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ORIGINAL CONTRIBUTION

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Polymer mediated peptide immobilization onto amino-containing *N*-isopropylacrylamide-styrene core-shell particles

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Abstract Monodisperse cationic core-shell latex particles have been prepared using a shot polymerization process, with *N*-(3-aminopropyl)-methacrylamide-hydrochloride (APMH) as the functional monomer. The final latexes were characterized with respect to final polymerization conversion, water soluble polymer formation, particle size and size distribution, surface charge density and electrokinetic properties. Then the covalent grafting of maleic anhydride-*alt*-methyl vinyl ether (MAMVE) copolymer onto aminated latex particles was investigated. The most efficient conditions to obtain derivatised particles with no alteration of the colloidal stability were to control both polymer amount/latex particles concentration ratio and the mixing method of the two species. The charge inversion of the hydrolysed MAMVE functionalized particles was demonstrated by measuring the electrophoretic mobility as a function of pH. Finally, the covalent binding approach was implemented with peptide-MAMVE conjugates, confirming the great potential of this promising methodology for the preparation of reactive latex particles bearing peptides.

Keywords Temperature sensitive · Core-shell latex · Reactive copolymer · Covalent coupling · Peptides

Introduction

Colloidal polymers such as polystyrene-based particles have largely been used as carriers for biomolecules (antibodies, antigens, enzymes, nucleic acids) in diagnostics applications. To avoid desorption of the adsorbed biomolecules from the solid phase, covalent grafting has long been investigated, leading to an irreversible binding of biological molecules onto functional particles [1]. The covalent immobilization is particularly well adapted for low molecular weight molecules such as peptides, which represent only the active part of the protein they are derived from. In this case, the immobilization process should occur with minimal alteration of the biorecognition properties of the peptide via a careful control of the site of occurrence of the grafting reaction. To achieve the control, specific reactions can be used such as described for chemical ligation of peptides [2]. Reactive groups such as aminooxy [3] or hydrazide [4] can be introduced on the peptide by solid phase synthesis and can react specifically with electrophiles.

Using standard amine chemistry, Ladavière et al. [5] controlled the site of reaction by taking advantage of attractive electrostatic interactions between the negative charges of poly(maleic anhydride-*alt*-methyl vinyl ether) (MAMVE), induced by hydrolysis during the chemical reaction, and a positively charged tag positioned at the N-terminus of the peptide. The so-called positive tag was composed of three contiguous lysine or arginine residues. This last approach was selected in the present work on the

basis of (i) a straightforward chemistry used leading to the formation of stable amide bonds, (ii) the observed increase in sensitivity when using polymeric bioconjugates [6].

As a matter of fact, in order to enhance their passive adsorption onto solid supports, peptides have previously been grafted onto polymers. For instance, Böcher et al. [7] reported the immobilization, via the ϵ-amino groups, of peptides onto periodate-activated dextran and the adsorption of the resulting conjugate to the inner wall of 96 well microtiter plates. A decrease of non-specific interactions was obtained. Taking advantage of the specificity of the reaction of thiols with maleimides, Gegg and Etzler [8] reported the directional coupling of peptides, bearing an N-terminal cysteine onto maleimide-derivatized polylysine. The adsorption of the peptide-polymer conjugate allowed an increase of the sensitivity and the specificity of Enzyme Linked Immunosorbent Assays (ELISA). In another field of application, to obtain imaging contrast agents, a peptide corresponding to a fragment of HIV-1 TAT protein was covalently linked to iodoacetamide-derivatized crosslinked dextrans deposited onto magnetic iron particles [9]. However, to our knowledge, no attempt at covalently binding polymer-peptide conjugates onto the solid support and mainly onto stimuli-responsive colloidal particles has ever been reported.

Thus, in order to allow the covalent linkage of the poly(maleic anhydride-*alt*-methyl vinyl ether)-peptide conjugate, an aminated colloidal dispersion was needed. Moreover, in order to reduce the non-specific interactions with proteins from the sample sera during the tests, particles had to feature a hydrophilic surface. Reactive polystyrene particles can be obtained with a great variety of functional groups as reported by Pichot [10] but the obtained interfaces still display an hydrophobic character when they are of an amine type [11]. Therefore, core-shell nanospheres bearing a hard polystyrene core and a fully hydrated shell, as reported by Duracher et al. [12], could meet the requirements for use as solid phases. The objectives of this work were (i) to prepare hydrophilic poly(styrene-*co*-*N*-isopropylacrylamide-*co*-*N*-(3aminopropyl)-methacrylamide) core-shell particles by emulsifier-free emulsion polymerization and (ii) to establish the MAMVE-mediated immobilization of peptides onto latex particles, the anhydride copolymer acting as a macromolecular binding agent.

The ultimate goal of this work is to develop a peptide based agglutination test for rapid and inexpensive diagnostic of Chagas disease.

Experimental part

Materials Water was of milli-Q grade (Millipore S. A. France) and was boiled for 2 h under nitrogen stream before use in the latex preparation. Styrene monomer (from Jansen) was distilled. *N*-Isopropylacrylamide (NIPAM) (from Kodak) was recrystallized from hexane/toluene (60/40 v/v) mixture. 2–2′azo-bis(2-Amidino propane) dihydrochloride (V50) initiator (from Wako) was recrystallized using water/acetone mixture (50/50 v/v). *N,N′*-methylenebiscacrylamide (MBA) (from Aldrich) was used as a crosslinker agent and *N*-(3-aminopropyl)-methacrylamide hydrochloride (APMH) (from Kodak) were used as received. *N*-Succinimidyl 3-(2 pyridyldithio) propionate (SPDP) (from Pierce), dithiothreitol (DTT) (from Aldrich), fluorescamine (from Aldrich) and poly(maleic anhydride-*alt*-methyl vinyl ether) (MAMVE, $M_W = 67{,}000$ g/mole) (from Polysciences Inc) were used without further purification. The peptide was obtained from Eurogenetec (Seraing, Belgium) and was used after dissolution in deionized water. The sequence H_2N-RRRSPPVSAPAKAAAPPAAARS AEPHVG-COOH corresponds to an antigenic domain of TC 40 protein of *Trypanosoma cruzi*, a parasite responsible for Chagas's disease. The first three arginine residues constitute the positively charged tag used to favour an oriented binding, as already reported [5].

Preparation of latexes All latexes were synthesized by soap free emulsion polymerization in a 100-ml thermostated glass reactor equipped with a glass anchor type agitator, condenser and nitrogen inlet. All polymerizations were carried out at 70 °C under stirring rate of 200 rpm. The polymerization conversion and water-soluble polymer formation were gravimetrically determined.

The batch polymerization was carried out using 50 g water, 5 g styrene, 0.5 g NIPAM and 0.05 g V50. The shot polymerizations were performed by adding an aqueous mixture containing (NIPAM, APMH, MBA and V50) of a given composition (see Table 1) to a batch styrene-NIPAM copolymerization when the overall conversion had reached less than 60%. The polymerization protocol was adapted from Duracher's work [12].

Latex characterization Prior to the colloidal characterization, latexes were cleaned by repetitive centrifugation and redispersion cycles. The hydrodynamic particle sizes were measured using quasi-elastic light scattering (Zetasizer 3000 HS, Malvern Instrument). The transmission electron microscopy (TEM, Hitachi S 800, CMEABG at Claude Bernard University, Lyon I, France) was used to examine the particles morphology and particle size distribution.

The surface charge densities of latexes were determined using SPDP (3-(2 pyridyldithio) propionic acid *N*-hydroxysuccinimide ester) and fluorescence methods as described in [13]; the principles of both chemical titration methods are depicted in the reaction scheme (Fig. 1).

Both methods are based on the chemical reaction between the amine groups onto the aminated latex particles and either 3-(2 pyridyldithio) propionic acid *N*-hydroxysuccinimide ester or fluorescamine. After reaction of SPDP onto the latex particles, and washings to remove the excess reagent, a subsequent reduction of the disulfide bond by Dithiotreitol allowed quantification.

Fluorescamine becomes fluorescent on reaction with a primary amines; thus, titration of the amount of functional monomer remaining in the continuous phase can be achieved. Then, by

Table 1 Experimental conditions for the preparation of functionalized core-shell latexes via shot process. The shots were performed above 60% batch conversion

P(St/NIPAM/APM)	CL-04/CL-05/CL-06	CL-07
NIPAM (g)	1.267	0.500
MBA (g)	0.017	0.017
APMH (wt% to NIPAM)	4/5/6	10
V50 (g)	0.030	0.025

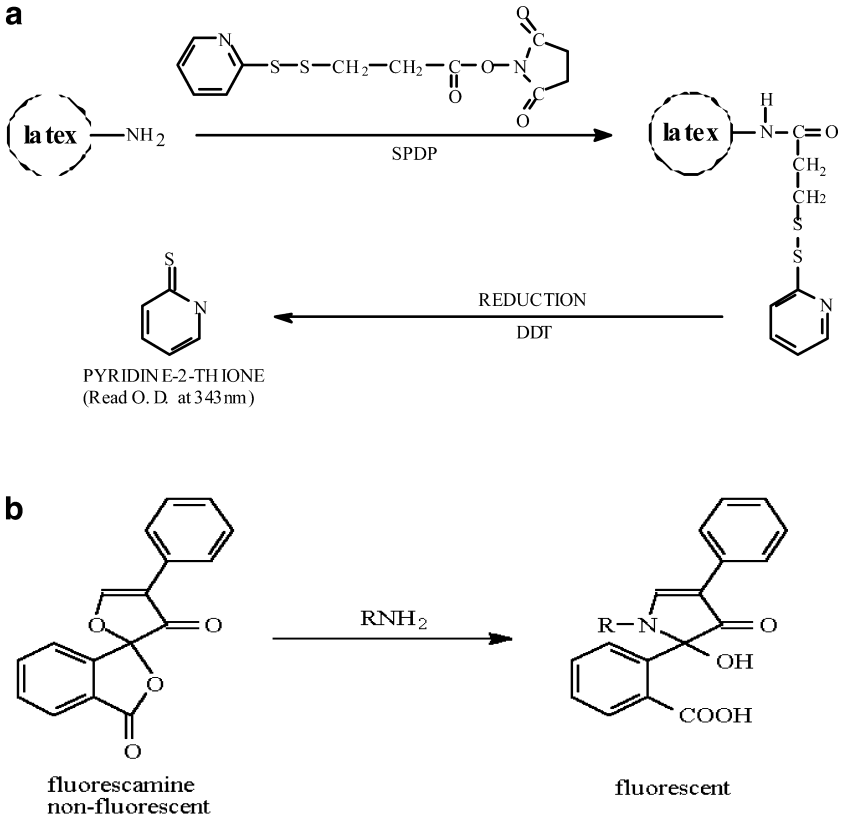

Fig. 1 Surface charge density determination using: **a** SPDP titration; **b** fluorescence quantification of amine groups methods (DDT: dithiotreitol)

difference, the amount of functional groups at the surface of the particles can be calculated.

The electrophoretic mobility measurements were performed (using Zetasizer 3000 HS) in 10^{-3} mol/l NaCl solution as a function of pH.

Covalent grafting of maleic anhydride-alt-methyl vinyl ether copolymer onto latex particles The covalent coupling of copolymer MAMVE onto the amino-functionalized latex particles is based on the chemical reaction between the available amino groups on the particles and the anhydride groups of the copolymer. The principle of the covalent reaction is described in Fig. 2.

Covalent grafting of maleic anhydride-alt-methyl vinyl ether copolymer-peptide conjugates onto latex particles Peptide-MAMVE copolymer conjugates were obtained by reacting 72 μmol/l of peptide in 10 mmol/l sodium phosphate buffer pH 6.8 in the presence of MAMVE at 0.72 μmol/l (final concentrations). The polymer was dissolved in DMSO and the final amount of DMSO in the coupling medium was 5 vol.%. After 10 min reaction time, half of the sample was quenched with excess ammonia in order to calculate the coupling yield of peptide to the polymer as reported in [5] to be used as a reference for the determination of the grafting efficiency. To the other half (500 μl), a mixture of 280 μl of 0.5% solids of latex and 720 μl of buffer were added. Incubation time was 3 h at 37 °C.

The conjugates were analysed by Size Exclusion Chromatography using an Ultra-Hydrogel column UH 500 (Waters Corporation, Milford), a Kontron HPLC 422 pump, a Kontron HPLC autosampler 465 and a Kontron UV Diode Array detector (Kontron Instrument, Saint Quentin en Yvelines, France). Chromatographic analyses were run in a 0.1 mol/l phosphate buffer, pH 6.8, at a flow rate of 0.5 ml/min. Detection was achieved by measuring the absorbance at 220 nm wavelength corresponding to the peptide. The grafting yields (Y%) were determined from the ratio 'R' of the area of the peak of the conjugate in the supernatant to the area of the peak of the reference conjugate using Eq. (1) [5]:

$$Y\% = (1\ R) \times 100 \quad (1)$$

Results and discussion

In this work, the following strategy was used. First, the synthesis of amino-functionalized core-shell latexes was investigated. Then, after colloidal characterization (particle size distribution and interfacial charge density) the reactivity of these particles toward poly(maleic anhydride-*alt*-methyl vinyl ether) (MAMVE) was studied and analysed in terms of colloidal stability and interfacial charge modification. Finally, the polymer mediated binding of peptides was undertaken.

Synthesis and colloidal characterization of the latex particles

Poly(styrene-*co*-*N*-isopropylacrylamide) core-shell particles were prepared by soap-free emulsion polymerization using a two-step shot process. First, a batch polymerization was carried out and, after a polymerization time of 4 h, a mixture of (NIPAM, MBA,

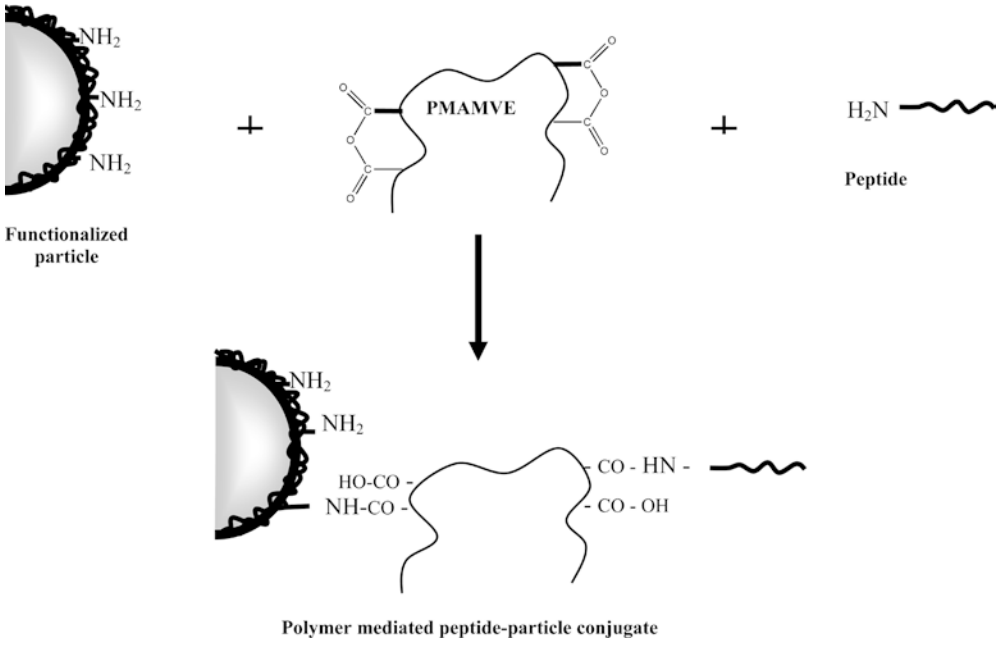

Fig. 2 Schematic representation for the chemical grafting process of peptide onto latex particle via the reactive copolymer (MAMVE)

APMH, V50) was added to allow the formation of the functionalized hydrophilic shell (Table 1).

Particle size analysis

All latexes were visualized by TEM and found to be spherical in shape and narrowly size distributed with low polydispersity indexes, between 1.004 and 1.01, as also reported by Duracher et al. [12] by using aminoethyl methacrylate hydrochloride (AEMH) as a functional monomer. Such behaviour reflects the absence of secondary nucleation during the polymerization processes. QELS was used to monitor the particle size change of the final latexes upon varying the temperature. The expected volume phase transition temperature was observed at around 32 °C with an amplitude between the shrunken and extended state, the hydrodynamic thickness of the thermally-sensitive shell part of the hydrogel shell, ranging from 20 nm to 80 nm.

As expected, particles CL-01 obtained by a single step procedure were smaller in diameter than CL-04 through CL-07 (Table 2) prepared from shot process. With increasing concentration of the functional monomer in the recipe (from CL-04 to CL-07), a decrease in the particle mean size was observed. Interestingly, the hydrodynamic thermally-sensitive thickness layer decreased as well but in a non-monotonous way. The decrease is quite sharp from the 70–80 nm range for CL-04 and CL-05 down to 23–26 nm range for CL-06 and CL-07. It is worth noting that the water soluble polymer content is much higher in the presence of the aminated monomer (an increase from 1.8 to ca. 13%).

Table 2 Final conversion and particle size determined by QELS

Latex code	APMH (w/w%)	Conversion (%)	WSP[a] (%)	$D_{20\,°C}$ (nm)	δ^e (nm)	σ^c μmol/g	σ^d μmol/g
CL-01[b]	0	85	1.8	343	27	16.8	0
CL-04[d]	4	93	ND	526	69	20.0	ND
CL-05[d]	5	99	14.0	469	79	20.7	55
CL-06[d]	6	85	14.0	394	26	35.2	78
CL-07[d]	10	88	11.0	358	23	26.6	52

[a]Water soluble polymer
[b]Batch polymerization
[c]Surface charge density determined using SPDP
[d]Shot polymerization
[e]Thermally-sensitive thickness layer $\delta = (D_{20\,°C} - D_{50\,°C})/2$
[f]Surface charge density determined by fluorescence methods
ND = Not determined

These results suggest that the APMH monomer, under the hydrochloride form, could act as a transfer agent as was observed by Meunier et al. [14] and by Duracher et al. [12] with the aminoethyl methacrylate hydrochloride (AEMH). Such a transfer reaction would cause the formation of shorter polymer chains as already discussed in [15]. When captured onto the particles, these oligomers led to the formation of a smaller hydrophilic thermally sensitive shell, explaining the decrease in diameter observed at 20 °C, when the hydrogel layer is extended. Moreover, the formation of shorter and charged oligomeric chains disfavoured their incorporation onto the particle as shown by the increased water

soluble polymer content observed for CL-05 through CL-07.

Surface charge density determination and electrophoretic mobility analyses

The use of SPDP, an agent that can react with the amino groups of APMH and the amidine groups of V50 (initiator), allows quantifying the total of available groups that provide charges to the surface. The amount of amine groups incorporated to the particles was determined indirectly by measuring the residual amine groups [NH_2] in the supernatant (fluorescence method). Thus, the fluorescence quantification method gave access to the amount of incorporated monomer, by difference from what was measured from the serum. In each case, the concentration of incorporated functional monomer was higher than that of available monomer obtained by the SPDP method, suggesting that some of the APMH in the shell layer of the particles might not be accessible for further reaction. The results obtained are reported in Table 2.

A thorough investigation, by atomic force microscopy and scanning electron microscopy, of the surface morphology of latexes obtained by the shot process, with AEMH as the functional monomer, performed by Duracher et al. [12], showed a raspberry-like structure of the surface. So, one can imagine that part of the functional monomer may be buried into the crosslinked poly(NIPAM) shell and, therefore, is not available for reaction with SPDP. Interestingly, the titration for a 4% AEMH monomer [14] and 4% APMH give similar results of 23 and 20 μmol/g respectively, confirming that both monomers have identical behaviour in the functionalization process.

Figure 3 shows the electrophoretic mobility variations as function of pH (0.001 mol/l NaCl) for the functional core-shell latexes. All the latexes displayed positive values of electrophoretic mobilities in the acidic region, which is evidence of the presence of cationic groups at the particle surface (amino and/or amidino groups). The isoelectric point (IEP) for amino-functionalized latex particles is close to 10, corresponding to the pKa of amine, but between 7 and 8 for latexes obtained without functional monomer, due to the presence of amidine groups [14, 16, 17]. The negative values of electrophoretic mobilities for pHs higher than 10 can be explained by the presence of anionic surface charges resulting from the hydrolysis of amidine groups [18].

Coupling of maleic anhydride-*alt*-methyl vinyl ether copolymer

The first approach consisted in reacting a polymer solution in DMSO with latex particles in various buffers.

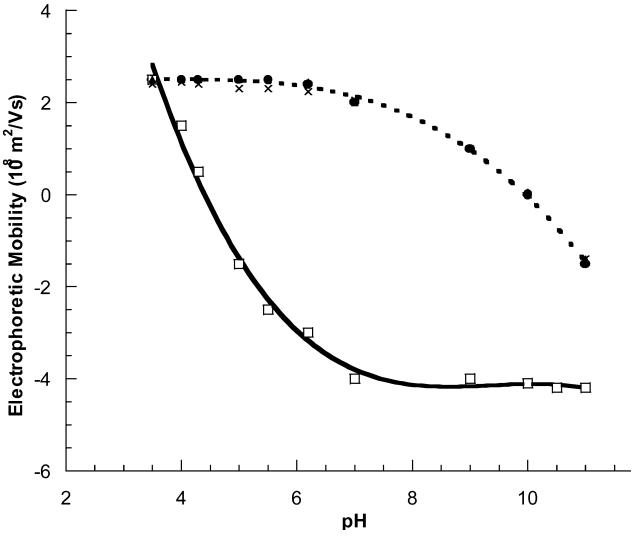

Fig. 3 Electrophoretic mobilities as a function of pH in 10^{-3} mol/l NaCl and at 20 °C of core-shell latexes (CL05(*crosses*), CLO6 (*dots*) and CL07 (*filled squares*) data fitted with *dashed line*) and CL07/MAMVE (*open squares*) fitted with *continuous line*

In 10 mmol/l buffers (sodium phosphate pH 6.8 or sodium borate 9.2), no flocculation of the latexes was observed, which was not the case with a 100 mmol/l buffer. On adding 25 μl of polymer solution in DMSO, at concentrations ranging from 5 to 30 g/l, to 475 μl of latex particles at 0.5% solids, the colloid flocculation was always observed. The reverse addition, colloidal particles to the polymer solution, led to better results. There was a polymer concentration range (between 8 and10 g/l) for which the particles were not too aggregated (ca. 900 nm in particle size) via a bridging flocculation process. It is worth noting that neither DMSO alone, nor the hydrolysed polymer, bearing no more anhydride moieties, led to flocculation of the colloid.

The results were not satisfactory in term of colloidal stability, so, in a second approach, the latex suspension at 0.5% solids was added to a diluted solution of polymer dissolved in the 10 mmol/l sodium phosphate pH 6.8 buffer, used for dilution of the latex. This procedure was used in order to hydrolyse some of the anhydride groups onto the polymer, as we suspected that inter-particle bridging arose from a too highly reactive polymer. So, MAMVE was dissolved in DMSO and then diluted in the phosphate buffer until DMSO constituted no more than 5% of the total volume. Practically, 25 μl of a MAMVE solution in DMSO was diluted in 500 μl of phosphate buffer and incubated for 10 min at 37 °C prior to addition of 475 μl of latex. No aggregation of the latex was observed, as reported in Table 3 so long as enough polymer was present in the reaction mixture. Interestingly, all attempts at increasing the solids of the latex led to flocculated material. In fact, the polymer (MAMVE)/latex ratio proved a key

Table 3 Polymer-latex conjugates obtained in a phosphate buffer (CL-07)

[MAMVE]		$D_{20\ °C}$(nm)	GA[a] (mg/m^2)
mg/l	(μg)		
43	(25)	585	6.6
8.7	(5)	483	1.3
5	(3)	385	0.8
1.7	(1)	Flocculation	0.27

[a]When total grafted amount is calculated by considering the particle size at 20 °C

parameter governing the colloidal stability (i.e. electrosteric stabilization) and preventing the bridging flocculation process. When the polymer concentration was in deficit (i.e. less than 0.8 mg/m^2) in the medium, referring to the available particle surface to be functionalized, inter-particle bridging occurred, leading to the formation of aggregates as observed for the lower polymer concentration (1.7 mg/l corresponding to 0.27 mg/m^2).

Figure 3 shows the variations of the electrophoretic mobilities as a function of pH for CL-07, the parent latex and the MAMVE derivatized colloid. The latter displayed a negative electrophoretic mobility (i.e., zeta-potential) all over the pH range investigated, thus proving that the amino-groups had reacted with the functional polymer and that carboxyl groups, from the hydrolysed reactive copolymer, were present at the surface. A further proof of the functionalization of the particles by the copolymer is the decrease in the zero charge point value from 10 to ca. 5 which corresponds to the expected pKa of carboxyl groups. In addition, the colloidal stability of latex particles bearing grafted copolymer was improved when the grafted amount was higher than 0.8 mg/m^2 (assuming smooth spherical particles at 20 °C). In fact, the modified particles were quite stable at alkaline pH in a moderate salinity for low surface coverage and highly stable up to 1 mol/l NaCl for high polymer concentration, whereas, the bare CL-07 latex was flocculated. A similar observation has also been pointed out by Duracher [19] in the case of chemical grafting (~1 mg/m^2) of MAMVE onto amino ethyl methacrylate containing *N*-isopropylacrylamide-styrene copolymer latex particles.

Covalent coupling of MAMVE-peptide conjugates onto reactive latex particles

The conjugations of the peptides to the polymers were achieved as described earlier [5] and coupling yields of 80% have reproducibly been obtained. As the molar ratio peptide to polymer is about 100, this means that an average of 80 peptide molecules were coupled per polymer chain. Kinetics experiments, performed by quenching the reaction with large excess of ammonia as reported in [5], revealed that the covalent coupling reaction occurred faster at pH 9 than at pH 6.8. It took around 10 min for the reaction to be complete in a 100 mmol/l phosphate buffer [5], 3 min. in the 10 mmol/l buffer used in the present investigation and, at pH 9, the reaction was instantaneous. This can probably be explained by: (i) an increase in reactivity of the primary amine as these groups are less protonated at higher pHs, and (ii) fast hydrolysis of MAMVE anhydride groups to carboxylate creating attractive electrostatic interactions with peptide molecules favouring the chemical coupling reaction.

The polymer mediated peptide immobilization onto latex particle proved successful as seen from Table 4. The immobilization yields of the peptide-polymer conjugate were higher at acidic pH than at alkaline pH (runs 1 and 3 compared to 5 and 7) confirming that electrostatic interactions were essential for the grafting reaction to occur. Eventually, one might also suggest that, in an alkaline medium, hydrolysis of the anhydride moieties of the polymer was too fast to allow an efficient grafting reaction. Increasing the salt concentration up to 150 mmol/l in the immobilization mixture resulted in an improvement of the grafting process as shown by comparing run 3 to 1 or run 7 to 5. This can result either from an increase in the particle charge density due to the salt-induced collapse of the NIPAM shell [20] or from a reduction of inter-conjugate

Table 4 Grafting of polymer-peptide conjugates onto aminated core-shell latex particles CL07

Run	Nature of the peptide-polymer conjugate	Coupling conditions	% Conjugated immobilized	% Peptide coupled
1	Non-hydrolyzed	10 mmol/l phosphate pH 6.8, 150 mmol/l NaCl	95	71
2	Hydrolyzed		80	58
3	Non-hydrolyzed	10 mmol/l phosphate, pH 6.8	80	65
4	Hydrolyzed		67	67
5	Non-hydrolyzed	50 mmol/l tris, pH 9, 150 mmol/l NaCl	73	53
6	Hydrolyzed		75	56
7	Non-hydrolyzed	50 mmol/l tris, pH 9	59	45
8	Hydrolyzed		54	45

molecule repulsive interactions, allowing a denser loading of the particles with the MAMVE-peptide counterpart. It is interesting to notice that the amount of peptide molecules per particles was found to be in between 2×10^5 and 3×10^5.

In order to have an idea of the contribution of covalent and electrostatic interactions to the immobilization process, grafting experiments were run in which the remaining anhydride groups of the conjugate had been hydrolysed prior to adding the cationic latex particles. At pH 6.8, more conjugate was grafted on the colloidal particles when some anhydride groups were still available on the polymer than with a fully hydrolysed conjugate, suggesting an important contribution of covalent bonds in the immobilization process (compare runs1 to 2 and runs 3 to 4). These covalent bonds should be formed before complete hydrolysis of the anhydride moieties of the conjugate molecules. At pH 9, no difference in the binding efficiency was observed whether the conjugate was fully hydrolysed or not, hence suggesting a major role played by electrostatic interactions. To confirm that, a control experiment was run with a non-hydrolysed conjugate but with latex particles featuring a low surface amino groups density and a negative global charge (as examined via electrokinetic study). No grafting reaction was evidenced (results not shown), which suggested that electrostatic interactions were essential to allow the particles and the peptide-MAMVE conjugate to interact prior to the formation of the covalent bond. Finally, for the hydrolysed and for the non-hydrolysed conjugates, the presence of salt improved the immobilization yields, for the reasons already discussed above.

Conclusion

Monodisperse aminated core-shell latex particles were obtained via a shot process based on the use of *N*-isopropylacrylamide (NIPAM, a hydrophilic monomer) as a co-monomer of styrene for the core of the particle. A mixture of NIPAM, methylene bis acrylamide (MBA), a crosslinker, and *N*-(3-aminopropyl)-methacrylamide hydrochloride (APMH) as a functional monomer was used for the synthesis of the shell. It was shown that the functional monomer influenced the radical polymerization process because of its chain transfer ability. As a result, the poly(NIPAM) shell got thinner as the functional monomer content increased in the polymerization recipe. Moreover, more water-soluble oligomers were formed in the presence of functional monomers than with NIPAM only.

To achieve an efficient functionalization of the latex particles by the reactive MAMVE copolymer, without alteration of their colloidal stability, it was essential to operate in a diluted medium at low ionic strength. The mixing methodology of the reactants proved to be of paramount importance, the colloid must be added to the polymer solution to prevent flocculation.

Finally, the polymer-mediated immobilization of peptides was achieved and was found to be principally governed by electrostatic interactions between the cationic latex and the negatively charged conjugate. The grafting yields were higher in acidic media in which the latexes displayed an elevated positive surface charge density, and lower in alkaline media. Moreover, in the presence of 150 mmol/l sodium chloride, the grafting efficiency was improved to reach 95% of the amount of conjugate involved in the reaction. This result was explained by the decrease in intra and inter molecules repulsive interaction and by a conformational modification of the poly(NIPAM) shell.

Further work will be devoted as well to: (i) investigate the influence of the amount of peptide molecules bound per polymer chain on the grafting efficiency onto the particles (in the present work conjugates bore an average of 80 peptide molecules per polymer chain); (ii) determine the ratio of covalent vs non-covalent binding for the non-hydrolysed polymers; (iii) assess the stability of the particle/polymer/peptide conjugates and their performances in diagnostic assays.

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References

1. Pichot C, Delair T, Elaïssari A (1997) Polymer colloids for biomedical and pharmaceutical applications. In Asua JM (ed) Polymeric dispersions: principles and applications. Serie E335 ed. Kluwers Academic, Netherlands, pp 515–539
2. Tam JP, Xu J, Eom KD (2001) Biopolymers 60:194
3. Admczyk M, Gebler JC, Reddy RE, Yu Z (2001) Bioconjugate Chem 12:139
4. Ollivier N, Olivier C, Gouyette C, Huynh-Dinh T, Gras-Masse H, Melnyk O (2002) Tetrahedron Lett 43:997
5. Ladavière C. Lorenzo C, Ela A, Mandrand B, Delair T (2000) Bioconjugate Chem 11:146
6. Charles MH, Charreyre MT, Delair T, Elaïssari A, Pichot C (2001) STP Pharma 11:129

7. Böcher M, Böldicke T, Kieβ M, Bilitewski U (1997) J Immunol Methods 208:191
8. Gegg CC, Etzler ME (1993) Anal Biochem 210:309–313
9. Wunderbaldinger P, Josephson L, Weissleder R (2002) Bioconjugate Chem 13:264
10. Pichot C (1995) Pol Adv Technol 6:427
11. Bouali B, Ganachaud F, Chapel JP, Pichot C, Lanteri P (1998) J Colloid Interface Sci 208:81
12. Duracher D, Sauzedde F, Elaïssari A, Perrin A, Pichot C (1998) Colloid Polym Sci 276:219
13. Ganachaud F, Mouterde G, Delair T, Elaïssari A, Pichot C (1995) Pol Adv Technol 6:480
14. Meunier F, Elaissari A, Pichot C (1995) Pol Adv Technol 6:489
15. Ganachaud F, Sauzedde F, Elaïssari A, Pichot C (1997) J Appl Polym Sci 65:2315
16. Nabzar L, Duracher D, Elaïssari A, Chauveteau G, Pichot C (1998) Langmuir 14:5062
17. Sauzedde F, Ganachaud F, Elaïssari A, Pichot C (1997) J Appl Polym Sci 65:2331
18. Ganachaud F, Bouali B, Veron L, Lantéri P, Elaïssari A, Pichot C (1998) Colloid Surf A Physicochem Eng Aspects 137:141
19. Duracher D (1999) PhD thesis, Claude Bernard University, France
20. Duracher D, Sauzedde F, Elaïssari A, Pichot C, Nabzar L (1998) Colloid Polym Sci 276:920