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A Nanoparticulate Raloxifene Delivery System Based on Biodegradable Carboxylated Polyurethane: Design, Optimization, Characterization, and *In Vitro* Evaluation

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ABSTRACT: Biodegradable carboxylated polyurethanes with three molecular weights were synthesized to prepare a nanoparticulate sustained delivery system of raloxifene hydrochloride, the drug with poor bioavailability. The nanoparticles were prepared by coprecipitation method. Optimal conditions for the preparation of nanoparticles were obtained using Box–Behnken design. Independent factors were ratio of polymer to drug, M_w of polymer and speed of magnetic stirrer. Dependent variables include zeta potential, polydispersity index (PdI), particle size, and loading efficacy (LE). Results of the fractional factorial design based on an analysis of variance demonstrated that the model for particle size, zeta potential, PdI and loading efficacy was statistically significant. The size of nanoparticles in design experiments were 46–96 nm in diameter and had entrapment efficiency of 84–92%. The nanoparticles were evaluated for *in vitro* release and showed a sustained release profile (24.19% ± 4.35% after 4 weeks), following the Fickian diffusion-based release mechanism. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39668.

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INTRODUCTION

In the coming years, many of the permanent prosthetic polymeric systems used for temporary therapeutic applications will be replaced by biodegradable polymers that could help the body in repairing and regenerating the damaged tissues. Biodegradable polyurethanes are one of the important classes of polymers which have been investigated as scaffolds for tissue regeneration and as controlled/sustained release drug delivery vehicles.[1] The susceptibility to the biodegradation of polyurethanes is related to the inherent feature of their chemistry. Hydrolytic degradation of biodegradable polyurethane occurs from aliphatic ester linkages in polyester–urethanes.[2] The properties of polyurethane are influenced by several parameters such as polymer molar mass and the type of ionic groups in polymer.[3] As the conventional polyurethane is insoluble in aqueous media, incorporating ionic groups such as carboxylated group can improve their physical properties like solubility.[4] The behavior of polyurethanes such as viscosity, conductivity and light scattering are changed by polyurethane molar mass and solvent polarity and distribution of ionic group in the polymer chain.[5–7] The synthesized carboxylated polyurethane can be dissolved in co-solvent system of acetone/water easily due to large anionic field strength of the carboxylated ($-COO^-$) groups.[8] This effect is created by dissociation of ionic groups in polar solvents resulting in the expansion of the ionomer coil.[5–7] One of the most interesting biopharmaceutical applications for biodegradable polyurethanes is related to the use of this type of material to control the release of drugs.[9] In addition, the polycaprolactone (PCL), presenting in the soft segment of polyurethanes, shows permeability to small molecules such as therapeutic agents.[10] Previously some biodegradable polymer coatings like collagen, polyanhydrides and polyurethanes have been applied for sustained delivery of drugs.[11–13] Polyurethane based nanoparticles possess several advantages over other systems, including the site-specific distribution of drugs within the body via cell-specific targeting of the nanoparticle delivery system and the ability to control the kinetics of drug release.[14] Biocompatibility of polyurethane as well as biodegradability of them has been confirmed by *in vitro* and *in vivo* study.[15–17] Polyurethanes (PURs) are widely used in biomedical fields due to their

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excellent mechanical properties. Only a few studies have been reported so far for the preparation of nanoparticles for drug delivery systems using biodegradable polyurethane.[17,18]

Raloxifene hydrochloride-methanone, [6-hydroxy-2-(4hydroxy phenyl) benzo[b] thien-3-yl]-[4-[2-(1-piperidinyl) ethoxy] phenyl]-hydrochloride is an estrogen agonist/antagonist, commonly referred to a selective estrogen receptor modulator (SERM) that belongs to the benzothiophene class of compounds. It is indicated for treatment and prevention of osteoporosis in postmenopausal women and reduction in the risk of invasive breast cancer in postmenopausal women with osteoporosis and postmenopausal women at high risk of invasive breast cancer.[19] It has poor bioavailability due to hepatic first-pass metabolism and poor water solubility.[20] The drug exhibits high interindividual and intraindividual variability (30%) of most pharmacokinetic parameters[21] and this fact makes it attractive for further disposition and controlled drug delivery. The reduction in particle size of prepared nanoparticles leads to increasing in drugs bioavailability and reducing in toxic result for the patients.[22] Controlled-release formulations of different medicines have been used to reduce the adverse effects of drugs and maintain clinical remission of diseases.[23,24] Moreover, oral bioavailability of Raloxifen is 60% and absolute bioavailability is 2% which is the basis for preparation of raloxifen-polyurethane sustained release nanoparticles. Raloxifene current dosage form is tablet of 60 mg and making such a nanoparticulate delivery system in an injectable form will support the controlled release delivery of raloxifene in the body for the longer period of the time.[25]

In the present study, three molecular weights of biodegradable carboxylated polyurethanes were synthesized and were applied as carriers of raloxifene. The purpose of this study was to prepare raloxifene nanoparticles with co-precipitation method and optimization of nanoparticles to result in controlled delivery of raloxifene. The dissolution behavior of the drug from these nanoparticles was evaluated.

EXPERIMENTAL

Materials

Poly (caperolacton diol) (PCL, M_n = 2000) and 1,4-butandiol (BD, Purity: > 99.7 %) were purchased from Aldrich–Chemical. Dibutiltin dilaurate (DBTDL) that used as catalyst, dimethylol propionic acid (DMPA), isophorone diisocyanate (IPDI) and triethylamine (TEA) were obtained from Merck Chemical. Raloxifene hydrochloride was purchased from SOLMAG S.p.A (Milano, Italy) as a yellowish crystalline powder with molecular mass of 510.05, melting point of 260°C and water content of 0.602%. All the other materials and solvents used for the analytical methods were of analytical grade.

Instrumentation

The ^{1}H NMR spectra of three molecular weights of caboxylated polyurethanes was measured by ^{1}H NMR Bruker spectrometer operating at a frequency of 400 MHz for protons. The FT-IR spectra of the samples were measured using Nicolet Magna (IR 550, Hayward, CA). The made tablets from potassium bromide (KBr) and sample were scanned, in transmission model, in the spectral region of 400–4000 cm, using a resolution of 4 cm and 32 coadded scans. GPC measurements in THF were performed using a model Shimadzu 6-A (Japan) equipped with Four Jordi Flash (high-speed) columns (Waters Ultrastyragel 10^3 Å) and a Waters 590 HPLC pump at a flow rate of 1 mL min^{-1}. Weight average molecular masses were calculated based on polystyrene standards (1,250,000; 400,000; 200,000; 43,000; 17,600; 6930; 2610; 982; and 472 Da). Thermo gravimetric analysis (TGA) measures the amount and rate of change in sample weight as a function of temperature or time. Thermal analysis on the samples was determined with Diamond TG/DTA (Perkin–Elmer Pyris, America) from room temperature to 700°C at heating rate of 20°C min^{-1} under N_2 atmosphere. The size analysis, polydispersity index and zeta potential of the nanoparticles were determined using a Malvern Zeta sizer Nano ZS (Malvern Instruments, UK). The morphology was investigated using MIRA II LMU, TESCAN FESEM (Czech). The nanoparticles were initially coated by gold. This operation in DC-magnetron sputtering was carried out at an argon pressure of 6 kV at a current of 6 mA. Thermo grams were obtained using DSC (Mettler-Toledo, Greifensee, Switzerland). The DSC scans were performed on 6–7 mg of sample, under nitrogen atmosphere, over the temperature range from −30 to 300°C and at a heating rate of 10°C min^{-1}. X-ray diffraction (XRD) studies on the samples were performed using an X-ray diffractometer (Siemens, D5000, Munich, Germany) with a horizontal goniometer. The samples were placed in the sample holder and scanned at a rate of 1° min^{-1} from 0° to 90°. HPLC (1260 infinity, Agilent technologies, Santa Clara, CA) was applied to analyzing the release profile and loading efficacy and loading capacity. Freeze-drier (Christ, Alpha 2-4 LD, Germany) was used for lyophylization process (contained 0.04 mbar pressure and −50°C temperature and 48 h time).

Synthesis of Carboxylated Polyurethane

Carboxylated polyurethanes (CPUs) with three molecular weights were synthesized in glass polymerization tube under nitrogen atmosphere without using a condenser (Table I). The reaction was carried out by a two-step procedure in DMF as 40% solution[3] (Scheme 1). Isocyanate (NCO) terminated prepolymers were prepared by reacting poly caperolacton diol as a polyol with IPDI dissolved in DMF at 75°C for ~4 h. The chain extension reaction was catalyzed with DBTDL (0.03 wt % based on prepolymer) and carried out at 95°C with DMPA for 48 h and BD was added to reaction at 80°C for 24 h. Polymers were precipitated by pouring DMF solutions into distilled water. The polymers were washed with distilled water for 24 h in order to dialyze unreacted monomers and were dried under vacuum at 50°C until ^{1}H signals of water and DMF in ^{1}H NMR spectra disappeared and white powder was obtained. CPUs were prepared by neutralization of carboxylic groups with TEA. The dried polyurethanes were dissolved in acetone/water in 15/1 ratio and mixed with a stoichiometric amount of a triethyl amine dissolved in methanol.

The neutralization was carried out at 60°C for 4 h. The solvents were evaporated with rotary evaporator at 50°C to give white crystal powder. Carboxylated polyurethanes are designated as follows:

B_1, B_2, and B_3 are CPUs with high, medium, and low molecular weights, respectively.

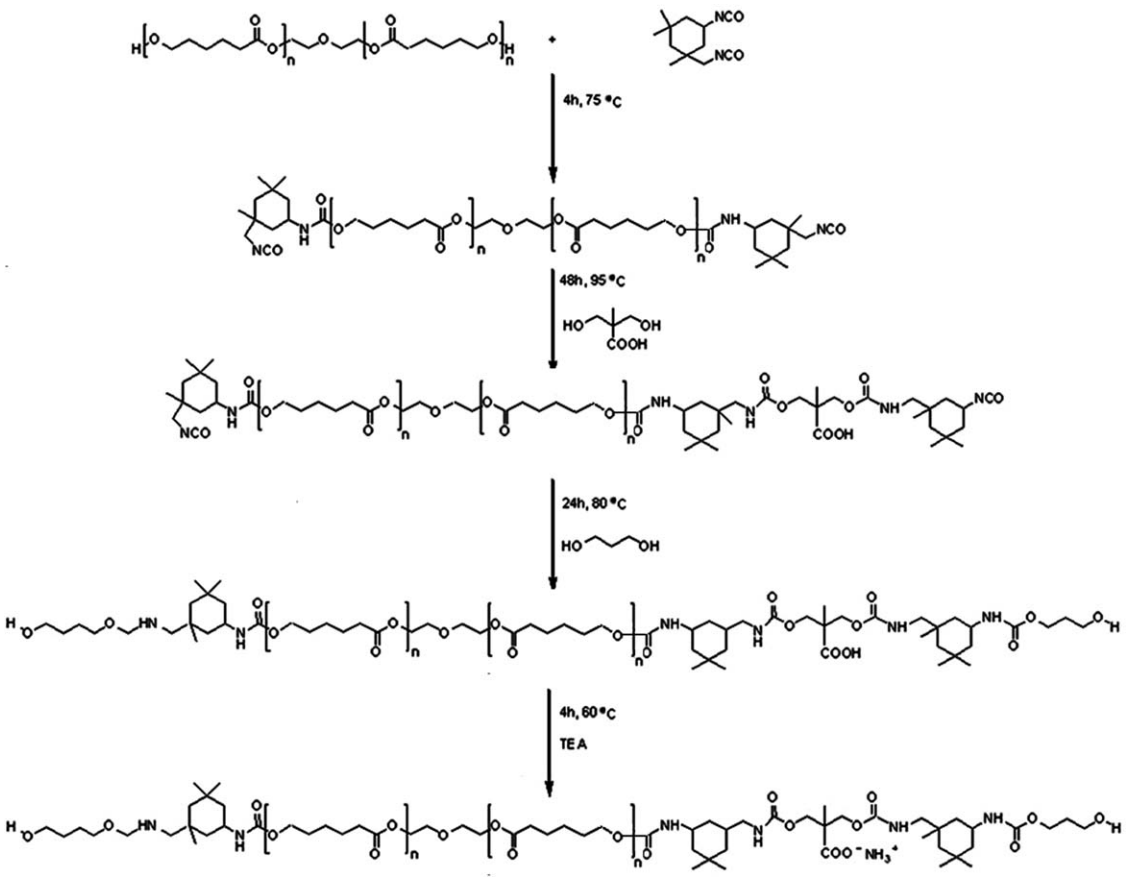
Table I. Mole Ratio of Monomers and GPC Results of Synthesized CPUs

Carboxylated polyurethane (CPU[a])	PCL diol[b]	DMPA[c]	IPDI[d]	BD[e]	TEA[f]	M_n (g mol^{-1}) (GPC)
B_1[g]	0.8	6	9	2.2	6	29,180
B_2[h]	0.5	6	9	2.5	6	22,979
B_3[i]	0.2	6	9	2.8	6	18,581

[a] CPU: carboxylated polyurethane.
[b] PCL diol: polycaprolactone diol.
[c] DMPA: dimetylol propionic acid.
[d] IPDI: isophorone diisocyanate.
[e] BD: butane diol.
[f] TEA: triethyl amine.
[g] B_1: CPU with high molecular weight.
[h] B_2: CPU with medium molecular weight.
[i] B_3: CPU with low molecular weight.

Preparation of Nanoparticles

Raloxifene-loaded carboxylated polyurethane nanoparticles (NPs) were prepared using the coprecipitation method which can be applied for poorly water soluble drugs.[22] Different formulation variables such as concentration ratio of polymer/drug (1 : 4, 1 : 7, 1 : 10 mg mL^{-1}), molecular weight of polymer, speed of magnetic stirrer (250, 600, 1000 rpm) were varied, and the effect on particle size, poly dispersity index, zeta potential, and entrapment efficiency were studied. Only one parameter was changed at a time in each set of experiments. The NPs were prepared by dissolving raloxifene hydrochloride (R-HCl) (5 mg) in 2 mL of acetone/water (15/1), the CPU in determined amounts was added to the same solution and sonicated for 2 min and the solution was added drop wise to 10 mL water at 30 min. Stirring was continued for 2 h to allow complete evaporation of the organic solvent. To remove polymer aggregates, the NPs were filtered by a microfilter with pore size of 1.2 μm (Millex AP, Millipore) and the obtained suspension was



Scheme 1. Synthesis route of CPU.

centrifuged at 14,800 rpm for 45 min, supernatant was alienated, and the free drug present in supernatant was measured using HPLC method by applying C8, 150 × 4.6 mm^2, 5 μm column, UV detection of 280 nm, and phosphate buffer pH 2.5 and acetonitrile (67 : 33) as mobile phase.[26] The collected sediment was resuspended in 1 mL deionized water and lyophilized using freeze-drier explained in instrumental section.

Loading Efficacy and Capacity

The loading efficacy (LE) and capacity (LC) of the nanoparticles were determined by HPLC method as mentioned in preparing the nanoparticles and calculated as follow:

$$\text{LE}\,(\%) = \frac{\text{Total amount of drug added} - \text{Free drug}}{\text{Total amount of drug added}} \times 100 \quad (1)$$

$$\text{LC}\,(\%) = \frac{\text{Total amount of drug added} - \text{Free drug}}{\text{Weight of nanoparticles}} \times 100 \quad (2)$$

In Vitro Drug Release Studies

In vitro release of R-HCl from NPs was evaluated by the dialysis bag diffusion technique reported by Yang et al.[27] The studies of release were performed in 0.1% polysorbate 80 in water to create a sink condition, since maximum solubility of drug in polysorbate solution is 1.5 mg mL^{-1}. The polysorbate solution nanoparticulate dispersion equivalent to 20 mg of R-HCl was placed in a dialysis bag (cut-off 12,000 Da; Himedia, Mumbai, India), which was previously soaked overnight in water and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 20 ml of 0.1% polysorbate 80 which was in water bath shaker and stirred at 100 rpm and maintained at 37°C ± 2°C. The receptor compartment was covered to prevent the evaporation of release medium. Samples were withdrawn at regular time intervals (30 min, 1, 2, 4, 8, 12, 24, 48, and 72h, 1, 2, 3, and 4 weeks) and the same volume was replaced by fresh release medium. All measurements were performed in triplicate (n = 3). The samples were analyzed by HPLC method as applied for LE and LC determination. The percentage of drug released at each time point was calculated according to eq. (3).

$$\text{Drug release}\,(\%) = \frac{\text{Drug in solution}\,(\mu\text{g/ml})}{\text{Initial drug in nanoparticles}\,(\mu\text{g/ml})} \times 100 \quad (3)$$

Assessing Release Kinetic and Mechanism by using Mathematical Model

Korsmeyer–Pepas model is usually used in case of an exponential relationship between release and time.[28] It is a powerful tool to distinguish between different release mechanisms, including Fickian diffusion, Non-Fickian transport, case II (relaxation or swelling controlled) transport, and supper case II (erosion controlled) transport. The model is presented by the following equation:

$$\frac{M_t}{M_\infty} = K_p t^n$$

in which M_t/M_∞ is the fractional drug release, K_p is the model constant, t is the time, and n is the release exponent which its value characterizes the release mechanism.

The mean of all experimental data were fitted by Sigma Plot 10 software to assess the mathematical models of drug release.

Statistical Analysis

Response surface methodology (RSM) that is a collection of mathematical and statistical techniques was employed to determine the optimum conditions. In this study as the response is affected by multiple variables and modeling, analysis and optimization of responses is needed. Box–Behnken design, one of the major RSM techniques, was used for designing of experiments. Box–Behnken is an independent quadratic design with the advantage of investigating three independent factors with fewer numbers of experiments.[29] In this study, one series of experiments is designed for preparation of nanoparticle. For the experiments the independent variables are the concentration of CPU to R-HCl (A), molecular weight of CPUs (B) and speed of stirring (C). Their values are given in Table II. According to Box–Behnken design, using Design-Expert software (version 8.0.6.1, Stat-Ease, MN) 17 experiments were done; 5 of these experiments are replicates of the center point and the other 12 are the midpoints of the edges of the process space. The aforementioned software was used for the analysis and graphical representations of the obtained data. The results of experiments are reported as mean ± SD (n = 3). The difference between the groups was tested using two way analysis of variance (ANOVA), followed by Bonferroni posttests using Graph Pad Prism 5.0, and the differences were considered to be statistically significant when $P < 0.05$.

RESULT AND DISCUSSION

Characterization of Synthesized Polyurethane

Gel Permission Chromatography (GPC). The effect of amount of poly (caperolacton diol) on the molecular weight was investigated by reducing the poly (caperolacton diol) and replacing it with butane diol chain extender. As shown in Table I, addition of butane diol instead of polycaperolacton diol from B_1 to B_3, results in a reduction in the anticipated molecular weight that would be found at the time of polycondensation.

The ^{1}H-NMR Analysis. The carboxylated polyurethanes were dissolved in DMSO as a solvent and analyzed. The ^{1}H NMR of CPUs in three molecular weights are shown in Figure 1. Chemical shifts at 0.948–0.998, 1.28–1.33, 3.35–3.37, 3.96–3.97, and 7.086–7.1142 ppm regions are corresponded to CH_3 and CH_2 in polymer backbone as well as, CH_2O ether bonds, RCOO—C—H bond and $NHCOO^-$ in spectra, respectively.

Optimization of the Preparation of Nanoparticles Procedure

The optimization step of the nanoparticle procedure was performed using a Box–Behnken design. Several variables that could potentially affect the zeta potential, polydispersity index (PdI), nanoparticle size and LE were chosen as: ratio of CPU to drug (A), molecular weight of CPUs (B) and speed of stirring (C). All suggested models were acceptable because of R square above 0.8. Table II shows the responses as analytical signals for nanoparticles.

Zeta Potential. Zeta potential is one of the major physicochemical properties of nanoparticles that determine the stability of nanoparticles in aqueous medium. The preparation of NPs is based on ionic interaction of positively charged amino groups of R-HCl with the negatively charged CPU in neutral aqueous

Table II. Results of Box–Behnken Experimental Design (Mean ± SD)

Trial number	A[a]	B[b]	C[c]	Zeta (mV)	PdI[d]	Size (nm)	LE (%)[e]
1	4	1[f]	600	−49.30 ± 4.75	0.44 ± 0.11	76.10 ± 12.37	83.00 ± 5.10
2	10	1	600	−50.90 ± 6.93	0.36 ± 0.52	73.20 ± 7.53	86.27 ± 3.90
3	4	2[g]	200	−44.20 ± 3.93	0.34 ± 0.03	51.20 ± 14.90	86.42 ± 8.42
4	10	2	1000	−43.70 ± 6.67	0.17 ± 0.13	96.98 ± 5.12	87.30 ± 5.70
5	7	2	600	−39.60 ± 2.54	0.10 ± 0.01	74.36 ± 8.31	91.62 ± 4.92
6	7	2	600	−38.60 ± 7.59	0.11 ± 0.01	73.10 ± 5.20	90.01 ± 7.89
7	10	3[h]	600	−49.10 ± 5.23	0.21 ± 0.13	83.20 ± 9.59	85.30 ± 3.37
8	7	2	600	−38.10 ± 3.82	0.12 ± 0.01	74.10 ± 19.70	91.21 ± 3.64
9	7	1	1000	−50.10 ± 5.37	0.22 ± 0.27	69.11 ± 17.34	91.00 ± 4.49
10	7	1	200	−56.03 ± 2.68	0.25 ± 0.12	77.27 ± 6.29	88.33 ± 5.88
11	7	2	600	−37.10 ± 4.39	0.10 ± 0.01	73.41 ± 4.11	90.83 ± 6.51
12	4	2	1000	−51.30 ± 3.84	0.45 ± 0.04	46.05 ± 10.38	87.67 ± 3.49
13	7	3	1000	−45.20 ± 3.25	0.27 ± 0.06	79.10 ± 5.91	87.64 ± 5.79
14	7	2	600	−37.20 ± 2.16	0.12 ± 0.09	73.58 ± 13.46	91.80 ± 2.40
15	10	2	200	−53.50 ± 2.67	0.33 ± 0.08	81.40 ± 31.71	85.26 ± 10.42
16	7	3	200	−44.00 ± 3.13	0.36 ± 0.03	53.19 ± 14.37	87.10 ± 3.66
17	4	3	600	−47.00 ± 5.13	0.26 ± 0.11	64.09 ± 8.57	84.04 ± 7.59

[a] A: the concentration of carboxylated polyurethane to raloxifene.
[b] B: molecular weight of carboxylated polyurethane.
[c] C: speed of stirring.
[d] pdl: polydispersity index.
[e] LE: loading efficacy.
[f] 1: high molecular weight of carboxylated polyurethane.
[g] 2: medium molecular weight of carboxylated polyurethane.
[h] 3: low molecular weight of carboxylated polyurethane.

solution. CPUs without negative charge, as a negative control, could not complex with R-HCl. The effects of concentarion ratio and molecular weigh of synthesized CPU on zeta potential of nanoparticles are shown in Figure 2(a). At constant molecular weight of polymer, negative surface charge is decreased, in consequence to concentration ratio increasing from 4 to 7 mg mL^{-1}. This can be due to electroestatic interaction between polymer and drug. It is assumed that in the coprecipitation method, by increasing the concentration ratio, the medium (water) creates some limitations for the process of drug encapsulating into polymer, thus with enhancing the concentration ratio from 7 to 10 mg mL^{-1}, the water does not provide the conditions for encapsulating the drug within the polymer; hence, the negative charged polymers are not involved with the drug, electroestatic interaction between CPU and R—HCl is reduced and negative surface charges are increased. On the other hand, in constant concentration ratio of CPU/R—HCl, by increasing the molecular weight of polymer from low (B_3) to medium, the negative surface charge is reduced and after that by increasing the molecular weight to highest level (B_1), the negative charge is increased. It can be explained that if the molecular weight of the polymer is above or below a certain level, the large amounts of drug molecules are not encapsulated in the polymer; therefore, the negative charges of polymers will not be in the interaction of encapsulated drug and surface charges of formed nanoparticles enhance.

The 3D response surface plots of zeta potential variation due to changes in speed of stirring demonstrated that at constant molecular weight of CPU and constant concentration ratio, in lower speed of stirring such as 200 rpm, the zeta potential is decreased namely becomes more negative (Supporting Information Figures S1, S2). Since the drug loading is reduced in lower speed of stirring, the drug contents that can interact with the polymer is reduced and the negative surface charge of polymer are not involved in the electroestatic interactions with the R-HCl and finally zeta potential of nanoparticles is decreased. On the other side, in high speed of stirring, the electroestatic interactions between loaded drug and polymer are disrupted by the water as an external medium in the process of preparation of nanoparticles, thus the negative charges of polymer remain free and zeta potential is decreased. It has been indicated that the particles with low negative zeta potential have minimum toxicity, because cytotoxicity studies of nanoparticles have shown that particles with high positive or negative zeta potential values may significantly reduce the cell viability.[30] The lowest negative zeta potential value is related to B_2 (CPU with medium molecular weight) while the ratio of CPU/R-HCl and the speed of stirring are kept at 8.5 mg mL^{-1} and 1000 rpm, respectively.

Polydispersity Index (PdI). PdI, an index that represents the homogeneity of nanodispersions, is ranged from 0 to 1.[31] It was observed that the molecular weight of CPU, speed of stirring

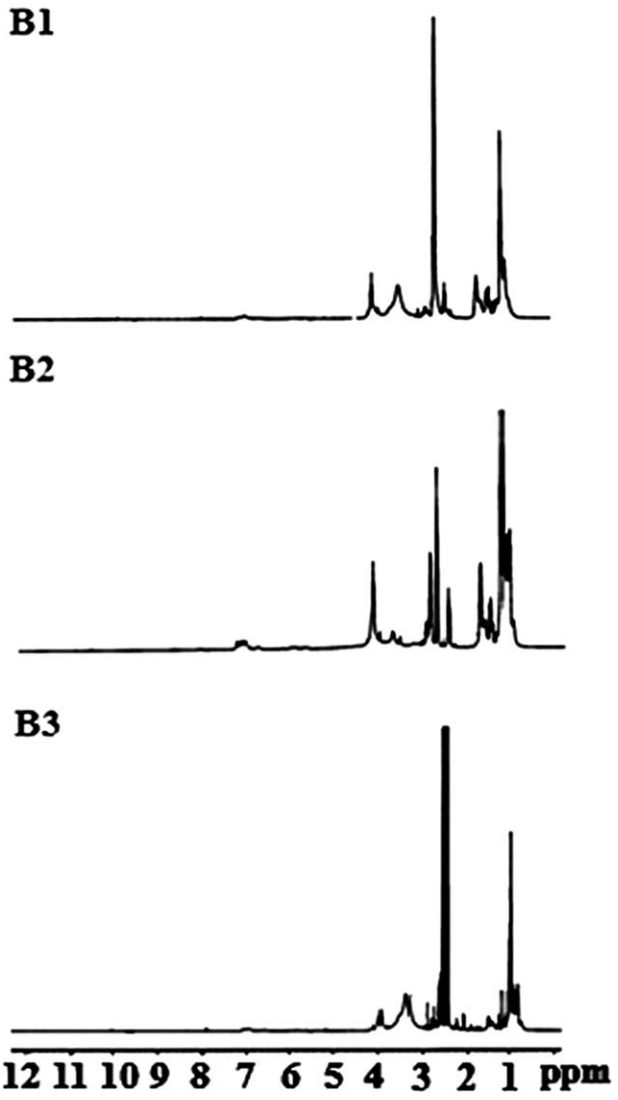


Figure 1. ^{1}H NMR spectra of carboxylated polyurethanes.

and the ratio of CPU/R-HCl have significant effects on the PdI of nanoparticles. The model was quadratic. The 3D response surface plots of PdI variation due to changes in concentarion ratio and molecular weight of synthesized CPUs on polydispersity index of nanoparticles are shown on Figure 2(b). High uniformity or lowest PdI for nanoparticles is obtained while ratio of polymer to drug was 7 mg mL^{-1} and speed of stirring was 600 rpm (Supporting Information Figures S3, S4) and molecular weight was considered to be medium (B_2).

Particle Size. The influence of concentration ratio of CPU/R-HCl and molecular weight of CPU on particle size was investigated and is shown in Figure 2(c). The model was linear and the results showed that particle size is independent to molecular weight of polymer and speed of stirring and it is just dependent to concentration ratio. As the ratio of polymer to drug is increased, the particle size is increased. It is assumed that in higher concentration ratio up to 7 mg mL^{-1}, electrostatic interaction between polymer and R-HCl increases and consequently negative surface charge decreases, so repulsion force between particles decreases; therefore, tendency of aggregation and producing larger particles enhances. Subsequently increasing in particle size when the concentration ratio increases from 7 to 10 mg mL^{-1}, is justified by polymer rigidity in high concentration ratio and aggregation of particles.

Loading Efficacy (LE). Calculated LE% of nanoparticles in the range of 83−91.62% depended on all three variables (A, B, and C). The effects of concentration ratio of CPU/R-HCl and molecular weight of CPU on loading efficacy are shown in Figure 2(d). It is obvious that drug entrapment is occurring due to electrostatic interactions and avoids contacting with the medium (water) in used coprecipitation method.[32] A second-order significant effect of concentration ratio on LE% has been observed. As it is demonstrated, at constant CPU molecular weight, by increasing the concentration ratio from 4 to 7 mg mL^{-1}, LE% was increased until the maximum LE% was obtained. Further increases in concentration ratio from 7 to 10 mg mL^{-1}, cause decrease in LE% of particles[33] have explained that increasing the ratio of CPU to R-HCl may cause rigidity of polymer and consequently decrease of LE%. Nanoparticles prepared from B_2 showed the maximum LE% while minimum entrapment has been observed for nanoparticles that prepared by both high and low molecular weights polymers. At constant molecular weight of polymer and fixed concentration ratio, by increasing the speed of stirring, the LE% is increased (Supporting Information Figures S5, S6). The percentage of loading efficacy for all CPUs confirms that R-HCl is mainly entrapped in the nanoparticles. The highest percentage of loading efficacy is observed for B_2, concentration ratio of 7 mg mL^{-1} and speed of stirrering of 600 rpm.

Model Validation

After determination of optimum condition of physicochemical properties of NPs at ratio of polymer to drug of 6.76 mg mL^{-1}, molecular weight of polymer to drug of 2 (medium) and speed of stirring of 670 rpm by Box–Benkehn design, the trials were repeated five times. Statistical student t test was performed in order to compare actual and predicted data. The significant level was considered as 0.05. The optimum results of trials was obtained with loading efficacy of 89.40 ± 3.32, loading capacity of 11.46 ± 0.40, poly dispersity index of 0.16 ± 0.04, mean diameter of 72.23 ± 2.71 nm and zeta average of −39.70 ± 3.00 mV, that confirm predicted data by Box–Benkehn design.

FT-IR Analysis

Figure 3 shows the FTIR spectra of CPU (B_2), R-HCl and NPs. Six main regions for characterization of CPU [Figure 3(b)] were assigned as follows: (a)—A broad absorption band of the N—H stretching mode in 3300–3600 cm. (b) Aliphatic C—H stretching mode of 2850–3000 cm. (c) The carbonyl (C═O) stretching absorption band of urethane free at 1710 cm. (d) C—O—C stretching absorption band at 1160 cm corresponding to the ether oxygen of soft segment. (e) C—N—H stretching absorption band at 1541–1542 cm. (f) C—O—C asymmetrical stretching absorption at 1230 cm.

Possibility of interactions between the CPU and R-HCl was determined by FTIR spectroscopy. As shown in Figure 3(a), besides to characteristic peaks at 2956, 2946, 2750, 2690, 2570,

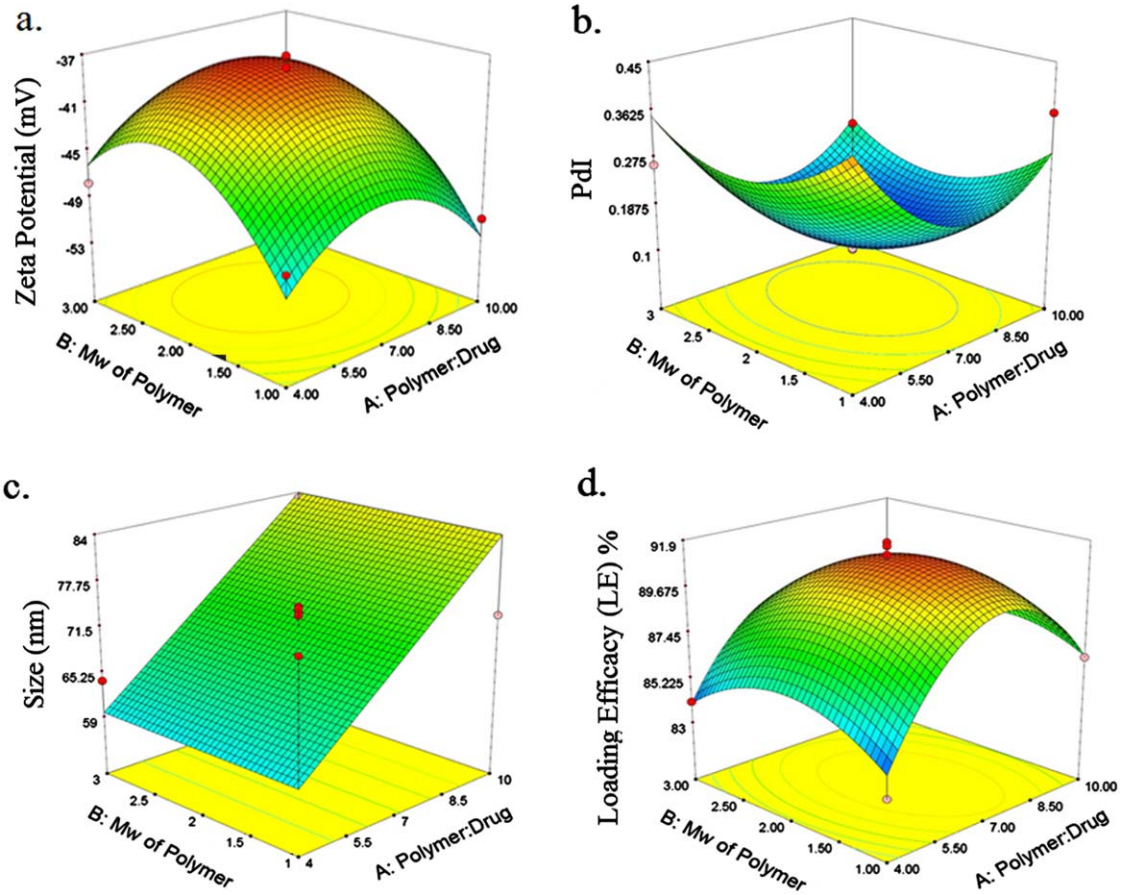


Figure 2. The 3D plots of the effects of ratio of polymer to drug and molecular weight of polymer on (a) zeta potential, (b) PdI, (c) particle size, (d) loading efficacy of nanoparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and 2540 cm, there is the most important double peak at 3140–3215 cm due to its functional N—H and Ph—OH bonds in the structure of R—HCl. It can be considered that physicochemical interactions during the preparation of the nanoparticles like hydrogen bonds between the carriers and drug will automatically lead to frequency shifts or splitting in absorption peaks.[22] Polyurethanes have a lot of N—H groups and ester groups that are able to interact with N—H and Ph—OH bonds in raloxifene. The presence of abroad peak at 3281 cm in FT-IR spectrum of NPs has confirmed the hydrogen bond interaction between CPU and R—HCl beside of ionic interactions [Figure 3(c)].

Nanoencapsulation of Raloxifene Hydrochloride in Carboxylated Polyurethanes

The nanoparticles of raloxifene hydrochloride are prepared for the first time using carboxylated polyurethane which probably would increase the drug's bioavailability. Earlier studies reported that lower doses can be administrated to the patient by nanoparticle formulations for intravenous, oral or pulmonary administration, so it can reduce the toxicity effects of drug and improve the bioavailability of poorly water soluble drugs.[34]

Dynamic Light Scattering (DLS)

Particle size distribution, polydispersity index and zeta potential for nanoparticles were obtained through dynamic light scattering. Zeta potential and particle size are two important characteristics of nanoparticles. The particle size distribution and polydispersity index of nanoparticles in optimum condition are 73.58 and 0.116 nm, respectively. As the particle size for bone application is 85–150 nm,[22] so it can be resulted that the prepared nanoparticles is accepted by bone cells.

The average zeta potential of the NPs is −37.2 mV. The stronger repellent interaction between polymer and drug lead to higher absolute zeta potential and higher stable nanoparticles.[31] These results suggest the presence of R—HCl molecules possibly into the nanoparticles and confirm this claim that the drug with positive charge is encapsulated in CPU with minus charge as a carrier.

Scanning Electron Microscopy (SEM)

The SEM micrograph of the NPs is shown in Figure 4 that is in agreement with the dynamic light scattering measurements. Particle size distribution and size diameter were determined using CLEMEX® particles image analysis software package. It seems the shape of nanoparticles is discrete spherical which assign them suitable features to be used as injectable delivery system.

Differential Scanning Calorimetry (DSC), Thermal Gravimetric Analysis (TGA), and X-ray Diffractometery (XRD)

DSC is a technique to access physicochemical state of the drug in system of nanoparticles and investigate its thermal properties.

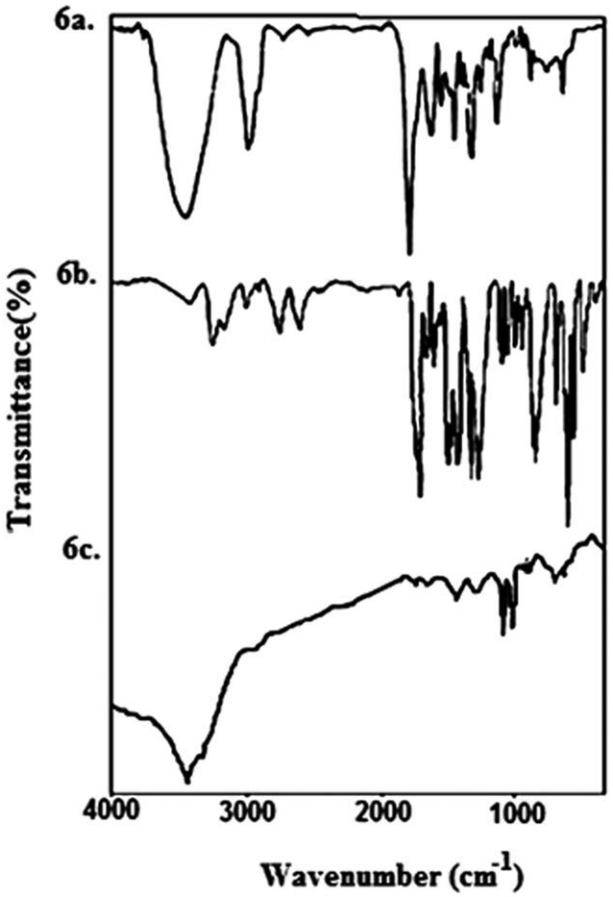


Figure 3. FT-IR spectrum of (a) R-HCl, (b) B_2, (c) NPs (ratio of polymer to drug: 6.76 mg mL^{-1}, molecular weight of polymer: 2, speed of stirring: 670 rpm).

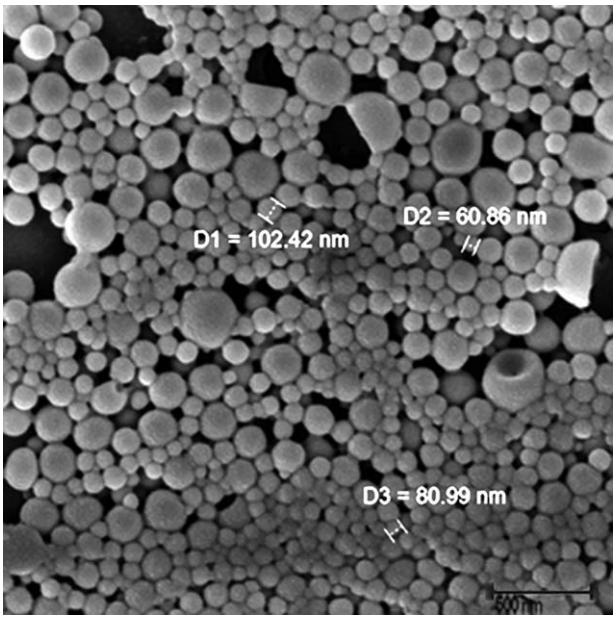


Figure 4. SEM micrographs of NPs (ratio of polymer to drug: 6.76 mg mL^{-1}, molecular weight of polymer: 2, speed of stirring: 670 rpm).

If pattern of drug loaded nanoparticles correspond to those of individual component (polymer or drug), there is no interaction between drug and polymer. If in prepared formulation of nanoparticles one or two new peaks corresponding polymer or drug appear or disappear or shift in location, interaction between two components occurs.[35,36] If the drug will be in solid solution state in polymeric nanoparticles or microsphere there is not any obvious endotherm.[37] DSC thermograms of R—HCl, B_2 and NPs are shown in Figure 5(a). Data were analyzed using STAR^e software. Pure R—HCl shows an endothermic melting peak at 270°C indicating its crystalline nature, while CPU shows an endothermic peak at 80°C due to dissociation of urethane soft segment hydrogen bond.[38] The thermogram of NPs showed R—HCl peak shifted from 270 to 220°C, suggesting a decrease in its T_g. This negative deviation may be ascribed to physical interaction,[39,40] such as hydrogen bonding[35,41] between the carbonyl group of CPU and the NH group of R—HCl. This claim is confirmed by TGA results, as the NPs were extremely stable and decomposed at 270°C [Figure 5(b)]. Moreover the crystal structure of the CPU, R—HCl, and NPs was determined by XRD [Figure 5(c)]. In NPs, disappearing the peak of R—HCl indicated that it was entrapped mainly in an amorphous phase in the CPU whereas the diffractogram of R—HCl exhibits sharp peaks, indicating the crystalline nature of it and CPU shows the amorphous nature.

In Vitro Release Study

Current studies on the *in vitro* release of R—HCl from optimum nanoparticles are shown in Figure 6. Using these raloxifene nanoparticles will result controlled release system with higher bioavailability and reduced toxicity effects in human body. The nanoparticles showed a controlled release of drug from the beginning. Crystalline nature of the carrier is one of the important factors that affects the dissolution rate of R—HCl in the medium.[22] Because of the fact that CPU is not in crystalline form, the microchannel structure is not created by the polymer and fast release is not observed. The ordinate of the plot was calculated on the basis of cumulative amount of R—HCl released, with respect to the amount of R—HCl in CPU. The percentage of release of R—HCl from CPU was 24.19% ± 4.35% after 4 weeks. Moreover, the release rate is seen to be highly dependent on the kind of soft segment of carboxylated polyurethane. The CPU made from polycaprolactone diol showed a much slower rate of release as compared to those made from polypropylene glycol with the same chemical composition in our previous study. The release data were examined by Pepas mathematical model and the extent of fitness in this model showed a Fickian release profile with $n = 0.5$ and $R^2 = 0.98$.

CONCLUSIONS

Raloxifene hydrochloride loaded carboxylated polyurethane nanoparticles were fabricated by coprecipitation method. For this purpose biodegradable carboxylated polyurethanes in three molecular weights were synthesized. The effects of some parameters such as ratio of polymer to drug, molecular weight of polymer and also speed of stirring on zeta potential, PdI, particle size and loading efficacy were investigated. Three determined

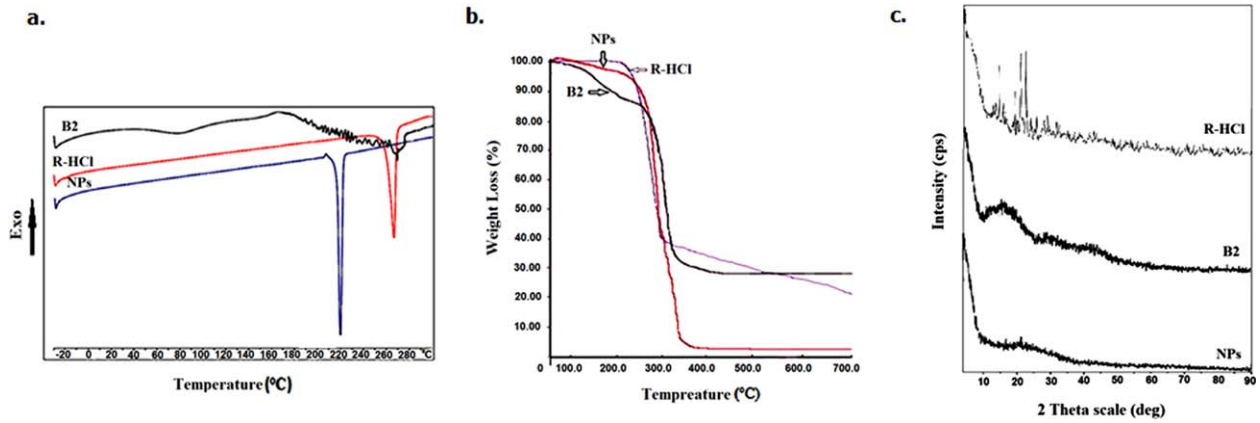


Figure 5. (a) DSC thermographs, (b) TGA thermograms and (c) XRD pattern of R-HCl, B_2 and NPs (ratio of polymer to drug: 6.76 mg mL^{-1}, molecular weight of polymer: 2, speed of stirring: 670 rpm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

parameters had significant effects on zeta potential, PdI and loading efficacy, so the model was quadratic for them. The particle size was just dependent to ratio of polymer to drug, as the ratio of polymer to drug increased; the size of nanoparticles was increased, so the model for particle size was linear. The negative deviation of the peak of raloxifene in DSC results of nanoparticles indicated that there is strong hydrogen bond between R—HCl and CPU. This claim was confirmed by the broad peak at 3281 cm in FT-IR spectrum of drug loaded nanoparticles. The drug was found dispersing in CPU in amorphous state, which was confirmed from XRD results. The results of SEM for the nanoparticles showed the nanoparticles have spherical shape.

A controlled release of R—HCl from nanoparticles was observed during the *in vitro* dissolution rate study All these results indicate that R—HCl loaded biodegradable carboxylated polyurethane are promising sustained drug delivery system and these types of CPUs can be widely used as effective carriers for controlled release drug delivery.

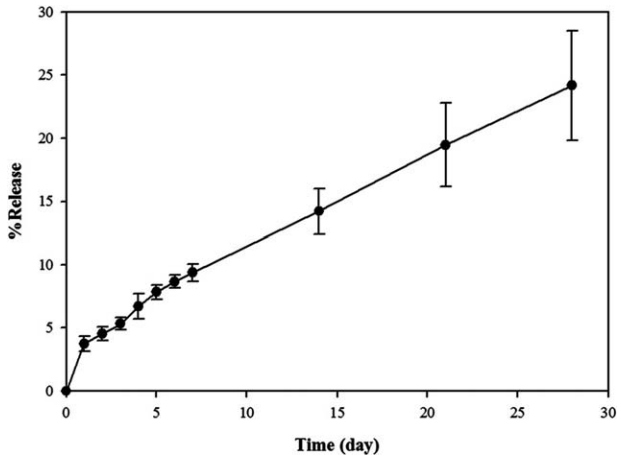


Figure 6. Release profile of R—HCl from NPs (ratio of polymer to drug: 6.76 mg mL^{-1}, molecular weight of polymer: 2, speed of stirring: 670 rpm), data is shown as mean ± SD of three samples.

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