Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2017, **27**, 419–420

Mendeleev Communications

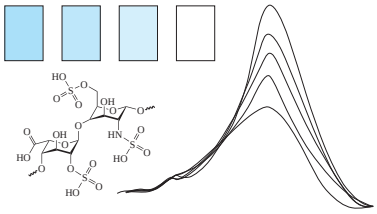
Colorimetric sensor for the determination of low-molecular-weight heparin

Mikhail A. Gavrilenko*[a] and Nataliya A. Gavrilenko[b]

[a] *Tomsk State University, 634050 Tomsk, Russian Federation. Fax: +7 3822 93 6444; e-mail: dce@mail.ru*
[b] *Tomsk Polytechnic University, 634050 Tomsk, Russian Federation. Fax: +7 3822 42 3951; e-mail: gavrilenko@mail.tsu.ru*

DOI: 10.1016/j.mencom.2017.07.035

A colorimetric sensor was proposed for the visual and solid phase spectrophotometric detection of low-molecular-weight heparin in concentration ranges of 40–120 and 5–40 mg dm^{-3}, respectively, with a detection limit of 2.0 mg dm^{-3} using a color destruction reaction of toluidine blue immobilized in an optically transparent polymethacrylate matrix.



Current optical sorption spectrometric methods include the preconcentration of substances from a liquid phase on a sensor, the chemical conversion of the substances with a change in their spectral characteristics in the visible range, and the detection of this change.[1,2] Transparent polymers, such as Nafion and PVC membranes and a modified polymethacrylate matrix (PMM), are promising optical sensors.[3–13] PMM was used as a solid-phase extractant for the extraction of phenols[14] and metal ions[15] from aqueous solutions. Moreover, PMM extracts many organic substances, such as amines, dyes and vitamins, from water.[7,16] Toluidine blue (TB) was applied as a modifying reagent to determine low-molecular-weight heparin (LMH) in biological samples.[17–19] The main aims for the LMH control are venous thrombosis, pulmonary embolism, unstable angina, acute myocardial or ischemic stroke.[20,21] LMH is used in surgical interventions for the prevention of blood coagulation during extracorporeal circulation.[22,23]

Chemical methods for the detection of LMH were developed; for example, the waterborne polyurethane WPU/LMH blend coatings effectively controlled the release of LMH, as determined by a TB method.[24] The properties of the metachromatic dye TB were used to colorimetrically determine the amount of LMH covalently coupled to Sepharose.[25] The nitrocellulose membranes can be stained with reversible staining for immunological detection.[26,27] LMH can also be transferred to membranes with a limit of detection of approximately 0.1–0.5 μg after reversible staining. Gold nanoparticles with a fluorophore were applied to the ultrasensitive detection of LMH.[28]

We used a transparent PMM to immobilize TB without losing its analytical response to the concentration of LMH. This makes it possible to measure optical characteristics by solid phase spectrophotometry with high precision. Here, we consider the immobilization of TB in a PMM and the subsequent determination of LMH in pharmaceuticals.

The PMM is a special material[29] containing functional groups, which can extract both the reagent and analyte. Transparent 10 × 10 cm polymethacrylate plates (thickness, 0.60 ± 0.04 mm) were prepared by the radical block polymerization of methacrylate, polyethylene glycol PEG 400 and alkaline earth metals at 60–70 °C for 3–4 h. Then, these plates were cut as 6.0 × 8.0 × 1.0 mm working platelets (~0.05 g) intended for analyses. We used an initial PMM as a reference standard when measuring optical qualities of the PMM with an immobilized reagent after contact with analyte solution. TB was immobilized into the PMM by sorption from a 1×10^{-5} M aqueous solution for 10 min to obtain a light blue sensing element. In the presence of LMH, the sensor changes to colorless because TB can bind glycosaminoglycans, which are negatively charged in aqueous solution.[30]

To determine LMH, we placed 10 ml aliquot portions in 50 ml flasks, added 0.9% NaCl solution (10 ml) and diluted with distilled water to the mark. Next, the solutions were transferred to conical flasks, and the PMMs modified with TB were dipped; the contents were stirred with a mechanical mixer for 10 min; then, the plates were taken out and dried between sheets of filter paper, and the absorption was measured at 631 nm.

The diversity of sorption sites in the PMM causes the complex of electrostatic and hydrophobic interactions with the sorbed substance. The relative contribution of the interactions depends on the chemical nature of the sorbed molecules. Sorption mechanism is based on the role of PMM with PEG as a solid polymer extractant.[31] Accordingly, the organic molecules were adsorbed by dissolving into a polymeric material, whose hydrophobicity depends on the amount of PEG injected into PMM.

PEG 400 was used for the immobilization of TB, the formation of an LMH/TB complex and low non-specific protein adsorption. PMM showed increased hydrophilicity after PEG modification.[32,33] TB sorbed quantitatively at low concentrations (10^{-5}–10^{-6} M). The analytical signal intensity changes with TB concentration in PMM (Figure 1).

The selectivity was measured at an LMH concentration of 10 mg dm^{-3}, pH 6.8 and the variable concentrations of concomitant substances. LMH determination was not prevented by 100-fold increase in amounts of proteins, amino acids and phosphates and 10-fold increase in amounts of sulfates and chlorides.

The analytical performance of the PMM for LMH detection under optimal conditions was evaluated. The linearity range was between 5 and 120 mg dm^{-3} with a regression coefficient of 0.995. The color intensity of PMM was proportional to the analyte concentration in a range of 40–120 mg dm^{-3}. The spectrophotometric detection limit was 2.0 mg dm^{-3}. This method was used to

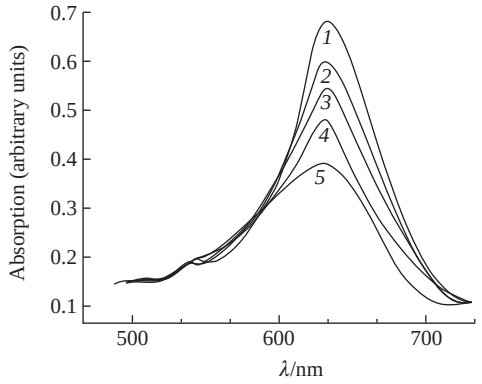


Figure 1 Absorption spectra of TB immobilized in the PMM after reactions with LMH at various concentrations: (*1*) 0, (*2*) 4, (*3*) 10, (*4*) 20, and (*5*) 30 mg dm^{-3}.

Table 1 Determination of LMH (n = 5, P = 0.95).

Sample	Labeled amount, mg dm^{-3}	Found / mg dm^{-3}
Clexane 6000 IU (Sanofi Winthrop Industrie, France)	60.0	61±8 (+VIS)
Hemapaxan 6000 IU (Italfarmaco, Italy)	60.0	61±8 (+VIS)
Sodium heparin solution (Deko, Russia)	31.7	32±4 (+VIS)
Clexane 2000 IU (Sanofi Winthrop Industrie, France)	20.0	20±3
Physiological solution spiked with Enoxaparin sodium	6.0	6.0±1.2
Substrate blood plasma spiked with Enoxaparin sodium	6.0	6.6±1.6

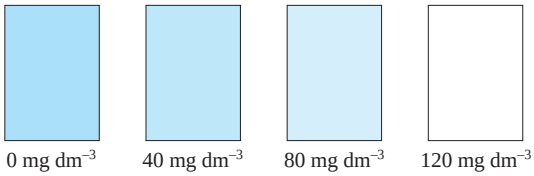


Figure 2 Visual responses to various concentrations of LMH.

determine LMH in biological fluid samples and pharmaceutical products (Table 1).

The semiquantitative visual detection of LMH is possible at concentrations above 40 mg dm^{-3} (Figure 2).

In conclusion, the solid phase extraction and spectrophotometric determination of LMH using PMM modified with TB has been proposed. The procedure developed is simple, rapid and reliable in an LMH concentration range of 5–120 mg dm^{-3}; it is characterized by a low detection limit, which can be reduced by increasing the solution volume or the sensing element. The developed method demonstrates satisfactory reproducibility and accuracy.

This study was supported by the Russian Foundation for Basic Research (project no. 16-43-700353).

References

1 C. McDonagh, C. S. Burke and B. D. MacCraith, *Chem. Rev.*, 2008, **108**, 400.
2 F. R. P. Rocha, I. M. Raimundo, Jr. and L. S. G. Teixeira, *Anal. Lett.*, 2011, **44**, 528.
3 V. N. Zaitsev and M. F. Zui, *J. Anal. Chem.*, 2014, **69**, 715 (*Zh. Anal. Khim.*, 2014, **69**, 787).
4 N. C. Dias, M. D. Porter and J. S. Fritz, *Anal. Chim. Acta*, 2006, **558**, 230.
5 A. Molina-Díaz, J. F. Garcia-Reyes and B. Gilbert-López, *Trends Anal. Chem.*, 2010, **29**, 654.
6 S. Matsuoka and K. Yoshimura, *Anal. Chim. Acta*, 2010, **664**, 1.
7 N. A. Gavrilenko, S. V. Muravyov, S. V. Silushkin and A. S. Spiridonova, *Measurement*, 2014, **51**, 464.
8 M. A. Gavrilenko and N. A. Gavrilenko, *Mendeleev Commun.*, 2015, **25**, 159.
9 K. N. Mikhelson and M. A. Peshkova, *Russ. Chem. Rev.*, 2015, **84**, 555.
10 A. A. Vavilova, R. V. Nosov and I. I. Stoikov, *Mendeleev Commun.*, 2016, **26**, 508.
11 O. G. Nikolaeva, E. N. Shepelenko, K. S. Tikhomirova, Yu. V. Revinskii, A. D. Dubonosov, V. A. Bren and V. I. Minkin, *Mendeleev Commun.*, 2016, **26**, 402.
12 S. Yu. Zaitsev, D. O. Solovyeva, I. S. Zaitsev and M. S. Tsarkova, *Mendeleev Commun.*, 2016, **26**, 40.
13 O. A. Inozemtseva, Y. E. Salkovskiy, A. N. Severyukhina, I. V. Vidyasheva, N. V. Petrova, H. A. Metwally, I. Y. Stetciura and D. A. Gorin, *Russ. Chem. Rev.*, 2015, **84**, 251.
14 M. A. Gavrilenko, N. A. Gavrilenko and G. M. Mokrousov, *J. Anal. Chem.*, 2006, **61**, 216 (*Zh. Anal. Khim.*, 2006, **61**, 234).
15 N. A. Gavrilenko and O. V. Mokhova, *J. Anal. Chem.*, 2008, **63**, 1038 (*Zh. Anal. Khim.*, 2008, **63**, 1141).
16 M. A. Gavrilenko and N. A. Gavrilenko, *Mendeleev Commun.*, 2006, **16**, 117.
17 L. D'Ilario, I. Francolini, A. Martinelli and A. Piozzi, *Dyes Pigments*, 2009, **80**, 343.
18 Y. Byun, H. A. Jacobs and S. W. Kim, *J. Biomed. Mater. Res.*, 1996, **30**, 423.
19 J. Hirsh, R. Raschke, T. E. Warkentin, J. E. Dalen, D. Deykin and L. Poller, *Chest*, 1995, **108**, 258S.
20 E. Chalayer, A. Bourmaud, F. Tinquaut, F. Chauvin and B. Tardy, *Thromb. Res.*, 2016, **145**, 119.
21 A. G. G. Turpie, *J. Thromb. Thrombolysis*, 1996, **3**, 145.
22 R. E. Peverill, *Lancet*, 2000, **356**, 593.
23 J.-Y. Lo, L. L. Minich, L.Y. Tani, J. Wilkes, Q. Ding and S. C. Menon, *Am. J. Cardiol.*, 2016, **16**, 31430.
24 Y.-Q. Song, Y.-L. Gao, Z.-C. Pan, Y. Zhang, J.-H. Li, K.-J. Wang, J.-S. Li, H. Tan and Q. Fu, *Chin. J. Polym. Sci.*, 2016, **34**, 679.
25 P. K. Smith, A. K. Mallia and G. T. Hermanson, *Anal. Biochem.*, 1980, **109**, 466.
26 N. Volpi and F. Maccari, *Methods Mol. Biol.*, 2015, **1312**, 119.
27 Y. Takegawa, K. Araki, N. Fujitani, J.-I. Furukawa, H. Sugiyama, H. Sakai and Y. Shinohara, *Anal. Chem.*, 2011, **83**, 9443.
28 J. Zhao, Y. Yi, N. Mi, B. Yin, M. Wei, Q. Chen, H. Li, Y. Zhang and S. Yao, *Talanta*, 2013, **116**, 951.
29 G. M. Mokrousov, N. A. Gavrilenko, N. G. Garber and M. A. Gavrilenko, *Russ. J. Phys. Chem.*, 2001, **75**, 1023 (*Zh. Fiz. Khim.*, 2001, **75**, 1132).
30 L. Tan, S. Yao and Q. Xie, *Talanta*, 2007, **71**, 827.
31 P. Kingshott, H. Thissen and H. J. Griesser, *Biomaterials*, 2002, **23**, 2043.
32 H. Chen, Y. Chen, H. Sheardown and M. A. Brook, *Biomaterials*, 2006, **26**, 7418.
33 Y. Feng, H. Tian, M. Tan, P. Zhang, Q. Chen and J. Liu, *Trans. Tianjin Univ.*, 2013, **19**, 58.

Received: 3rd October 2016; Com. 16/5061