A DYNAMIC THEORY OF THIN LAYER CHROMATOGRAPHY

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(Received June 14th, 1966; modified April 21st, 1967)

INTRODUCTION

Thin layer chromatography takes place in sheets of a porous sorbent. The components of a solution form chromatographic spots which move and gradually spread in the plane of the chromatoplate. The spreading of the spots in thin layer chromatography occurs in two dimensions, unlike column chromatography where a chromatographic zone spreads only along the direction of movement.

Thus thin layer chromatography represents a two-dimensional process. Nevertheless up to now only theories of one-dimensional chromatography have been used for its description[1].

Such an approach is incorrect even in a first approximation and gives only a qualitative idea of the character of the chromatographic process in thin layers. Quantitative results can be obtained only on the basis of a two-dimensional theory.

BASIC EQUATIONS OF THE DYNAMIC THEORY OF THIN LAYER CHROMATOGRAPHY

In order to develop a dynamic theory of thin layer chromatography we use the following assumptions:

1. A substance moves as a chromatographic spot (zone) with a velocity $\vec{v}$, which is a definite fraction of the eluent velocity $\vec{u}$: $\vec{v} = R_F\vec{u}$ where R_F is a coefficient determined by the properties of the moving substance and by the volume ratio of the mobile to the immobile phase.

2. The process of thin layer chromatography is accompanied by spreading due to diffusion of the substance in the mobile phase. This spreading proceeds at the same rate both along the direction of movement and in the transverse direction. It is characterized by an effective diffusion coefficient D_l.

3. Thin layer chromatography is a non-equilibrium process because the adsorbent grains are of finite size. Owing to this it takes a definite time to establish thermodynamic equilibrium between the solid grains and the moving liquid phase. This effects additional spreading of the spot in the direction of the movement. It is possible to describe this phenomenon in a quantitative way using BRESLER's theory[2], its main parameter τ represents a time lag in the establishment of equilibrium between the mobile and immobile phases.

With these assumptions we can deduce a basic differential equation for the spreading of the spots in non-ideal ($D_l \neq 0$) and non-equilibrium ($\tau \neq 0$) two-dimensional chromatography. For this purpose the flow ($\vec{I}$) of a certain component should be examined at an arbitrary point of the plate.

$$\vec{I} = \beta\vec{v}C + \alpha\vec{v}m - \beta D_l \nabla C \qquad (1)$$

where C and m are the concentrations of the substance in the mobile and immobile phases, β and α are the relative areas occupied by the mobile and immobile phases respectively ($\beta + \alpha = 1$).

The first two terms in eqn. (1) correspond to the substance transfer due to the overall movement of the zone with a velocity $\vec{v}$. The third term reflects the diffusion flow in the liquid filling the pores of the adsorbent. Its dependence on the velocity of the zone is insignificant and can be neglected.

Let us express m through C by means of a sorption isotherm, confining ourselves to the case of a linear equation (it fits well our actual conditions since low concentrations of the substance chromatographed are used):

$$m = kC \tag{2}$$

where k is the sorption coefficient.

Since in chromatography there is a kinetic lag in the establishment of equilibrium between the sorbent and the solution, expression (2) should be corrected. This can be done by means of the lag parameter τ. When the zone moves with a velocity v, the concentration in the adsorbent at a point with the coordinate (x) corresponds to the equilibrium concentration in the solution at the point ($x + v\tau$), as if the adsorbent grain was in equilibrium with the solution which had been adjoining it τ seconds ago. Therefore instead of eqn. (2) we can write:

$$m(x,y,t) = kC(x + v\tau,y,t) \tag{3}$$

Expanding the right-hand side of eqn. (3) into a series and discarding terms of second order with respect to $v\tau$ we obtain:

$$m(x,y,t) = k\left[C(x,y,t) + v\tau \frac{\partial C}{\partial x}\right] \tag{4}$$

The balance equation is known to be:

$$\operatorname{div} \vec{I} = \beta \frac{\partial C}{\partial t} - \alpha \frac{\partial m}{\partial t} = -(\beta + \alpha k) \frac{\partial C}{\partial t} \tag{5}$$

Combining eqns. (1), (4) and (5) we obtain the fundamental differential equation of two-dimensional chromatography:

$$\frac{\partial C}{\partial t} + v \frac{\partial C}{\partial x} = R_F \left(D_l \nabla^2 C + \frac{1 - R_F}{R_F} v^2 \tau \frac{\partial^2 C}{\partial x^2}\right) \tag{6}$$

where:

$$R_F = \beta/(\beta + \alpha k) = v/u \tag{7}$$

It has been taken into account that the continuity equation $\operatorname{div} \vec{v} = 0$ was valid (which is really correct).

Now we must formulate initial and boundary conditions. We can assume that

at $t = 0$ the whole substance occupied a narrow region δ with a concentration C_0. In this case initial and boundary conditions may be expressed as:

$$C \mid_{t=0} = \begin{cases} C_0 & 0<x,y<\delta \\ 0 & \delta<x,y<\infty \end{cases} \quad C \mid_{t\to\infty\to 0}; \tag{8}$$
$$C \mid_{x,y=0} = 0;$$

Equation (6) can be transformed to canonical form by substituting new variables

$$\xi = \frac{x}{R_F\left(D_l + \frac{1 - R_F}{R_F} v^2\tau\right)^{1/2}}; \quad \eta = \frac{y}{(R_F D_l)^{1/2}}; \tag{9}$$

and assuming that:

$$C(\xi,\eta,t) = W(\xi,\eta,t) \exp\left\{\frac{v}{2 R_F\left(D_l + \frac{1 - R_F}{R_F} v^2\tau\right)}\left[\xi R_F^{1/2}\left(D_l + \frac{1 - R_F}{R_F} v^2\tau\right)^{1/2} - \frac{v}{2} t\right]\right\} \tag{10}$$

We finally get:

$$\frac{\partial W}{\partial t} = \nabla^2 W \tag{11}$$

The function $W(\xi,\eta,t)$ must satisfy the following initial and boundary conditions:

$$W \mid_{t=0} = \begin{cases} W_0 & 0<\xi,\eta<\delta \\ 0 & \delta<\xi,\eta<\infty \end{cases} \quad W \mid_{t\to\infty\to 0}; \tag{12}$$
$$W \mid_{\xi,\eta=0} = 0;$$

The solution of eqn. (11) with the conditions of eqn. (12) is a well known boundary value problem of heat conductivity. We will consider the fundamental solution of eqn. (11) in the case when $t \gg \tau$ and $\delta \to 0$ (this always occurs in practice). It can be expressed as:

$$W = \frac{A}{4\pi t} \exp\left\{-\frac{1}{4t}\left[(\xi - \xi_0)^2 + (\eta - \eta_0)^2\right]\right\} \tag{13}$$

where A is a constant.

Using eqns. (9) and (10) and taking into account $x_0 = y_0 = 0$ and also the conditions of normalization:

$$\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} C(x,y,t)\mathrm{d}x\mathrm{d}y = q,$$

(where q is the total amount of substance in the spot) we finally obtain:

$$C(x,y,t) = \frac{q}{4\pi R_F t\left[D_l\left(D_l + \frac{1-R_F}{R_F}v^2\tau\right)\right]^{1/2}} \exp\left\{-\tfrac{1}{2}\left[\frac{(x-vt)^2}{2\left(D_l + \frac{1-R_F}{R_F}v^2\tau\right)R_F t} + \frac{y^2}{2D_l R_F t}\right]\right\} \quad (14)$$

Equation (14) represents the concentration distribution in a chromatographic spot. It is a two-dimensional Gaussian distribution. Comparing eqn. (14) with a standard distribution:

$$C(x,y,t) = C_m \exp\left\{-\tfrac{1}{2}\left[\frac{(x-vt)^2}{\sigma_x^2} + \frac{y^2}{\sigma_y^2}\right]\right\} \quad (15)$$

we obtain expressions for its basic parameters:

$$C_m = \frac{q}{4\pi R_F t\left[D_l\left(D_l + \frac{1-R_F}{R_F}v^2\tau\right)\right]^{1/2}} \quad (16)$$

$$\sigma_x^2 = 2\left(D_l + \frac{1-R_F}{R_F}v^2\tau\right)R_F t \quad (17)$$

$$\sigma_y^2 = 2D_l R_F t \quad (18)$$

Here C_m is the maximal concentration in the center of the chromatographic spot, σ_x,σ_y are the standard deviations characteristic for the spreading of spots.

COMPARISON OF THEORY WITH EXPERIMENT

Expression (14) shows that the maximum concentration within the spot corresponds to the zero value of the exponent. Hence we can write: $x_{max} = vt$ where v is the velocity of the spot movement, a characteristic value $v = u/[1 + (\alpha/\beta)k]$.

Considering expressions (17) and (18) it is obvious that the spreading of the spot increases with R_F and t. Consequently the maximal sensitivity of analysis is obtained with the minimal values of R_F and t. This is necessary for the determination of microcomponents. To realize this possibility it is appropriate to use conditions of elution chromatography for the microcomponent, and displacement chromatography for the macrocomponent.

An example is given by Fig. 1, where we see the chromatogram of DNP-glycine and DNP-alanine in the presence of a 20,000 times excess of dinitrophenol.

Substituting in eqn. (14) for $C(x,y,t)$ a value C_{min}, which is the minimal concentration of the microcomponent which still can be detected, and taking the logarithms of both parts of the equation, we arrive at the formula:

$$\frac{(x-vt)^2}{4\left(D_l + \frac{1-R_F}{R_F}v^2\tau\right)R_F t \ln C_m/C_{min}} + \frac{y^2}{4D_l R_F t \ln C_m/C_{min}} = 1 \quad (19)$$

Equation (19) describes the shape of the spot in plane (x,y). It is the equation of an ellipse with the center in point $x=vt, y=0$ and with half axes:

$$a = 2\left[R_F t \ln C_m/C_{min}\left(D_l + \frac{1 - R_F}{R_F} v^2\tau\right)\right]^{1/2}; \quad b = 2\,(D_l R_F t \ln C_m/C_{min})^{1/2}.$$

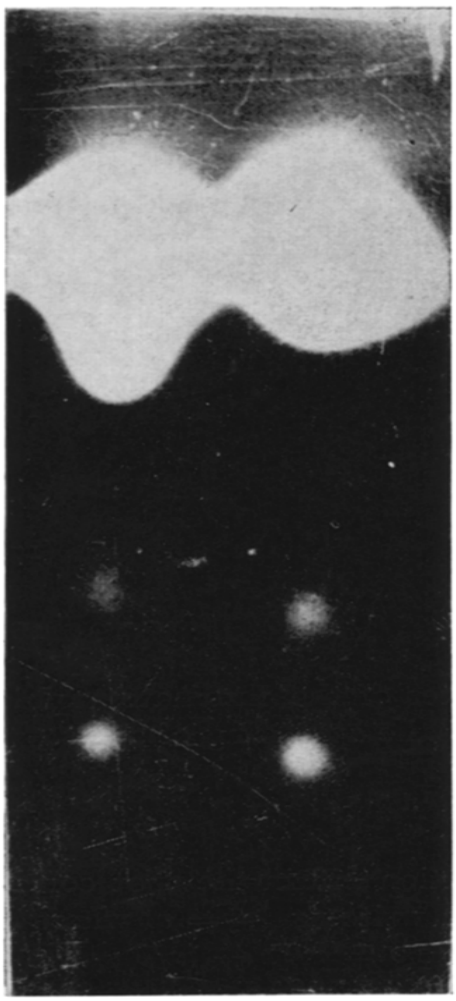

Fig. 1. Elution and displacement chromatography of DNP-glycine, DNP-alanine (0.05 μg); dinitrophenol (1000 μg) on silica gel "KSK" (large pore size). The eluent is chloroform–benzyl alcohol–acetic acid (70:30:3).

It is easy to show that in both limiting cases when $v \to 0$ ($R_F = 0$) and $v = u$ ($R_F = 1$) the ellipse becomes a circle with a radius $r = (D_l R_F t \ln C_m/C_{min})^{1/2}$. On the other hand, when $R_F = 1/2$, the chromatographic spot has the shape of an ellipse with the maximal value of eccentricity, *i.e.* we will observe the picture shown in Fig. 2a.

The physical meaning of this phenomenon is clear. When the velocity is small ($v \to 0$), the spot moves slowly and therefore is only slightly kinetically spread. When the velocity is high ($v = u$), the substance does not participate in the sorption processes and therefore there is no kinetic spreading.

The second term in eqn. (17) evidently approaches 0 if the grains become very

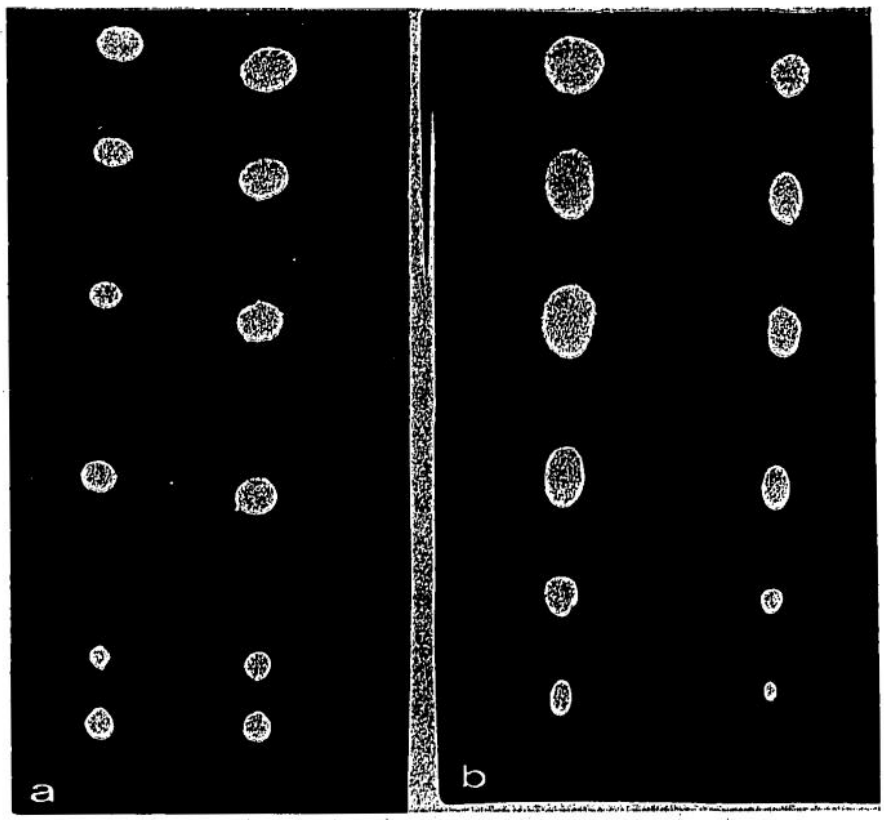

Fig. 2. Contact U.V.-photographs of thin layer chromatograms of DNP-amino acids on silica gel "KSK" of various grain diameters: (a) $d_p = 2.5\ \mu$; (b) $d_p = 25\ \mu$. Chromatographic eluent is the same as in Fig. 1.

small ($\tau \to 0$). Consequently $\sigma_x \to \sigma_y$, *i.e.* the spots on the chromatogram acquire a round shape (see Fig. 2b).

The dependence of spreading on the diameter of the silica gel grains d_p is of particular interest. This dependence is easily obtained by substituting this quantity for t and τ in expression (17).

It has been shown[2] that $\tau = d_p^2/60D$, in the case when τ is entirely determined by the diffusion within the grains (with a diffusion coefficient D).

Further, if we consider the layer of silica gel on the plate as a bundle of capillaries with a small diameter d_p we can express the velocity of the solvent as $u = Md_p$ and therefore $t = R_s/Md_p$ (where M is a term dependent on the density, viscosity, and surface tension of the eluent, and R_s is the distance from the origin to the front of developing fluid.

Finally we obtain:

$$\sigma_x^2 = \frac{2\,D_l R_s R_F}{Md_p} + \frac{MR_F^2 R_s\,(1 - R_F)}{30\,D}\,d_p^3 \qquad (20)$$

Since the first term reflects a hyperbolic dependence on d_p and the second one a parabolic dependence, the total function σ_x^2 first diminishes with d_p, passes through a minimum and then begins to increase with further decrease of the grain size. This relationship is based on quite obvious physical phenomena. When the grain size

decreases the time lag τ diminishes, hence, the influence of the kinetic factors upon the spreading becomes less important. At the same time, owing to an increase in hydrodynamic resistance of the layer the movement of the liquid slows down, the time of experiment increases and, consequently, diffusion spreading increases. Evidently, an optimal grain size should exist when the combined influence of both factors is reduced to a minimum.

Experimental data on the influence of the diameter of silica gel grains upon the separation coefficient of two substances:

$$K_R = \frac{R_F'' - R_F'}{\sigma_x'' + \sigma_x'} R_s$$

and upon the sensitivity of the analysis:

$$E_m/q = \varepsilon/2\,\pi\sigma_x\sigma_y$$

reveal an optimum grain size between 2.5 and 7.5 μ (Figs. 3 and 4).

By finding the expression for d_p in eqn. (20) corresponding to $\sigma^2_{x(\min)}$ and substituting empirical values of D_l (restricting ourselves to molecular diffusion), M, R_F and R_s, we can calculate the optimal grain diameter d_p and we find it in the range of 2–5 μ, *i.e.* in satisfactory agreement with experimental data.

By using experimental values of R_s, R_F, t, d_p, σ_x and σ_y (as halfwidths of Gaussian peaks at the height of 0.607 C_m) it is possible to determine the time lag τ. In the case of chromatography with a constant velocity it is easily calculated from eqns. (17) and (18):

$$\tau = (\sigma_x^2 - \sigma_y^2)t/2\,R_s^2(R_F^2 - R_F^3) \qquad (21)$$

By integrating the expression (15) in the limits $\pm\infty$ we obtain an expression for the total amount of substance in the chromatographic spot:

$$q = \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} C_m \exp\left[-\frac{1}{2}\left(\frac{x^2}{\sigma_x^2} + \frac{y^2}{\sigma_y^2}\right)\right] dx\,dy = 2\,\pi C_m\sigma_x\sigma_y \qquad (22)$$

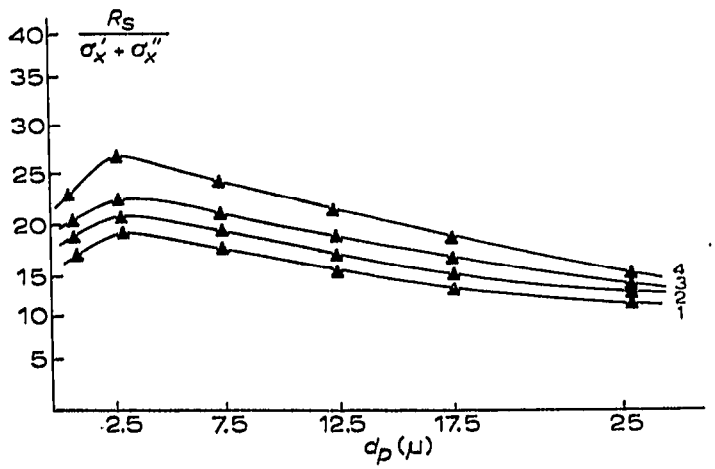

Fig. 3. Dependence of $R_s/(\sigma_x' + \sigma_x'')$ for DNP-glycine and DNP-alanine on d_p of silica gel "KSK" on plates with various values of R_s. (1) R_s = 3 cm; (2) R_s = 4 cm; (3) R_s = 5 cm; (4) R_s = 6 cm. Chromatographic eluent is the same as in Fig. 1.

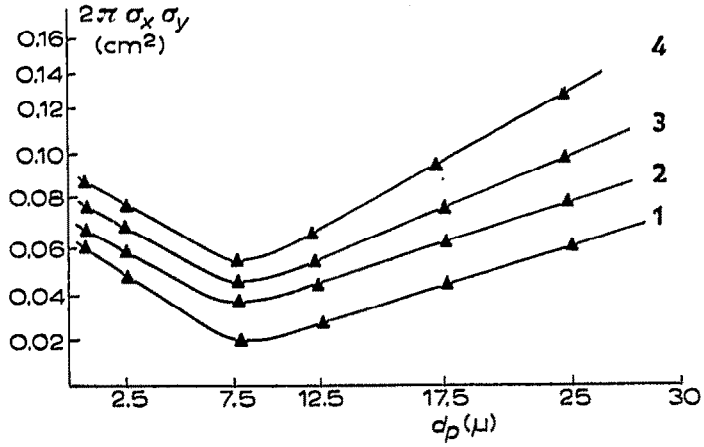

Fig. 4. Dependence of $2\pi\sigma_x\sigma_y$ for DNP-glutamic acid on d_p of silica gel "KSK". Chromatographic eluent and symbols are the same as in Fig. 3.

Thus by way of determining the maximal concentration in the spot and the standard deviations of the Gaussian distribution we can find the amount of substance in the chromatographic spot.

Practically these measurements can be carried out by means of a two-coordinate densitometer. In this case eqn. (22) can be transformed into:

$$q = 2\pi E_m\sigma_x\sigma_y/\varepsilon \tag{23}$$

where E_m is the measured extinction at the center of the spot (maximum of the peak), ε is the molar extinction coefficient of the substance. Comparing the spots for various quantities of the same substance eluted in identical conditions ($\sigma_x'' = \sigma_x'$; $\sigma_y'' = \sigma_y'$) we can calculate the amounts of substance using the simpler expression:

$$q'/q'' = E_m'/E_m'' \tag{24}$$

i.e. using only the ratio of extinctions measured in their maxima. The precision in the measurement of the parameters of densitometric curves, σ_x, σ_y, C_m, is the better, the smaller the dimensions of the photometric field are.

If we take photographs of the chromatograms, the spots reveal an elliptical shape described by eqn. (19), the limiting concentration C_{min} being determined by the sensitivity threshold of the photographic material. The area of this ellipse with halfaxes:

$$a = \sigma_x(2\ln C_m/C_{min})^{1/2}; \quad b = \sigma_y(2\ln C_m/C_{min})^{1/2}$$
$$S = \pi ab = 2\pi\sigma_x\sigma_y(\ln q - \ln 2\pi\sigma_x\sigma_y C_{min}). \tag{25}$$

is proportional to the logarithm of the total amount of substance in the spot. Since it is much easier to measure the length and the breadth of the spot on the photograph ($2a$ and $2b$, see eqn. 25) instead of σ_x, σ_y, C_m, it is advisable to use eqn. (25) for the determination of the total amount of substance in the spot. In particular, for two spots of the same substance (with σ_x and σ_y identical) it follows from eqn. (25):

$$A\ln(q_1/q_2) = S_1 - S_2 \tag{26}$$

where $A = 2\pi\sigma_x\sigma_y$ is a constant for the conditions used.

If partly overlapping spots are obtained it is possible to separate them on the photograph and to measure them quantitatively by selecting a higher sensitivity threshold C_{min}. This is convenient since it requires far less time than is necessary for working out new conditions of chromatography. Evidently this method is usable only to a certain limit C_{max} determined by the contrast properties of the photographic paper.

The solution of eqn. (14) was obtained for the case of constant velocity of the spot (v = const.). Nevertheless in practice the rate of the process varies with time (or with the distance from the origin to the front of the eluent R_s).

But we can divide the time into intervals small enough to consider the velocity as being constant during each of them. Then it is possible to apply eqn. (14) to these intervals and to come to the final result by summation.

The data mentioned above were obtained by means of ascending chromatography.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Prof. S. E. BRESLER for helpful discussion and valuable advice.

SUMMARY

A dynamic theory of thin layer chromatography has been worked out. It is a theory of two-dimensional non-ideal and non-equilibrium chromatography.

A basic differential equation has been derived and its fundamental solution obtained. It follows from this solution that the concentration of the substance in the chromatographic zone is described by a two-dimensional Gaussian distribution. Its degree of anisotropy depends on the nonequilibrium character of the process (τ value).

On the basis of this theory the peculiarities of thin layer chromatography are discussed. The influence of experimental conditions upon the efficiency of separation and sensitivity of detecting a substance on the plate is predicted and verified. The most important factor is the grain size. Dependence of kinetic spreading (the elongation of the chromatographic spots) on R_F has been shown. This value passes through a maximum when $R_F = 0.5$.

It was shown that it is possible to measure τ, the kinetic lag parameter, directly from chromatograms.

A precise method for the determination of small amounts of substances in chromatographic spots was worked out on the basis of the theory. The measurement is carried out by means of two-coordinate densitometry. Another variation of this method involves determination of the area of the chromatographic spot by a photographic technique.

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