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Coupling development and elution, a new thin-layer chromatography technique

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Abstract

Three methods of coupling development and elution were studied in this paper. (1) A new mode of solvent supplementation and eluate collection was developed for descending development. By using a new distributor and collector in descending development, components can be separated and eluted continuously. (2) The same effect can be realized with a slope distributor [Su et al., *J. Planar Chromatogr.* 14 (2001) 203] and a collector by horizontal development. (3) In-situ elution can be used to treat a developed silica plate, which can elute the separated components to the receptor without scraping them off. These three methods can be used individually, and the in-situ elution can be used with other modes of development.
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Keywords: Instrumentation; Thin-layer chromatography

1. Introduction

Although the thin-layer chromatography (TLC) scanner has been used for a long time, its results are unreliable sometimes. It may be a better choice to remove the samples from the silica layer and elute them quantitatively if we can reduce the complexity of this method. So we have some ideas for coupling separation and elution. With the assistance of gravity, descending development seems a matter-of-course.

Theoretically, descending development has the potential elution power due to the downward movement by the force of gravity. But considering supplying the solvent, it seems too difficult to use this method in TLC. Lapp and Erali (see Ref. [1]) first run a descending thin-layer chromatography on a loose-layer basis. In 1957 Stanley and van Nier (see Ref. [1]) were the first to use descending development with bound layer. Another method [2,3] of descending development used capillary action to supply solvent. In practice, it is difficult to elute the components out of the layer using the methods mentioned above. Pelick [4] used a descending instrument, which combined a distributor and a silica plate in a sealed cylinder. The whole instrument seems too complex to be used.

Here, we designed a simple and practical descending development instrument, which can separate the components, and then remove them out of the layer. After further study, we found that the key factor of the system is the collector but not the mode of

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development and that horizontal development can also realize these functions with a special collector. Naturally, we found another new technique, in-situ elution, which removes the separated components out of the layer by eluting instead of scraping off spots.

2. Experimental

2.1. The descending development instrument

The instrument is composed of four parts: (1) a distributor, (2) a collector, (3) a chamber and (4) a receiving system. Besides, some part of the silica plate should be scraped off before being used. The whole instrument and each part are described below in detail.

2.1.1. Distributor

The distributor is a key part of the descending development. It is a piece of glass (5.0×2.0×0.3 cm) with a slope edge and two holes, through which two polyethylene pipes are threaded, as shown in Fig. 1. The underlying pipe is used to deliver the solvent and the upper pipe is used to balance the air pressure. Generally, the solvent is stocked in the interspaces between the cover distributor and the silica layer.

The silica layer should be scraped to form a rectangle window at the upper part, and the under part should have a trapezoid shape, as shown in Fig. 2a. The distributor covers the plate tightly by two nippers as shown in Fig. 2b and c. As the distributor is like a cover plate, we call it a cover distributor to differentiate it from other types of distributors.

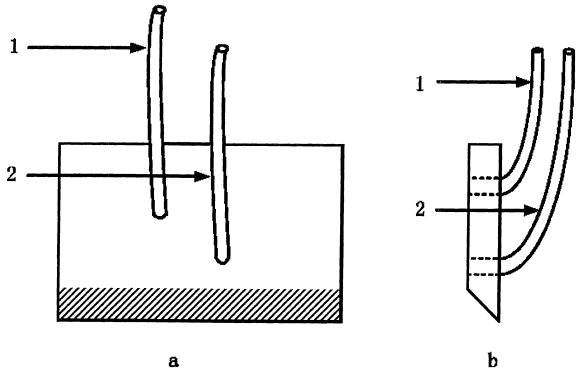

Fig. 1. Construction of the cover distributor (all the schematic diagrams, not drawn to scale) (a) front view (b) side view. 1=A pipe used to balance the air pressure, 2=a pipe used to deliver the solvent.

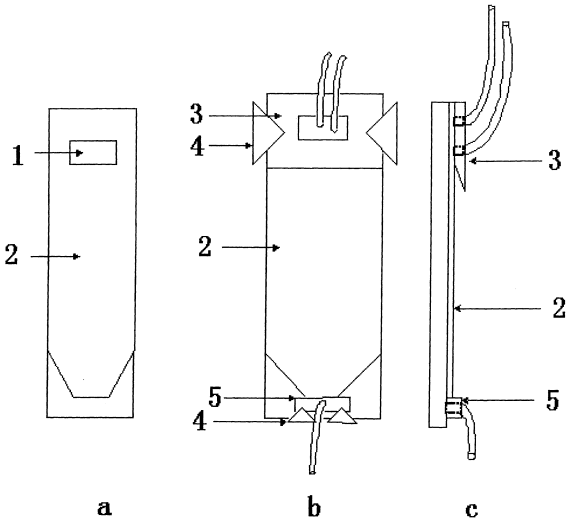

Fig. 2. Construction of the cover distributor, silica plate and the cover collector. (a) Silica plate, (b) front view of the silica plate with the cover distributor and the cover collector, (c) side view of the silica plate combining the cover distributor and the cover collector (without nippers). 1=Window, 2=silica layer, 3=cover distributor, 4=nipper, 5=cover collector.

2.1.2. Collector

The collector is a key part to remove the eluate from the layer. It is a piece of glass (2.0×0.5×0.1 cm) with a hole through which a polyethylene pipe is threaded, as shown in Fig. 3. Before development, two nippers clamp the collector to the bottom side of the silica plate as shown in Fig. 2b and c. As the

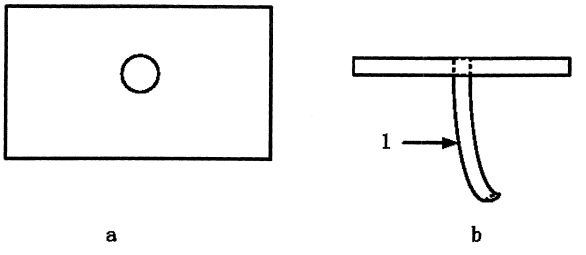

Fig. 3. Construction of the cover collector. (a) Top view, (b) side view. 1=Pipe used to deliver eluate.

collector is like a cover plate, we call it a cover collector to differentiate it from other types of collector. The upper side of the cover collector should attach to the bottom side of the silica layer. As soon as the eluent reaches the edge of the layer, the solvent is sucked into the capillary slab between the collector and the glass plate of the silica plate immediately, and then removed to the receptor by the decreasing air pressure. The pipe should thread the hole on the cover and connect it to the negative air pressure system.

2.1.3. Chamber

The chamber is modified from the traditional sandwich chamber. Five pieces of glass (3 mm thick) were glued together to build a rectangle box (9×3×4 cm) by epoxy resin (Fig. 4). Two holes were drilled in the upper side of the chamber. Those are used to thread the pipes of the cover distributor out of the chamber. A piece of glass is used as the cover of the chamber. A hole was drilled in it, through which the pipe of the cover collector was threaded.

2.1.4. Receiving system

The receiving system is composed of a refitted aspirator pump, a buffer bottle and a receptor as shown in Fig. 5a. The original use of the aspirator pump is to supply goldfish with oxygen. We refitted the pump by changing the place of the diaphragm and made it a micro aspirator pump, which can continue working for a long time. If the pump is not available, the eluent can also be sucked into the receptor by another simple device as shown in Fig. 5b.

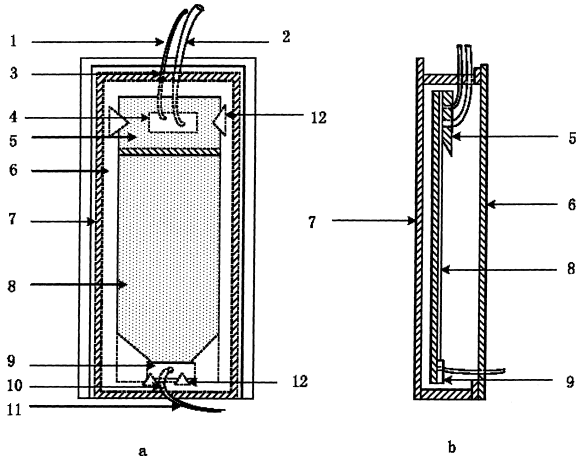

Fig. 4. Construction of the descending development chamber and the silica plate. (a) Front view, (b) side view. 1=Pipe used to balance air pressure, 2=pipe used to deliver solvent, 3=a hole in the upper side of the chamber, 4=''window'' on upper unit of the chamber, 5=cover distributor, 6=the cover of the chamber, 7=chamber, 8=silica plate, 9=cover collector, 10=a hole in the cover of the chamber, 11=the pipe of the collector used to deliver the eluate, 12=nippers.

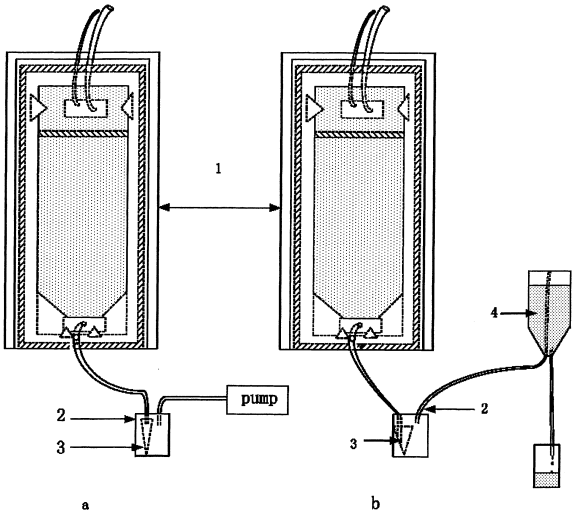

Fig. 5. Construction of the chamber, receptor and negative pressure system. (a) Descending development with aspirator pump, (b) descending development with hydraulic negative pressure. 1=Chamber, 2=buffer bottle, 3=receptor, 4=water bottle.

2.2. Couple separation and eluting at horizontal development

The half-way development device [5] can be used in horizontal development to couple separation and elution. The device and the operation were described before [5]. The cover collector is mentioned above, and the whole instrument is shown in Fig. 6.

2.3. In-situ elution

In-situ elution is a method to remove spots by eluting but not scraping. Similar methods are mentioned above. The difference is that the former couples the process of separation and elution, while

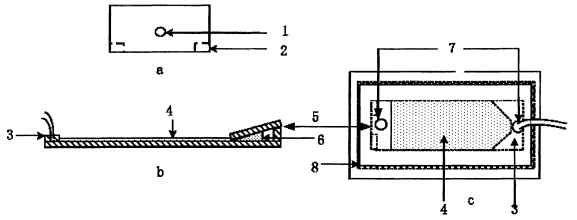

Fig. 6. Construction of the horizontal development. (a) Slope distributor, (b) side view of the slop distributor, the silica plate and the cover collector, (c) top view of chamber and silica plate. 1=A hole used to deliver solvent, 2=a small piece of glass, 3=cover collector, 4=silica plate, 5=slope distributor, 6=solvent, 7=two holes in the cover of the chamber.

the latter develops first, then elutes the interested spots.

A schematic diagram of the method is shown in Fig. 7: after development, if the distance between the two spots is not far enough, it can be pushed away by the funnel distributor [6] as shown in Fig. 7a. The layer will be scraped into a suitable shape, and the arrangement of slope distributor and cover collector are shown in Fig. 7b.

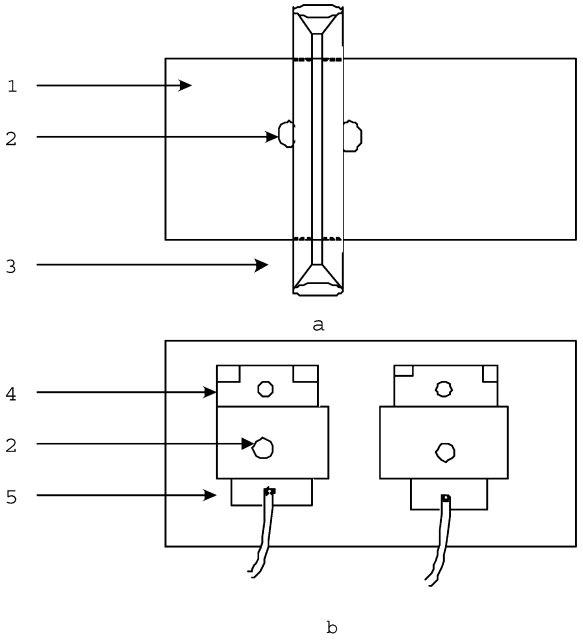

Fig. 7. Schematic diagram of in-situ elution. (a) The funnel distributor used to push spots further far away, (b) the slope distributor and the cover collector. 1=Silica plate, 2=sample spot, 3=funnel distributor, 4=slope distributor, 5=cover collector.

3. Examples

3.1. Reagents and instrument

The dyes of disperse yellow, disperse blue, disperse red were provided by Shenyang Chemistry Institute, China. Methanol (HPLC grade) was purchased from Tianjin Concord Tech, China. Diethyl ether and chloroform (analytical-reagent grade) were purchased form Shenyang Reagent Factory, China. A UV-9100 spectrophotometer was purchased from Beijing Rily Analytical Instrument.

3.2. Operations

The newly spread silica plates were continuously developed with methanol for about 2 h to remove dirt. The prewashed silica plates were dried at 110 °C for 0.5 h, then they were placed in a desiccator. The subsequent treatment of the silica plate and the construction of cover distributor, cover collector, silica plate and chamber were described before. It should be noticed that no matter whether descending or horizontal development is applied, the solvent should flow in the direction as that of the step of continuous development for removing dirt.

About 30-mg of disperse yellow, disperse blue and disperse red were weighed precisely and solved with chloroform to 50, 25, and 10 ml as the stock solutions. Volumes of 2.0, 2.5, and 1.5 ml of three stock solutions were mixed together.

The 6-μl mixture was applied to the silica plate. Gradient development was applied in the course of the analysis. The gradient solutions are as follows (I) 0.5 ml of diethyl ether–chloroform (9:1); (II) 0.5 ml of diethyl ether–chloroform (1:3); (III) chloroform–methanol (5:1) volume as needed. As soon as the eluent reaches the end edge of the silica layer, the aspirator pump begins to work. The eluent collected before the first component reaches the end edge should be cast away. After one component is collected, the corresponding receptor should be replaced. The eluate solution was dried under air stream and the residues were solved with methanol to 0.6 ml, and then were measured at the maximum wavelength of each. Volumes of 2.0, 2.5 and 1.5 μl of the stock solutions were solved with methanol to 0.6 ml, and used as the standard solutions.

Table 1
Result of determination of the three dyes (n=5)

	Disperse yellow (λ 440 nm)			Disperse blue (λ 570 nm)			Disperse red (λ 505 nm)		
	Absorbance	RSD	Recovery (%)	Absorbance	RSD	Recovery (%)	Absorbance	RSD	Recovery (%)
Standard solution	0.046	0.012	–	0.029	0.008	–	0.026	0.003	–
Descending development	0.043	0.015	93.48	0.027	0.014	93.10	0.025	0.019	96.15
Horizontal development	0.044	0.016	95.65	0.027	0.026	93.10	0.027	0.017	103.84
In-situ	0.045	0.010	97.83	0.029	0.029	100.00	0.026	0.028	100.00

4. Results and discussion

(1) The results of the three methods are shown in Table 1. The accuracy and precision are satisfactory.

(2) Though the instrument is designed for descending development, the solvent is moved mainly by capillary action and gravity is not the major factor affecting the velocity of development. We tried to increase the level of the solvent in the distributor, but the solvent osmosed the silica layer easily. Other designs to increase gravity had various problems. This is why we chose this simple and easily self-made device, which can also reduce the even distance.

(3) We originally expected to realize auto-elution without the help of any outer force except gravity. Many different types of collectors were studied, but the results were unsatisfactory. Because gravity was not the major factor as mentioned above and the capillary action sharply reduced when the solvent reached the end edge of the silica layer, so, not enough eluate would be collected in the receptor. A lot of new types of capillary devices were designed in our laboratory, which could not meet the requirements. All of them could easily remove the samples out of the silica layer, but the following difficulty is still how to remove the eluate from the capillary devices to the receptor.

At last we were aware of the necessity of the outer force. The receiving system we used is the simplest and the most effective one among all those designed and the cost is very low.

(4) If the silica layer is not smooth, the solvent will leak from the slit between the cover distributor and the layer, and the even distance will be longer. We often met this problem, as the silica plates were made by hand and there was no slope on the distributor at the beginning. In order to resolve it, we ground one edge of the distributor to form an acute angle as shown in Fig. 1b. In this way, the solvent leaked from the distributor be collected and distributed by capillary action between the slope edge and silica layer immediately, so that the even distance is very short. It seems that the leak of a little solvent may be a good thing.

(5) At the initial stage, we did not think it was possible to couple development and elution in horizontal development. With the new receiving system and the slope distributor, we found that horizontal development could work well as shown in Fig. 8a and c, but if we used a cover distributor in horizontal development as shown in Fig. 8b and d, it did not work well. Obviously, the higher level of the solvent was very important, or collection would be fail even in the negative air pressure system.

(6) Compared with horizontal development, descending development can improve the velocity of development. We used three kinds of solvents to study it, as shown in Fig. 9 chloroform–diethyl ether (3:1), ethanol, and chloroform. The results indicate: (i) in general, the velocity of descending development is faster than that of horizontal development.

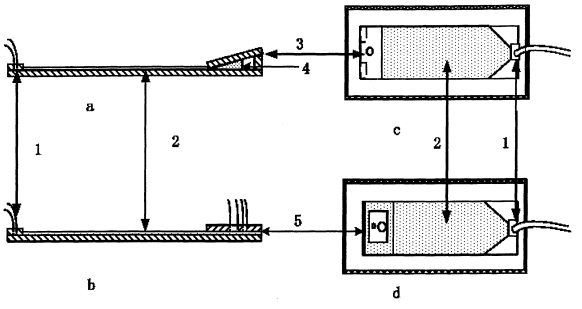

Fig. 8. Comparison of the horizontal development using different distributor. (a) (b) Side view, (c) (d) top view. 1=Cover collector, 2=silica plate, 3=slope distributor, 4=solvent, 5=cover distributor.

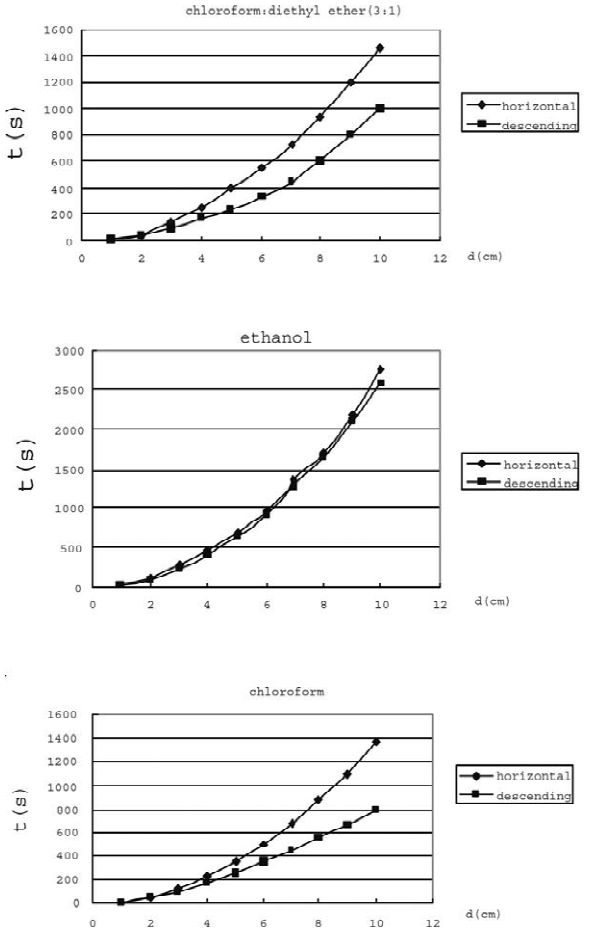

Fig. 9. Comparison of descending and the horizontal development using chloroform–diethyl ether (3:1), ethanol and chloroform as the solvent.

When using the high viscosity solvent, the diversity was not evident, but the total time was reduced; (ii) after 3 min, the velocity of these two modes declined obviously.

(7) No matter whether descending or horizontal development is used, the subsequent step is always the same as in continuous development, after eluent reaches the end edge of the silica layer. In this step, the velocity of the solvent is slower than that of the step that the solvent front does not reach the end edge of the silica plate. We measured the velocity of disperse red (R_F=1) using the chloroform and ethanol as solvents after the silica plate was saturated with the correspondence solvent. We found that the velocity of the solvent was almost steady.

(8) The silica plate always contains some impurities that have absorption in the UV–Vis region. So the silica plate should be prewashed before the development. We placed the silica plates into the continuous development chamber and methanol was used as the solvent. After 2 h, the silica plates were dried at 110 °C for 0.5 h. The prewashed silica plates were put into the desiccators.

(9) Coupling development and elution is a new technique of TLC. Compared with the classical Tswett method that uses column chromatography, the mobile phase instantaneous velocity of our method is slower. But the total time of our experiment is shorter than that of column chromatography. Another advantage of the column chromatography is that it is suited to dealing with massive samples. However, planar chromatography is a flexible technique to retreat separated spots [5–7]. Besides this, planar chromatography can separate many different samples at the same time. This paper only gave examples of treating single samples as mentioned above. Virtually, many samples can be dealt with at the same time. The sizes of the distributor, silica plate, chamber and the collector can also be changed to meet the need. In descending development, the silica plate should be cut apart according to the number of the samples first, then the sequential process continued. In practice, four samples have be separated with a 10×20 cm silica plate. It is possible that more samples can be treated with bigger silica plates by horizontal development. Our pump can supply enough power.

5. Conclusions

Combination of the development and elution was studied in this paper. Another similar technique, in-situ elution was also introduced. Descending development is faster than horizontal development, while horizontal development is simpler than descending development in device and operation. In-situ elution is flexible and the elution time is very short, so it is suitable for many purposes.

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