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Highly Dispersed Polymer Suspensions with a Narrow Particle Size Distribution for Biotechnology

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Abstract: Polymer suspensions with narrow particle size distribution and a diameter of 0.2-0.4 um was synthesized by two different routes, suitable for use in Biotechnology: emulsion polymerization in conditions of surfactant synthesis at the interface and without surfactant in the presence of the polymer which is incompatible with formed polystyrene and participating in the formation of structural and mechanical barrier in the interfacial adsorption layer of particles.

Key words: Polymer suspensions, narrow particle size distribution, diagnostic test systems, synthesized, incompatible

INTRODUCTION

The relevance of the synthesis of polymeric suspension with a narrow particle size distribution is related to their various applications in various fields of science and technology. In particular such suspensions are used as reference standards in electronic and optical microscopy for counting aerosol particles. Now a days, they are used as bioligands carriers (live cells and viruses) for creating diagnostic test systems (Gritskova *et al*., 1996).

Complexity of solving this problem lies in the fact that there are stringent requirements that are imposed to the polymer dispersion used in biotechnology (Volkova *et al*., 2012). They must have a predetermined diameter and a narrow particle size distribution (coefficient of variation-not >5-10%), the stability in saline (temperature 25-40°C, pH-6.5-8.5, the ionic strength -0.1-0.15 M) and stability properties during storage time for at least 6 months.

This study discusses ways of obtaining highly dispersed polystyrene suspensions with particle diameter of 0.2-0.4 μm in the conditions of the emulsion polymerization of styrene with an emulsifier (surfactant) formed at the interface with or without an emulsifier in the presence of the polymer which is incompatible with formed polystyrene and a stabilizing polymer suspension.

Experimental part: Styrene is a technical product purified from the stabilizer by 5% aqueous sodium hydroxide solution, washed with water until neutral reaction is obtained, dried over calcined calcium chloride and double-distilled in vacuum. Using a fraction boiling at t = 41°C (10 mmHg) d_4^{20} = 0,906 g, cm^{-3}, n_q^{20} = 1,5450. Potassium persulfate ($K_2S_2O_8$) is used without further purification containing 99.9% of primary substance.

PMMA - $\bar{M}_w$ = 128000 Da, synthesized by the method of anionic polymerization. Fatty acids stearic acid, lauric acid, used with a mark "chemically pure".

Potassium hydroxide, lithium hydroxide, barium hydroxide, calcium hydroxide, zinc hydroxide used mark "pure for analysis" without further purification.

MATERIALS AND METHODS

Stability of emulsions was studied in a field of centrifugal forces following the procedure described by Kulichikhin (2012).

The particulate composition of the initial emulsions and polymeric suspensions was studied by electrophoretic light scattering particle analyzer Zetasizer Nano ZS of "Malvern" company of United Kingdom by the method of the manufacturer.

The formation of microemulsions studied in a graduated tube of 10 mL in static conditions (without stirring) with gentle layering of the organic phase to the aqueous phase and with the introduction of surfactant to monomeric phase or the aqueous phase. After that the system was kept for 1 day and the thickness of the layer of the microemulsion was determines.

Interfacial tension at the interphase boundary was measured by stalagmometric method (Kulichikhin, 2012). Kinetics of polymerization was measured by dilatometry.

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The molecular weight of the polymer was calculated by the Mark-Houwink-Kuhn: $(n) = k_{14}^{a}$ where (c) intrinsic viscosity which was determined by the method of K and a constants for the polymer-solvent system at a certain temperature.

RESULTS AND DISCUSSION

In the synthesis of the polymer suspensions, three possible mechanisms for the formation of polymer-monomer particles are considered: formation from the micelles of emulsifier, formation from the microdroplets of the monomer and from the mechanism of homogeneous nucleation (Gritskova *et al*., 2011a, b; Kirutina, 2008; Khomikovskiy, 1958). Using conventional method for producing an emulsion by emulsifying the monomer using aqueous solution of surfactant and initiation of polymerization the formation occurs by all three mechanisms that lead to the formation of polymer suspensions with a wide particle size distribution (Gritskova *et al*., 2011a, b).

The narrow particle size distribution in the polymer suspensions may be obtained when forming the polymer-monomer particles by the one mechanism of microdroplets monomer. For this purpose, it was suggested to polymerize a monomer subject to an intensive mass transfer of surfactant from one phase to another which leads to the formation of high concentrations of surfactant at the interphase boundary from the phase side in which the surfactant is less soluble, more often from the monomer phase (Gritskova *et al*., 2011a, b). Significant reduction of interfacial tension (up to 1-5 mN m^{-1}) due to the surfactant mass transfer across the interface into the phase in which the surfactant is readily soluble and due to high temperature polymerization results in initiating polymerization to form a highly dispersed emulsion and formation of polymer-monomers particles predominantly from monomer microdroplets. At full conversion of monomer there is a formation of polymeric suspension with significantly narrower particle size distribution than the polymeric suspension synthesized in the usual manner. These conditions are implemented when adding a surfactant in the phase in which it is less soluble (Gritskova *et al*., 2011a, b) and during the synthesis of the surfactant at the interphase boundary (Gritskova and Prokopov, 2001).

For synthesis of the polymer suspensions with a predetermined particle diameter it was necessary to study the influence of the main process parameters on particle diameter and size distribution during the emulsion polymerization of styrene in the absence of surfactant and during its synthesis at the interphase boundary. These parameters include: the volume ratio of the monomer aqueous phase, the temperature, nature of initiator and concentration of surfactant. A mandatory condition for the synthesis of polymeric suspensions was their stability during polymerization, the full monomer conversion, a narrow particle size distribution and stability in saline solution wherein the immunochemical studies performed.

In carrying out the polymerization under the conditions of surfactant synthesis at the interphase boundary the initial emulsion is usually prepared by pre-dissolving a long chain carboxylic acid in the monomeric and an alkali-in aqueous phase (Gritskova and Prokopov, 2001). In this case, a highly dispersed monomer emulsion is obtained due to the strong decrease of the interfacial tension at the interphase boundary as a result of the neutralization reaction and the formation of a surfactant. Emulsifier which is formed at the interphase boundary is distributed according solubility between the monomeric and aqueous phases which leads to an intense monomer microemulsion. It should be emphasized that the reduction in interfacial tension occurs to a much lower value than that was observed during the adsorption of the emulsifier from the aqueous phase on the surface of droplets in the preparation of the monomer emulsion by emulsifying an aqueous solution of the same emulsifier with the same concentration.

This is illustrated by the data for changing the interfacial tension at the interface of styrene solution of stearic acid aqueous solution of metal hydroxides selected from the series of carboxylic salts for the study (Fig. 1), and the volume of the microemulsion formed at the interface in the synthesis of these salts (Fig. 2).

It is seen that in the synthesis of the potassium salt of stearic acid at the interphase boundary from 1, 2 lower than during adsorption from an aqueous phase of potassium oleate (15 mN/m). The microemulsion of monomer in adsorption of potassium salt of stearic acid from the aqueous phase to the interphase boundary is not formed as potassium stearate is practically insoluble in styrene.

In order to find the polymerization conditions under which particle formation would occur by one mechanism from monomer microdroplets, the composition of styrene emulsion obtained in the synthesis of water-soluble salts of carboxylic acids in the boundary of the monomer/aqueous phase was studied. These studies showed that the emulsions contain microdroplets of monomer with a diameter of about ~10 μm, microdroplets with a diameter of ~0.04 μm and a surfactant micelle size of ~0.005 μm with a commonly used surfactant concentration of ~2-5% by weight based on the monomer phase ~100 times greater than the Critical Micelle Concentration (CMC). This means that it is possible to exclude micelle from the emulsion only when the

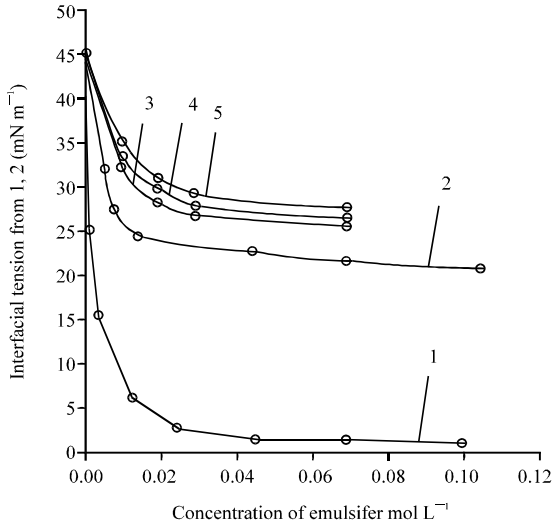

Fig. 1: Modifying the interfacial tension at the interphase boundary of aqueous sodium hydroxide/styrene solution of stearic acid; 1) Potassium hydroxide; 2) Lithium hydroxide; 3) Barium hydroxide; 4) Calcium hydroxide; 5) Zinc hydroxide. T = 20°C, time = 20 sec

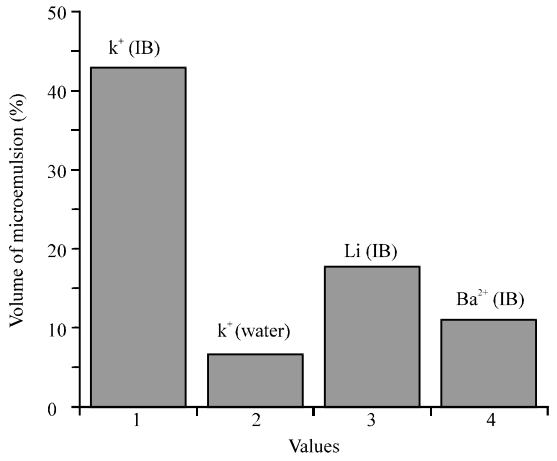

Fig. 2: The volume of styrene microemulsion in the synthesis of various salts of stearic acid in Interphase Boundary (IB) and potassium stearate adsorption from an aqueous phase

concentration of surfactant which synthesized at the interphase boundary in the aqueous phase decreases, i.e., it must be below the CMC of surfactant studied (i.e., <0.5 wt.% based on monomer).

In the static (without stirring) conditions concentration changes of laurate and potassium stearate in an aqueous phase during their synthesis on the boundary of the monomer/aqueous phase was determined. The results are shown in Table 1.

Table 1: Change of emulsifier concentration in the aqueous phase during its synthesis on boundary of styrene/aqueous KOH solution

Surfactant	The total concentration of surfactant which is formed as a result of complete neutralization of the acid	The concentration of the surfactant after synthesis in aqueous phase
Potassium laurate	0.15	0.08
CCM 0.22 (%)	0.25	0.10
	0.50	0.20
	4.00	1.00
Potassium stearate	0.15	0.02
CCM 0.05 (%)	0.25	0.04
	0.50	0.12
	4.00	1.40

Table 2: Colloid-chemical properties of potassium stearate

Method of administration of potasium stearate	$G_{max}\times10^6$ (mol m^2)	S_{min} (nm^2)	G (mN m^2 mol^{-1})
In the aqueous phase	3.6	0.45	30
Synthesis in the interphase boundary	8.4	0.22	67

It can be seen that the concentration of surfactant in the aqueous phase ~2-fold lower than the concentration of surfactant, calculated from the condition of complete deacidification. This means that part of the surfactant is consumed for the stabilization of the resulting microdroplets of the microemulsion. CMC of emulsifier in the aqueous phase is achieved only when the concentration of acid in the monomer is >0.5% by weight based on styrene.

Colloid-chemical properties of potassium stearate were determined when added to the aqueous phase and for synthesis in the interphase boundary. The results are shown in Table 2.

It can be seen that in the synthesis of potassium stearate at the interphase boundary there is a considerable increase in its surface activity (G), increase in its maximum adsorption (G_{max}) and decrease in the area occupied by the surfactant at the interphase boundary (S_{min}). The obtained results are explained by the different conditions of the formation of interfacial adsorption layer on the surface of the emulsion droplets. In the synthesis of the surfactant at the interphase boundary the interfacial layer is first formed from molecules of stearic acid and the neutralization reaction takes place in the surface layer containing carboxyl acid molecules oriented towards the interphase boundary. Thus, acid molecules and salt molecules formed as a result of the neutralization reaction are involved in the formation of the adsorption interfacial layer of the emulsion droplets.

During emulsification of the monomer by aqueous solution of the potassium salt of stearic acid the interfacial layer on the surface of the emulsion droplets is formed by adsorption of surfactant from the aqueous phase.

Stability of the emulsion obtained in the synthesis of the surfactant at the interface boundary is much higher

than that obtained by emulsifying the monomer in aqueous solution of potassium stearate due to the presence in it of a large volume of the microemulsion.

Thus in order to avoid the formation of surfactant micelles in the aqueous phase and their participation in the formation of polymer-monomer particles the concentration of surfactant in the monomer should not exceed 1% by weight.

Such a low concentration of surfactant may be insufficient to stabilize polymer-monomer particles formed at the initiation of polymerization. In this regard, we studied the effect the volume ratio of the monomer/aqueous phase on the stability of the reaction system and kinetics of styrene polymerization in the synthesis of potassium stearate at the interphase boundary.

It was found that the reaction system is stable (no coagulum was formed) only when the volume ratio of the monomer/aqueous phase is equal to 1:10, respectively, the concentration of potassium persulfate equal to 1% by weight based on styrene and the surfactant concentration of 0.5% by weight based on the monomer. Kinetic curves of the conversion-time are shown in Fig. 3.

It can be seen that during the formation of the potassium salt of stearic acid at the interphase boundary the polymerization proceeds without an induction period at a high speed until substantially complete conversion of monomer. Stability of the suspension particles is provided by the formation of not only structural mechanical barrier in the interfacial layer but also electrostatic stabilization, as evidenced by the high value of æ-potential of the particles suspension which even after the fourfold purification in ultrafiltration cell constitutes ~50 mV (Table 3). The average diameter of the polymer particles was = 93 nm and their size distribution was much narrower than already observed in the synthesis of polymeric suspensions in the presence of potassium stearate under equal conditions. Polymer suspension remained stable during storage period of 3 month.

The situation is different in the polymerization of styrene under the same conditions in the formation of barium, calcium and zinc salts of carboxylic acids of the same concentration at the interphase boundary. It turned out that in this case the average particle diameter is virtually independent of the nature of the metal salt and constitutes ~800 nm.

The average particle diameter formed by the polymerization of monomers in the synthesis conditions of salts of stearic acid at the interphase boundary is independent of monomer conversion, wherein the suspension polymer characterized by a narrow distribution of particle by diameters. This means that from the moment of the formation of polymer-monomer particles solid interfacial layer is formed on their surface which keeps the particles from coalescing and polymerization proceeds in a discrete volume of polymer-monomer particles as in microreactors.

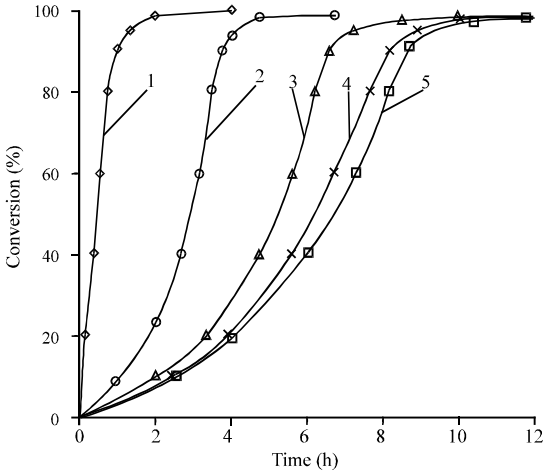

Fig. 3: Dependence of the polymer conversion on the polymerization time in the conditions of formation of the emulsifier at the interphase boundary. The polymerization temperature is 70°C, the volumetric phase ratio monomer/water 1:10 and the concentration of potassium persulfate 1% by weight based on the monomer, the concentration of emulsifier 0.5 wt% based on the monomer; Emulsifier: 1-Potassium stearate; 2-lithium stearate; 3-barium stearate; 4-calcium stearate; 5-zinc stearate

Table 3: Polymerization of styrene in the synthesis conditions interfacial salts of stearic acid

Surfactant	$W_0 \times 10^2$ (mol $L^{-1/s}$)	$MM \times 10^{-5}$	d (nm)	æ-potential (mV)
Potassium stearate	7.3	3.3	93	-50.0
Barium stearate	5.5	6.7	700	-29.5
Calcium stearate	5.4	6.6	800	-25.7
Zinc stearate	4.9	6.2	850	-23.2

Temperature of polymerization is 70°C, the volumetric phase ratio 1:10, the concentration of potassium persulfate based on monomer 1 wt.%, Emulsifier concentration of 0.5 wt% ased on the monomer

Under these conditions, we were not able to obtain a polymer suspensions with particle diameter of about 0.2 μm.

We could synthesis them during the polymerization of styrene in the synthesis conditions of lithium salts of stearic acid which are characterized as having low solubility in water as compared with the potassium salts of stearic acid. Polymerization was carried out at a concentration of lithium stearate of 0.5% by weight based on the aqueous phase, the volume ratio of the monomer/aqueous phase = 1:10 potassium persulfate concentration of 1% by weight based on the water.

The above results suggest that the polymeric suspension synthesized during a synthesis of lithium stearate at the interphase boundary at its concentration in the aqueous phase equal to 0.5% and the volume ratio of the monomer/aqueous phase equal to 1:10, respectively, meet the requirements for use in biotechnology and can be recommended for use.

The microphotos of particles of polystyrene suspension and their size distribution are shown in Fig. 4.

Further this paper presents the results of experiments on the polymerization of styrene in the presence of PMMA (Mw = 125000), initiated by potassium persulfate.

Kinetic laws of styrene polymerization in the Presence of Polymethylmethacrylate (PMMA) which was conducted at various volumetric ratios of monomer/aqueous phase, polymer concentration of 3% by weight based on the monomer, the concentration of potassium persulfate equal to 1% by weight based on the monomer and temperature of 70°C are shown in Fig. 5.

Table 4 presents data on the effect of the volume ratio of the monomer/aqueous phase to the rate of polymerization, average particle size and stability of the reaction system. Figure 6 shows microphotos and particle size distribution of obtained polystyrene suspensions.

During the polymerization of styrene the diameter of polymer particles is larger due to the fact that the styrene monomer is more hydrophobic than methyl methacrylate, and interfacial tension from 1, 2, etc., at the boundary of a styrene/aqueous phase is greater than at the boundary of methyl methacrylate/water phase.

Figure 7 and Table 5 present data on the effect of the concentration of initiator, potassium persulfate, on the properties of the polymer suspensions obtained by polymerizing styrene in the presence of PMMA (M_w = 125000).

Polystyrene suspension with particle diameters of 0.3-0.4 μm and a narrow size distribution were prepared by polymerizing styrene in the presence of methyl methacrylate monomer with the low monomer content (the volume ratio of the monomer/aqueous phase = 1:25) and at a concentration of initiator, potassium persulfate, 2% by weight based on the monomer. In other cases, the average particle size was 0.5-0.7 μm. These results can be explained as follows.

During the polymerization of the monomers in the presence of polymers which are incompatible with formed during polymerization, interfacial layer formed from polymers which are characterized by low surface activity, high Hamaker constant value and form thin films with properties that can be characterized as a gel. Steric

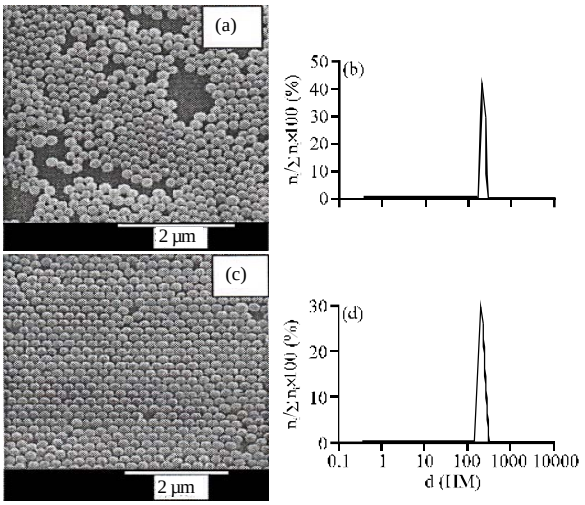

Fig. 4: Microphotos and distribution of polystyrene particle according to the size obtained by the polymerization of styrene in the synthesis conditions of lithium stearate at the interphase boundary

Table 4: The effect of volumetric ratio of phases monomer/water on the characteristics of the polymerization process and the properties of polystyrene suspensions

Phase ratio	Conversion (%)	Polymerization rate (W % min^{-1})	Coagulum content (%)	Polydispersity (D_w/D_n)	Particle diameter (D_n μm)	Stability 0.15 (NaCl)
1:25	99.2	4.7	Absent	1.01	0.30	+
1:9	99.8	4.3	Absent	1.01	0.51	+
1:6	99.1	4.2	Absent	1.09	0.53	+
1:4	95.2	3.0	Absent	1.11	0.62	+

Table 5: The effect of initiator concentration on the characteristics of the polymerization process and the properties of polystyrene suspensions. The volume ratio of the monomer/aqueous phase of 1: 9. The polymerization temperature is 70°C, the concentration of PMMA (Mw = 125 000) of 3% by weight based on the monomer

Concentration of PP weight on monomer (%)	Monomer conversion (%)	Polymerization rate (W % min^{-1})	Coagulum content (%)	Average particle diameter (Dn μm)	Polydispersity (D_w/D_n)	Stability 0.15 NaCl
0.2	99.1	2.0	Absent	0.71	1,022	+
0.5	99.5	3.7	Absent	0.72	1,016	+
1.0	99.8	4.3	Absent	0.51	1,003	+
2.0	99.8	6.0	Absent	0.40	1,009	+

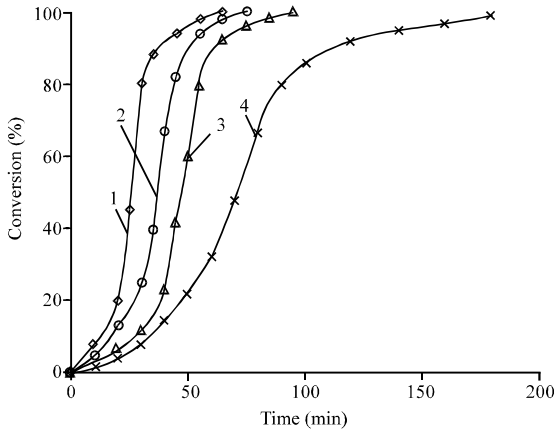

Fig. 5: Conversion-time curves obtained by polymerizing styrene in the presence of PMMA (Mw = 125000). Temperature 70°C [PMMA] = 3% by weight based on the monomer [potassium persulfate] = 1 wt% based on the monomer phase, volume ratio of monomer/water: 1-1:25, 2-1: 9, 3-1: 6, 4-1: 4, respectively

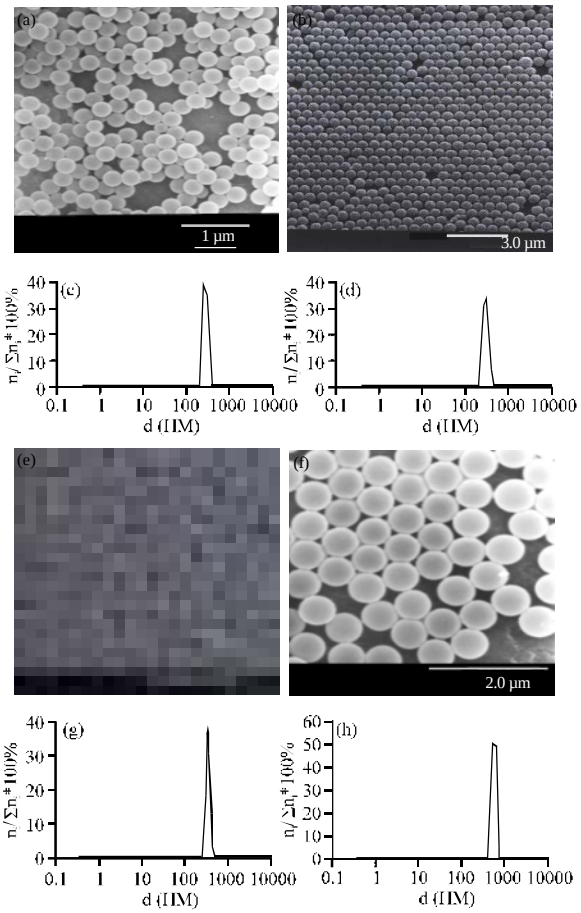

Fig. 6: Microphotos and particle size distribution of samples of polymer microspheres obtained by polymerizing styrene in the presence of PMMA (Mw = 125000). Temperature 70°C (PMMA) = 3% by weight based on the monomer, (potassium persulfate) = 1 wt% based on the monomer, phase volume ratio of monomer/water: 1-1:25, 2-1: 9, 3-1: 6, 4-1: 4, respectively

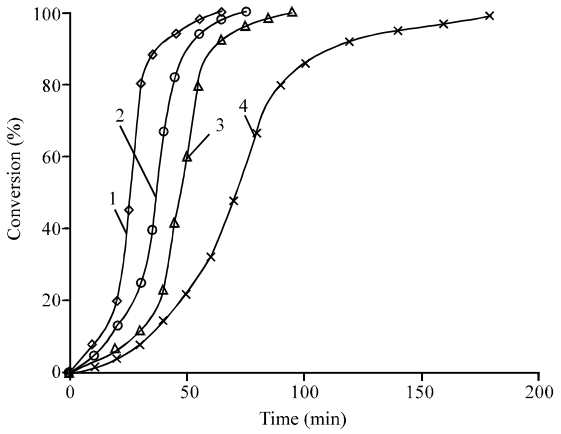

Fig. 7: Conversion-time curves obtained by polymerizing styrene in the presence of PMMA (M_w = 125000). The volume phase ratio monomer/water 1:9, temperature 70°C,(PMMA) = 3 wt.% based on the monomer (potassium persulfate) (wt.% based on monomer): 1) 2%, 2) 1%, 3) 0.5% and 4) 0.2%

stabilization of the polymer particles was effective only in systems where the number of particles was 1010 mL of suspension, i.e., increase in the number of particles with increased monomer concentration leads to a loss of stability of the reaction system.

This is probably due to the fact that with an increase in the number of particles the process of coagulation becomes noticeable due to increased probability of their mutual encounter. In the presence of the polymer (as opposed to surfactant), it is impossible to achieve a low interfacial tension at the interphase boundary.

Under these conditions, the capillary pressure in the particles is large which leads to a migration from the bulk of the monomer polymer-monomer particles to the contact zone of coagulation. This eliminates the likelihood of separation of the particles in the gradient of shear stresses generated during the stirring of the reaction mixture.

CONCLUSION

Thus, conditions for the synthesis of polystyrene suspensions with a narrow particle size distribution and a diameter of 0.2-0.4 μm were defined, suitable for use in Biotechnology.

Polystyrene suspension with particle diameters of 0.2 μm can be obtained with low monomer content in the emulsion in the synthesis conditions of salts of stearic acid at the interphase boundary; or with a diameter of 0.3-0.4 μm in the presence of additives (3%) of polymethylmethacrylate, incompatible with formed polysterene and participating in the formation of structural and mechanical barrier of Rebinder in interfacial adsorption layer of particles.

REFERENCES

Gritskova, I.A., N.I. Prokopov, A.A. Chalih and V.R. Cherkasov, 1996. The Synthesis of Functional Monodisperse Polymeric Microspheres for Immunochemical Studies. Progress of Chemistry, 65 (2): 178-192.

Gritskova, I.A., M. Haddazh and G.I. Litvinenko, 2011a. Theoretical study of the emulsion polymerization of styrene. Effect of initial dispersion system on the rate of polymerization and polymer-monomer distribution according to the size. High Molecular Compound, 53 (4): 642-656.

Gritskova, I.A., M. Haddazh and G.I. Litvinenko, 2011b. Theoretical study of the emulsion polymerization of styrene. The impact of the initial dispersion system on the molecular weight distribution of polystyrene. High Molecular Compounds, 53 (5): 808-816.

Gritskova, I.A. and N.I. Prokopov, 2001. Features of Heterophase Polymerisation of Styrene in the Formation of Surfactants at the Iinterphase Bpundary. Progress Chem., 70 (9): 890-901.

Kirutina, O.P., 2008. A Study of the Initial Stage of a Heterophase Polymerization of Styrene. Report of Thesis. Cand. Chem. Sci., Moscow, pp: 147.

Khomikovskiy, P.M., 1958. Kinetics and Topochemical Features of Emulsion Polymerization. Progress of Chem., 27 (2): 1025-1055.

Kulichikhin, V.G., 2012. Workshop on Colloidal Chemistry. Moscow, pp: 288.

Volkova, E.V., I.A. Gritskova, S.A. Gusev, A.D. Lukashevish, A.N. Levshenko, L.A. Zlidneva and K.O. Sochilina, 2012. Development of polystyrene microspheres for immunofluorescence analysis. Biotechnology, 4: 4-77.