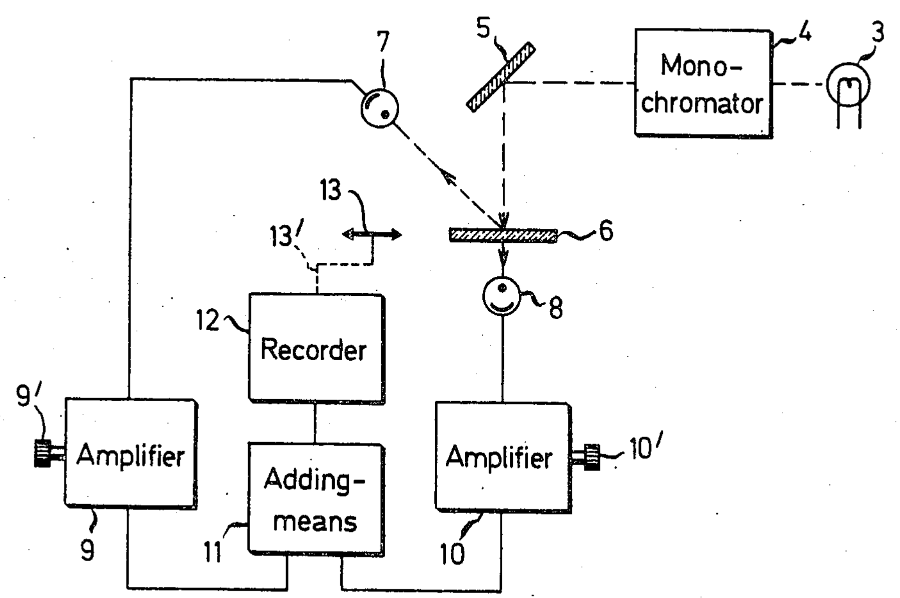


Fig.1

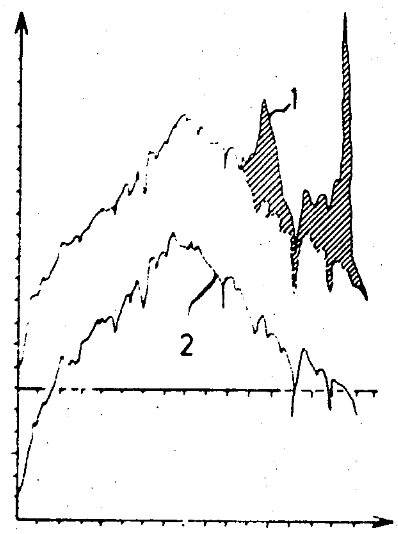


Fig.2

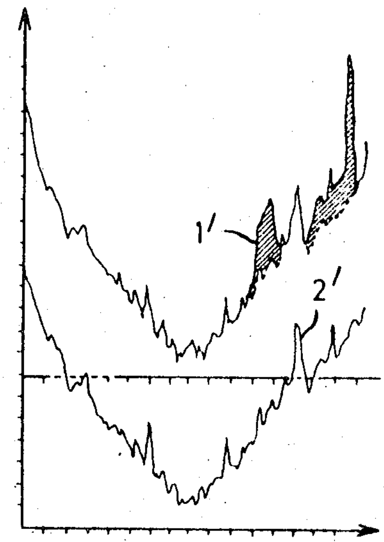
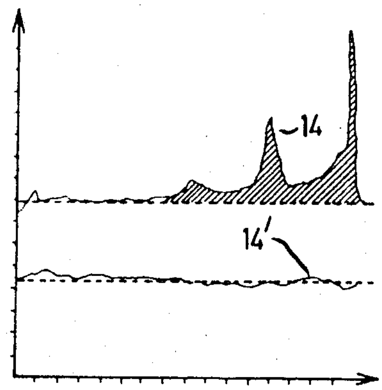


Fig.3



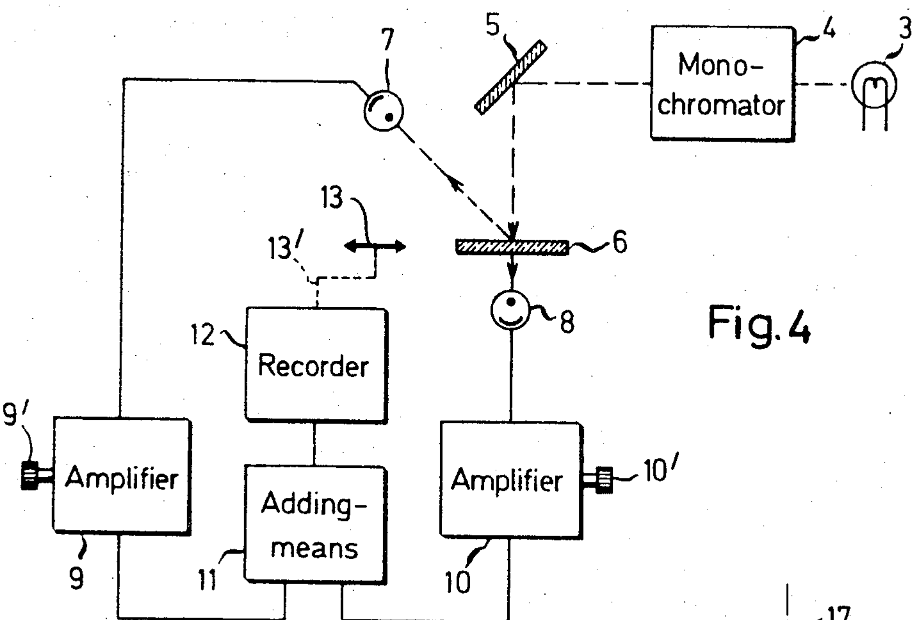


Fig.4

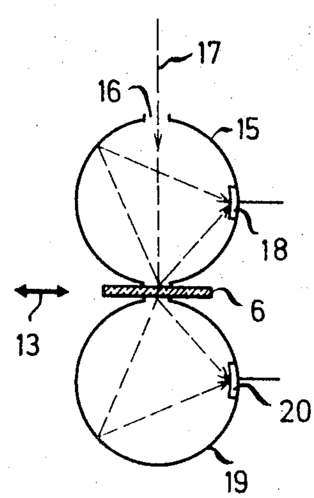
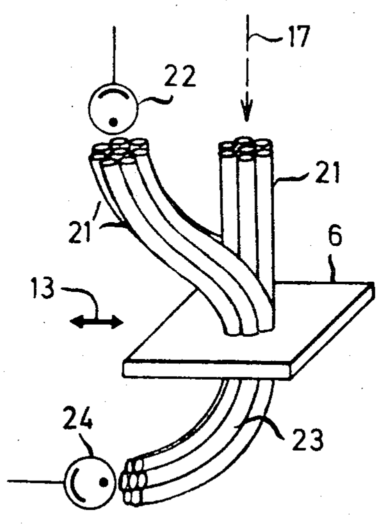


Fig.5

Fig.6



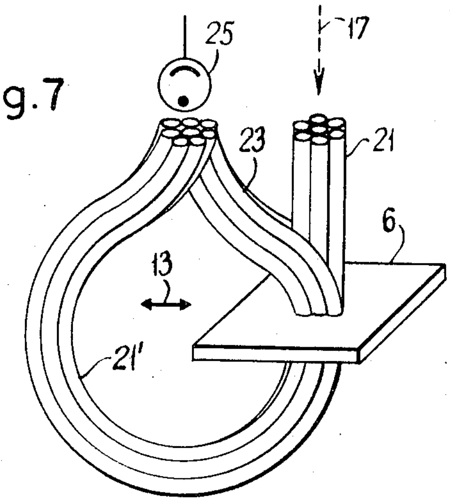


Fig.7

METHOD OF PHOTOMETRICALLY PLOTTING LIGHT SCATTERING OBJECTS

This invention relates to a method and means for photometrically plotting light-scattering objects. This object is presented, for instance, when plotting pherograms of chromatograms.

The method according to this invention and its mode of operation will be described hereinafter by way of the example of plotting thin layer-chromatograms, without thereby restricting the range of application of the said method.

Thin layer-chromatography is a modern analytical method of separating mixtures of organic and inorganic substances.

A thin layer of sorption medium consisting of fine-granular light-scattering material, such as silica gel, which is called the separating layer, is applied on a carrier plate or support, generally consisting of glass. The solution of the substance mixture which is to separate is applied as a spot or as a band at a specific position on the separating layer. Thereafter, the plate is placed in a chamber containing a suitable fluid medium. In the ensuing development, the fluid medium travels across the separating layer, as by capillary forces, thereby separating the substance mixture. After termination of the development, the different substances of the mixture are found at different positions of the separating layer, at which point it is necessary to evaluate the developed thin layer-chromatogram qualitatively and quantitatively.

To evaluate the thin layer-chromatogram, it is prior art to first detect the substance spots, then to scratch off the separating layer in the area of the individual substance spots, to elute the substances fromthe sorption medium, and thereafter to determine the same qualitatively or quantitatively. This method of evaluation is quite complicated and not always applicable, as a recovery loss cannot be avoided,

It is also prior art to directly plot thin layer-chromatograms. To this end, measurement is made of variation in light absorption in the course of scanning the chromatogram with a light beam of constant intensity; alternatively, in the case of fluorescent substances, the emission of the substance zone is observed.

The light-absorption measurement technique is generally applicable in the case of objects which cause no, or only a small amount of, light scattering, in which case a transmission measurement is made. In the case of light-scattering objects, for instance thin layer-chromatograms, it is necessary prior to a transmission measurement to make the object transparent by means of a liquid of a suitable index refraction. Due to the resulting elution effects, such a method of plotting thin layer-chromatograms is hardly recommendable.

In recent years, there has been a change to use of reflectance measurements in the plotting of thin layer-chromatograms. To this end, the chromatogram is scanned by a monochromatic light beam in the direction of separation, and the intensity of the diffusely reflected measuring radiation is measured with a photoelectric detector and recorder, for instance as a function of position, by a suitable recorder. If light-absorbing substance spots are found on the separating layer, the reflected radiation will be attenuated in those wavelength ranges in which absorption bands would occur for transmission measurements of corresponding solutions. The intensity of the diffusely reflected measuring radiation is therefore indicative of the absorption caused by the substance. The absorption-position curve obtained by the scanning can be evaluated quantitatively by means of the Kubelka-Munk-function.

In the described plotting by measurement of the diffusely reflected measuring radiation, the effect being measured is always degraded by a superimposed light-scattering function, which varies as a function of position on the separating layer; in the specific case of small substance volumes, this superimposed function becomes very detrimentally noticeable and deteriorates the limit of detection. This position-dependent function is unspecific relative to the light-scattering substance and may result in a highly irregular base line of the curve being evaluated, making it difficult if not impossible in some cases to decide definitely whether one is observing an effect to be measured or an interference effect.

To avoid such ambiguity and to increase the measuring sensitivity, it has been proposed, as a step prior to the application of the substance under investigation, to record the base line of the absorption-position curve, and then, during the actual evaluation, to subtract from the measuring curve the base-line values thus obtained. This is of course possible in a careful operation since the structure of the separating layer is preserved. The execution of this proposal, however, is unduly complicated and requires a very accurate operation.

It is an object of the present invention to provide an improved method and means of the character indicated.

It is a specific object to provide a method and means for photometrically plotting light-scattering objects, in particular thin layer-chromatograms, which method and means substantially improve the measuring accuracy and the limit of detection as compared with conventional method, while premitting a quick plotting.

Briefly stated, the method according to the invention is characterized by making simultaneous measurements of the diffusely reflected and of the transmitted radiation, for each plotted position of the object, the two signals thus obtained being means of a recorder.

As investigations carried out in connection with the invention have shown, there is a close relationship between the transmission and reflectance base lines, in the case of a substance-free light-scattering background. A change (due to scattering) in the degree of reflectance results in a change (also due to scattering) in the degree of transmission, and both effects are homologous in good approximation. In the summing operation of the invention, the signal components due to scattering of the reflectance signal therefore offset or compensate the signal components due to scattering of the transmission signal, and the base line of the measuring curve is thus effectively straightened.

If an absorbing substance is applied on the light-scattering carrier, both the degree of reflectance and also the degree of transmission will be thereby reduced. Absorption specifically related to the substance thus influences the reflectance and of these signals, the effect being measured is enhanced.

It is thus seen that, in the plotting of thin layer-chromatograms, the novel method has the advantage that the making of the measurement inherently and automatically eliminates scattering effects related to position of the separating layer and unspecific to the substance of the light scattering, while, at the same time the actual measuring signal specific to the substance is amplified.

In order as completely as possible to attain compensation of the reflectance and transmission signals due to scattering (using the addition technique of the invention), it is necessary that prior to the addition the amplitudes of both signals assume the same level. This is advantageously accomplished by reducing the sensitivity of one detector with the respect to the other. Generally, the sensitivity of the transmission detector is reduced as the latter almost completely detects the measuring radiation transmitted by the object, whereas the reflectance detector detects the diffusely reflected measuring light only in a limited solid angle.

By these measures, base-line variations attributable to the separating layer are reduced to the order of magnitude of electronic instability (noise) of the whole system.

The device for carrying out the novel method is advantageously designed so that the measuring radiation impinges vertically on the surface of the object being plotted, that a first photoelectric detector is arranged above this surface and a second one below the object, and that both detectors are connected with an arrangement for adding the signals. This arrangement is preferably in connection with a recorder recording the resultant absorption-position curve.

It is advantageous to connect the photoelectric detectors with the adding arrangement through independently variable amplifiers.

For the detection of the reflected and the transmitted light it is preferred to employ photometer balls or fiber-optical equipment.

The invention will now be more fully described with reference to FIGS. 1 to 6 of the accompanying drawings in which:

FIG. 1 is an absorption-position curve of a thin layer-chromatogram, recorded by measuring the reflected radiation as well as the associated base line;

FIG. 2 is the absorption-position curve of the same thin layer-chromatogram, recorded by measuring the transmitted radiation as well as the associated base line; of the invention, together with the associated base line; for carrying out the method of the invention;

FIG. 5 is a schematic diagram showing one embodiment of a first device, in which photometer balls are provided for the integral detection of the radiation; and

FIGS. 6 and 7 are schematic perspective diagrams showing further detection devices, in which fiber-optical equipment is provided for the supply of the measuring light as well as for the detection of the reflected and the transmitted radiation.

In FIG. 1, reference numeral 1 designates an absorption-position curve obtained by recording reflected radiation, in the plotting of a thin layer-chromatogram. As can be seen, the effect being measured has superimposed thereon a light-scattering function depending upon local position on the separating layer.

The base line associated with curve 1 is referenced 2, and the desired data is represented by the difference between photometric curves, suggested by the cross-hatched area. The base line is seen to be irregular, and the sufficiently accurate determination of the hatched area is very difficult when plotting.

If the same thin layer-chromatogram (whose absorption-position curve, obtained by reflectance measurement, is illustrated in FIG. 1) is plotted for transmission measurements, then the absorption-position curve referenced 1' in FIG. 2 will be obtained. The associated base line is referenced 2', and the desired data is again represented by the difference between these curves, suggested by the cross-hatched area.

As a comparison of FIGS. 1 and 2 shows, the base lines 2 and 2', resulting from effects unspecific to the substance, are approximately homologous. However, absorption specifically related to the substance influences curves 1 and 1' in the same sense.

According to the invention, a device as schematically illustrated in FIG. 4 may be used to determine the absorption-position curve. Light from a source 3 is monochromatized in the monochromator 4 and, via a reflecting mirror 5, impinges upon a thin layer-chromatogram plate referenced 6. Measuring light impinges upon the separating layer of the chromatogram 6 at an angle of 0° to the surface normal. Diffusely reflected radiation is measured by means of a photoelectric detector 7 while a detector 8 simultaneously measures transmitted radiation. The signal generated by the detector 7 is amplified by means 9 and is then supplied to an adding stage 11. In like manner, the latter also receives the signal output of detector 8 after amplification at means 10. The two amplifiers 9 – 10 are preferably independently variable, as suggested by manually adjustable elements 9' – 10', so that the amplitudes of signals unspecific to the substance can be caused to assume the same level. The additive signal provided by means 11 is supplied to a suitable recorder 12. The arrow symbol 13 will be understood to suggest means for traversing the chromatogram plate 6 and the described optical system with respect to each other, and the synchronizing connection 13' to the recorder 12 assures proper correlation of the summation signal with traverse position.

The absorption-position curve recorded by the recorder 12 is illustrated at 14 in FIG. 3. The base line associated with this curve is also illustrated in FIG. 3 and is referenced 14'. Again, the desired data is the difference between curves 14 – 14', but since the base line curve 14' is so nearly flat, the curve 14 alone can be relied upon to yield the desired data. As can be seen, the base line 14' has now been flattened to such extent that variations due to irregularities of the separating layer are only in the order of magnitude of electronic instability of the whole system (i.e. system noise). Thus, evaluation of curve 14 is relatively simple and can be carried out with great accuracy.

Aside from the indicated advantages, the method of the invention will be seen to extend the limit of detection, inasmuch as deflections of the curve 14 (insofar as they are taken into consideration for an evaluation) are attributable only to effects specific to the substance.

In the embodiment illustrated in FIG. 5, a photometer ball 15 is arranged above the chromatogram plate 6; photometer ball 15 has an opening 16 for passage of the measuring radiation 17 therethrough. Radiation diffusely reflected by the separating layer of the plate 6 is detected by photoelectric means 18, in accordance with the various reflections within the photometer ball 15, as suggested by dashed lines. Below plate 6, another photometer ball 19 including a photoelectric detector 20 is arranged to respond to transmitted light, the radiation passing through the separating layer of the chromatogram plate 6 reaching the detector 20 after several reflections within the photometer ball 19, again as suggested by dashed lines. In the embodiment illustrated in FIG. 5, a substantially integral detection of both the reflected and the transmitted radiation is achieved, thereby further enhancing the accuracy of measurement, as compared with the photocell arrangement in FIG. 4.

In FIG. 6, reference numeral 6 again designates a chromatogram plate. The measuring radiation 17 is directed via a bundle of glass fibres 21 to the local area or position of the plate 6 being plotted. The bundle 21 is divided at this position, and the partial bundle 21' directes the diffusely reflected light to a photocell 22. Below the point of impingement of the measuring light 17 on plate 6, another bundle of glass fibers 23 is arranged to receive the transmitted radiation, dirdcting the same to a second photoeldctric detector 24.

It is also possible, as shown in FIG. 7, to modify the device thus far described in such a manner that only a single photoelectric detector serves the function of adding both light components. In such a modified device fiber-optical bundles 21' and 23 are suitably converged at a common end, adjacent said single detector, e.g. detector 25 only. Balance between base lines for the reflected function, as compared with the transmitted function, could be achieved (a) by appropriate length selection for the direct-transmission bundle (21') as compared to length of the diffuse-reflection bundle (23), or (b) by use of filtering means (not shown) selectively applicable to one with respect to the other of the bundles.

It will be understood that, in the embodiment illustrated in FIG. 4, it is possible to eliminate the two amplifiers 9 – 10, in which case, the sensitivity of the transmission detector 8 is designedly reduced to such extent that signals unspecific to the substance and supplied by the two detectors 7 and 8 have the same amplitude. In this situation, any signal amplification can be accomplished after the addition has been effected.

With the novel method, calibration curves are obtained which are almost linear at least in the range of small substance volumes. Therefore, the novel method is particularly useful for the plotting of thin layer-chromatograms in which only very small substance volumes are available.

We claim:

1. The method of photometrically evaluating light-absorbing zones resulting upon the separation of mixtures of substances in thin layers of light-dispersing material, which comprises irradiating light upon one localized area of the material, performing a traverse of said area over one such zone, simultaneously photometrically and additively measuring diffusely reflected light and transmitted light at said area in the course of the traverse, and recording the additive value as a function of traverse position.

2. The method of claim 1, in which the irradiating light is substantially monochromatic.

3. The method of photometrically plotting light scattered by a light-scattering object, which comprises irradiating light upon one surface of the object, simultaneously photometrically measuring diffusely reflected light and transmitted light for a given location on the object, and adding the simultaneously measured values; the given location being one of a succession involved in a transverse of the object, the added values being plotted as a function of traverse position, and the base-line amplitudes of the two measuring signals being caused to assume the same level, prior to adding the simultaneously measured values.

4. The method of claim 3, in which the base-line amplitudes are equalized by reducing the sensitivity of one photomdtric measurement with respect to that of the other photometric measurement.

5. Apparatus for photometrically plotting light scattered by a light scattering object, comprising a transpardnt support for the object, means for light-irradiating a limited surface area of the object normal to the support; photoelectric-detector means comprising a first detector on one side of the support for responding to diffusely reflected light from said area of the object, and a second detector on the other side of the support for responding simultaneously to directly transmitted light at said local area; means adding the outputs of said detectors, means for transversing said detector means and said support with respect to each other, and recorder means synchronized with traverse displacement and responsive to the added outputs of said detectors.

6. Apparatus according to claim 5, in which separate signal amplifiers are interposed between each detector and said adding means.

7. Apparatus according to claim 6, in which at least one of said amplifiers is selectively variable, whereby base-line amplitudes of the amplifier outputs may be caused to assume the same level for supply to said adding means.

8. Apparatus according to claim 6, in which both said amplifiers are selectively variable.

9. Apparatus according to claim 5, in which said light-irradiating means includes a monochromator.

10. Apparatus according to claim 5, in which said detectors are photometer balls disposed above and below said support, in vertically aligned relation, each photometer ball including a photoelectric cell.

11. Apparatus according to claim 5, in which said detector means includes fiber-optical means disposed on said one side of said support for conducting reflected light to said first detector.

12. Apparatus according to claim 5, in which said detector means includes fiber-optical means disposed on said other side of said support for conducting transmitted light to said second detector.

13. Apparatus according to claim 5, in which said light-irradiating means includes a source of light and fiber-optical means between said source and said one side of said support.

14. Apparatus for photometrically plotting light scattered by a light-scattering object, comprising a transparent support for the object, means for light-irradiating the object normal to the support, and detection means comprising fiber-optical means having a first fiber-optical bundle with ends adjacent one side of the support for responding to diffusely reflected light from a local area of the object, said fiber-optical means having a second fiber-optical bundle with ends adjacent the other side of the support for responding to directly transmitted light at said local area; and means including photoelectric means responsive to the sum of the light components transmitted by said first and second bundles.

15. Apparatus according to claim 14, in which said light-irradiating means includes a further fiber-optical bundle with ends on said one side of said support and terminating normal thereto at said local area.

16. Apparatus according to claim 14, in which said last-defined means is a single photoelectric cell.

17. Method for the photometric evaluation of an object having zones resulting upon the separation of mixtures of substances in thin layers of light-dispersing material, characterized by the fact that the diffusely reflected and the transmitted radiation are photometrically measured simultaneously at the same localized area of the object to be evaluated, that the localized area and the object are traversed with respect to each other to develop the simultaneous radiations as a function of traverse position, and that the two radiations thus obtained are added, the proportion of reflected-to-

transmitted radiation thus added being adjusted to substantially equalize the base lines for the respective reflected and transmitted radiation functions.

18. The method of photometrically plotting a thin layer of light-scattering material which comprises at different positions zones of absorbing materials, consisting of the steps of scanning said layer by a light beam, measuring simultaneously the diffusely reflected and the transmitted intensity of said light beam, adding these simultaneously measured values, and plotting the resulting sum-signal as a function of the position of said scanning light beam.

* * * * *