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Quasi-continuous videodensitometric registration of chromatograms on a plate during its development process

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1. Introduction

Thin-layer chromatography (TLC) is the simplest, sufficiently efficient and highly economical method of liquid chromatography [1]. However, TLC is characterized by particular drawbacks, among which the most significant is that the processes of separation and detection are divided in time and space [1,2]. In traditional TLC an experimenter obtains analytical results only after development of the whole chromatographic plate, independently on that whether the compounds (zones) were separated in the beginning or only at the end of the experiment. Quasi-continuous registration of separation results is possible during the process of their development [3,4]. The proposed variant of detection is based on the simultaneous carrying out of separation on a plate and registration of the obtained chromatograms using the videodensitometer. Using on-line videodensitometric detection in TLC permits: 1) to continuously obtain the information on separation; 2) in a number of cases to sharply reduce the duration of the experiment conducting (since often analyzed compounds are separated earlier than an experiment is finished, what is especially significant when analyzing colorless (registered in UV-light) compounds).
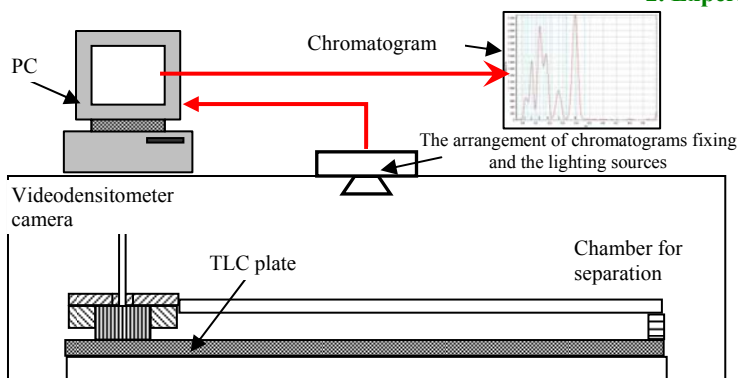
2. Experiment and results

Fig.1. The schematic of the experiment conducting.

The research was conducted on the plates Silica gel 60 F_{254} "Merck". As the mobile phase (MPh) toluene was used, as the object for separation the dye mixture - Test Dye Mixture III (CAMAG) – was used. All the chromatograms were obtained using the videodensitometer and the software "Sorbfil" (IMID) (the determination error is 3-7%).

For carrying out the continuous registration of the separation results the video camera was installed over the TLC plate, which was connected to the computer (the program «Videodensitometer»). Using the given approach (Fig.1) it is possible to directly observe the process of separation and to see the change in the efficiency of separation (Fig. 2). The chromatograms with separation have been registered at every cm of eluent front migration (that is at 1, 2, 3, 4, 5, 6, 7 cm).

At the Fig.2 there is an example of chromatograms obtained directly during the process of dyes separation within the quasi-continuous registration of the results at the different distances of the MPh front. The distance traversed by the MPh front increases, the pattern of separation improves: for example, at 3 cm the separation into 5 zones is observed, and at 4 cm – already complete separation of the mixture into all the 6 components is observed. Within the further development of the plate the pattern of separation does not principally change, however, the separation factor markedly improves. Thereby, the complete separation of the analyzed mixture components in the given example was achieved already at 4 cm, and it was possible not to carry out the further development. It allows substantially reducing the experiment duration (by 42%).
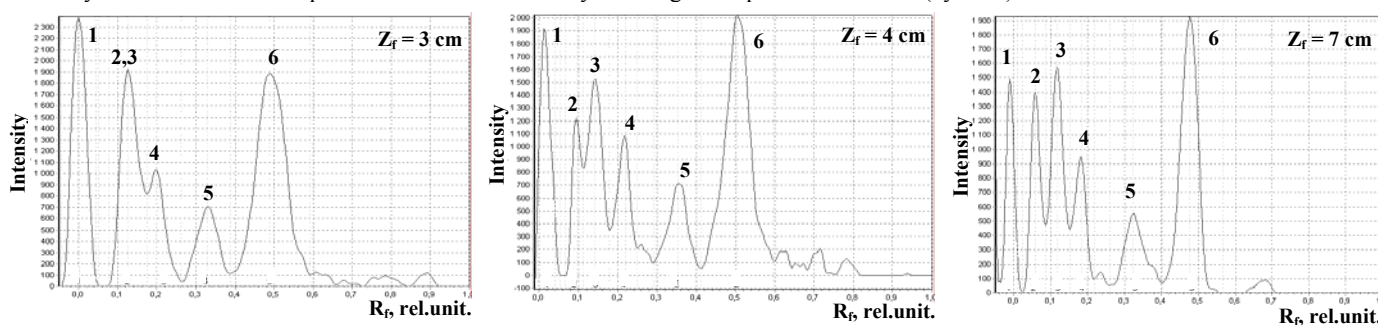

Fig .2. The change of the chromatograms within the continuous results registration in linear TLC.
(MPh is toluene, 1- Ciba FII, 2 - Indophenol, 3 - Ariabel Red, 4 - Sudan Blue, 5 - Sudan II, 6 – Dimethylaminoazabenzene).

Table 1. The characteristics of the chromatograms sequentially obtained within the quasi-continuous registration of the results of separation of dye mixture (MPh is toluene).

Compound	Characteristic	Position of mobile phase front, Z_f, cm (t, min)		
		4 cm (11.0 min)	5 cm (13.7 min)	7 cm (19.1 min)
Ciba FII	R_f	0.06	0.05	0.05
	N	69	96	168
	S	9	9	8
Indophenol	R_f	0.10	0.10	0.11
	N	102	138	282
	S	12	11	10
Ariabel Red	R_f	0.15	0.16	0.15
	N	187	224	424
	S	14	15	16
Sudan Blue	R_f	0.23	0.23	0.22
	N	272	360	578
	S	17	17	18
Sudan II	R_f	0.36	0.35	0.35
	N	456	548	778
	S	12	11	10
Dimethylaminoaza-benzene	R_f	0.52	0.52	0.51
	N	530	668	918
	S	36	37	38

3. Conclusion

The way of videodensitometric detection in TLC has been proposed, which permits, first, to practically continuously obtain the information on the process of separation, second, to sharply reduce the duration of experiment conducting at the expense of that in some cases the compounds of interest are separated earlier than the very experiment is finished, what is especially significant when analyzing colorless compounds that give a signal in UV-light, and to reduce the contribution of manual labor - no plate drying stage before detection.

References

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