Thermoresponsive poly[oligo(ethylene glycol) methacrylate]s and their bioconjugates – synthesis and solution behavior

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DOI: dx.doi.org/10.14314/polimery.2017.298

This work is dedicated to the memory of our dear colleague Prof. Andrzej Duda

Abstract: Growing interest for polymers containing oligoethylene side groups was observed within last years. Amongst these polymers the poly[oligo(ethylene glycol) methacrylate]s (POEGMAs) became most important. These polymers are biocompatible. Many of these polymers are thermoresponsive, when dissolved in water or physiological media, exhibit a narrow phase transition range and show only a small hysteresis. This review describes the synthesis and self-organization of homo- and copolymers of OEGMAs of different macromolecular topology. Data about the synthesis of thermoresponsive conjugates of POEGMAs with biologically active species and the possibility to use such conjugates for the synthesis of nanocarriers are discussed.

Keywords: thermoresponsive polymers, poly[oligo(ethylene glycol) methacrylate]s, aggregation, bioconjugates.

Termoczułe poli(metakrylany glikoli oligoetylenowych) i ich biokoniugaty – synteza i zachowanie w roztworze

Streszczenie: W ciągu ostatnich kilkunastu lat znacząco wzrosło zainteresowanie polimerami zawierającymi boczne łańcuchy glikolu oligoetylenowego. Wśród polimerów tego typu największe znaczenie uzyskały poli(metakrylany glikoli oligoetylenowych) (POEGMA). Polimery te są biokompatybilne. Wiele z polimerów POEGMA wykazuje termoczułość w roztworach wodnych i w płynie fizjologicznym, a ich przejście fazowe jest wąskie i charakteryzuje się nieznaczną histerezą. Przegląd obejmuje syntezę i samoorganizację homo- i kopolimerów POEGMA o różnej topologii makrocząsteczek. Zawiera także dane o otrzymywaniu termoczułych koniugatów POEGMA z substancjami aktywnymi biologicznie i możliwość wykorzystania takich biokoniugatów do otrzymywania nanonośników.

Słowa kluczowe: polimery termoczułe, poli(metakrylany glikoli oligoetylenowych), agregacja, biokoniugaty.

During last decades the interest paid to the polymers having oligo(ethylene glycol) side groups has increased significantly. Many of these polymers are thermoresponsive when dissolved in water or in media simulating body fluids.

So far, polymers containing oligo(ethylene glycol) groups connected to the chains of poly(vinyl ether) [1], polynorbornene [2], polylactide [3], polystyrene [4, 5], polyacrylate [5, 6] or polymethacrylate [7, 8] have been synthesized. The cloud point temperature (T_{CP}) of these polymers decreases with increasing hydrophobicity of the main chain: poly(vinyl ether) < polyacrylate, polymethacrylate < polynorbornene < polylactide < polystyrene and increases with the length of the hydrophilic OEG substituent. Also the end group exercises some influence (the more hydrophobic the end group, the lower the T_{CP}).

The poly[oligo(ethylene glycol) methacrylate]s (POEGMAs) are the most frequently studied and described polymers in this group [9–16].

These polymers have many advantages compared with the widely applied poly(*N-iso*propylacrylamide) (PNIPAM). The phase transition occurs in a narrow temperature range and exhibits a small hysteresis. The influence of external factors, like polymer or salt concentration *e.a.* upon T_{CP} is frequently negligible [15, 16].

The studies of the interactions of POEGMAs with a number of cell lines (HepG2, Caco-2, HT29-MTX-E12, NIH/3T3, HUVEC, J774.A1) [17–20] indicate that these

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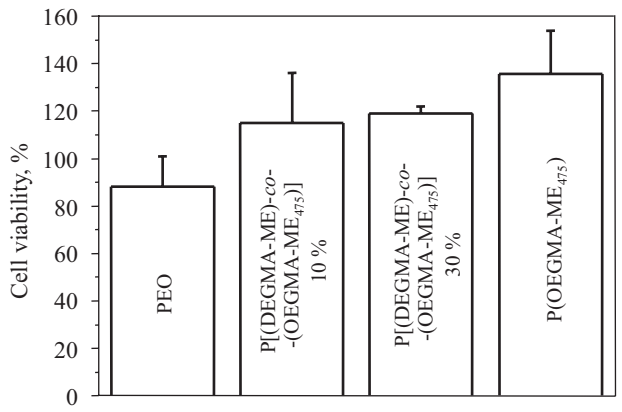

Fig. 1. Viability of HepG2 cells at 37 °C in the presence of the polymers PEO, P[(DEGMA-ME)-*co*-(OEGMA-ME$_{475}$)] with OEGMA-ME$_{475}$ content of 10 and 30 % and P(OEGMA-ME$_{475}$); polymer concentration 5 g/dm^3; adapted with permission from Lutz J.-F., Andrieu J., Üzgün S. *et al.*: *Macromolecules* 2007, *40*, 8540, copyright (2007) American Chemical Society [17]

polymers are non-toxic in a wide range of concentrations, sometimes up to 10 g/dm^3. Such properties are likely to be due to the presence of biocompatible PEG units, which may constitute up to 85 wt %. The viability of cells increases with increasing content of OEG units in the chains (Fig. 1) [17].

Growing interest paid to these thermoresponsive polymers is driven also by their potential applications, mostly in biomedicine. The biocompatibility, relatively easy formation of bioconjugates, temperature dependent water solubility, and self-organization seem to open simple and clean route to design biomedical devices.

Synthesis of poly[oligo(ethylene glycol) methacrylate]s

POEGMAs are obtained in the polymerization of monomers containing OEG units [11]. One of the first published papers described the synthesis of poly[oligo(ethylene glycol) monomethyl ether methacrylate] *via* the atom transfer radical polymerization (ATRP) [21].

Homo- and copolymers of oligo(ethylene glycol) methacrylates are generally obtained from monomers having OEG substituents of different length (from one to several dozens of OEG units). The majority of monomers is commercially available. They may be polymerized using different techniques:

– controlled radical polymerizations (CRP): atom transfer radical polymerization [22–24], radical polymerization with reversible addition-fragmentation chain transfer (RAFT) [8, 25], nitroxide-mediated radical polymerization (NMP) [19–26], or cobalt-mediated radical polymerization (CMRP) [27],
– free radical polymerization (FRP) [28],
– group transfer polymerization (GTP) [29, 30],
– anionic polymerization [31, 32].

Using these techniques a number of random, gradient, block and graft copolymers of linear, brush-like, dendritic and star topology have been obtained (Table 1).

T a b l e 1. Thermoresponsive homo- and copolymers of oligo(ethylene glycol) methacrylates and their conjugates with biologically active species

Monomer	Polymer	Synthesis	Topology	Comments	Ref.
DEGMA-ME	P(DEGMA-ME)	RAFT	Linear	Influence of molar mass and pH upon T_{CP}	[8]
		Anionic polymerization	Linear	T_{CP} in different solvents	[31]
	P(DEGMA-ME) P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]	ATRP	Linear	Thermoresponsiveness, aggregation to mesoglobules	[39]
	P(DEGMA-ME) PGEMA-*b*-P(DEGMA-ME)	ATRP, RAFT	Linear	Thermoresponsiveness, aggregation to vesicles	[40]
	P(DEGMA-ME) P[(DEGMA-ME)-*co*-(TEGMA-ME)] P(DEGMA-ME)-*b*-P(TEGMA-ME)	ATRP	Linear	Influence of composition and molar mass upon T_{CP}, self-organization	[23]
	P(DEGMA-ME) P(DEGMA-ME)-*b*-P(S/TEGMA-ME)	Anionic polymerization	Linear	T_{CP} in different solvents, influence of molar mass upon T_{CP}	[32]
	P(DEGMA-ME) P(DEGMA-ME)-*b*-P(OEGMA-ME$_{300}$) P(DEGMA-ME)-*b*-P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]	ATRP	Linear	Micelles, multimicellar aggregates	[22]
	P(DEGMA-ME)-GRKFG-Dns	ATRP	Linear bioconjugate	Thermoresponsiveness, aggregation to mesoglobules	[46]

T a b l e 1. (cont.)

Monomer	Polymer	Synthesis	Topology	Comments	Ref.
DEGMA-ME	P(DEGMA-ME)-$(GPO)_7$GG-P(DEGMA-ME)	RAFT + coupling	Linear bio-conjugate	Thermoresponsiveness, aggregation	[44]
	$(GPO)_7$GG-P(DEGMA-ME)	RAFT + coupling	Linear bio-conjugate	Thermoresponsiveness, aggregation	[45]
	PArOx-P(DEGMA-ME) PArOx-P[(DEGMA-ME)-*co*-(OEGMA-ME_{300})]	ATRP	Star	Kinetics of polymerization, thermoresponsiveness, aggregates	[47]
	P(DEGMA-ME) P(DEGMA-ME)-*b*-P(OEGMA-ME_{475})	ATRP	Linear	Thermoresponsiveness, protein conjugate	[48]
	P(DEGMA-ME) P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	ATRP	Linear	Thermoresponsiveness, influence of composition and concentration upon T_{CP}	[7]
	P[EBIEM-*g*-(DEGMA-ME)] P(EBIEM)-*g*-P[(DEGMA-ME)-*co*-(TEGMA-ME)] P(EBIEM)-*g*-[P(DEGMA-ME)-*b*-P(TEGMA-ME)]	ATRP	Comb	Influence of composition and concentration upon T_{CP}, aggregation, DLS	[49]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{300})] P[(DEGMA-ME)-*co*-(OEGMA-ME_{300})]-*met*--enkephalin-Dns	AGET ATRP	Linear	Thermoresponsiveness, aggregation, nanoparticles, degradation	[43]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{300})]-(-/C_{60})	ATRP	Linear	Multimicellar aggregates	[50]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{300}/OEGMA--ME_{475})]	AGET ATRP miniemulsion	Linear	Thermoresponsiveness, cross-linking, microgels	[51]
	sPEG-P[(DEGMA-ME)-*co*-(OEGMA-ME_{300}/OEGMA-ME_{475})]	ATRP	4-arm star	Thermogels	[12]
	DPE-P(DEGMA-ME) DPE-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	CWP, ATRP	Dendritic--linear	Thermoresponsiveness, core-shell particles	[52]
	CBI-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	ATRP	Linear	Influence of surfactants	[53]
	P(DEGMA-ME)-*co*-P[(OEGMA-ME_{475})-*co*--EGDMA]	DE-ATRP	Hyperbranched	Thermoresponsiveness, cytotoxicity, photoinduced cross-linking	[24]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})-*co*-EGDMA]	RAFT	Hyperbranched	Thermoresponsiveness, influence of salts upon solution behavior, aggregation	[25]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	ATRP	Linear	Thermoresponsiveness, aggregation, mesoglobules, cross-linking	[54]
		ATRP	Linear	Influence of molar mass, composition and salt content upon T_{CP}, comparison with PNIPAM	[15]
		CMRP, Click	Linear	Thermoresponsiveness, influence of end groups	[27]
	P[(DEGMA-ME)-*co*-AMA]	ATRP	Linear	Thermoresponsiveness, aggregation, cross-linking, degradation	[55]
	EC-*g*-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})] EC-*g*-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]--PDMAEMA	ATRP, Click	Comb	Self-organization to micelles, thermo- and pH-responsiveness	[56]

T a b l e 1. (cont.)

Monomer	Polymer	Synthesis	Topology	Comments	Ref.
DEGMA-ME	NBD-P[(DEGMA-ME)-*co*-(OEGMA-ME$_{475}$)]--RhB$_2$	ATRP	Linear	Thermo- and pH--responsiveness, fluorescence	[57]
	P2VP-*b*-PEO-*b*-P[(DEGMA-ME)-*co*-(OEGMA--ME$_{475}$)]	Anionic polymerization, ATRP, Click	Linear	Self-organization to micelles, thermo-, pH- and light-responsiveness of hydrogels	[58]
	PSPMA-*b*-P(DEGMA-ME)	ATRP	Linear	Thermo- and light-responsiveness, micelles and reversed micelles	[41]
	PDMAEMA-*b*-P(DEGMA-ME)	RAFT	Linear	Thermoresponsiveness, mono- and multilayer vesicles	[59]
	PS-*b*-P(DEGMA-ME)	Anionic polymerization	Linear	–	[60]
	PLG-*g*-P(DEGMA-ME)	ROP, ATRP	Comb	Self-organization to micelles, core-shell particles	[61]
	PEDI-P(DEGMA-ME)	ATRP	Linear-dendritic	Self-organization to micelles	[62]
TEGMA-ME	P(TEGMA-ME)	Anionic polymerization	Linear	T_{CP} in different solvents	[31]
		ATRP	Linear	Thermoresponsiveness	[48]
	P(TEGMA-ME) P[(DEGMA-ME)-*co*-(TEGMA-ME)] P(DEGMA-ME)-*b*-P(TEGMA-ME)	ATRP	Linear	Influence of molar mass and composition upon T_{CP}, self-organization	[23]
	P(TEGMA-ME) P(DEGMA-ME)-*b*-P(S/TEGMA-ME)	Anionic polymerization	Linear	T_{CP} in different solvents, influence of molar mass and composition upon T_{CP}	[32]
	P(EBIEM)-*g*-P[(DEGMA-ME)-*co*-(TEGMA-ME)] P(EBIEM)-*g*-[P(DEGMA-ME)-*b*-P(TEGMA-ME)]	ATRP	Comb	Influence of copolymerization upon T_{CP}, aggregation, DLS	[49]
TEGMA-EE	P(TEGMA-EE)	RAFT	Linear	Influence of molar mass, composition and pH upon T_{CP}	[8]
		Anionic polymerization	Linear	T_{CP} in different solvents	[31]
	P(TEGMA-EE) P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)]	ATRP	Linear	Thermoresponsiveness, aggregation to mesoglobules	[39]
	trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)] trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)]-*b*--P(OEGMA-ME$_{475}$)	AGET ATRP	Linear/star	Thermoresponsiveness, aggregation	[42]
OEGMA--ME$_{300}$	P(OEGMA-ME$_{300}$)	AGET ATRP	Linear	Kinetics of polymerization	[63]
		RAFT	Linear	Influence of end groups, molar mass, solvents upon T_{CP}	[64]
		ATRP	Linear	Thermoresponsiveness	[48]
	P(OEGMA-ME$_{300}$) P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]	ATRP	Linear	Thermoresponsiveness, aggregation to mesoglobules	[39]

T a b l e 1. (cont.)

Monomer	Polymer	Synthesis	Topology	Comments	Ref.
OEGMA--ME$_{300}$	P(OEGMA-ME$_{300}$) P[(OEGMA-ME$_{300}$)-*co*-S]	NMP	Linear	Kinetics of polymerization	[26]
	P[(OEGMA-ME$_{300}$)-*co*-AN] P[(OEGMA-ME$_{300}$)-*co*-AN]-*b*-PS	NMP	Linear	Kinetics of polymerization, cytotoxicity	[19]
	P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)] P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]-*met*--enkephalin-Dns	AGET ATRP	Linear	Thermoresponsiveness, aggregation, nanoparticles, degradation	[43]
	P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]-(-/C$_{60}$)	ATRP	Linear	Micelles, multimicellar aggregates	[50]
	P(DEGMA-ME)-*b*-P(OEGMA-ME$_{300}$) P(DEGMA-ME)-*b*-P[(DEGMA-ME)-*co*--(OEGMA-ME$_{300}$)]	ATRP	Linear	Micelles, multimicellar aggregates	[22]
	PArOx-P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]	ATRP	Star	Kinetics of polymerization, thermoresponsiveness, aggregation, DDS	[47]
	P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]	AGET ATRP miniemulsion	Linear	Thermoresponsiveness, cross-linking, microgels	[51]
	sPEG-P[(DEGMA-ME)-*co*-(OEGMA-ME$_{300}$)]	ATRP	4-arm star	Thermogels	[12]
	SP-PCL-*b*-P(OEGMA-ME$_{300}$)	ROP, ATRP	Star	Micellar aggregates, DDS	[65]
	PE-*b*-P(OEGMA-ME$_{300}$)	CSP, ATRP	Linear	Self-organization, micelles, crystallization	[66]
	P(OEGMA-ME$_{300}$)-*b*-PS-*b*-P(OEGMA-ME$_{300}$)	ATRP	Linear	Self-organization to spherical and cylinder--like micelles	[67]
	EC-*g*-P(OEGMA-ME$_{300}$)	ATRP	Comb	Micelles	[22]
	DPE-P(OEGMA-ME$_{300}$)	CWP, ATRP	Dendritic--linear	Thermoresponsiveness, core-shell particles	[68]
OEGMA--ME$_{350}$	P(OEGMA-ME$_{350}$) P[(OEGMA-ME$_{350}$)-*co*-(MMA/nBuMA)]	FRP	Linear	Thermoresponsiveness, self-organization to micelles	[28]
	P(OEGMA-ME$_{350}$) P(OEGMA-ME$_{350}$)-*b*-PBzMA	GTP	Linear	Thermoresponsiveness, stabilization of emulsion	[29]
	P(OEGMA-ME$_{350}$) P[(OEGMA-ME$_{350}$)-*co*-(BzMA/DMAEMA/nBuMA/THPMA)]	GTP	Linear	Study of copolymerization, hydrolysis	[30]
OEGMA--ME$_{475}$	P(OEGMA-ME$_{475}$)	RAFT	Linear	Influence of molar mass and pH upon T_{CP}	[8]
		ATRP	Linear	Kinetics of copolymerization	[69]
		ATRP	Linear	Thermoresponsiveness, influence of composition and concentration upon T_{CP}	[7]
		AGET ATRP	Linear	Kinetics of copolymerization	[63]
	P(OEGMA-ME$_{475}$) P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)]	ATRP	Linear	Thermoresponsiveness, aggregation to mesoglobules	[39]
	P[(OEGMA-ME$_{475}$)-*co*-HOPEGMA$_{375}$-EGDMA]	DE-ATRP	Hyperbranched	Thermoresponsiveness, photocross-linking, mechanical parameters	[70]

T a b l e 1. (cont.)

Monomer	Polymer	Synthesis	Topology	Comments	Ref.
OEGMA-ME_{475}	P(OEGMA-ME_{475}) P(DEGMA-ME)-*b*-P(OEGMA-ME_{475})	ATRP	Linear	Thermoresponsiveness	[48]
	P(OEGMA-ME_{475}) P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	CMRP, Click	Linear	Thermoresponsiveness, influence of end groups	[27]
	CBI-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	ATRP	Linear	Surfactants	[53]
	DPE-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	CWP, ATRP	Dendritic--linear	Thermoresponsiveness, core-shell particles, DDS	[52]
	sPEG-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	ATRP	4-arm star	Thermogels	[12]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	AGET ATRP miniemulsion	Linear	Thermoresponsiveness, cross-linking, microgels	[51]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})-*co*-EGDMA]	DE-ATRP	Hyperbranched	Thermoresponsiveness, cytotoxicity, photocross--linking	[24]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})-*co*-EGDMA]	RAFT	Hyperbranched	Thermoresponsiveness, influence of salts upon solution behavior, aggregation	[25]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	ATRP	Linear	Thermoresponsiveness, aggregation, mesoglobules, cross-linking	[54]
	P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]	ATRP	Linear	Influence of salts, molar mass and concentration upon T_{CP}, comparison with PNIPAM	[15]
	trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME_{475})] trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME_{475})]-*b*--P(OEGMA-ME_{475})	AGET ATRP	Linear/stars	Thermoresponsiveness, aggregation	[42]
	P[(OEGMA-ME_{475})-*co*-$OPGMA_{430}$]	ATRP, FRP	Linear	Biodegradable, thermoresponsive microparticles for cell transport	[71]
	P(OEGMA-ME_{475})-*b*-PCL-*b*-P(OEGMA-ME_{475})	ATRP	Linear	Aggregation to micelles	[72]
	EC-*g*-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})] EC-*g*-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]--PDMAEMA	ATRP, Click	Comb	Self-organization to micelles, thermo- and pH--responsiveness	[56]
	NBD-P[(DEGMA-ME)-*co*-(OEGMA-ME_{475})]--RhB_2	ATRP	Linear	Thermo- and pH--responsiveness, fluorescence, FRET	[57]

AN – acrylonitrile, AMA – 2-aminoethyl methacrylate, 2VP – 2-vinylpyridine, BzMA – benzyl methacrylate, CBI – cholesterol, CL – ε-caprolactone, CSP – chain shuttle polymerization, CWP – chain walking polymerization, DDS – drug delivery systems, DE-ATRP – deactivation enhanced ATRP, DMAEMA – 2-(dimethylamino)ethyl methacrylate, Dns – dansyl, DPE – dendritic polyethylene, EBIEM – 2-(2-bromo*iso*butyryloxy)ethyl methacrylate, EC – ethyl cellulose, EGDMA – ethylene glycol dimethacrylate, GEMA – 2-glucosyloxyethyl methacrylate, HOPGMA – oligo(propylene glycol) methacrylate, LG – L-glutamine, NBD – 7-nitrobenzofurazan, nBuMA – *n*-butyl methacrylate, RhB_2 – rhodamine B, PArOx – poly(arylen-oxindol), PEDI – dendritic polyester initiator, SP – porphyrin multi-initiator, sPEG – PEG multi-initiator, SPMA – spiropyran-containing methacrylate, THPMA – tetrahydropyranyl methacrylate.

ATRP is the technique most frequently used for the synthesis of POEGMAs, as shown in Table 1.

Becer *et al.* [8] used RAFT for the synthesis of a library of POEGMAs (Fig. 2).

2-Cyano-2-butyl dithiobenzoate was used to polymerize the depicted monomers. Amongst the obtained homopolymers the polymers of MAA, MEOMA and $OEGMA_{1100}$ were not thermoresponsive in water solutions. Polymers of DEGMA-ME, TEGMA-EE and OEGMA-ME_{475} were thermoresponsive with a cloud point in water of 20.6, 93.7 and 20.0 °C (for 5 g/dm^3 solutions). A strong influence of pH upon the cloud point temperature was observed.

Badi and Lutz [12] used ATRP for the synthesis of star polymers with DEGMA-ME and OEGMA-ME_{475} arms. 4-arm poly(ethylene glycol) modified with ATRP initiating groups was applied to initiate the process (Fig. 3).

The obtained macromolecules consisted of a hydrophilic PEG core to which a thermoresponsive

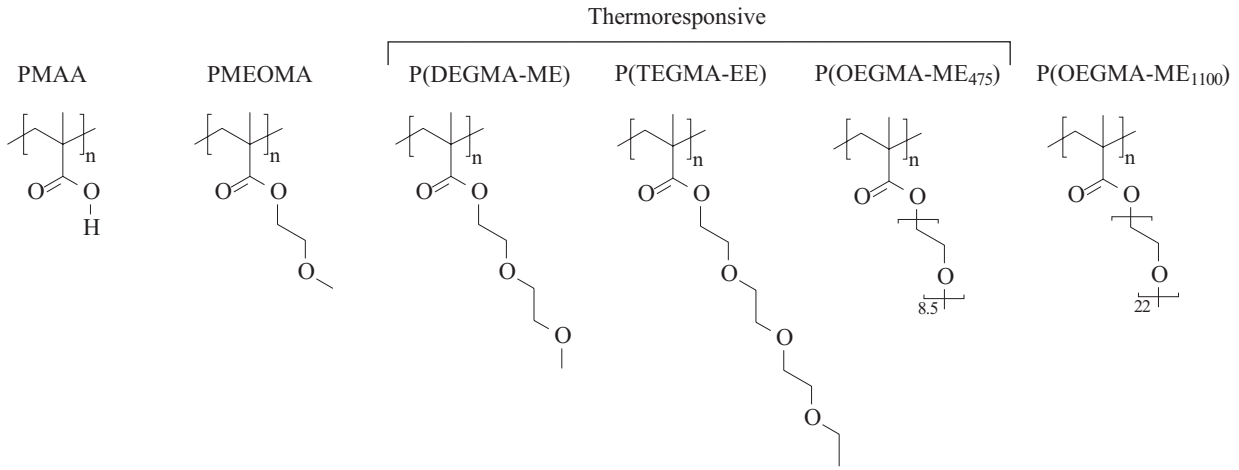

Fig. 2. Polymers from POEGMAs library

poly[(DEGMA-ME)-*co*-(OEGMA-ME_{475})] shell was attached. This made reversible thermogel formation near the physiological temperature possible.

Next to the above discussed other, commercially not available monomers have been studied, like di(ethylene glycol) monomethyl ether methacrylate, tetra(ethylene glycol) monoethyl ether methacrylate or tetra(ethylene glycol) monomethyl ether methacrylate [11, 31]. Ishizone first obtained the homopolymers of these monomers by anionic polymerization [31]. Cloud points of 0.2 wt % water solutions were determined to 4 °C for poly[di(ethylene glycol) monoethyl ether methacrylate], 68 °C for poly[tetra(ethylene glycol) monomethyl ether methacrylate], and 42 °C for poly[tetra(ethylene glycol) monoethyl ether methacrylate].

Thermoresponsive poly[oligo(ethylene glycol) methacrylate]s in water solutions

Thermoresponsiveness of POEGMAs is due to the amphiphilic character of their chains. The hydrophilic oligo(ethylene glycol) side groups may form hydrogen bonds with water molecules, while the unipolar backbones of the main chains enter hydrophobic interactions. Above the transition temperature the hydrogen bonds break, the hydrophobic interactions start to prevail.

The cloud point temperatures of POEGMAs containing 2 to 9 ethylene oxide units in the side group increase with increasing length of the side group (Table 2), those having longer oligo(ethylene glycol) side groups are water soluble in the whole range of temperatures. POEGMAs with hydroxyl terminated side groups are water soluble at all temperatures.

Like other thermoresponsive polymers POEGMAs form mesoglobules – spherical, colloidally stable aggregates of several to several hundred nanometers – when their dilute water solutions are heated. The sizes of such aggregates depend upon several parameters – the concentration of the solution, molar mass and composition of the copolymer, presence of additives [35–38]. Mesoglobules are stable only above T_{CP} of the polymers, what

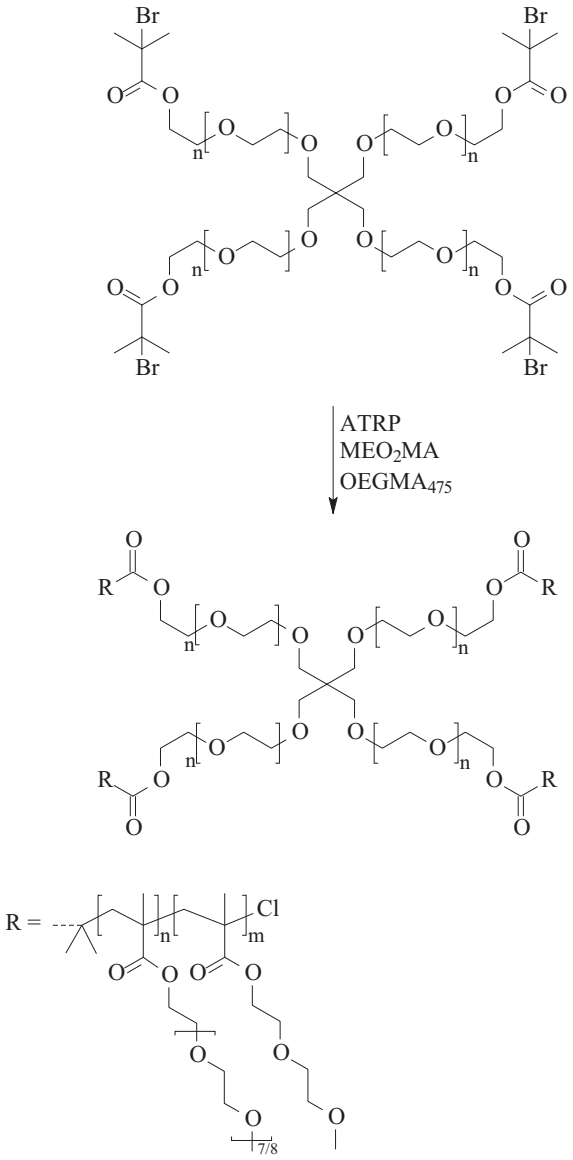

Fig. 3. ATR copolymerization of DEGMA-ME and OEGMA-ME_{475} initiated with a 4-arm star PEG macroinitiator; according to [12]

T a b l e 2. Cloud point temperatures of poly[oligo(ethylene glycol) methacrylate]s

Polymer	Cloud point temperatures (T_{CP}) in water	Reference
P(DEGMA-ME)	T_{CP} ~ 26 °C (c = 0.2 g/dm^3); T_{CP} ~ 28 °C (c = 3 g/dm^3)	[7, 32]
P(TEGMA-ME)	T_{CP} ~ 47 °C (c = 1 g/dm^3)	[23]
P(TEGMA-EE)	T_{CP} ~ 24 °C (c = 3 g/dm^3); T_{CP} ~ 22 °C (c = 5 g/dm^3)	[8, 33]
P(OEGMA-ME$_{300}$)	T_{CP} ~ 66 °C (c = 5 g/dm^3)	[34]
P(OEGMA-ME$_{475}$)	T_{CP} ~ 90 °C (c = 5 g/dm^3)	[8]

limited the range of techniques used for their characterization [light scattering, atomic force microscopy (AFM), cryo-transmission electron microscopy (TEM)].

Trzebicka *et al.* [39] studied the aggregation of POEGMAs in water solutions. In Fig. 4a the apparent hydrodynamic radius of the aggregates formed in the solutions of P(TEGMA-EE) as a function of temperature are shown.

Below the cloud point temperature the polymers are water soluble. Dynamic light scattering (DLS) indicates the presence of single chains, particle size ranging from 0.5 to 1.2 nm. As the solutions are heated, the radius of the particles increases drastically at the cloud point and remains stable, when this temperature is exceeded. Increasing concentration leads to the formation of larger aggregates. The distribution of sizes remain monomodal and relatively narrow (Fig. 4b).

Incorporation into the chain of OEGMA monomers having OEG side chains of different length permits to obtain copolymers of exactly defined T_{CP}. Lutz and Hoth [7] were the first to show (using DEGMA-ME and OEGMA-ME$_{475}$ monomers) that T_{CP} depends upon the composition of the copolymer in a linear way, what opens the route to a control over T_{CP} by simply changing the composition of the monomer mixture (Fig. 5).

The linear change of T_{CP} with the composition of POEGMA copolymers was also confirmed by Trzebicka *et al.* [39] for TEGMA-EE and OEGMA-ME$_{475}$ copolymers. The authors synthesized by ATRP a series of copolymers

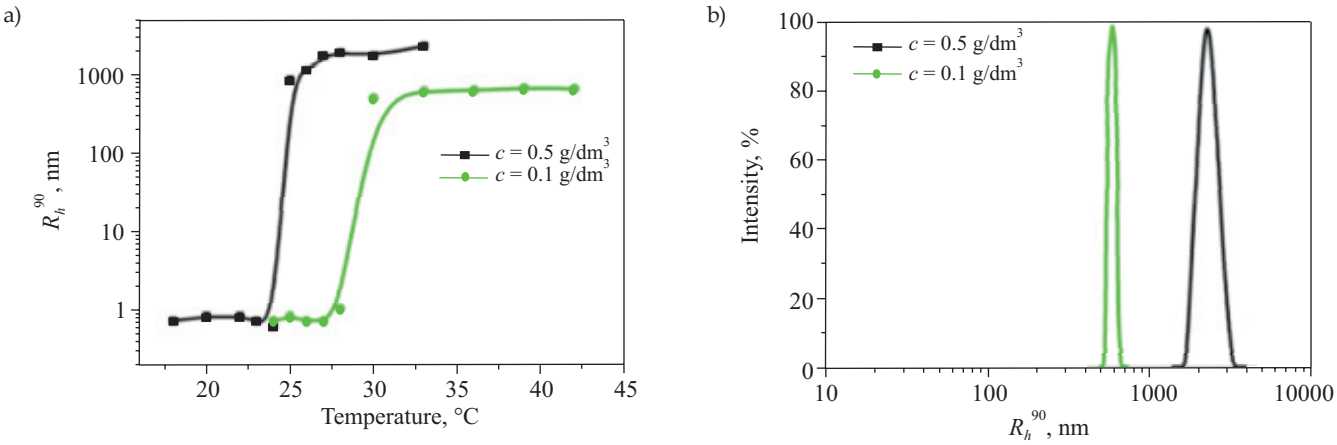

Fig. 4. Aggregation of OEGMAs: a) apparent hydrodynamic radius (R_h^{90}) as function of temperature for water solutions of P(TEGMA-EE), b) size distribution of formed mesoglobules

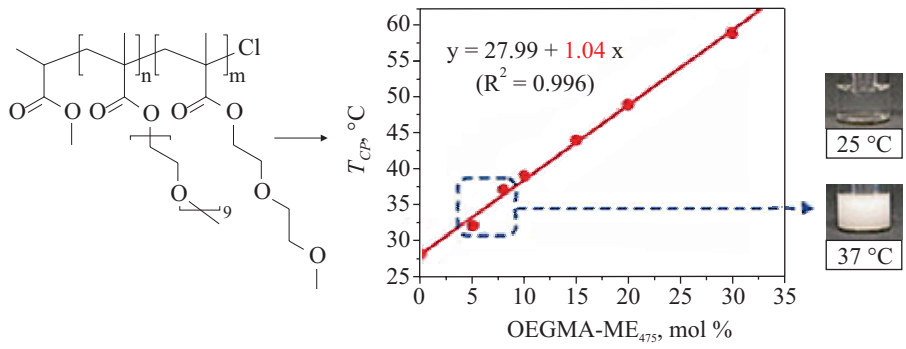

Fig. 5. Cloud point temperature T_{CP} of P[(DEGMA-ME)-*co*-(OEGMA-ME$_{475}$)] copolymers as function of OEGMA-ME$_{475}$ content; reprinted with permission from Lutz J.-F.: *Journal of Polymer Science Part A: Polymer Chemistry* 2008, *46*, 3459, copyright (2008) Wiley-VCH GmbH & Co. KGaA, Weinheim [20]

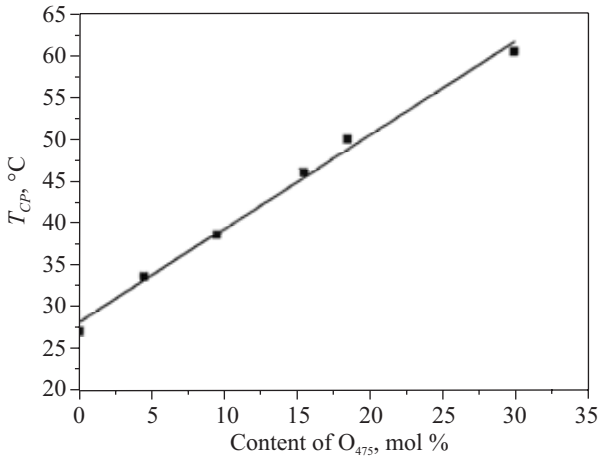

Fig. 6. Cloud point temperature T_{CP} of the copolymers P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)] as function of the content of OEGMA-ME$_{475}$; reprinted with permission from Trzebicka B., Szweda D., Rangelov S. *et al.*: *Journal of Polymer Science Part A: Polymer Chemistry* 2013, *51*, 614, copyright (2013) Wiley-VCH GmbH & Co. KGaA, Weinheim [39]

with increasing fraction of the more hydrophilic OEGMA-ME$_{475}$ comonomer and observed a linear increase of T_{CP} with increasing content of the more hydrophilic comonomer (Fig. 6).

Data concerning the solution behavior of POEGMAs, also their self-organization to nano/microparticles, multimicellar aggregates, vesicles, nano/microgels, core-shell particles, and thermogels are collected in Table 1.

Pasparakis and Alexander [40] studied double hydrophilic copolymers built from a poly(2-glucosyloxyethyl methacrylate) block and a thermoresponsive P(DEGMA-ME) block. The copolymers were synthesized using ATRP and RAFT (Fig. 7a).

The DLS measurements have shown that at 15 °C the copolymers existed in water solutions as single chains. At 20 °C the copolymers P1 and P2 (see Fig. 7b) organized themselves to vesicles of 251 nm and 500 nm diameter, respectively. At 37 °C the diameter of the vesicles decreased

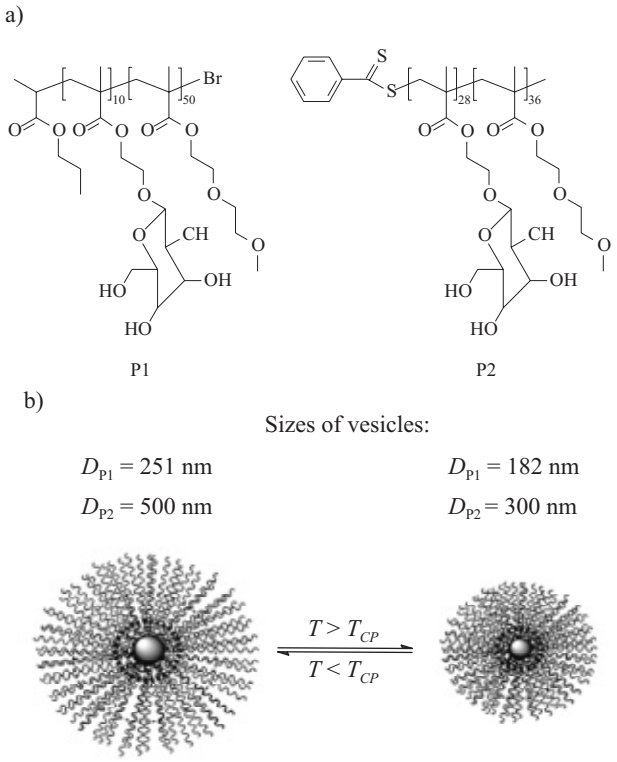

Fig. 7. Thermoresponsive block copolymers: a) synthesis of PGEMA-*b*-P(DEGMA-EE) using ATRP – P1 and RAFT – P2, b) thermoresponsiveness of homo- and copolymers, their self-organization and response of PGEMA-*b*-P(DEGMA-ME) vesicles to temperature stimulus

to 182 nm and 300 nm, what was assigned to the phase transition of the DEGMA-ME blocks.

Jin *et al.* [41] obtained a new photo- and thermoresponsive block copolymer, containing a poly(spiropyran-containing methacrylate) and a poly(DEGMA-ME) block. Changes of temperature and of the wavelength of incident light lead to formation of reverse micelles: micelles having a P(DEGMA-ME) or PSPMA block (Fig. 8). Obtained micelles were tested as smart carriers of a model compound – coumarin 102.

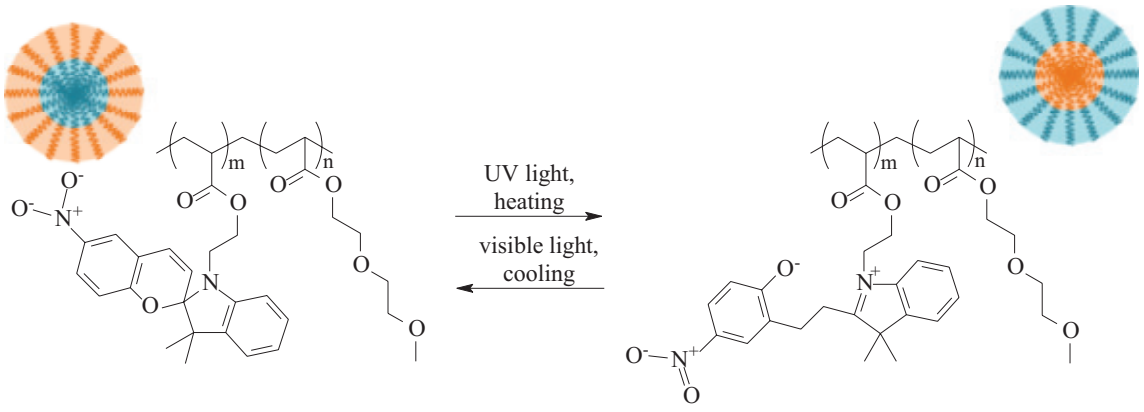

Fig. 8. Self-organization of the block copolymer PSPMA-*b*-P(DEGMA-ME) to micelles in water solution under the influence of light and temperature stimulus

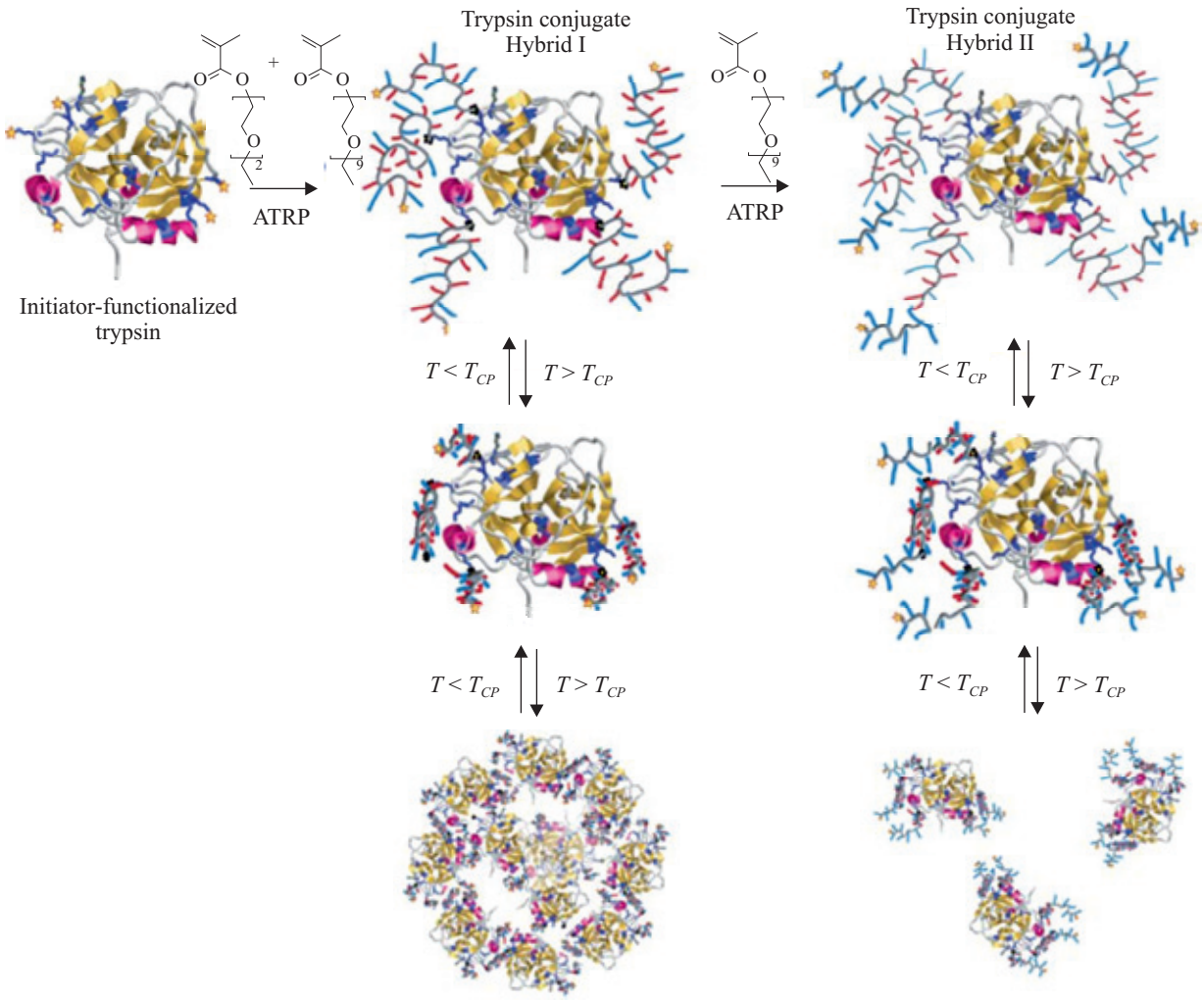

Fig. 9. Schematic structures and solution behavior of the conjugates trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)] (Hybrid I) and trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)]-*b*-P(OEGMA-ME$_{475}$) (Hybrid II); adapted from Yasayan G., Saeed A.O., Fernandez--Trillo F. *et al.*: *Polymer Chemistry* 2011, *2*, 1567 with permission of The Royal Society of Chemistry [42]

Thermoresponsive poly[oligo(ethylene glycol) methacrylate] bioconjugates

Polymer bioconjugates are intensively investigated because of their potential applications in biotechnology, medicine or nanotechnology. The use of conjugates of thermoresponsive polymers is of special interest, as such conjugates behave similar to biomolecules, intrinsically sensitive to temperature and other environmental stimuli. The linking of a thermoresponsive chain to a biomolecule opens also new possibilities to use the self-organization of such conjugates to construct nanocarriers.

The bioconjugates are available using two approaches: polymerization initiated by a modified biomolecule or coupling, covalent or *via* biorecognition.

Also the copolymers of oligo(ethylene methacrylate)s were used to build conjugates with biologically active molecules (Table 1).

A detailed study of thermoresponsive conjugates of trypsin was carried out by Yasayan *et al.* [42]. AGET ATRP of TEGMA-EE and OEGMA-ME$_{475}$ initiated by a modified trypsin made two bioconjugates available: trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)] and trypsin--P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)]-*b*-P(OEGMA-ME$_{475}$) (Fig. 8). The behavior of the conjugates in solution depended very strongly upon the ionic strength. The influence of temperature upon the morphology of obtained structures was studied by cryo-TEM and DLS. Below T_{CP} both conjugates did not aggregate and retained their spherical structure. As the temperature was increased above T_{CP}, so formed the conjugate trypsin-P[(TEGMA-EE)-*co*-(OEGMA--ME$_{475}$)] aggregates of R_h = 150 nm (where R_h is the hydrodynamic radius), while P[(TEGMA-EE)-*co*-(OEGMA--ME$_{475}$)]-*b*-P(OEGMA-ME$_{475}$) did not aggregate. The size of the last bioconjugate decreased from 5 nm to 4 nm, the decrease caused by the temperature induced transition of the P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)] block (Fig. 9).

Above T_{CP} the trypsin-P[(TEGMA-EE)-*co*-(OEGMA--ME$_{475}$)] conjugate precipitated, what caused a decrease of its enzymatic activity. To the contrary, the enzymatic activity of trypsin-P[(TEGMA-EE)-*co*-(OEGMA-ME$_{475}$)]-*b*--P(OEGMA-ME$_{475}$) conjugate increased.

Szweda *et al.* [43] obtained novel polymeric nanocarriers of a therapeutic peptide, the *met*-enkephalin, *via* thermal

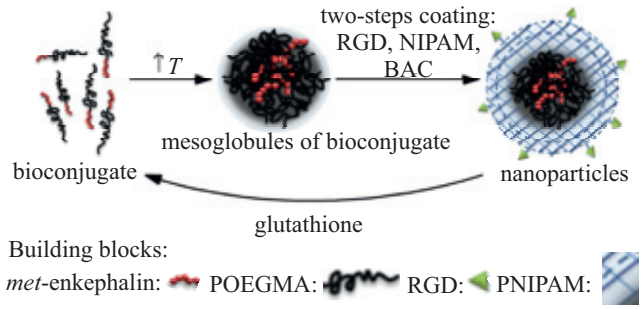

Fig. 10. Synthesis of degradable thermoresponsive nanocarriers of *met*-enkephalin equipped with the tropic peptide RGD; adapted with permission from Szweda R., Trzebicka B., Dworak A. *et al.*: *Biomacromolecules* 2016, *17*, 2691, copyright (2016) American Chemical Society [43]

aggregation of thermoresponsive bioconjugate. The thermoresponsive bioconjugate P[(DEGMA-ME)-*co*-(OEGMA-ME_{300})]-*met*-enkephalin-dansyl was synthesized using AGET ATRP applying a modified *met*-enkephalin as initiator. The thermally induced aggregation of the bioconjugate in presence of SDS, a surfactant, led to small nanoparticles, the mesoglobules of *ca.* 30 nm diameter. These nanoparticles were covered with a PNIPAM shell cross-linked with a degradable *N*,*N'*-bis(acryloyl)cystamine (BAC), containing disulfide bridges. During the formation of the shell RGD, a tropic peptide, was attached (Fig. 10). The authors studied the degradation of obtained nanoparticles in the presence of glutathione at pH = 7. The particles disintegrated to single polymer chains within 20 hours.

Krishna *et al.* [44] observed a strong influence of temperature upon the structure of particles obtained from a triblock bioconjugate, where the middle block consisted of a collagen-like peptide [$(GPO)_7GG$] and side blocks of P(DEGMA-ME). Using microscopy the authors followed the influence of temperature upon the morphology of the particles (Fig. 11).

Below 35 °C the single macromolecules of the bioconjugate of chain-rod-chain structure were observed. At 35 °C the bioconjugate formed spherical vesicles of *ca.* 30 μm diameter. An increase of the temperature to 65 °C caused the formation on empty spherical structures of *ca.* 100 μm diameter. Above 75 °C the macromolecules rearranged themselves to fiber-like structures. Lou *et al.* [45] observed a similar behavior of the diblock bioconjugate $(GPO)_7GG$-P(DEGMA-ME). Above T_{CP} of the P(DEGMA-ME) block, vesicles of 50–200 nm diameter were formed, stabilized by a hydrophilic shell of the peptide block.

CONCLUSIONS

The variety of available or easily accessible monomers make the polymers of oligo(ethylene glycol) methacrylates a flexible material for designing macromolecules exhibiting thermoresponsiveness, good biocompatibility, capable of self-organization to particles of desired sizes and stabilities. They may easily be linked with biologically active species, thus opening the route to a promising class of carriers.

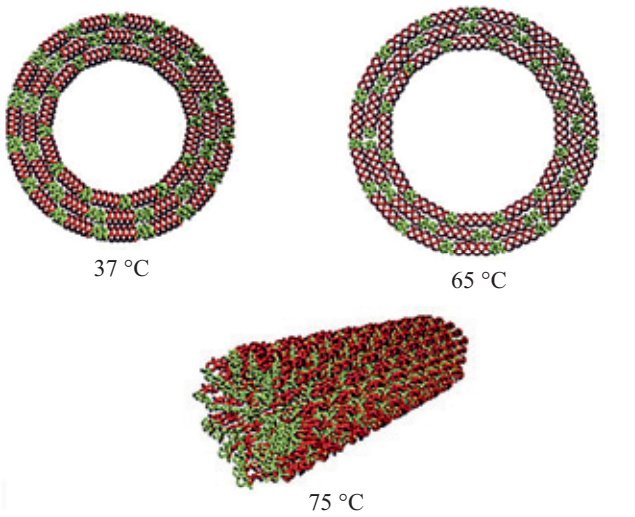

Fig. 11. Self-organization of the bioconjugate P(DEGMA-ME)-$(GPO)_7$GG-P(DEGMA-ME) to different structures at 37, 65 and 75 °C; adapted from Krishna O.D., Wiss K.T., Luo T. *et al.*: *Soft Matter* 2012, *8*, 3832 with permission of The Royal Society of Chemistry [44]

The concise review of such conjugates, presented in this paper, may be of help when planning experiments or research tasks in this area.

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