Digest Journal of Nanomaterials and Biostructures Vol. 1, No. 4, December 2006, p. 129 - 138

BIOMEDICAL APPLICATIONS OF POLY[(N-ACYLIMINO)ETHYLENE]S. GELS, INTERPENETRATING POLYMER NETWORKS

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The synthesis and characterization of smart or multifunctional polymers is a domain that received considerable attention worldwide from both academic and industrial communities. This paper describes some new polymeric materials envisaging biomedical uses, designed with the contribution of poly[(N-acylimino)ethylene] (PROZO) macromonomers, pointing on hydrogels synthesis and characterization. They were prepared by the copolymerization of methyl methacrylate/2-hydroxiethyl methacrylate (HEMA) and poly[(N-acetylimino)ethylene] (PMOZO) or poly[N-propionylimino)ethylene] (PEOZO) multifunctional macromonomers of unsaturated polyester type. Some preliminary data on thermosensitive poly[(N-acylimino)ethylene]/poly(2-hydroxiethyl methacrylate)/ poly(N-isopropylacrylamide) (PNIPAM) gels are included. The structure and properties of the prepared hydrogels were characterized by elemental analysis, differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA) and electron microscopy. Equilibrium swelling experiments were also performed. PROZO-PMMA/polyurethane and PROZO-PHEMA/polysiloxane interpenetrated polymer networks (IPNs) were obtained by sequential interpenetration alternative, using the developed synthetic route for PROZO/PMMA and PROZO/PHEMA gels in the first reaction stage. The possible applications of these polymeric materials in the biomedical domain are mentioned considering the evidenced peculiarities and their non/low toxicity.

Keywords: Functional polymers, Gels, Copolymers, Interpenetrating polymer networks

1. Introduction

Poly(2-alkyl-2-oxazoline)s have captured the scientific attention for a long time, both from theoretical and applicative point of view, due to the high reactivity and versatility of 2-alkyl-2-oxazoline (ROZO) monomers, which give rise to a large range of reactions: living cationic polymerization, zwitterionic copolymerization, coupling reactions [1-3]. They have as result a large number of new compounds which may be exploited in various applications: nonionic emulsifiers, adhesives, dispersants, controlled drug delivery systems, hybrid enzymes, blood anticoagulants, antibacterial agents, bioseparation systems etc. Most of the studies carried out since their first synthesis, reported in the 1950's, focused on the kinetic and polymerization mechanism as well as on the structure and properties of linear homo- and copolymers, functional derivatives, polymers with a complex structure (hiperbranched polymers, liquid-crystalline polymers etc.), gels. However, relatively few studies have been carried out on interpenetrated polymer networks (IPNs) based on PROZO [4].

In the following we present some results on the preparation of new functional materials based on poly[{N-acylimino)ethylene] macromonomers [5, 6], pointing on the preparation of (multifunctional) polymer gels and their use in the synthesis of IPNs. The aim of our research was to find polymer materials with new performances, which make them able to be used in the biomedical area. Emphasizing the synthesis of tailor–made materials, detailed studies on the effect

of the composition and structural features of the formed polymers on their properties were performed.

2. Experimental

Materials

2-hydroxiethyl methacrylate (HEMA, Aldrich) and methyl methacrylate (MMA, Merck) were purified by vacuum distillation. N-isopropylacrylamide - NIPAM (Aldrich) was recrystallized from benzene/n-hexane mixture. α, α'-Azoisobutyronitrile (AIBN, Merck), used as initiator, was purified by recrystallization from methanol just before use. N,N-dimethylformamide (DMF, Merck) was dried by usual methods, vacuum distilled and then stored on molecular sieves. Ethanol, methanol, anhydrous diethyl ether, acetone, solvents of analytical grade, were used as received. Tin octanoate (Aldrich), crosslinking catalyst, was used without further purification. The PROZO macromonomers, poly(ester-urethane) (PU) and poly(ester-siloxane)urethane (PUS) were synthesized according to literature data [5, 6, 7].

Gels synthesis

Hydrogels were obtained by the free–radical polymerization (precipitation technique) of selected vinyl monomers and PROZO macromonomer, which acts also as a crosslinker, in ethanol, at 60°C, for 20h, under inert atmosphere (Ar), with 2 % molar AIBN relative to total monomer. The crude products were washed with diethyl ether, acetone, stored in vacuum, weighed, washed with methanol and water to remove unpolymerized monomer or macromonomer and water-soluble fractions. After drying, the resulted gels were subjected to characterization.

Preparation of interpenetrating polymer networks

The IPNs based on PROZO were obtained by sequential interpenetration alternative, i.e., a mixture of PMOZO/PMMA gel (12.5 wt% PMOZO, DP_{PMOZO}=20), partially crosslinked polysiloxane prepolymer (gravimetric ratio polysiloxane: PMOZO/PMMA gel=70:30) and crosslinking catalyst (tin octanoate), in chloroform, was cured at 60ºC.

The preparation of semi-interpenetrating polymer networks (s-IPNs) based on PROZO and polyurethane involves the radical copolymerization of the PEOZO multifunctional macromonomer (DP_{PEOZO}=14,4; f=88%) with MMA (initiator: AIBN; DMF, 50ºC, 24h) in the presence of partially crosslinked poly(ester-urethane) (PU) or poly(ester-siloxane)urethane (PUS), followed by a curing at 70ºC, for 24h and 4h at 120ºC, in vacuum. In a typical recipe 10g PU /PUS and 30 ml DMF were mixed and purged with inert gas (N_2). 1.12g PEOZO multi-functional macromonomer were added and the mixture was stirred for 3h in inert atmosphere. Then MMA (1.2 ml) and AIBN (0.02g) were added to the resulted clear solution. The reaction mixture was maintained at 50°C, under stirring, for 24h, when the s-IPN/DMF system was poured in Petri glass wears and cured for 24h at 70ºC, under air flow and 4h at 120ºC, in vacuum.

Characterization

The characteristics of the PROZO macromonomers (polymerization degree, functionalization efficiency) were obtained from ^{1}H-NMR spectra, performed with a Bruker AC250 apparatus.

The IR spectra were recorded with a Specord M 90 instrument. The thermal behavior of the samples was determined using a Mettler Toledo Star System.

The morphology of the synthesized gels and IPNs was studied by transmission electron microscopy (TEM) with a TESLA-BS-513 apparatus or by scanning electron microscopy (SEM) using a Jeol JSM-5400 scanning electron microscope. The samples for TEM observation were film

casted from their diluted solution in appropriate solvents on carbon or collodium covered grids. For SEM investigation the samples were used after platinum coating for 30 s.

Swelling experiments

Disks of 10 mm in diameter (~ 0.6g) were cut and subjected to swelling investigation. The equilibrium swelling ratio α_e was defined as the weight of the absorbed water (W_{H2O}) per weight of dried gel (Wp). Equilibrium swelling weights for the gels in water at various temperatures and time periods were measured gravimetrically after wiping carefully excess water from the gel surface using filter paper.

3. Results and discussion

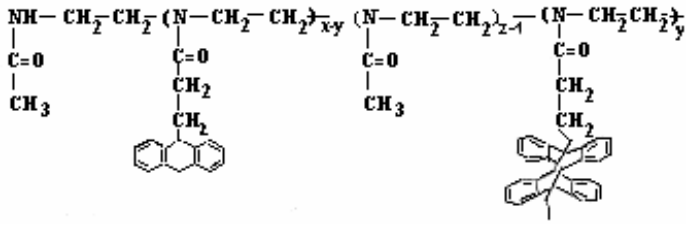
PROZO based hydro- and amphigels

The inclusion of multifunctional macromers of UPE type in a copolymerization system together with appropriate monomers yielded hydro- or amphigels.

Hydrogels are three-dimensional hydrophilic polymer networks capable of imbibing a large amount of water or biological fluids, yet insoluble in water, but swellable when immersed. The numerous applications of the hydrogels, particularly in medical and pharmaceutical areas (microbiological culture media, drug delivery systems, components of biomedical devices, i.e. as hemodialysis membranes), emerged in an increased interest for the synthesis and characterization of such materials, if possible with controlled properties.

Poly[(N-acylimino)ethylene]s as components of gels offer the possibility of tuning properties depending on the substituent of the N-acyl group, which can induce hydrophilicity or hydrophobicity of the resulted material. Different crosslinking approaches were applied [1-3]. Usually, the gelation was achieved by: 1) PROZO modification (i.e. by partial hydrolysis) followed by a crosslinking reaction of the functional prepolymers with polyfunctional compounds; 2) random copolymerization of 2-substituted-2-oxazolines with bisoxazoline monomers; 3) specific reactions of functionalized PMOZO (i.e. photodimerization of photosensitive pendant groups or coordination of metal ions to reactive inserted groups [8]). Recently Rueda and coworkers [9, 10] reported the synthesis of new hydrogels by the copolymerization of PMOZO bis(macromonomers) with N-vinylpyrrolidone or by the initiation of the copolymerization of the MOZO and bisoxazoline with a "macroinitiator", consisting of a random copolymer of chloromethylstyrene with styrene or methyl methacrylate.

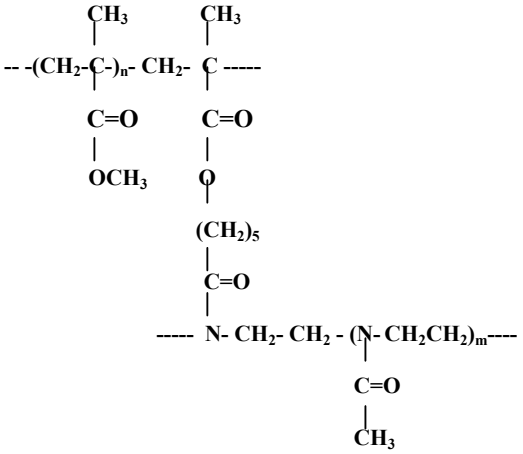
Considering the literature data we have prepared first PMOZO hydrogels (**1**) by the UV irradiation of presynthesized or *in situ* formed random copolymers of 2-methyl-2-oxazoline and 2-(9-anthrylethyl)-2-oxazoline (AEOZO) [11], the crosslinking being the result of a photodimerization process of the anthryl substituent groups [12].



1

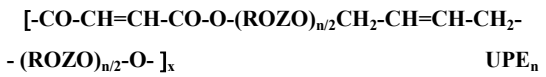
Amphigels with the structure **2** (δ = 10.61-11.47 $(cal/cm^3)^{1/2}$), characterized by a relatively high equilibrium sweeling degree, both in water (~ 80%) and in organic solvents (acetone ~ 370 %, ethanol – 420 %), were prepared by photocopolymerization of a bifunctional monomer, 2-(5-

methacryloyl–penthyl)-1,3-oxazoline [13], with MMA, followed by a subsequent copolymerization with MOZO [14].



2

However, the mentioned synthetic routes gave materials with a random structure and composition, a better design of this materials being required in order to obtain a controlled response. With this aim we used in the synthesis of gels the multifunctional PROZO macromonomers **3** with an unsaturated polyester structure (UPE type) [5].



3

Two different methacrylic monomers were used: HEMA and MMA. Their free radical copolymerization with the multifunctional PMOZO macromonomer UPE_{21} gave generally crude products in a high yield (80-95 %). The content in acrylic units was slowly higher, due to the higher relative reactivity of the comonomer as compared to PMOZO macromonomer. The nearly similar com-position with the comonomer mixture composition in the feed is in accordance with the high yield of the reaction. Tables 1 and 2 summarize the copolymerization data.

Table 1. Copolymerization data for the multifunctional PMOZO macromonomer – HEMA system

No.	$HEMA_0$ $PMOZO_0$+ $HEMA_0$	M[a]	3[b]	S	PHEMA[c] PMOZO+ PHEMA in copolymer
	g/g	ml	g	ml	g/g
1	0.75	0.6	0.2	3.0	0.75
2	0.66	0.4	0.2	2.1	0.65
3	0.50	0.4	0.4	1.6	0.60
4	0.33	0.1	0.2	0.7	0.20

a – monomer M – HEMA

b – PMOZO macromonomer of UPE type (DP_{PMOZO}=21, Mn = 2025)

c – from elemental analysis (N%) data

Table 2. Copolymerization data for the multifunctional PMOZO macromonomer – MMA system.

No.	$\frac{MMA_0}{MMA_0+PMOZO_0}$ g/g	M[a] ml	3[b] g	S ml	$\frac{PMMA^c}{PMMA+PMOZO \text{ in copolymer}}$ g/g
5	0.00	-	0.4	0.2	0.00
6	0.15	0.1	0.5	0.6	0.20
7	0.25	0.2	0.6	1.3	0.35
8	0.35	0.2	0.4	1.3	0.50
9	0.50	0.4	0.4	2.7	0.65
10	0.67	0.6	0.3	4.0	0.80
11	0.80	0.6	0.2	4.1	0.87
12	1.00	0.4	-	2.7	1.00

a – M-MMA
b – PMOZO macromonomer of UPE type (DP_{PMOZO}=21, Mn = 2025)
c – from elemental analysis (N%) data

The composition of the final gel is that calculated for the extracted samples. As can be seen from the data included in Table 3, a relatively large amount (10-50 wt%) was removed by washing with methanol and water, suggesting that the initial product is like a semi-interpenetrated polymer network, due to the presence of water-soluble components, i.e. unpolymerized PMOZO macromonomer and water-soluble copolymer fractions. The content in PMOZO in the final product is diminished (Table 3). As an example, for the samples synthesized by the copolymerization of MMA with UPE type macromonomers with a DP_{PMOZO} of 7, 21 and 40 the content in PMOZO after extraction was modified from 50 wt% to 14 wt%, 13 wt% and 15 wt%, respectively.

The composition of the resulted gels was also confirmed by the IR investigation data and by the thermal behavior.

Table 3. PMOZO/PHEMA copolymer composition before and after washing with water and methanol.

Code	η[a] %	Weight loss %	$\frac{PHEMA^b}{PMOZO+PHEMA \text{ in copolymer}}$ g/g before extraction	g/g after extraction
1	95	33	0.83	0.75
2	90	45	0.76	0.65
3	87	60	0.50	0.60
4	75	65	0.39	0.20

a – global yield before washing with water or methanol
b – from elemental analysis data (N%)

The IR spectra include signals specific to PMOZO sequences (1650 cm^{-1}, tertiary amide groups) and to the polyvinyl sequences (1730 cm^{-1}, esteric group).

By comparison with the original components the TGA plots are modified, as shown in Fig. 1. For the PMOZO/PMMA as for the PMOZO/PHEMA gels three degradation stages can be observed: 100-250 °C (5-15 % - water evaporation), 250-380 °C (~ 60 % - scission of the substituent groups) and 400-500 °C (20% - chain degradation and crosslinking side reactions)

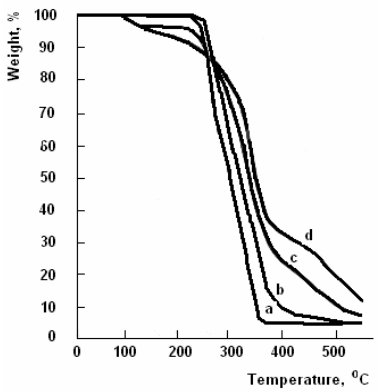


Fig 1. TGA plots for : a – PMMA, b – PMOZO, c – sample 7 and d –sample 10 (Table 2).

The properties of the synthesized gels (i.e. flexibility, swelling behaviour) are dependent on comonomer nature, on composition and on the dimension of the PMOZO inserted sequences, which are modeling the shape and area of the separated microphases and the crosslinking density.

For PMMA/PMOZO gels two distinct transition glass temperatures can be observed, suggesting a phase separation in the material; as for the PMOZO/PHEMA gels, two Tg values were registered for the crude products and only one Tg was evidenced for most final, extracted samples (Fig. 2, Table 4). The forced compatibilization in the last case is the result of the dense packing of the network in the final gel and is facilitated by the presence of hydroxyl groups in the methacrylic moieties. The two Tg values in the original samples are in agreement with a structure of semi-interpenetrated polymer network type, as mentioned before.

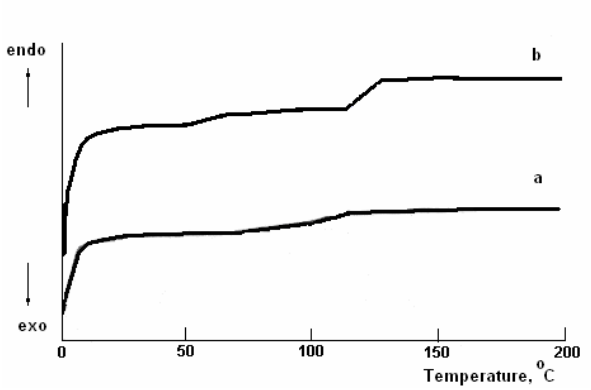


Fig. 2. Typical DSC plots for: (a) PMOZO/PHEMA hydrogel (sample 2) and (b) PMOZO/PMMA amphigel (sample 9).

Table 4. Thermal behavior of the PMOZO/PHEMA gels.

Sample	**Tg**	
	before extraction	**after**
	°C	
1	70, 130	110
2	75, 118	105.5
3	82	93
4	68, 118	80, 110

PMOZO/PMMA gels are swelling in both water and methanol. In methanol the α_e values are higher with the increase of the DP_{PMOZO} in the macromonomer, i.e. 20 % for a DP_{PMOZO} of 7, 31

% for DP_{PMOZO} of 20 and 40 % for a DP_{PMOZO} of 40. The same behavior can be observed in water (Figure 3). It is explained by a lower crosslinking density for higher polymerization degrees of the PMOZO inserted sequences (Tables 5 and 6).

The use of PROZO macromonomers with a controlled structure in the synthesis yielded an ordered microphase separated morphology [15].

Thus, materials with prerequisite properties can be obtained by an appropriate selection of the comonomers, recipe and synthesis conditions.

Table 5. The swelling behavior of the PMOZO/PMMA amphigels as a function of composition

PMMA PMMA+ PMOZO g/g	0	0.2	0.35	0.5	0.8	0.86
α_e in methanol (%)	140	260	130	120	55	30

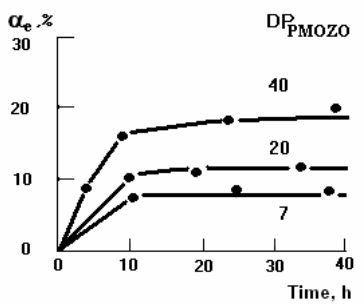


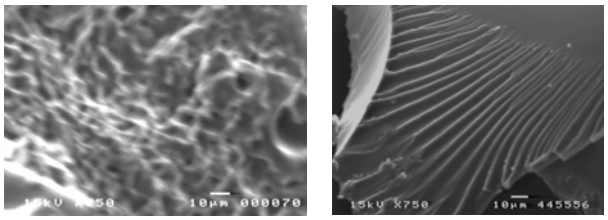
Fig. 3. Variation of the equilibrium swelling degree in water for the PMOZO/PMMA gels with the length of the inserted PMOZO sequences (PMOZO content ~ 15 %).

Table 6. Equilibrium swelling degree of PMOZO/PHEMA hydrogel samples in water.

PHEMA PHEMA+ PMOZO g/g	0.75	0.65	0.60	0.20
α_e (%)	100	85	70	350

The amphigels based on PMOZO are known as appropriate materials for membranes of high selectivity. On the other hand, taking into account the facile modification of the two sequences by hydrolysis yielding to the generation of different reactive, pH sensible groups, able to immobilize biocompounds (NH- and -COOH), we may easily imagine the application of such materials with tailored architecture in the controlled, selective release/capture of substances (e.g. for environmental reasons, or drug delivery) as response to external stimuli (e.g. pH).

The copolymerization of UPE type macromonomers (with PMOZO or PEOZO chains, named UPE^M and UPE^E, respectively) with N-isopropylacrylamide in the presence or absence of HEMA was also performed (weight ratio 1:1, and respectively 1:1:1). It was found that all samples present thermosensitivity, i.e. LCST in the therapeutic domain (27.5-38.0ºC). The SEM observation evidenced a porous, ordered structure, with nearly uniform large channels, which is expected to give rise to an improved response rate (Figure 4). The ordered structure was attributed to a self-assembling process of the initial formed microgel particles (narrow size distribution, core-shell structure).



a b

Fig. 4. Typical SEM micrographs for: (a) PMOZO/PHEMA gel (sample 3) and (b) PEOZO/PHEMA/PNIPAM gel.

As compared to PNIPAM gel they are characterized by a lower toxicity level, multifunctionality (thermo-sensitivity, HO- reactive groups) and faster response. Such multifunctional, smart materials may be used as a polymer support in controlled drug delivery systems, for sensor or display technologies. Further characterization studies are in progress.

Interpenetrating polymer networks

Our goal was to develop, based on the synthesized thermoplastic gels, biocompatible materials possessing good physico-mechanical properties. With this aim we have added polysiloxane or polyurethane to the mentioned PMOZO/PMMA gels, which are brittle materials, in order to form a (semi-) interpenetrating polymer network (s-IPN, IPN) structure, with an improved morphology together with thermoplastic processability. An IPN is an intimate combination of two polymers, both in network form, one of which is synthesized and/or crosslinked in the immediate presence of the other [16]. The forced compatibilization of both polymers gives the possibility to obtain (especially) an improved mechanical behavior and an increased functionality of the resulted complex material. The most popular and well-studied system of this type is cross-polyurethane-inter-cross-poly(methyl methacrylate) (PU-PMMA) [17]. As far as we know, there is only one earlier report focusing on the synthesis of such materials having as one component polyethylenimine derivatives. It deals with the preparation of a simultaneous semi-interpenetrating polymer network of crosslinked PMMA and poly(2-benzyl-2-oxazoline) [4], by a solvent – free reaction process. Note that PEOZO and PMOZO are immiscible with PMMA in their blends, but their amphipathic copolymers may act as compatibilizers [1-3]. PMOZO/PMMA gel (12.5 wt% PMOZO), OH functionalized polysiloxane (gravimetric ratio polysiloxane:PMOZO/PMMA gel=70:30) and crosslinking catalyst were cured together. The resulted crosslinked casted film is soft, flexible, transparent, permeable to oxygen, with improved wetting ability (swelling degree of 6 % in water and of 56 % in ethanol). The maintenance of the transparency in the interpenetratred polymer network was explained by the compatibilization of the two materials (a single glass transition, i.e. Tg = -82.5 ºC), confirmed also by the structure visualised by TEM (Figure 5), which evidenced a mutually relative uniform distribution of the implied polymeric materials at a submicronic level. The forced compatibilization due to the interpenetration of the polymer networs is favored by the presence of OH groups in the polysiloxane moieties, giving rise to hydrogen bonds development. In accordance with the optical transparency it was found that the polysiloxane sequences are partially crystallized, a melting peak being observed in the registered DSC plot at –47.5 ºC. Thus, optimized materials may envisage the use for optical lenses or controlled drug delivery systems.

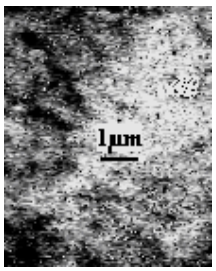


Fig. 5. TEM micrograph of the PSiO/PMMA-PMOZO interpenetrating polymer network.

A semi-interpenetrated network was also prepared by a sequential interpenetrating method involving the radical copolymerization of the PROZO multifunctional macromer with MMA in the presence of polyesterurethane (PU) or poly(ester-siloxane)urethane (PUS), followed by curing to achieve networks stability. A poly[(N-propionyl)ethylenimine] PEOZO macromer with a structure of unsaturated polyester was selected, considering the advantages offered by the lower Tg comparative to PMOZO, and the specific thermosensitive properties (it possesses a LCST at 36ºC), complementary to its biocompatibility. The interpenetration of the the elastomer chains with the PEOZO/PMMA network yielded an increased flexibility, as can be seen in Figure 6.

The peculiar mechanical features and the low toxicity recommend such materials for surgical dressing and blood vessels.

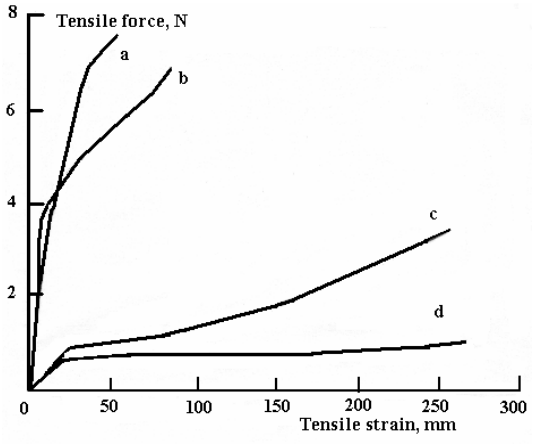


Fig. 6. Stress vs strain diagrams for:a - PU; b - PUS; c - PUS:PEOZO/PMMA (80:20); d - PU: PEOZO/PMMA (80:20).

In conclusion, the versatility of PROZO macromonomers allowed the preparation of new smart polymeric materials. Their physico-mechanical characteristics together with the multifunctionality and low/no-toxicity recommend them for biomedical applications, like on-off switches for controlled drug release, immobilization of enzymes biosensors, biocompound separation and gene carriers.

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