COLORIMETRIC SENSORS FOR HEPARIN DETECTION BY TOLUIDINE BLUE AND MALACHITE GREEN DYES

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КОЛОРИМЕТРИЧЕСКИЕ СЕНСОРЫ НА ГЕПАРИН С КРАСИТЕЛЯМИ ТОЛУИДИНОВЫЙ СИНИЙ И МАЛАХИТОВЫЙ ЗЕЛЕНЫЙ

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Аннотация. *Мониторинг гепарина в крови используется для контроля дозировки препаратов и для оценки достаточности антикоагулянтной терапии. При курсовом приёме препаратов, содержащих гепарин, сопряженных с риском кровотечения и опасностью передозировки рекомендуется проводить лабораторный контроль терапии при слабой и умеренной почечной недостаточности , при пониженной массе тела или ожирении, при кровотечениях неясного генеза. Рассмотрена количественная сорбция различных форм толуидинового синего и малахитового зеленого, сорбированных на полиметакрилатной матрице в качестве чувствительного элемента при определении концентрации гепарина в растворах. Чувствительный полимерный элемент использовали для определения гепарина в растворах с концентрациями 50–210 мг/л. Показано, что коэффициенты чувствительности сорбционного фотометрического определения красителя пропорциональны значениям их молярных коэффициентов поглощения в водных растворах. Пределы обнаружения могут быть снижены на порядок при увеличении объема раствора на стадии сорбции и, соответственно, увеличения объема чувствительного элемента. При концентрациях свыше 10^{-5} моль/л возможно визуальное полуколичественное детектирование гепарина в растворе по снижению и исчезновению окраски красителя в полимерной матрице.*

Currently sorption-optical methods are developed successfully. These include concentration of substances from the liquid phase on the sensitive element which is accompanied with a change in spectral characteristics in the visible diapason. We have examined the quantitative sorption of various forms of toluidine blue (TB) and malachite green (MG) adsorbed on polymethylmethacrylate matrix (PMM) in the process of determination of the heparin concentration in a solution. Polymethylmethacrylate (PMMA), one of the commonly used polymers, is widely used to fabricate optical analytic systems. There have been advantages for the application of PMMA in analysis due to such properties as hydrophobicity and compatibility with additives. Bulk modification is an effective way to improve the analytical properties while retaining the transparent properties of the polymer.

Various modifications of the PMMA matrix have been obtained using different techniques like physical adsorption, biomolecule adsorption, chemical modification and so on [1-3]. The extraction mechanism is based on the PMM role as a solid polymeric extractant. In accordance with this mechanism, organic molecules sorbed into hydrophobic PMM. PMMA have unique advantages over other polymers, for their physical and chemical properties can be easily changed by molecular design.

The introduction of polyethylene glycol (PEG) into the PMMA has attracted considerable attention as a means of minimizing waste sorption, because of their low interfacial energy, non-adhesive property and high diffusion. The technique was described [4] where PEG was immobilized into PMM on polymerization stage.

Combined matrix PMMA and PEG, having a polymethacrylate backbone and hydrophilic PEG chains, has been synthesized and applied for the design of transparent color sensors. The properties of the new modified PMM such as attachment, functionality, and sorption properties are characterized by various techniques.

The results of investigation of dyes sorption into transparent matrix are used in the field of technology for sensors, catalysis, analytical chemistry and drug discovery. A large number of experimental studies of dyes sorption to various sorbents have been performed in order to understand the underlying mechanism of the sorption process. This simple method allows the study of a wide range of PMMA-PEG polymers with well-defined the optical sensor properties.

PMMA was synthesized via free radical block polymerization, using benzoyl peroxide as initiator. The structure of the dye is shown in Fig. 1. Immobilization of analytical reagents into a polymeric matrix has been carried out in a static mode. The solution was placed in a constant temperature bath and stirred with the help of a stirrer at 120 rpm.

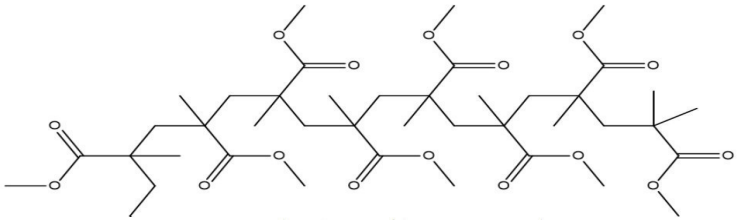

Fig. 1. Structure of the PEG-PMMA matrix

Sorption of dyes into PMM depends on the nature, structure and polarity. Increasing the degree of extraction of hydrophobic dyes with increasing evidence of the significant role of hydrophobic interactions between the sorbent and sorbate. Equilibrium sorption time and degree of dye extraction depend on the forms of its existence in the solution.

The molecular form of the dye was sorbed by PMM completely and 40% of the anionic form was sorbed during 15–20 minutes. The sorption of neutral and charged forms of TB on the concentration in the aqueous solutions with pH 4 was researched. The equilibrium concentration of the dye, the recovery rate and the value of limiting sorption were calculated in this work. Both neutral and charged forms of TB in low concentrations ($<10^{-6}$ M) were sorbed completely. The ionic form of the dye was sorbed on 50 % and neutral form on 90 % at concentrations > 10^{-5} M. The optimal pH was below 4.0 because the proteins anionic groups (carboxyl group complicate the interpretation of results greatly) lose their charge. TB binds with nucleic acids at pH 3.0.

Sensitive polymer element with TB immobilized was used to determine concentrations of heparin in the solutions with 50-210 mg/L. Response factors for sorption-photometric determination of dye was proportional to the value of the molar absorption coefficients in aqueous solutions. Detection limits can be reduced by increasing the volume of the solution or increase the sensing element.

Semiquantitative visual detection is possible in a solution of heparin at concentrations above 10^{-5} mol/L. This shows as decrease or disappearance of the TB color into PMM (Fig. 2).

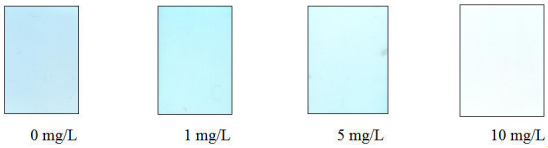

Fig. 2. The visual response to the presence of various concentrations of heparin

MG also triphenylmethane dye is highly soluble in water and used as a therapeutic agent. MG dye is used extensively in medical diagnostic. Previously MG has been analyzed using spectrophotometry, HPLC-MS and capillary electrophoresis. Selective solid phase extraction is not used for MG from biological samples of fluids before. A method for the trace analysis of MG with colorimetric measurements based on solid-phase extraction (SPE) by transparent PMM is described for TB. The investigation was aimed to study the adsorption capacity of PMM for MG removal from aqueous solutions for the solid phase extraction of MG, different parameters such as adsorbent dose, effect of pH, initial dye concentration and contact time.

The quantity of sorbed PMM reagent is proportional to intensity of its painting and it depends on conditions of modification: pH, concentration of the reagent in solution and durations of processing. The kinetic profiles of sorption at different temperatures are similar. This suggests that the sorption mechanism is mainly due to the formation of an inclusion complex through host–guest interactions.

Solid-phase extraction on PMM allowed a 290-fold enrichment of the dyes if 10 mL sample volume is used with extraction efficiencies 92 %. The method enables the determination of MG to 0.1–5 µg/L combined with a fast and easy sample-preparation (pH-adjusting prior to SPE). Extraction and determination of MG in biological liquid samples confirmed the applicability and reproducibility of the method. Batch adsorption results indicated that Langmuir isotherm described the adsorption isotherms better.

This work was supported by the Russian Science Foundation project 14-19-00926.

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