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Antiseptic Nanocapsule Formation via Controlling Polymer Deposition onto Water-in-Oil Miniemulsion Droplets

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Summary: The stable nanodroplet was prepared by inverse miniemulsion with an aqueous antiseptic solution dispersed in an organic medium of solvent/nonsolvent mixture containing an oil-soluble surfactant and the polymer for shell formation. The change in gradient of the solvent/nonsolvent mixture, obtained by heating at 50 °C, led to the precipitation of the polymer in the organic phase and deposition onto the large interphase of the aqueous miniemulsion droplets. The monodisperse polymer nanocapsule, with the size range of 80–240 nm, dispersed in cyclohexane phase was achieved as a function of surfactant concentration. By variation of polymer content, molecular weight and type, an encapsulation efficiency of 20–100% was obtained as detected by proton-nuclear magnetic resonance spectroscopy measurement. The nanocapsule could be easily transferred into water as continuous phase resulting in aqueous dispersion with nanocapsule containing the antiseptic agent as an aqueous core. The encapsulated amount of the antiseptic agent was evaluated to indicate the durability of the nanocapsule's wall. Additionally, the different types of polymer having glass transition temperature ranging from −60 to 100 °C have been successfully used. Currently, the research work on the incorporation of nanocapsules onto natural rubber (NR) latex in order to prepare NR latex glove containing the antiseptic agent nanocapsules is carried out. By using the simple and versatile layer-by-layer (LbL) technique based mainly on an electrostatic interaction between oppositely charged species, the deposition of nanocapsules onto NR latex film has successfully been fulfilled.

Keywords: core-shell polymers; Inverse miniemulsion; nanocapsules; nanoprecipitation

Introduction

The development of nanocapsule or submicron-sized hollow particle, which in principle possess a central cavity core surrounded by a polymeric shell, as effective drug delivery device has been a major goal in delivering the drug at the optimal rate and dose regimen. It is of great interest to encapsulate the antiseptic chlorhexidine digluconate salt, a hydrophilic drug model, in nanocapsule since this agent, a dicationic surfactant, has gained attention for its use in the cleansing solution owing to its activity against Gram-positive and Gram-negative bacteria.

To deal with the encapsulation of water-soluble guest molecule, the well-defined nanocapsule with aqueous core has to be, ideally, prepared in a single step and the shell forming should be stable and has high structure perfection. However, available protocols for preparation of aqueous core nanocapsule are still limited to water-in-oil (w/o) interfacial polymerization.[1,2] The physical adsorption of polyelectrolyte onto

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a solid template, which was then removed, has been successfully achieved by using the layer-by-layer (LbL) technique.[3]

Therefore, the idea is to use stable nanodroplet consisting of the encapsulating antiseptic agent for a further encapsulation by modified nanoprecipitation of a polymer. The monodisperse aqueous nanodroplet is firstly prepared from a w/o or inverse miniemulsion process, which generally provides critically stabilized small droplets dispersed in a continuous phase, by ultrasonicating a two-phase system.[4–6] The droplet size ranging from 30–500 nm is principally governed by the type and amount of surfactant used as compatibilizer. One key characteristic of miniemulsion is that no effective material exchange should occur between the droplets, the so called Ostwald ripening effect. In the case of inverse miniemulsion, the salt or chlorhexidine digluconate, which is an extremely hydrophilic component, presented in aqueous miniemulsion droplets plays an important role of building up an osmotic pressure inside each droplet.[7,8]

In the present study, a modified nanoprecipitation of poly(methyl methacrylate) (PMMA) from the continuous phase and its deposition onto the aqueous nanodroplets has been established to fabricate the aqueous core antiseptic nanocapsules. It has been shown for the first time that after redispersion in water, aqueous dispersions with nanocapsules consisting of the aqueous cores were obtained. The well-defined aqueous core-PMMA shell morphology was investigated under transmission electron microscopy (TEM). The effect of PMMA content and its molecular weight (M_w) on the nanocapsule size and encapsulation efficiency has been studied. Besides the use of PMMA with high glass transition temperature (T_g) of 100 °C, various types of polymer nanocapsules having different physical properties, i.e., poly(methyl acrylate) (PMA) and semicrystalline poly(ε-caprolactone) (PCL) possessing T_gs of 10 and −60 °C, respectively, have been achieved. The adsorption of PMMA latex particles on NR sheet via LbL technique was investigated as a model for preparation of special NR latex medical glove containing nanocapsules.

Experimental Part

Materials

All chemicals, including chlorhexidine digluconate purchased from Sigma (20% in water); surfactants, i.e., sodium dodecyl sulphate (SDS), Brij® 52 (hydrophilic-lipophilic balance or HLB: 5.3) and Brij® 72 (HLB: 4.9) from Aldrich (GC) and solvents, i.e., acetone, dichloromethane, cyclohexane, tetrahydrofuran (THF), ethanol and other reagents, acrylamide (AAm), hexadecane from Fluka (purum), deuterated water and pyrazine from Merck (GC), riboflavin from Sigma, were used without further purification. PMMA having M_w of 71 460 g/mol from BASF (practical), of 350 000 g/mol from Acroes (practical) and of 996 000 g/mol from Aldrich (standard); Lubrizol U, poly(isobutylene-succinimide pentamine) with M_w of poly(isobutylene) of 950–2500 g/mol supplied from Lubrizol Ltd., were also used as received. The block copolymer emulsifier poly[(butylene-co-ethylene)-*b*-(ethylene oxide)], P(B/E-EO), with a molecular mass of P(B/E) block of 3700 g/mol and of the PEO block of 3600 g/mol, was synthesized starting from Kraton liquid (Shell).[9] PCL with M_w of 65 000 g/mol was purchased from Aldrich and PMA was synthesized by miniemulsion polymerization technique.[10] Commercial high ammonia (HA)-preserved NR latex (Rayong Bangkok Rubber Co. Ltd.; Thailand) was used.

Nanocapsule Preparation[10]

The organic continuous phase comprising dichloromethane (9.5 g), a known amount of P(B/E-EO) (%w/v: % ratio of the surfactant (g) to drug volume (ml)), and cyclohexane (12 g) was firstly prepared. An antiseptic agent, a chlorhexidine digluconate solution, (0.5 ml) was charged into the solvent mixture. A solution of PMMA, PCL

or PMA (100, 200, 300, 400 or 500 mg) in dichloromethane (0.5 g) was slowly dropped into the mixture, which was subsequently ultrasonicated for 2 min at 90% amplitude (Branson sonifier W450 1/2″ tip). Then, the temperature was raised to 50 °C in an open vessel with continuous mechanical stirring overnight. During evaporation of dichloromethane, cyclohexane was added to replace dichloromethane and also its evaporated volume.

Characterizations of Nanocapsules[10]

Size of the nanocapsules was characterized by applying a dynamic light scattering (Malvern; NanoZS). For determination of the encapsulation degree of the antiseptic agent, the nanocapsules were separated by centrifugation in a microcentrifuge (Eppendorf) and were carefully dried before dissolving in a mixture of THF and ethanol. Deuterated water and a known amount of pyrazine were applied as an external solvent and a calibration product for the quantitative analysis of the encapsulation efficiency by ^{1}H-NMR (Bruker, DRX 400 with 400.123 MHz). Mass of drug in nanocapsule was calculated from the area ratio of peaks at 7.66 to 9.07 ppm corresponding to aromatic-protons of the chlorhexidine digluconate and pyrazine, respectively. The morphology of the polymer nanocapsules, mounted on copper grid before coating with carbon, was investigated under TEM (Phillips EM 400).

$$\text{Encapsulation efficiency}(\%) = \frac{\text{mass of drug in nanocapsule} \times 100}{\text{mass of drug in formulation}} \quad (1)$$

The separated nanocapsules were finally re-dispersed in a 2% w/v aqueous SDS solution by stirring overnight for complete de-aggregation. The characterization procedures, mentioned above, were also exploited and the remaining amount of the drug in the nanocapsules after redispersion in water was evaluated.

Adsorption of PMMA Latex Particles on NR Sheet[11]

The surface of NR sheet ($1.5 \times 4 \times 0.1$ cm^3) was cleaned with methanol and Milli-Q water for 15 min each in an ultrasonic bath and then dried in air. The cleaned rubber strip was next treated with Ar plasma (100 W, 13.56 MHz under 0.5 Torr) for 10 s and then exposed to the atmosphere for 10 min. In the UV-induced graft copolymerization, the pretreated rubber was immersed into an aqueous solution of 5 wt% acrylamide (AAm) (40 ml) and 0.05 mM riboflavin (2 ml). The grafting reaction was performed by irradiation with UV 1000 W for 30 min.

PMMA latex was synthesized by using a soap-free emulsion polymerization batch process as described elsewhere. The NR-*g*-PAAm strip was immersed into a beaker containing PMMA latex (1–20 mg/ml) and NaCl (1×10^{-4}–1×10^{3} mM) for 1 to 60 min with pH varying from 2 to 12. The sample was subsequently washed with water and finally allowed to dry at room temperature. The amount of PMMA particles adsorbed per unit area of the film surface, or surface coverage (Cs), was determined under scanning electron microscope (SEM) by using the following equation:

$$\text{Cs}(\%) = (\text{N}/\text{N}_{\text{max}}) \times 100 \quad (2)$$

where N is the number of adsorbed particles per unit area; N_{max} is the maximum number of particles on the same area.

Results and Discussion

Nanocapsule Formation[10]

The requirement at the beginning of the process was to completely dissolve the polymer in the continuous phase consisting of the solvent/nonsolvent mixture. It was found that the maximum ratio of dichloromethane to cyclohexane, in order to dissolve the required polymer amount, was limited to 1:1.2. In this mixture, an aqueous solution of the chlorhexidine digluconate was miniemulsified to obtain small and stable droplets. Since the anti-

septic agent plays the role of the lipophobe so as to suppress the Ostwald ripening by building up an osmotic pressure inside the droplet in order to counteract with the Laplace pressure, individual nanodroplets were capable of keeping their inner compositions during a time interval of at least several days. The selective evaporation of dichloromethane at 50 °C, caused the descending of solubility value from 17.8 to 16.8 $(MPa)^{1/2}$ leading to polymer precipitation. Then, the polymer precipitated onto the nanodroplets on which the polymer shell was formed.

It could be thermodynamically explained that, the spreading or shell forming of polymer around the nanodroplet was dictated by the interfacial tension (γ) between the polymer shell, the continuous cyclohexane phase and the dispersed nanodroplet, called phase 3, 2 and 1, respectively. By applying the equilibrium state of three phases of equal density, a spreading coefficient (S) is defined as: $S_1 = \gamma_{23} - (\gamma_{12} + \gamma_{13})$. In conventional terms, with $\gamma_{12} > \gamma_{23}$ ($S_1 < 0$), phase 1 completely engulfed by phase 3, when $S_2 < 0$ and $S_3 > 0$, no engulfing occurs when $S_2 > 0$ and $S_3 < 0$; and S_1, S_2, $S_3 < 0$ leads to partial engulfing and formation of two-phase droplets with three interphases. At the end of evaporation, no residue of dichloromethane was detected in the cyclohexane phase by ^{1}H-NMR measurements. In addition, there was no polymer coagulum being visible, the precipitation in nanoscale of polymer on the surface of nanodroplet could be assumed and proven in the following part of this study.

Effect of Type and Concentration of Surfactant[10]

To stabilize aqueous nanodroplets dispersed in the mixture of dichloromethane and cyclohexane, the use of several oil-soluble surfactants were firstly attempted. Among non-ionic surfactants applied, the copolymer P(B/E-EO) turned out to be the most effective stabilizer and only 1% w/v of the copolymer was required to obtain stable droplets of 230 nm. The good stabilization effect was due to the miscibility between each block and one of the phases, i.e., the hydrophilic part or anchoring moiety of PEO and the aqueous or dispersed phase and the hydrophobic part or stabilizing moiety of P(B/E) and cyclohexane phase providing steric stabilization. During the nanocapsule formation, PEO chains could penetrate into PMMA matrix and also adsorbed on the PMMA shell. Size of PMMA nanocapsules as a function of PMMA and different P(B/E-EO) contents are shown in Figure 1.

The PMMA nanocapsule size depended mainly on the amount of P(B/E-EO) which agreed well with the miniemulsion principle. Comparing the samples prepared by using similar surfactant concentration, it was found that PMMA contents did not

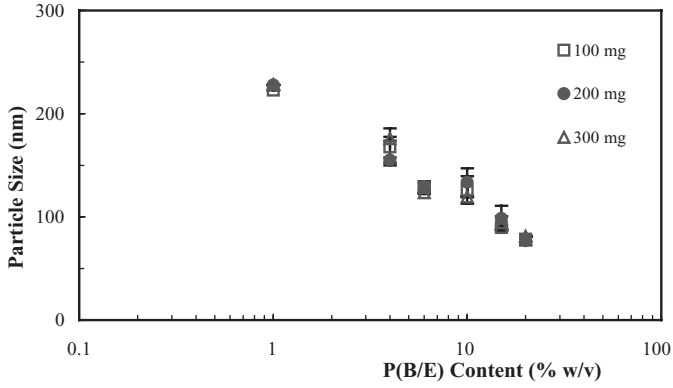

Figure 1.
Size of PMMA nanocapsules as a function of PMMA and different P(B/E-EO) contents.[10]

Table 1.
Size of nanocapsules, by applying different M_ws and contents of PMMA, dispersed in cyclohexane by using 4 %w/v of P(B/E-EO).[10]

[PMMA]	M_w: 71 460	335 000	996 000
mg	g/mol	g/mol	g/mol
	Size	Size	Size
	nm	nm	nm
100	168	120	146
200	155	138	133
300	176	135	141
400	151	139	154
500	142	170	154

significantly affect the nanocapsule size. This indicated that small nanodroplet size could be preserved throughout the process. The relatively low amount of P(B/E-EO) of 4% w/v was selected for use in the further experiments.

Effect of Amount and M_w of PMMA on Size and Encapsulation Efficiency of the Nanocapsules[10]

For determination of the encapsulation efficiency, the nanocapsules with different PMMA contents having different M_ws stabilized by 4%w/v of P(B/E-EO) were synthesized. The nanocapsule size was also investigated and is depicted in Table 1.

The results showed a slight change in size of the nanocapsules prepared by different amounts and M_ws of PMMA. In principle, comparing the three M_ws, at the same concentration, the sensitivity to precipitate upon the decrease of solvent solubility was contributed to high M_w of 350 000 and 996 000 g/mol. The fast precipitation of the polymer onto the antiseptic nanodroplets could be assumed and thus nanocapsules were formed. The amount of the antiseptic agent retained in the nanocapsules or encapsulation efficiency as a function of M_w of the PMMA and its amount determined by ^{1}H-NMR are displayed in Figure 2.

It was observed that with increasing PMMA content, the encapsulation efficiency of the nanocapsules for each M_w increased from 20 to 90% which indicated that the effectiveness of the nanocapsules for keeping the antiseptic agent relied mainly on M_w and the amount of PMMA.

Morphological Study

The morphologies of air-dried PMMA nanocapsules from cyclohexane were monitored by TEM as depicted in Figure 3.

A well-defined nanocapsule structure was detected and the nanocapsules were nearly spherical with continuous intact thin shells. The nanocapsules with the higher M_w PMMA appeared larger in the TEM, which was probably due to the high nanocapsules wall's strength. However, during the drying process, the deformation of the shell could take place due to the gravity effect and did

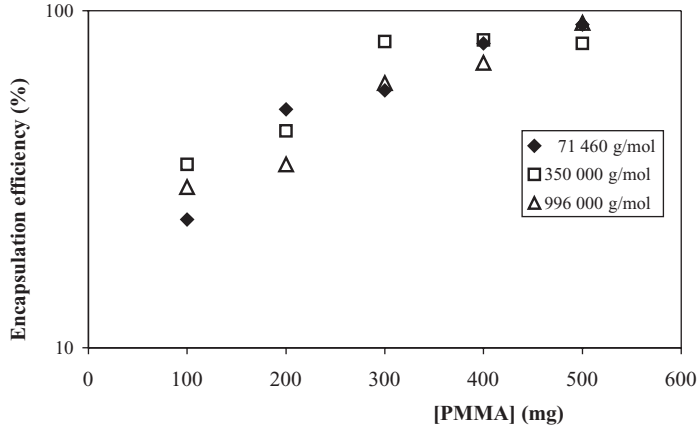

Figure 2.
Encapsulation efficiency of nanocapsules as a function of PMMA's M_ws and concentrations.[10]

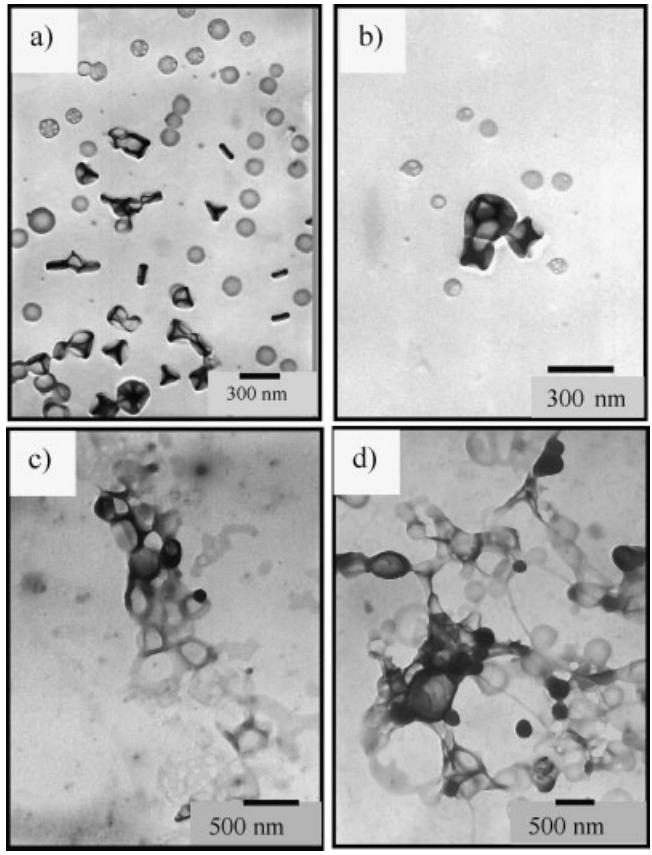

Figure 3.
TEM micrographs of PMMA nanocapsules (cyclohexane); PMMA of 71 460 g/mol at 1% w/v: a) 300 mg, b) 400 mg, and PMMA of 996 000 g/mol at 4% w/v: c) 100 mg, d) 500 mg.

not shrink while evaporating the water inside the nanocapsules.

Redispersion of PMMA, PMA and PCL Nanocapsules[10]

The dried nanocapsules were then redispersed in an aqueous solution of 2% w/v SDS in order to obtain a final dispersion having 0.5% solid content.

It was observed that the size of the redispersed nanocapsules in all samples was in the range of 234–330 nm which was about twice of that of the original one. This was possibly due to the swollen nanocapsules, taking place during the redispersion process, caused from an osmotic pressure difference between the nanocapsules and the continuous phase and the formation of small aggregates. The remaining amount of the antiseptic agent significantly increased when increasing the amount and M_w of PMMA used for the encapsulation as shown in Figure 4.

In the case of using PMMA with low M_w of 71 460 g/mol, the amount of retained antiseptic agent increased from 8 to 33% corresponding to the increase in PMMA concentration from 100 to 500 mg. It might be due to the increased shell thickness and hence the durability of the wall's nanocapsule. By using high M_w PMMA of 996 000 g/mol, the high content of antiseptic agent of 92% in the redispersed nanocapsules was achieved. From the strong entanglement and low molecular dynamic point of view, water sorption and penetration across the

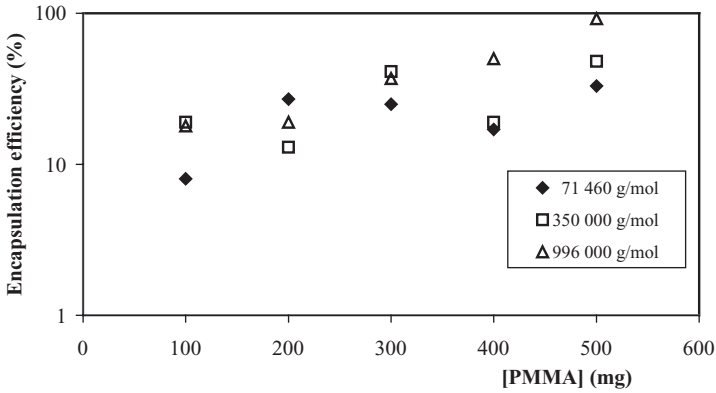

Figure 4.
The remaining amount of antiseptic agent of the redispersed nanocapsules as a function of PMMA's M_ws and concentrations.[10]

thin polymer barrier might be retarded. Furthermore, when applying PMMA having M_w of 996 000 g/mol, no leakage of the core material was detected indicating the high durability of the PMMA shell, i.e., the nanocapsules are stable enough to withstand the swelling effect. It can be confirmed by the morphology of the redispersed nanocapsules, as shown in Figure 5, that no change in the intact capsule morphology was observed after redispersion which indicated the formation of stable nanocapsules.

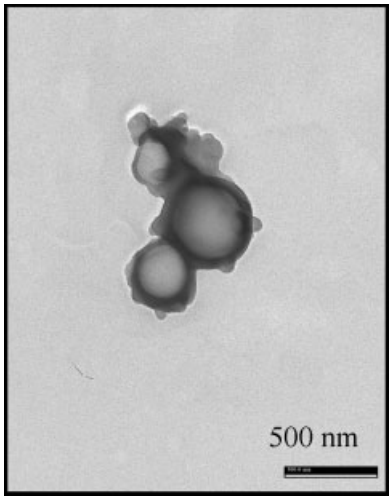

Figure 5.
TEM micrograph of redispersed PMMA nanocapsules using 400 mg of PMMA with M_w of 71 460 g/mol.

Apart from the preparation of PMMA nanocapsules, the modified nanoprecipitation technique was extended to prepare PMA and PCL nanocapsules. Data showed that with using 100–500 mg of PCL, the original size was in the range of 130–180 nm, while a size of 300–400 nm was detected after redispersion. Increasing the concentration reduced the difference between the encapsulation efficiency before and after redispersion of the nanocapsules at each PCL concentration. Keeping the PCL at 500 mg, the remaining amount of the disinfectant agent being as much as 52% from the original one of 87% was achieved. In the case of the PMA with using 100 and 200 mg, the resulting nanocapsule size was 150 and 190 nm, respectively. An encapsulation efficiency of 100% with both concentrations was detected. After redispersion the PMA nanocapsules, the aggregation with 460 and 260 nm in diameter, respectively, was measured. The remaining amount of the antiseptic agent was as high as 90%. It should be pointed out that the dissolution of high amount of PMA (300-500 mg) in the continuous phase took more time when compared to other polymers. The high viscosity of the PMA solution caused the formation of coagulum during evaporation of dichloromethane and, consequently, the polymer did not deposit onto the aqueous nanodroplets

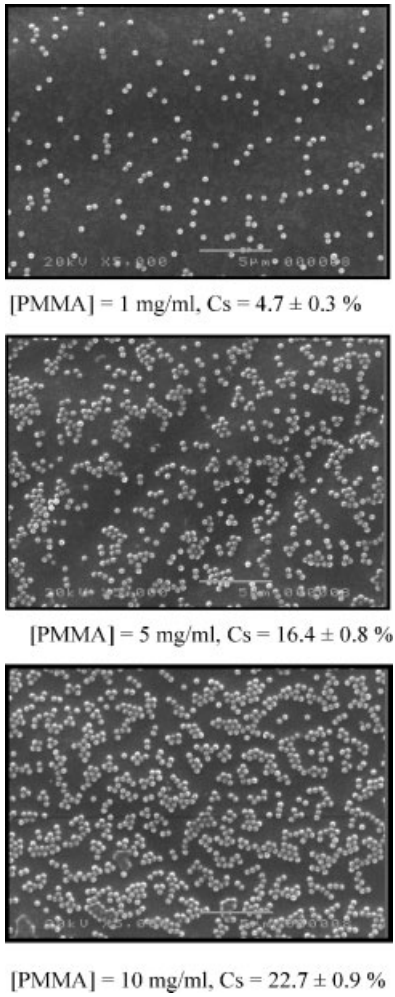

Figure 6.
SEM micrographs of PMMA particles deposited on NR-*g*-PAAm films as function of latex concentration (immersion time = 10 min, pH 4).

probably due to the low mobility of molecular chains.

Deposition of PMMA Latex on NR Sheet[11]

SEM micrographs of PMMA particles (an average diameter of 350 ± 5 nm) adsorbed on the NR-*g*-PAAm surface, when using different latex concentrations (immersion time = 10 min, pH 4), are shown in Figure 6. It was observed that the Cs increased with increasing latex concentration which could be explained that the high latex concentration favored the repulsion among similar charged particles providing the great collision between particle and surface of the substrate. The deposition of the polymer nanocapsules containing antiseptic agent onto NR or prevulcanized NR latex film has been currently proceeded.

Conclusions

The aqueous core nanocapsule formation by the controlled polymer nanoprecipitation onto inverse miniemulsion droplets containing the chlorhexidine digluconate is well established. The deposition of polymeric shell, i.e., PMMA, PCL or PMA, from the organic continuous phase onto the stable nanodroplets dispersed phase was achieved by changing the gradient of the solvent/nonsolvent mixture of dichloromethane/cyclohexane under mild evaporation. After redispersion of the nanocapsules in an aqueous medium, the stable nanocapsules with an aqueous core redispersed in the aqueous continuous phase was obtained for the first time. A well-defined nanocapsule structure has been investigated by TEM. The encapsulation efficiency of 20–100% depended mainly on the concentration, molecular weight and types of the polymer used. From the success of PMMA latex particles deposited on NR sheet via LbL, this technique will be used for incorporation of polymer nanocapsules into NR latex film.

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