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PREPARATION OF THE REACTIVE COPOLYMER OF N-VINYLCAPROLACTAM AND ITS CHEMICAL ATTACHMENT TO FINE SILICA

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NOTE

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ABSTRACT

Free radical polymerization of p-nitrophenyl acrylate (NPA) carried out in the solution of poly-N-vinylcaprolactam ($M_n = 9 \cdot 10^5$) in 1,4-dioxane results in the formation of graft copolymer. As found by covalent chromatography of PVCL-NPA, *ca.* 70% of PVCL was grafted with NPA. The grafted fraction of the polymer contains *ca*. 2 w/w % of NPA units used for its chemical attachment to aminopropyl-silica. Modification of inorganic carrier results in its temperature-depended aggregation in aqueous medium. The grafting reaction can be used in a chemical design of novel bioseparation materials.

INTRODUCTION

Temperature-responsive polymers have a serious potential in the chemical design of bioseparation materials like sorbents, membranes, reagents for conjugation, etc. [1-3]. In particular, grafting of temperature-responsive polymers to inorganic supports allows preparing chromatographic sorbents with their permeability and adsorptivity controlled by a temperature shift. Several methods for

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synthesis of such composite packings were described earlier and their chromatographic properties studied [2-5]. The existing grafting procedures most often exploit rather short polymeric chains of molecular weight below 8000 carrying activated end-groups [2, 3]. Theoretical analysis of coil-globule transitions in grafted chains predicts, however, stronger variations of the height and density of the polymer layers formed by longer macromolecules [6]. This is the reason for elaboration of long-chain temperature-responsive polymeric reagents. Graft copolymerization of *p*-nitrophenyl acrylate (NPA) to poly-N-vinylcaprolactam (PVCL) appears to be of value for the above purpose. PVCL, a polymer with a lower critical solution temperature (LCST) of 38°C, was used earlier to prepare temperature-modulated biosorbents based on agarose gels [1]. Implementation of the coating technique to a rigid inorganic carrier offers many ways to its further use in bioseparation.

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Materials

Fine silica gel Silasorb 600 with the particle size of 5 ± 2 μm (irregular shape) and pore diameter of <100 Å was a product of Lachema (Brno, Czech Republik). Affi-Gel 101 was purchased from Bio-Rad Laboratories (Richmond, VA, USA). 3-Aminopropyltriethoxysilane (APTES), monoethanolamine and poly-N-vinylpyrrolidone K30 (PVP, M_r = 40000) were purchased from Fluka (Buchs, Switzerland). *p*-Nitrophenylacrylate (NPA, m.p. 54°C) and 2,2-azobis-isobutyronitrile (AIBN) were supplied by VNII Polymerov (Dzerzhinsk, Russia). 14% w/v aqueous solution of poly-N-vinylcaprolactam (PVCL) of molecular weight $9 \cdot 10^5$ was a gift from Professor V. P. Zubov (Fine Chemical Technology Institute, Moscow, Russia). 1,4-Dioxane was dried over potassium hydroxide and distilled at 100-101°C; toluene was distilled at 110°C. All other solvents and reagents supplied by Reakhim (Moscow, Russia) were of analytical grade and used as received.

Methods

1. Graft Copolymerization

25 ml of dioxane and 2 ml of the PVCL aqueous solution (14% w/v) were mixed by shaking. An azeotropic mixture of dioxane and water, containing 82% dioxane (b.p. 88°C [7]), was removed under a reduced pressure on a rotavapor at 50°C, the solution volume decreased to *ca*. 6 ml. 20 ml of dioxane was then added and evaporation repeated. 386 mg (2 mmol) NPA and 20 mg AIBN

were dissolved in the PVCL solution in dioxane (20 ml) and free radical polymerization was started by heating the mixture to 70°C and nitrogen bubbling. After 10 hours, the reaction mixture was cooled, centrifuged at 10000 rpm for 15 minutes, and filtered off to remove the polymer fractions insoluble in dioxane. The insoluble product was collected, washed with diethyl ether and dried in an air flow (20 mg). Analysis of *p*-nitrophenol content performed as described in Section 5 proved the insoluble product to consist almost thoroughly of poly(NPA). The supernatant was poured dropwise to 200 ml diethyl ether at vigorous magnetic stirring to precipitate the soluble product of the reaction. The product was further purified by repeated precipitation from dioxane into diethyl ether and dried in an air flow (213 mg).

2. Covalent Chromatography of PVCL-NPA Copolymer

The prepared PVCL-NPA copolymer was analyzed by liquid chromatography on 0.9×15 cm glass column packed by Affi-Gel 101, the wide-pore cross-linked aminoalkyl agarose beads containing 7.6 μmol aminogroup per ml gel, in 1,4-dioxane. 2.8 mg PVCL-NPA (0.2 μmol *p*-nitrophenyl acrylate units) in 0.5 ml 1,4-dioxane was applied to the column, incubated for 30 minutes and eluted with dioxane at a flow rate of 0.15 ml/min; fractions of the eluate (1 ml) were collected. The separation profile is shown in Figure 3c. For reference, the same amounts of non-grafted PVCL and PVP were chromatographed on the same column with quantitative recoveries registered by their UV-absorbances at 240 nm (Figure 3a,b).

3. Chemical Modification of Silica

Silasorb 600 was chemically modified by 5% v/v solution of APTES in dry toluene at 100°C for 12 hours, washed by toluene, acetone, and dried in an air flow. The aminogroup content estimated with picric acid as described in reference [8] was 300 μmol/g carrier. For chemical attachment of the reactive copolymer, 400 mg aminopropyl-silica was added to 0.7% w/v solution (2 ml) of the copolymer in dimethylformamide, agitated by shaking and left at room temperature for overnight. The residual aminopropyls of the carrier were acylated by acetic anhydride (50 μl). The carrier was extensively washed by dimeth-ylformamide, then 50 μl monoethanolamine was added to amidate *p*-nitrophenyl ester groups of the chemisorbed copolymer. The carrier was washed by distilled water to neutral pH, the washings were collected and photometrically assayed for *p*-nitrophenylate to estimate the content of NPA-grafts in the carrier.

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4. Preparation of the Reference Sample of Modified Silica

The reference sample of the silica carrier was prepared according to the above coating technique, the only exception was that 0.7% w/v solution of PVCL in the mixture of dioxane and dimethylformamide (1:1) was taken to treat aminopropyl-silica.

5. Ammonolysis of Polymers: Determination of P-Nitrophenyl Esters

4.5 mg of the polymer was dissolved in 2 ml dimethylformamide, 0.5 ml of concentrated aqueous ammonia was added and the reaction ran for 24 hours at room temperature. After dilution with distilled water (1:50–1:2000) the concentration of *p*-nitrophenolate in the reaction mixture was determined photometrically (λ_{max} = 405 nm, ε = 15300 $M^{-1}cm^{-1}$) and the content of NPA-units in the polymer was calculated.

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6. Sedimentation Technique

Copolymer-coated silica or the reference sample (400 mg, the wet precipitate volume V_f = 0.8 ml) was suspended by shaking in 5 ml of distilled water contained in a vertically situated measured test-tube of I.D. *ca.* 8 mm. The suspension was thermostated at 20° or 50°C and the volume of precipitate (V_t) was visually estimated at times by the position of boundary between the precipitate itself and the aqueous phase containing precipitating particles. Non-modified particles formed the precipitates growing in volume with time and thus exhibited usual sedimentation mode. Conversely, the polymer-modified particles quickly stuck together at 20°C and precipitated in less than 1 minute, so that the aqueous phase above them became almost clear. The loose sediment thereby formed was getting more compact with time. In this case, therefore, the boundary slowly went down until the final volume V_f = 0.8 ml was attained.

7. Photometry and IR Measurements

Photometric assay of *p*-nitrophenolate was performed with a Specord UV-VIS instrument (Carl Zeiss Jena, Germany). IR spectrum of PVCL-NPA copolymer was recorded on a Perkin-Elmer 1710 Spectrometer (USA).

RESULTS AND DISCUSSION

Graft copolymerization of alkyl methacrylates with poly-N-vinylpyrrolidone (PVP) was previously described [9]; the authors found that free radical

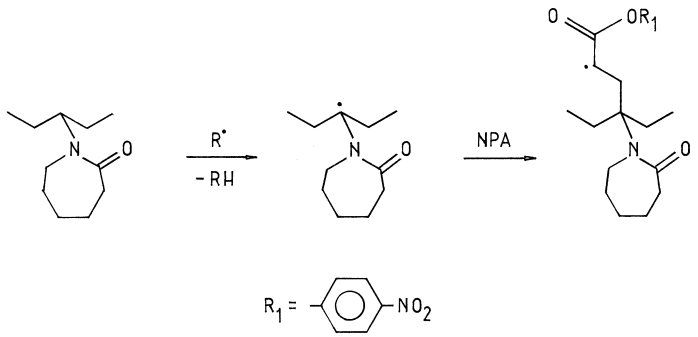

Figure 1. Hypothetical scheme of NPA grafting to PVCL.

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polymerization of hydroxyethyl methacrylate in aqueous solution of PVP resulted in the formation of graft copolymers. As also shown, the grafting was strongly promoted by the charge-transfer complex formation between PVP and methacrylic monomers [9]. The present study takes a similar approach to grafting of p-nitrophenyl acrylate (NPA) on poly-N-vinylcaprolactam (PVCL), a chemical homologue to PVP (see Figure 1).

PVCL-NPA copolymer was prepared as described in section 1 "Methods". The content of NPA-groups determined by ammonolysis of the copolymer was 1.4 w/w % or *ca*. 1 mol% of the monomer units. IR-spectrum of the copolymer illustrated in Figure 2 exhibits the absorbance bands of p-nitrophenyl ester carbonyl (1760 cm^{-1}) and nitrogroup (1340 and 1530 cm^{-1}), which are the most strong in the spectrum of poly(NPA) [5]. Due to the insolubility of poly(NPA) in the reaction medium, one can hardly presume its admixing to the reprecipitated PVCL-NPA copolymer (see Methods).

Figure 3 shows chromatograms of non-grafted PVCL, PVP, and PVCL-NPA on Afii-gel 101. Roughly speaking, the elution of both the non-reactive polymers obeyed the order typical of gel-permeation chromatography. In contrast, the high molecular weight portions of PVCL-NPA ($M_r > 40000$) were covalently bound by the carrier due to acylation of aminoalkyls by the esters of NPA-grafts (empty fractions 3-5, Figure 3c). Noteworthy, chemisorption of poly(NPA), a fast and easily controlled reaction, proceeds due to acylation of aminoalkyls by the pendant ester groups of the polymer as studied in detail elsewhere [10].

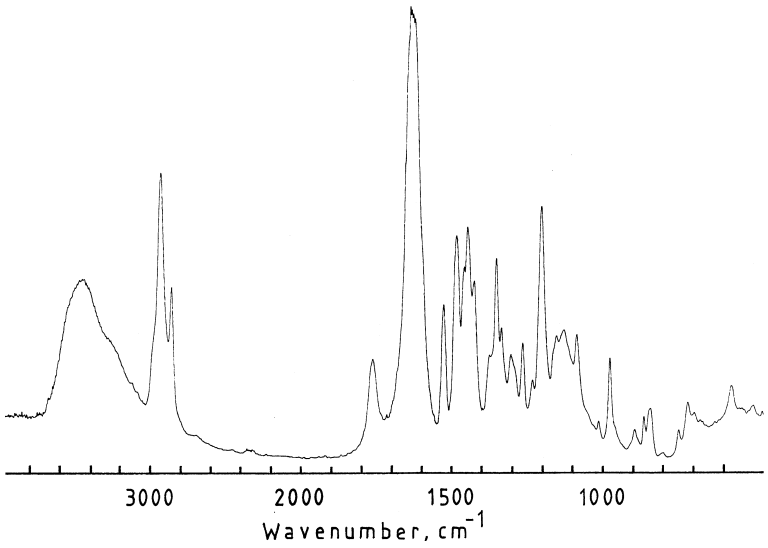

Figure 2. IR-spectrum of the PVCL-NPA copolymer.

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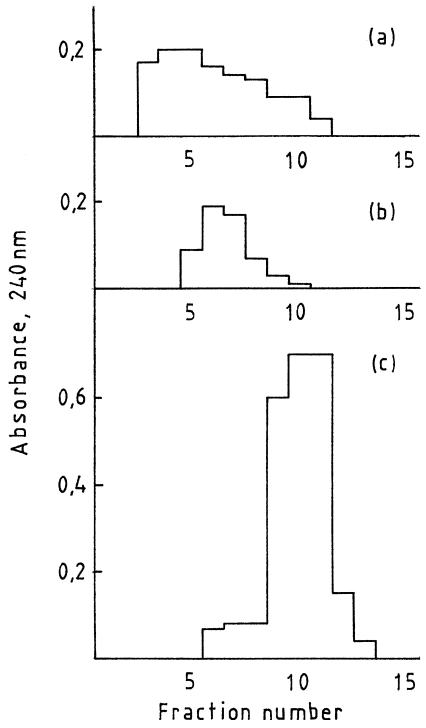

Figure 3. Chromatography of poly-N-vinylcaprolactam (M_n = 900000) (a), poly-N-vinylpyrrolidone (M_r = 40000) (b) and PVCl-NPA copolymer (c) on Affi-Gel 101 in 1,4-dioxane. Absorbance of the fractions 9-13 (c) belongs to *p*-nitrophenol.

The main objective of the study, preparation of high molecular weight reactive copolymer of PVCL, was, therefore, achieved. The lower molecular weight portions of PVCL –NPA were partially eluted: they were not grafted with NPA as could be estimated by their subsequent treatment by aqueous ammonia (fractions 6-8, Figure 3c). Judging by UV-absorbances of fractions 6-8 at 240 nm, ca. 70% of PVCL was grafted with NPA and remained bound to Affi-Gel 101. Thus, ca. 2w/w % -content of NPA-grafts per grafted PVCL molecules may be calculated. As molecular weights of poly(NPA) prepared by radical polymerization of the monomer in 1,4-dioxane are usually in the range of M_w = 6000-8000 and $M_w/M_n \approx 2$ (unpublished data), one can presume that a long-chain PVCL molecule of 900000 molecular weight contains one to several poly(NPA) grafts.

The clear evidence for true grafting of NPA to PVCL is given by chemisorption of the PVCL-NPA copolymer to the fine aminopropyl-silica as shown in Figure 4. Chemical attachment of the PVCL-NPA copolymer to the silica, carried out as described in section 3, yields the carrier with *p*-nitrophenyl ester group content of 0.7 μmol/g. The coating procedure leads to a dramatic change in colloidal and sedimentation properties of the carrier. As illustrated in Figure 5, at 50°C (i.e. under conditions of the PVCL chains collapse above LCST) the copolymer-coated silica settles on the bottom in a manner typical of the parent carrier. In contrast, at 20°C the grafted chains are better solvated, whereas the

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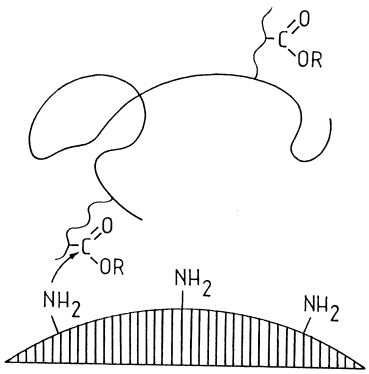

Figure 4. Chemisorption of PVCL-NPA copolymer onto aminopropyl-silica. The main chain of PVCL adsorbs via the shorter NPA-grafts prepared as shown in Figure 1 (R: *p*-nitrophenyl).

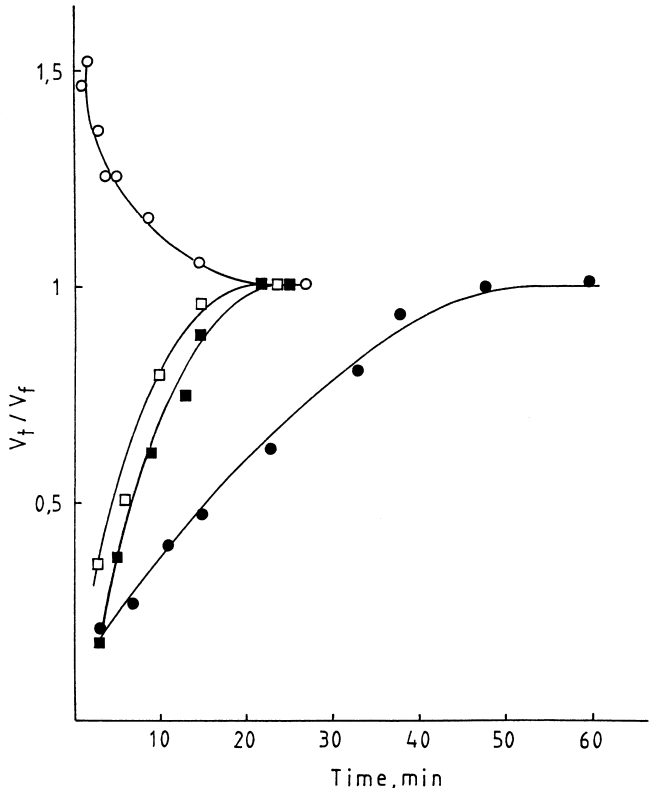

Figure 5. Sedimentation volumes of the modified silicas as a function of time; (○): PVCL-NPA-coated silica at 20°C, (□): 50°C; (●): reference sample at 20°C, (■): 50°C. For detail see sections 3 and 5 of the Methods.

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particles tend to aggregate and quickly form a loose sediment, which is getting more compact as time progresses. Such a behavior is quite reversible: the sedimentation mode changes into the opposite one each time when the ambient temperature ranges from 20° to 50°C or back.

To exclude the possibility of a physical adsorption of the long-chain PVCL to aminopropyl-silica, the reference sample of the carrier was prepared by its subsequent treatment with PVCL and acetic anhydride as described in section 3 of the Methods. No aggregation of the thus treated particles was registered, their sedimentation modes at 20° and 50°C differing in velocity dealt with changing viscosity of water (see Figure 4). This indicates to the absence of non-covalently adsorbed PVCL on the carrier surface. The distinctive sedimentation prop-

erties of the copolymer-coated silica may come, therefore, solely from the covalent attachment of the reactive PVCL-NPA copolymer. This uniquely proves, in turn, the grafting of NPA chains to PVCL in the course of polymerization, which allows use of the copolymer for further synthesis of temperature-modulated composite sorbents.

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REFERENCES

[1] I. Yu. Galaev, M. N. Gupta, and B. Mattiasson, CHEMTEC 12, 19-25 (1996).

[2] M. Gewehr, K. Nakamura, and N. Ise, *Makromol. Chem., 193*, 249-256 (1992).

[3] H. Kanagava, Y. Kashiwase, K. Yamamoto, Y. Matsushima, A. Kikuchi, Y. Sakurai, and T. Okano, *Anal. Chem., 69*, 823-830 (1997).

[4] K. Hosoya, E. Sawada, K. Kimata, T. Araki, N. Tanaka, and J. M. J. Frechet, *Macromolecules, 27*, 3973-3976 (1994).

[5] A. E. Ivanov, L. S. Zhigis, E. V. Kurganova, and V. P. Zubov, *J. Chromatogr. A, 776*, 75-80 (1997).

[6] E. B. Zhulina, O. V. Borisov, V. A. Pryamitsyn, and T. M. Birshtein, *Macromolecules, 24*, 140-149 (1991).

[7] A. J. Gordon and R. A. Ford, *The Chemist's Companion*, John Wiley and Sons, New York, London, Sydney, Toronto, 1972, Table 12.

[8] A. J. Alpert and F. E. Regnier, *J. Chromatogr., 185*, 375-392 (1979).

[9] O. V. Suberlyak, V. I. Skorokhoda, and I. G. Tkhir, *Vysokomol. Soedin B, 31*, 336-340 (1989); Chem. Abs.: 111:174774f.

[10] A. E. Ivanov, *Macromolecular Reports, A31, Suppl. 5*, 521-524 (1994).

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