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Interactions between fluorescein isothiocyanate and star-shaped polymer carriers studied by isothermal titration calorimetry (ITC)

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ABSTRACT

Isothermal titration calorimetry (ITC) technique was used to determine the thermodynamic parameters of reaction between fluorescein isothiocyanate (FITC) and novel star-shaped copolymers. Measurements were taken at 310.15 K using 0.64 g dm^{-3} NaCl, 0.5 g dm^{-3} $Na_2B_4O_7{\cdot}10H_2O$ in deionized water/methanol (60/40 v/v). Tested polymers belong to the innovative group of star-shaped copolymers with sugar core. Fluorescent FITC was chosen as one of the most reactive dye, which allowed monitoring the polymer behavior in physiological ionic strength conditions, as well as it was treated as the model biologically active substance. Analysis of the obtained calorimetric data allowed to determine the standard molar enthalpy of binding (ΔH°), the stoichiometry (n), the equilibrium constant (K), the standard Gibbs free energy change (ΔG°) and the standard entropy change (ΔS°), whose values allowed to obtain a wealth of information for the polymer-FITC binding, and identified the best carrier for the dye.

1. Introduction

Fluorescein isothiocyanate (FITC) is a yellow-orange dye with wide range of biological applications. Isothiocyanate group is strong electrophile of particular importance because of reactive properties towards amine and sulfhydryl groups, which are present in intracellular proteins. It can be used as a reagent for labeling of proteins and peptides to reagent tracing [1–4] or rapid identification of pathogens. FITC can be also immobilized into polymeric nanoparticles for its cell-labeling application [5]. Moreover, the antibacterial properties of FITC were proved by cysteine residue attack, which causes inhibition of ATP binding sites of P-ATPase [6]. Among various available dyes, FITC is the best choice because of its reactivity and strong emission intensity with pH-dependence [7].

Synthetic star-shaped copolymers belong to the group of carriers for biologically active substance [8–10]. Their usefulness in the controlled drug release [11–13] as well as bioimaging capabilities [14] have been proven at the same time. These properties result in wide application in both the treatment and diagnosis of a disease. In their three-dimensional globular structure can be distinguished a core and polymeric arms, which significantly affects their properties. Compared with the widely studied block polymers, star-shaped polymers are characterized by improved mechanical properties and lower values of the transition temperatures [15]. It has been shown that a single polymer macromolecule can be connected to a much larger amount of the drug with a high efficiency of entrapment of the active substance [16,17]. In addition, modified star-shaped copolymers facilitate penetration of biologically active substance across the cell membrane [18].

Isothermal titration calorimetry (ITC) is one of the best techniques to obtain data on reactivity and reversibility of the reaction. ITC is an excellent tool to get complete thermodynamic profile of studied phenomenon in a single experiment. The instrument characteristics include rapid response and fast thermal equilibration, which can detect even some very week interactions. The last feature distinguishes ITC among other techniques (nuclear magnetic resonance [19], fluoroscence, chromathography, electrophoresis, phase solubilization studies, potentiometry and circular dichroism spectroscopy), which are not as sensitive as the microcalorimetry. ITC technique has found many applications, among which there can be distinguished the following research: protein interaction, macromolecular assembly, study of kinetics, interaction of surface to nanoparticles, drug design and characterization, test of the chirality of crystals of chiral silicate zeolites, and other [20–23]. It was also used in thermodynamic studies on reaction of star-shaped polymers with cyclodextrins [24]. However, the study of interactions polymer-biologically active substance by the ITC is rare.

The aim of this study was to present the thermodynamic aspect of the reaction between copolymer and FITC via thiocarbamide

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Table 1
Chemical samples.

Chemical name	CAS Registry No.	Mass fraction purity	Source	Purification method
Fluorescein isothiocyanate isomer I	3326–32-7	0.95	Alfa Aesar	None
Sodium chloride	7647–14-5	1	POCH	None
Sodium tetraborate anhydrous	1303–96-4	>0.98	Fluka	None
Methyl alcohol	67–56-1	>0.99	Chempur	None
Methyl methacrylate	80–62-6	0.98	Sigma-Aldrich	Dried over molecular sieves
Glycidyl methacrylate	106–91-2	0.97	Sigma-Aldrich	Dried over molecular sieves
1,2-Diaminoethane	107–15-3	0.99	Alfa Aesar	None

bond using a microscale method of isothermal titration calorimetry (ITC). This kind of research may be one of the future ways to determine the reactivity of active sites in carriers with biologically active compounds. This is the first report of the investigation of the interaction of the biologically active dye and star-shaped polymers by ITC method, yet similar reports have considered drug–polymer interactions, for example, ITC was used to characterize the thermodynamic profile of doxorubicin-DC Bead™ particles interactions [25].

2. Experimental

Novel, pH-sensitive poly{2-hydroxy-3-[(2-aminoethyl)amine] propyl methacrylate-co-methyl methacrylate} (P(MMA-*co*-HAmPMA)) star-shaped copolymers with sugar core were studied in the reaction with FITC in the presence of sodium chloride in a concentration corresponding to the physiological conditions. The scheme of the reaction and the structure of studied star-shaped copolymers are shown in Fig. 1. After reaction with FITC the modified copolymer was endowed with fluorescent properties. Previously, these copolymers obtained in laboratory scale were investigated in order to characterize their biochemical properties using normal and colon cancer cells [26]. The modification of the polymer with FITC, under conditions similar to physiological, allowed to examine the degree of internalization of polymeric conjugates by the cells.

2.1. Materials

Fluorescein isothiocyanate (isomer 1, 95%) was purchased from Alfa Aesar. Fluorescein isothiocyanate solution was freshly prepared by dissolving the fluorescein isothiocyanate powder in the buffer (0.64 g dm^{-3} NaCl, 0.5 g dm^{-3} $Na_2B_4O_7{\cdot}10H_2O$ in 60/40 deionized water/methanol (v/v)). Content of 40% methanol in the buffer was necessary to dissolve the desired amount of FITC. All solutions were stirred at room temperature to obtain a homogenized dispersion.

2.2. Preparation of poly{2-hydroxy-3-[(2-aminoethyl)amine] propyl methacrylate-co-methyl methacrylate} (P(MMA-co-HAmPMA))

Poly{2-hydroxy-3-[(2-aminoethyl)amine]propyl methacrylate-*co*-methyl methacrylate} star-shaped copolymers were synthesized in our laboratory according to the procedures reported previously [27,28]. Briefly, three- and four-arm stars were obtained via atom transfer radical copolymerization (ATRP) of methyl methacrylate (MMA) with glycidyl methacrylate (GMA) initiated by the tri- (GI1), and tetrafunctional (GI2) α-D-glucopyranoside initiators, respectively. Next, the ring opening of pendant oxirane groups in GMA was performed by means of aminolysis with ethylenediamine. Reagents were obtained from commercial suppliers and used without further purification, detailed information is provided in Table 1.

The content of 2-hydroxy-3-[(2-aminoethyl)amine]propyl methacrylate units (F_{HAmPMA}) in three- (CI-CIII) and four-arm (CIV) stars depended on the GMA content in the initial copolymer. Copolymers were dispersed in the same buffer as FITC and stirred at room temperature to obtain a homogenized dispersion.

2.3. Nuclear magnetic resonance (NMR)

^{1}H NMR spectra of P(MMA-*co*-HAmPMA) solutions in $CDCl_3$ were collected on Varian Inova 600 MHz spectrometer at 298.15 K using TMS as an internal standard.

Molecular weights of CI-CIV ($M_{n,NMR}$) and content of amine functionalized units (F_{HAmPMA}), which are shown in Table 2, were calculated on the basis of NMR spectra with respect of full conversion of GMA units into HAmPMA after aminolysis with EDA as it was previously described in details [29].

2.4. Isothermal titration calorimetry

2.4.1. Theory

By ITC technique, heat evolved during process is measured. Due to the fact that the measurement cell is maintained at a constant temperature and pressure, energy (heat) represents the change in standard molar enthalpy (ΔH°) for all occurring phenomena. For the enthalpy of reaction, two additional experiments, in the same conditions as the primary measurements, were performed: FITC and polymer dilutions. Independent mathematical model [30], available in the calorimeter software, was used to calculate other thermodynamic parameters. The heat of occurring phenomena (q) for the mentioned model is given by Eq. (1).

$$q = \frac{[P_T] \cdot V \cdot H \cdot K \cdot [FITC]}{1 + K \cdot [FITC]} \quad (1)$$

where: $[P_T]$- the final concentration of the unbound form of P(MMA-*co*-HAmPMA) after the measurement, *[FITC]*- concentration of the unbound FITC, *K*- equilibrium constant, *V*- volume, *H*- enthalpy.

Using Eq. (2) as an integrated heat on time graph, the equilibrium constant (K), standard molar enthalpy change (ΔH°) and stoichiometry (n) were obtained.

$$\frac{dq}{d[FITC_T]} = \Delta H \cdot V \left(\frac{1}{2} + \frac{1 - X_R - r}{(X_R + r + 1)^2 - 4 \cdot X_R} \right) \quad (2)$$

where: $[FITC_T]$- total FITC concentration, $X_R = [FITC_T]/[P_T]$, $r = 1/K[P_T]$.

Table 2
The molecular weights $M_{n,NMR}$ and the content of amine pendant group F_{HAmPMA} for the tested copolymers.

Copolymer	$M_{n,NMR}$ (g mol^{-1})	F_{HAmPMA}
CI	14 700	0.56
CII	25 600	0.27
CIII	42 600	0.75
CIV	47 100	0.52