

EFFECT OF TEMPERATURE ON PARAMETERS OF FLUORESCENCE SPECTRA OF SOLVATOCHROMIC INDICATOR SOLUTIONS IN STATIONARY PHASES FOR GAS–LIQUID CHROMATOGRAPHY

V. I. Beketov,^a M. V. Belkov,^b N. B. Zorov,^a
S. N. Lanin,^a P. N. Nesterenko,^{a,*} and P. P. Pershukevich^b

UDC 535.37;535.34;543.544.43

Luminescence spectra of several polarity indicators, including Coumarin 153 and coronene that were previously studied at room temperature and Nile Red and Pigment Red 179, were recorded at 20 and 120°C in solutions of liquid stationary phases of various polarities. The effect of temperature on the spectral parameters sensitive to the stationary phase polarity was estimated for these indicators. The prospects for estimating the polarity of stationary liquid phases using luminescent methods at high temperatures was demonstrated.

Keywords: fluorescence spectrum, gas–liquid chromatography, liquid stationary phase, polarity, solvatochromic effect.

Introduction. The enormous numbers of stationary liquid phases (SLPs) for gas–liquid chromatography (GC) have a variety of properties and exist to solve a large array of actual problems in chromatographic analysis and a broad spectrum of compounds to be separated [1]. Characterization of the properties of deposited or immobilized SLPs that determine the selectivity of the separation is a labor-intensive and complex endeavor because intermolecular interactions between a dissolved compound and the SLP are extremely complicated. The retention of various compounds and, correspondingly, the selectivity of the phases, which is directly related to their polarity, cannot be reliably predicted because they cannot be described with adequate accuracy [2–4]. Furthermore, many organic compounds, including stationary phases and especially polar ones, can alter their characteristics upon immobilization to the surface of a solid carrier used as a support and under the separation conditions [5]. The mobility of molecules and SLP polymer chains in a thin surface layer and structural changes of a thin layer with simplified coordination of SLP functional groups to the support surface can increase with increasing temperature [6]. Therefore, the polarity of SLPs should be expected to change with varying temperature.

The fundamental problem consists of understanding the change of SLP characteristics, primarily the polarity, as a function of the layer thickness, the nature of the support or solid carrier, temperature variation, increased service or shelf life, and the ability to identify a change of physicochemical properties of such phases by analyzing luminescence spectra of compounds produced under variable conditions and adsorbed or dissolved model compounds.

A set of test compounds with known parameters must be chosen to determine the SLP polarity and to construct corresponding polarity scales. Their retention under GC conditions must be studied. This procedure is labor-intensive and requires considerable investments of time [2, 3]. The use of quantum-chemical descriptors to calculate SLP polarity is at least as complicated and inadequately accurate [7]. An alternative and simpler method is the use of a suitable spectrophotometric or fluorescent indicator for which the change of absorption spectrum parameters as a function of medium polarity is characterized [8]. Changes in emission spectra of indicators, which can be more indicative, are used more rarely for these purposes. The potential of a method for evaluating SLP polarity that is based on measurement of the fluorescence parameters of indicator compounds was demonstrated [9]. The present work continues the previous work [9] by studying the ability to use empirical spectroscopic polarity scales of solvents to evaluate the SLP polarity in GC at high temperatures.

*To whom correspondence should be addressed.

^aM. V. Lomonosov Moscow State University, Moscow, Russia; email: nes.pavel@gmail.com; ^bB. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, Minsk, Belarus. Translated from Zhurnal Prikladnoi Spektroskopii, Vol. 89, No. 3, pp. 330–335, May–June, 2022. Original article submitted March 30, 2022; <https://doi.org/10.47612/0514-7506-2022-89-3-330-335>.

TABLE 1. Wavelengths of Fluorescence Maxima of Used Indicators

Indicator	λ_{\max}^N , nm ^a	λ_{\max}^P , nm ^b	$\Delta\lambda_{\max}$, nm	$\log P^c$	Ref.
Coumarin 153	460	554	94	4.51	[9]
Coronene	428	429	1	7.64	[9]
Nile Red	554	634	80	4.38	[10]
Pigment Red 179	–	536	–	4.18	[11]

^aFor indicator solutions in cyclohexane.

^bFor indicator solutions in DMSO except for Pigment Red 179, in DMF.

^c P is the distribution coefficient of the compound between immiscible 2-octanol and H₂O.

Experimental. Fluorescence emission and excitation spectra of solutions of luminophores were measured on a Lumoscan spectrofluorimeter system equipped with MDR-12 and MDR-23 excitation and recording monochromators (LOMO, USSR). The excitation source was an XBO-150W/1 Xe lamp (OSRAM, Germany). Luminescence was recorded at an angle of 90° relative to the excitation axis, Light passed through a monochromator and impinged on a cooled FEU-100 photomultiplier (230–800-nm range) operating in photon-counting mode. The MDR-23–FEU monochromator recording system was corrected using a standard TRSh 2850 tungsten ribbon lamp. The spectral slit width for recording structured spectra was 1.2 nm; broadband, 2.4 nm. Emission spectra were given on scales of the number of quanta (quantum intensity) per wavelength λ with subtraction of the solvent spectrum. A cylindrical cuvette with the test solution was placed into a heating block and automatically maintained at the given temperature within $\pm 1^\circ\text{C}$ to obtain spectra in the temperature range from ambient ($\sim 20^\circ\text{C}$) to 150°C .

Electronic absorption spectra of solutions were recorded on a UV-3600 Plus spectrophotometer (Shimadzu, Japan) in rectangular quartz cuvettes with optical pathlengths of 2 or 5 mm and solution concentration $\sim 10^{-6}$ M. Final adjusted absorption spectra were obtained by subtracting the solvent spectrum from the solution spectrum (with spectra recorded in the same cuvette).

The fluorescent indicators were Coumarin 153 (C153), coronene, Nile Red, and Pigment Red 179 (*N,N'*-dimethyl-3,4,9,10-perylenetetracarboxyldiimide) of purity $\geq 95\%$.

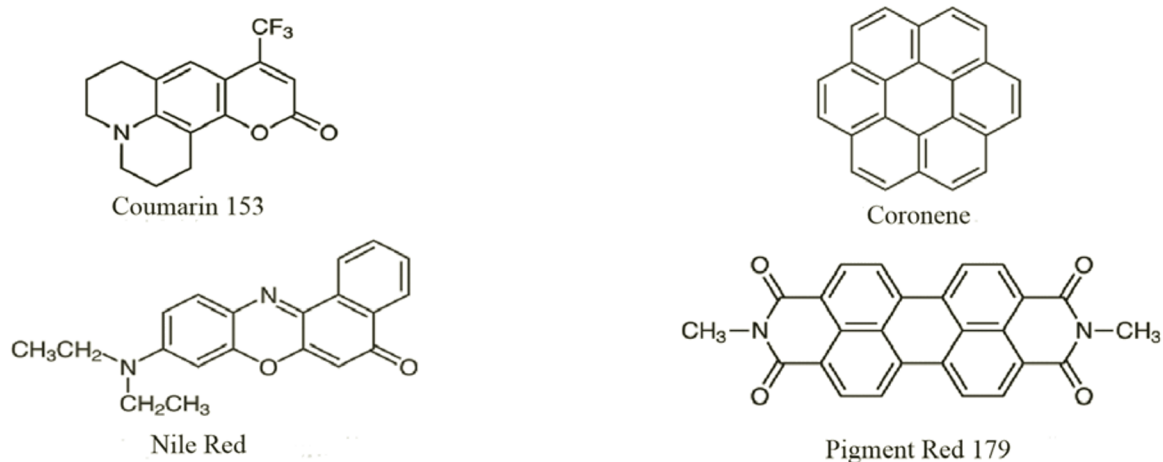


Table 1 lists the characteristics of the indicators. The polarity of the GC stationary phases squalane, polymethylsiloxane liquid PMS-100, polymethylphenylsiloxane liquid Lukooil MF (25% phenylsiloxane), Triton X-100, poly(ethylene glycol) PEG-250, dinonyl phthalate, poly(propylene glycol) 250 (60% Polypropylen-glykol MG 150 and 40% Polypropylen-glykol MG 425), di-(2-ethylhexyl)sebacate, di(ethylene glycol) succinate (DEGS), and 3,3'-oxydipropionitrile was estimated. Compounds of chemically pure, for chromatography, or analogous purity were used. Table 2 presents the polarities of the SLPs and their melting points.

TABLE 2. Parameters of Fluorescence Spectra of Perylene and Coronene Derivatives in SLP of Various Polarities at 20 and 120°C

SLP				Pigment Red 179						Coronene*	
Phase	T_{\max} , °C	Polarity		Spectrum center of gravity λ_{CW} , nm		Shift $\Delta\lambda_{CW}$, nm		Rel. shift $\Delta\lambda_{CW}/\Delta$		I_1/I_3	
		McReynolds $\Sigma^5\Delta I$ at 120°C	Thermo- dynamic – $\Delta G^E_{CH_2}$, J/mol								
		20°C	120°C	20°C	120°C	20°C	120°C	20°C	120°C		
Squalane	150	0	2426	540.7	534.0	0.0	0.0	0.00	0.00	0.08	0.06
PMS-100	200	224	2211	541.9	535.0	1.2	1.0	0.02	0.02	0.16	0.15
Lukoil MF	170	620	2242	552.9	549.0	12.2	15.0	0.22	0.25	0.17	0.17
Di-(2-ethylhexyl) sebacate	120	653	2178	564.9	556.0	24.2	22.0	0.45	0.37	0.48	0.46
Dinonyl phthalate	130	803	n/d	576.2	575.0	35.5	31.0	0.65	0.53	0.60	0.55
Poly(propylene glycol) 250	125	1419	n/d	588.8	583.0	48.1	49.0	0.89	0.83	0.71	0.69
3,3'-oxydipropionitrile	100	4427	1563	595.0	593.0	54.3	59.0	1.00	1.00	0.93	0.92

*Values I_1 and I_3 measured at ~428 and ~445 nm; n/d, no data.

Results and Discussion. An analysis of results for many SLPs (>20) showed that the most effective of the single-band model compounds were Coumarin 153, a perylene derivative, and Nile Red; of the compounds with a linear spectrum, coronene. Experimental fluorescence spectra of the model compounds used to evaluate the SLP properties at 20 and 120°C were summarized in the present work. The solvatofluorescent indicator Nile Red with a characteristic broad (80 nm) range of shifts of the fluorescence maximum upon increasing the solvent polarity from cyclohexane to DMSO was used for the evaluation [10]. This shift was insignificantly less than that of Coumarin 153 (94 nm) [9].

The potential of using Pigment Red 179, which is a symmetric perylenecarboxyldiimide, as the solvatochromic indicator was examined. Its structured fluorescence spectra in solutions of low-molecular-mass solvents were situated in the red region [12], where the influence of the intrinsic fluorescence of the used stationary phases was minimal. Resolved vibrational structure of the fluorescence spectra was mostly lost in the studied solutions. However, significant shifts of the fluorescence maxima wavelengths were observed depending on the nature (polarity) of the solvent. According to the literature [12, 13], the fluorescence spectrum of symmetric N,N' -dialkyl-substituted perylenecarboxyldiimides consists of three bands with maxima at 536, 574, and 628 nm for solutions in DMF and at 535, 577, and 628 nm for solutions in $CHCl_3$.

Figure 1 shows fluorescence spectra of solutions of Pigment Red 179, Nile Red, and coronene in SLPs of various polarities at 20 and 120°C. It is noteworthy that the temperature chosen to measure the fluorescence spectra of the solvatochromic indicators (120°C) corresponded to the standard temperature of chromatographic columns during measurement of McReynolds constants in GC [2]. The flexibility of polymer chains of many SLPs at 120°C can differ markedly from that at 20°C. This was reflected in the accuracy of the obtained spectral characteristics.

Table 2 presents the McReynolds constants and thermodynamic polarity, maximum temperatures used to study the SLPs, and complete data on the relative shifts of the band centers of gravity at 20 and 120°C for one of the three most effective single-band indicators, i.e., Pigment Red 179. The center of gravity of the band instead of the wavelength maximum (λ_{\max}) was given for this compound because vibrational structure appeared in solutions of SLP of low polarity. The parameters of spectra for the linear indicator of the second type (coronene) were the intensity ratio of two chosen narrow bands I_1/I_3 at wavelengths ~428 and ~445 nm, which increased as the SLP polarity increased at 20 and 120°C.

Figure 1a,a',b, and b' shows that fluorescence spectra of solutions of Pigment Red 179 and Nile Red shifted to long wavelength as the SLP polarity increased. Slight vibrational structure characteristically appeared in solutions of low

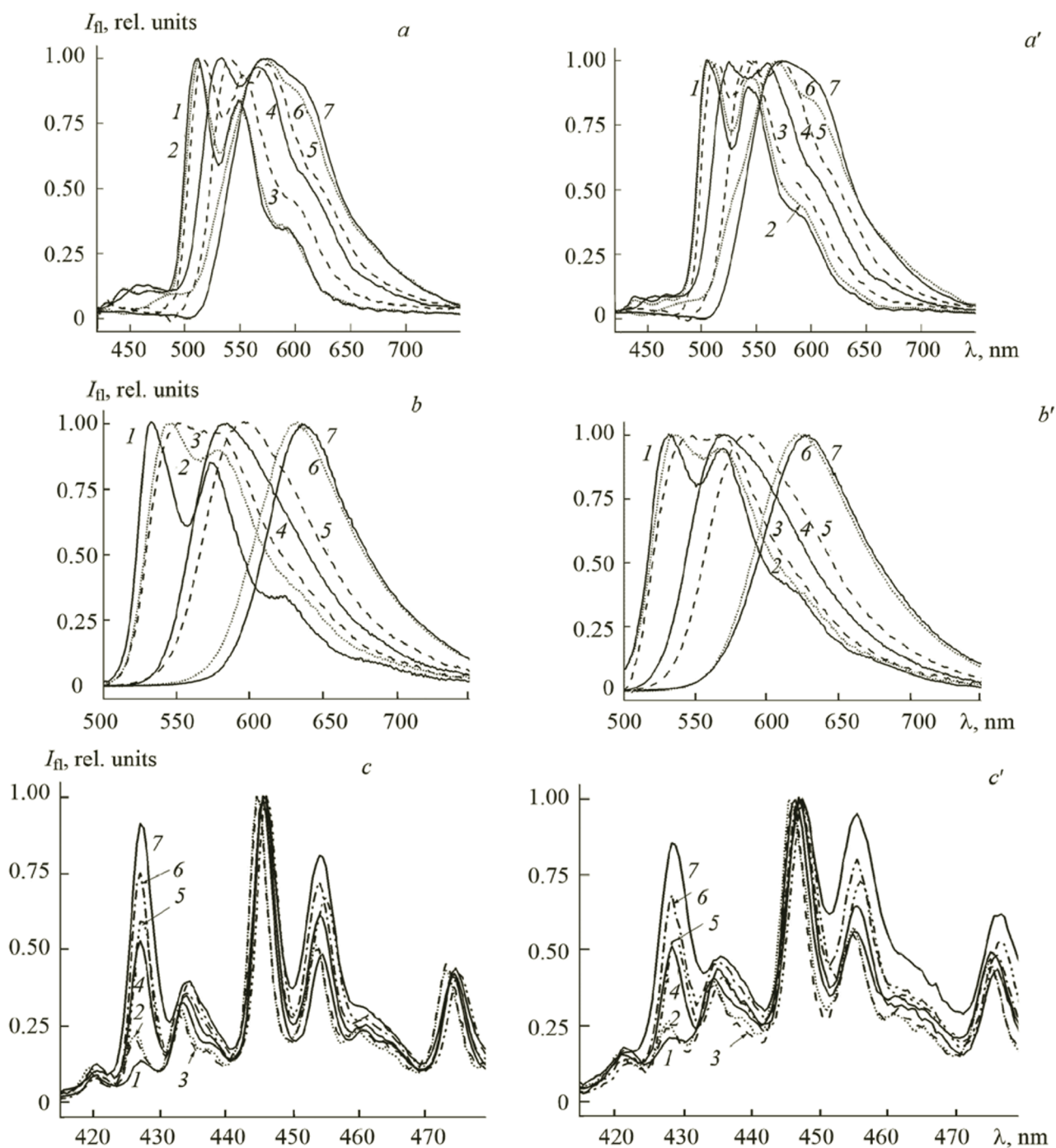


Fig. 1. Fluorescence spectra of solutions of Pigment Red 179 (a, a'), $\lambda_{\text{ex}} = 405$ nm; Nile Red (b, b'), $\lambda_{\text{ex}} = 470$ nm; coronene (c, c'), $\lambda_{\text{ex}} = 340$ nm at 20 (a–c) and 120°C (a'–c'): squalane (1); PMS-100 (2); Lukooil MF (25% phenylsiloxane) (3); di-(2-ethylhexyl)sebacate (4); dinonyl phthalate (5); poly(propylene glycol) 250 (6); 3,3'-oxydipropionitrile (7).

polarity and disappeared completely for high-polarity SLPs. The bands shifted by 1 to 9 nm to the blue spectral region as the temperature increased from 20 to 120°C, to different extents depending on the SLP (Table 2). Conversely, a slight shift (by 1.5–2.5 nm) to the red region occurred for solutions of coronene with an analogous temperature increase (Fig. 1c and 1c'). The ratio I_1/I_3 slightly decreased depending on the SLP (Table 2).

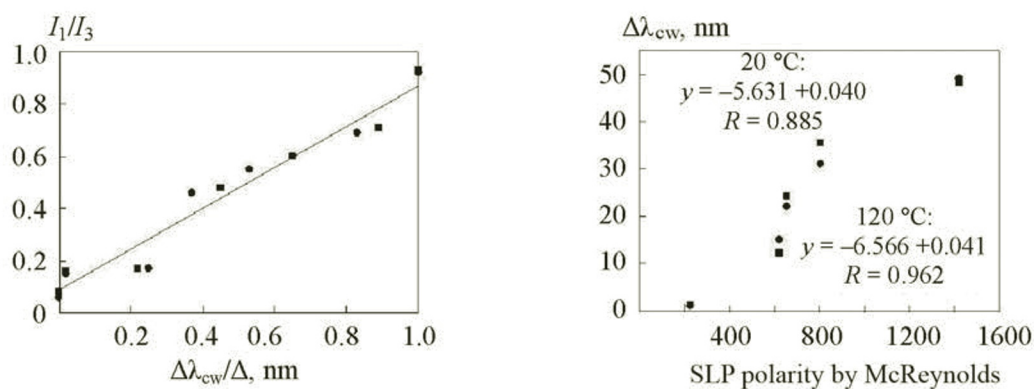


Fig. 2. Correlation dependence between relative shift $\Delta\lambda_{CW}/\Delta$ in spectra of Pigment Red 179 and intensity ratio I_1/I_3 in spectra of coronene obtained for solutions in SLP of various polarities at 20 (■) and 120°C (●).

Fig. 3. Correlation dependence between shift $\Delta\lambda_{CW}$ in fluorescence spectra of Pigment Red 179 obtained at 20 (■) and 120°C (●) with polarity expressed as McReynolds constants for various SLP.

The intensity fell (up to 30%) in various SLPs for the broad-band indicator as the temperature increased from 20 to 120°C. A barely noticeable broadening (washout) of bands occurred with an analogous temperature change for the indicator with a linear spectrum. The washout of the bands was greater in solutions of nonpolar SLP than of polar ones.

The results from evaluation of SLP polarity using Pigment Red 179 and coronene as fluorescent indicators correlated rather well with each other (Table 2). Figure 2 shows the correlation dependence between the spectral parameters of Pigment Red 179 and coronene in SLPs of various polarities, which was approximated well by a straight line with slopes 0.783 and 0.808 and correlation coefficients 0.965 and 0.952 obtained at 20 and 120°C. Analogous correlation dependences were observed for solutions of Coumarin C153 and Nile Red with the only difference that the slopes of the lines changed.

Figure 3 shows the correlation dependence between shift $\Delta\lambda_{CW}$ in fluorescence spectra of Pigment Red 179 obtained at 20 and 120°C and the polarity expressed as McReynolds constants in the range 200–1600 for various SLPs. As expected, the correlation obtained at higher temperature was stronger ($R = 0.962$). This agreed well with the McReynolds constants being calculated from chromatographic data obtained at 120°C [1]. The ability to use Pigment Red 179 as a solvatofluorescent indicator was demonstrated.

Conclusions. The indicator properties of solutions were well preserved as the temperature increased. However, they should be considered to increase the accuracy of the polarity determined from the indicator parameters. It was shown that Pigment Red 179 could be used as an effective indicator of the polarity of SLPs used in GC because its absorption band was distant from absorption bands of the SLPs and its fluorescence spectra were characterized by a strong dependence on the polarity of the SLPs.

Acknowledgments. The work was financially supported by grants of the Russian Foundation for Basic Research (No. 20-53-00025, Bel_a) and the Belarusian Republic Foundation for Basic Research (No. F20R-190).

REFERENCES

1. K. I. Sakodynskii, V. V. Brazhnikov, S. A. Volkov, V. Yu. Zel'vedenskii, E. S. Gankina, and V. D. Shatts, *Analytical Chromatography* [in Russian], Khimiya, Moscow (1993), pp. 108–110.
2. N. D. Loik and I. G. Zenkevich, *Vestn. S.-Pb. Univ. Fiz. Khim.*, No. 2, 97–103 (2008).
3. P. N. Nesterenko and E. P. Nesterenko, *Heliyon*, **7**, e07290 (2021).
4. M. H. Abraham, C. F. Poole, and S. K. Poole, *J. Chromatogr. A*, **842**, 79–114 (1999).
5. P. N. Nesterenko, A. I. Elefterov, D. A. Tarasenko, and O. A. Shpigun, *J. Chromatogr.*, **706**, 59–68 (1995).
6. C. F. Poole, *J. Chromatogr. A*, **1604**, 460482 (2019).
7. A. A. D'Archivio and A. Giannitto, *Int. J. Mol. Sci.*, **20**, 2120 (2019).
8. C. F. Poole and S. K. Poole, *J. Chromatogr. A*, **1184**, 254–280 (2008).

9. V. I. Beketov, M. V. Belkov, N. B. Zorov, S. N. Lanin, P. N. Nesterenko, and P. P. Pershukevich, *J. Appl. Spectrosc.*, **88**, 749–754 (2021).
10. L. G. Samsonova, N. I. Selivanov, and T. N. Kopylova, *Opt. Spektrosk.*, **116**, 79–84 (2014).
11. N. Pasaogullari, H. Icil, and M. Demuth, *Dyes Pigments*, **69**, 118–127 (2006).
12. F. Zhang, Y. Ma, Y. Chi, H. Yu, Y. Li, T. Jiang, X. Wei, and J. Shi, *Sci. Rep.*, **8**, 8208 (2018).
13. S. Icli and H. Icil, *Spectrosc. Lett.*, **29**, 1253–1257 (1996).