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Comparative Study of Ibuprofen and Indomethacin Loaded Poly(caprolactone) Nanoparticles : Physicochemical Properties

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Abstract

Nanotechnology is a straightforward strategy for the development of drug delivery systems. Poly(caprolactone) (PCL) has been used as a hydrophobic biodegradable polymeric core of nanoparticles for encapsulation of various kinds of self-problematic drugs. In this study, PCL was fabricated by ring opening polymerization and the nanoparticles were then obtained by solvent diffusion and evaporation method using Tween 80 and Span 80 as surfactants and Poloxamer 188 as a stabilizer. Ibuprofen and indomethacin were encapsulated into the PCL nanoparticles to observe the effect of molecular characteristics on the physicochemical properties of the nanoparticles. The results showed that the characteristics of encapsulated drugs profoundly affected the physicochemical properties of the nanoparticles. After incorporation of drug, indomethacin provided smaller particle size, less size distribution, and more negative surface charge as compared to ibuprofen, probably due to the individual effect on the polymer and surfactant during nanoprecipitation. Also, %yield, %entrapment efficiency and %drug loading of indomethacin-loaded nanoparticles were higher than those of ibuprofen-loaded nanoparticles at all drug:polymer ratios. The result indicated that the hydrophilic small molecular drug, ibuprofen, was less efficiently entrapped in the system than the hydrophobic larger molecular drug, indomethacin, due to the drug leakage during incorporation process by nanoprecipitation method. Thus, it can be concluded that the different physicochemical properties of drug profoundly have individual effect on the drug-loaded PCL nanoparticles.

Key words: indomethacin, ibuprofen, poly(caprolactone), nanoparticle

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INTRODUCTION

Over the past periods, nanotechnology has been enormously gained an attention in the field of drug delivery system. Stemming from the extremely small size of this system, the therapeutic efficiency of drug loaded in this system is enormously improved. Many attempts have been considerably utilized to fabricate the nanoparticles as effective and efficient nano-carriers to overcome the limitation of drug administration; for instances, to surpass the physiological barrier, to precisely deliver to the targeted tissue or organ, to controllably extend the desired residence time, to enhance the therapeutic benefit, and to minimize the undesirable side effect[1]. Several kinds of core shell particles have been fabricated to incorporate the drugs such as solid lipid, natural, and synthetic polymers[2-11] by the different methods developed for the achievement of highly effective carriers for each kind of drug. Among the synthetic polymers, polyester is one of interest for medical and pharmaceutical purposes due to its biodegradable and biocompatible properties. Poly(caprolactone) (PCL) is a good candidate in this group and has been remarkably applied for the development of carriers since it shows semi-crystalline, highly permeable, and slowly degradable both *in vitro* and *in vivo* properties[12-14]. The characteristics of carriers made from PCL can be desirably modulated by, for examples, covalently bonding or physically blending with other polymer etc. Up to present, the PCL carriers have been developed to deliver various kinds of pharmacological actives, including antibiotics[15], antifungals[16], vaccines[17], anticancer drugs[18,19], sunscreen agents[20], anticonvulsants[21], and DNA[22].

Ibuprofen (IB) and indomethacin (IN) (Figure 1) are weak acid non-steroidal anti-inflammatory drugs (NSAIDs) available in the market. Typically, these drugs are orally effective in medical treatment of osteoarthritis, rheumatoid arthritis, inflammations, and a variety of pains[23]. Nevertheless, the major side effect of such drug is known to irritate the GI tract. These drugs are classified according to their structure, into the different group. IB is classified in propionic acid derivatives and IN is acetic acid derivatives[24,25]. Regarding their structures, IN contains methylated indole ring which is not found in IB. This distinguished structure results in the different characteristics in molecular volume and numbers of functional group to form bonding[26].

In general, one consideration concerning the development of nanocarrier is the compatibility between incorporated drug and polymeric core referring to miscibility and/or interaction without alteration in chemical structure. Not only the nature of nanostructure core but also the molecular characteristic of drug can affect the properties of the system, such as stability, drug loading capacity, drug entrapment efficiency, and drug release profiles, proceeding through the interaction between drug and polymeric core involving van der Waals force, dipole-dipole interaction, and hydrogen bonding[27-29]. Therefore, it is in consideration that various characteristics of drugs and polymers can possess the interaction that leads to differences in the characteristics of the carriers including micro- and nanoparticles. To the best of our knowledge, the comparison between different structure of these two NSAIDs (IB and IN) affected the characteristics of PCL nanoparticles has not been studied. Therefore, the present study aimed to investigate the structural effect of these two drugs on the physicochemical characteristics of PCL nanoparticles after drug-incorporation. In this study, PCL was synthesized by ring opening polymerization (ROP) and its characteristic was then evaluated by NMR spectroscopy and gel permeation chromatography (GPC). The PCL nanoparticulate dispersion was prepared by solvent diffusion and evaporation technique using Tween 80 and Span 80 as a pair of surfactants, and Poloxamer 188 as a stabilizer. Subsequently, IB and IN at the various drug and polymer ratios were loaded into the PCL nanoparticles to study the effect of their molecular structures on the physicochemical properties of the nanoparticles.

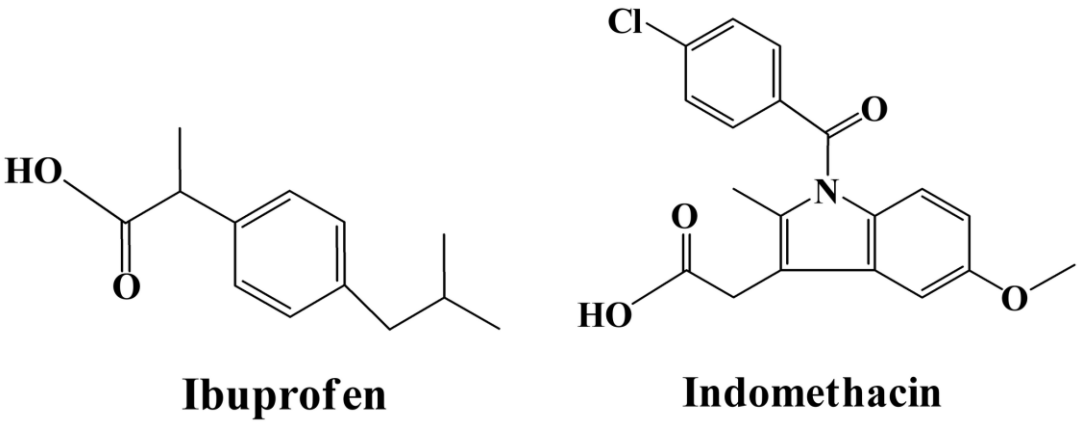

Figure 1. Structure of ibuprofen and indomethacin.

MATERIALS AND METHODS

Materials

ε-Caprolactone monomer (CL; Aldrich, Steinheim, Germany) was stirred over calcium hydride for 48 h and distillated prior to use. 1,4-Butanediol, poloxamer 188 (PL188), polysorbate 80 (Tween 80, TW80), and stannous (II) octanoate ($Sn(Oct)_2$) were obtained from Sigma-Aldrich, Steinheim, Germany. Sorbitan oleate (Span 80, SP80) was purchased from Croda Iberica SA, Barcelona, Spain. Milli-Q water was used by purification with a Synergy® (Millipore, Molsheim, France). Other organic solvents were used as received. Acetonitrile and methanol were of HPLC grade from Merck (Damstadt, Germany). Glacial acetic acid (100%) was purchased from VWR International S.A.S, Damstadt, Germany. Ibuprofen and indomethacin were kindly gifted from Vita Co. Ltd, Bangkok, Thailand, and Government Pharmaceutical Organization (GPO), Bangkok, Thailand, respectively.

Polymerization

CL monomer (1.14 g, 10 mmole) was polymerized by ROP using one-tenth molar equivalence (compared to mole of monomer) of 1,4-butanediol as an initiator and $Sn(Oct)_2$ as a catalyst[30-32]. The mixture in the reaction flask was evacuated for 15 min and the reaction was processed in an oil bath at 120°C for 6 h under argon atmosphere. The crude polymer was purified by precipitation in cold hexane and dried in vacuo overnight.

Polymer characterization

^{1}H-NMR spectrum was recorded in FT-mode with Bruker Avance 300 apparatus (Bruker corporation, Rheinstetten, Germany) using $CDCl_3$ as a solvent. Number- and weight-average molecular weights (M_n and M_w, respectively) of polymer were evaluated by a Water 150-CV GPC (Waters Corporation, Massachusetts, USA) equipped with refractive index detector. The polymer was eluted from 2 columns of PLgel 10 μm mixed B (Varian, Inc., California, USA) using tetrahydrofuran (THF) as a solvent. The molecular weight values were calculated relative to those values of polystyrene standards calibrated in the range of 4,490–1,112,000 g/mole.

Nanoparticle preparation

Fifty milligrams of PCL was dissolved in THF containing SP80. An organic solution was gently added to an aqueous phase containing TW80 and PL188 under magnetic stirring. The solvent was removed under reduced pressure. The final volume of dispersion was adjusted to the initial volume. The aggregates were filtered out through 0.45 μm cellulose acetate membrane filter (Millipore, Schwalbach, Germany). The lyophilized powder was obtained after freeze drying process. In case of drug loaded nanoparticles, an accurate amount of drug (Table 1) was initially added into polymer solution, and the loaded nanoparticles were prepared as previously described.

Table 1. Compositions of the preparation of the nanoparticles

Formulations	D:P Ratio[a]	Weight (mg)				
		PCL	TW80	SP80	PL188	Drug
Blank	0:10	50	25	25	25	-
IB/IN-NP-2	2:10	50	25	25	25	10
IB/IN-NP-3	3:10	50	25	25	25	15
IB/IN-NP-4	4:10	50	25	25	25	20
IB/IN-NP-5	5:10	50	25	25	25	25

[a]Drug:PCL polymer ratio

Nanoparticle characterization

The particle size, polydispersity index (PDI) and zeta potential of the obtained nanoparticles were measured by Photon Correlation Spectroscopy (PCS) using Zetasizer 3000 HSA (Malvern Instruments, Malvern, UK). The measurements were analyzed with HeNe laser (633 nm, 90^o angle) at 25^{o}C.

Drug loading and entrapment efficiency

The nanoparticle dispersion was transferred into Microcon Ultracel YM-30 tube (MW cut-off 30,000 Dalton) (Millipore, Schwalbach, Germany) and centrifugally filtered at 16,000 rpm for 10 min. The amounts of drug in the lyophilized powder and in the filtrate were analyzed by HPLC method. The difference of the amount of drug between in lyophilized powder and in filtrate was used to calculate the amount of drug incorporated in the nanoparticles. The yield, drug loading and entrapment efficiency were calculated according to the following equations (eq. 1-3):

Quantitative analysis of drug

The amount of drug was quantitatively analyzed by HPLC assay using HPLC spectroscopy (Shimadzu Corporation, Kyoto, Japan) consisting of an autoinjector SIL-10A and a pump LC-10AD (Shimadzu Corporation, Kyoto, Japan). The quantity of eluted drug through a reverse phase Hypersil ODS column, 5 µm particle size, 250×4.6 mm (Thermo Scientific, Massachusetts, USA) was detected at 264 nm for IB and 254 nm for IN using a UV detector (Shimadzu SPD-10AV, Shimadzu Corporation, Kyoto, Japan). The mixture of methanol: water: acetronitrile: acetic acid (35:55:10:1) was used as a mobile phase at the flow rate of 1.4 and 1.2 ml/min for IB and IN, respectively.

Data analysis

The data are present mean values± standard deviation (SD). Significance of difference was evaluated using Student's t-test and one-way ANOVA at the probability level of 0.05.

$$\%\ Yield = \frac{Amount\ of\ lyophilized\ nanoparticles}{Initial\ amount\ of\ total\ solid\ content} \times 100 \quad (1)$$

$$\%\ Drug\ Loading = \frac{Amount\ of\ drug\ in\ nanoparticles}{Amount\ of\ lyophilized\ nanoparticles} \times 100 \quad (2)$$

$$\%\ Entrapment\ Efficiency = \frac{Amount\ of\ drug\ in\ nanoparticles}{Amount\ of\ drug\ fed\ initially} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Synthesis of poly(caprolactone)

PCL was synthesized by ROP method using 1,4-butanediol and $Sn(Oct)_2$ as an initiator and a catalyst, respectively. Figure 2 demonstrates NMR spectrum of the synthesized PCL. The $M_{n,theo}$ and $M_{n,NMR}$ were calculated based on theory and ^{1}H-NMR integral value according to the eq. 4 and 5, respectively, as shown belows:

$$M_{n,theo} = \frac{[CL]}{[I]} \times 114 \quad (4)$$

$$M_{n,NMR} = \frac{2 \times I_A}{I_F} \times 114 \quad (5)$$

where [CL] and [I] are the molar concentrations of CL monomer and initiator, respectively. I_A and I_F are the integrals of methylene proton at 2.30 ppm (peak A, Figure 2) and 3.65 ppm (peak F, Figure 2), respectively. A factor of 114 refers to the molecular weight of CL monomer.

The molecular weight value was well agreed with the theoretical value as shown in Table 2. The value of $M_{n,GPC}$ was the highest among the other molecular weights due to the fact that this value was relatively calculated using polystyrene as a calibration standard. In general, the molecular weight determined from polystyrene calibration by GPC technique according to Mark-Houwink equation is approximately two times the actual molecular weight as previously reported[32,33]. The M_w/M_n indicated the broad molecular weight distribution of the synthesized polymer due to the transesterification during the polymerization at high temperature and such broad value was generally obtained when using $Sn(Oct)_2$ and bi-functional alcohol as a catalyst and an initiator, respectively[31,32].

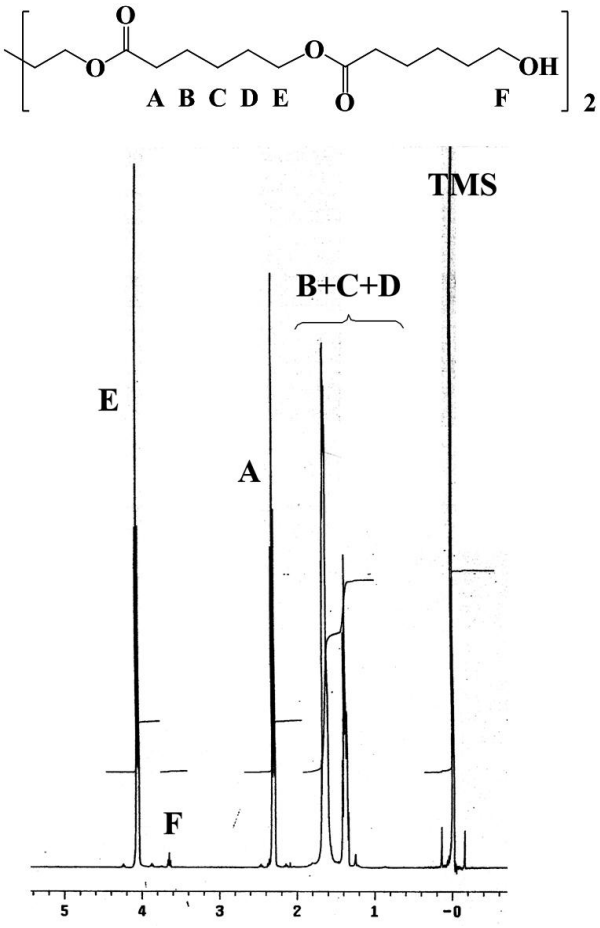

Figure 2. ^{1}H-NMR spectrum of the synthesized PCL

Table 2. The molecular characteristics of the synthesized PCL

Composition	$M_{n,theo}$	$M^{a}_{n,NMR}$	$M^{b}_{n,GPC}$	$M^{b}_{w,GPC}$	$M_w/M_n{}^{b}$
PCL	11,400	10,643	14,369	9,934	1.45

[a]determined by ^{1}H-NMR spectroscopy
[b]determined by GPC

Nanoparticle formation

To prepare nanoparticles of PCL having hydrophobic core, the stabilizer was essentially involved in the formulation. Typically, TW80 and SP80 have been used to stabilize emulsion in topical medication. In our preliminary study, it was found that the use of this pair of surfactants could not stabilize PCL nanoparticles in an aqueous dispersion. PL188, which is widely used as a steric stabilizer in the formation of polyester nanoparticles, was added in the formulation to additionally stabilize the PCL nanoparticles. The particle size of the blank nanoparticles was found to be 219 nm with negative surface charge (approx. -16 mV) (Table 3). Figure 3 illustrates the effect of D:P ratio on the particle size and zeta potential of the nanoparticles. After incorporation of IB, the particle size and PDI gradually increased when D:P ratio increased. In the meantime, increasing D:P ratio tentatively increased the particle size of the IN-loaded nanoparticles and the PDI increased with the increasing particle size. This might be due to the fact that increasing D:P ratio led to more amount of drug incorporated into the polymeric core of the nanoparticles as can be seen from %DL that at the constant amount of solid content, increasing the drug added resulted in the increase in %DL (Figure 4B). The particle size of IB-loaded nanoparticles was significantly larger than that of IN-loaded nanoparticles although IN displays the higher molar volume than IB indicating the bigger molecular size of IN (209.8 mL/mole for IN and 195.5 mL/mole for IB[34]). According to the molar volume, it was anticipated that the particle size of IN-loaded nanoparticles would be larger than IB-loaded nanoparticles. However, the opposite result was obtained probably due to the fact that the different characteristic of drug may have an individual effect on the polymer and SP80 during the incorporation process, based on the nanoprecipitation method, resulting in the slightly compacted polymeric core[35,36].

As compared to the surface charge of the blank nanoparticles, after loading IB, the surface charge of the IB-loaded nanoparticles was not affected by the presence of IB. On the contrary, IN suppressed the surface charge of the nanoparticles to be more negative than that of the blank nanoparticles and also IB-loaded nanoparticles (Table 3). The more negative charge of IN-loaded nanoparticles was presumably contributed to some extent of IN molecules which deposited nearby the surface of nanoparticles. Since a pair of surfactants was used to stabilize the nanoparticles during the nanoparticle formation, the molecule of IN might be inserted between the interface of nanoparticles thereby affecting the surface charge of particle. The more negative charge of drug-loaded nanoparticles has been previously reported with increasing D:P ratio and %DL, unfortunately no explanation has been stated[37,38]. From the results, the higher %DL of IN than IB at all D:P ratios led to the more negative charge of IN- than IB-loaded nanoparticles. Nonetheless, the more negative charge of nanoparticles did not correlate to the increasing %DL of both loaded drugs in nanoparticles which may be due to the increment in size distribution as particle size increased. Thus, it may be deduced that the characteristics of entrapped drug independently affected the surface charge of nanoparticles depending on amount of drug deposited on the surface of particle.

Table 3. The physicochemical characteristics of nanoparticles

Formulations	D:P Ratio[a]	Size[b] (nm)	PDI [b]	Zeta Potential [b] (mV)
Blank	0:10	219± 5	0.071±0.030	-16.2±6.4
IB				
IB-NP-2	2:10	257±9	0.036±0.009	-19.5±6.5
IB-NP-3	3:10	312±10	0.162±0.034	-14.3±6.4
IB-NP-4	4:10	391±4	0.453±0.040	-19.1±6.4
IB-NP-5	5:10	421±7	0.358±0.037	-17.3±6.5
IN				
IN-NP-2	2:10	278±8	0.035±0.019	-25.9±6.4
IN-NP-3	3:10	260±11	0.036±0.017	-27.6±6.5
IN-NP-4	4:10	309±10	0.198±0.037	-25.1±6.5
IN-NP-5	5:10	293±10	0.119±0.017	-24.1±6.5

[a] Drug:PCL polymer ratio
[b]The experiments were performed in triplicate and the values are expressed as mean±SD.

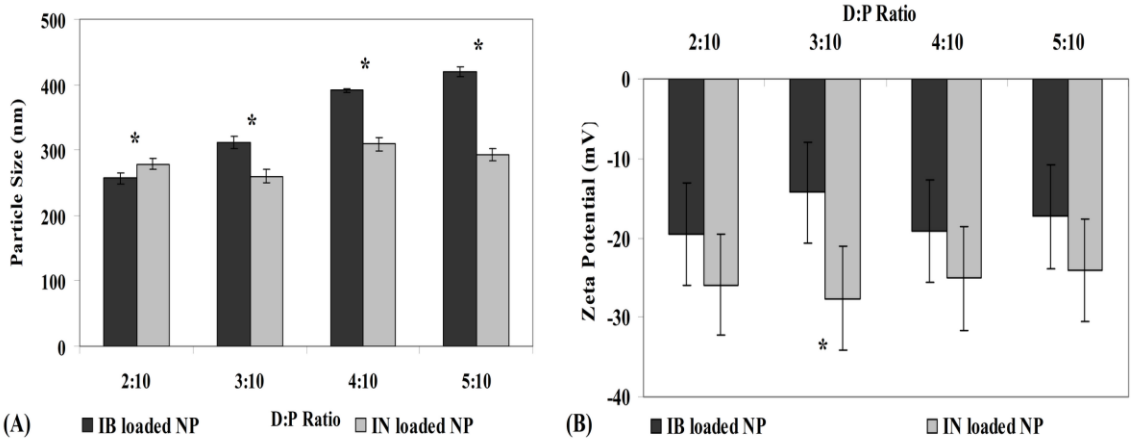

Figure 3. The effect of drug:PCL polymer ratio (D:P ratio) on the particle size (A) and zeta potential (B) of the ibuprofen- and indomethacin-loaded nanoparticles. *Statistically significant different comparing between the different drug at the same D:P ratio (n=3).

Drug loading and entrapment efficiency

Figure 4 shows the effect of D:P ratio on %yield, %DL, and %EE of the drug-loaded nanoparticles. From Figure 4B, it was found that the values of %DL of the two drugs loaded nanoparticles significantly increased when increasing the D:P ratio (*p*-value < 0.05) indicating the increase in the amount of drugs incorporated into the nanoparticles. However, %yield (Figure 4A) and %EE (Figure 4C) of the IB-loaded nanoparticles gradually decreased (*p*-value < 0.05) while these values of the IN-loaded nanoparticles increased and reached the maximum values at 3:10 ratio (*p*-value < 0.05). The decreases in %yield and %EE were due to the calculation of both values based on the total solid content at initial feeding. Increasing D:P ratio increased the total solid content, whereas the amount of drug entrapped was not linearly increased with D:P ratio. From the results, the D:P ratios of IB- and IN-loaded nanoparticles, at which the highest %EE

and %yield were obtained, were found to be 2:10 and 3:10, respectively. The significant difference in loading capacity and entrapment efficiency between IB and IN could be explained by the great difference in an aqueous solubility of both drugs. Due to the higher solubility of IB in water (approx. 0.23 mg/mL[5]), the IB molecules were preferable more dissolved in water during the nanoparticle formation and less partitioned into hydrophobic core resulting in the less amounts entrapped in hydrophobic core. In contrast, IN showed the higher entrapment in hydrophobic core due to the less solubility in water (~ 0.016 mg/mL[39]). The obtained result is consistent with the previous report on the lower drug loading and entrapment efficiency of the hydrophilic drug, such as ibuprofen and ketoprofen, in nanoparticles using nanoprecipitation method as compared to the lipophilic drug, i.e. indomethacin and cyclosporine[40,41]. Therefore, it can be deduced that the solubility of drug greatly affected the loading capacity and entrapment efficiency rather than the other characteristics when the preparation of drug-loaded nanoparticles based on the nanoprecipitation technique.

In the current work, the nanoprecipitation method was applied to prepare the nanoparticles. With this technique, it has been well-documented that it is basically based on the interfacial turbulence and the diffusion process of organic phase into water phase[42,43]. Once the polymeric solution gently added into aqueous phase, the solvent diffuses to aqueous medium taking together with the dissolved polymer and then the hydrophobic polymer solidifies leading to the hydrophobic polymeric core of nanoparticles. Therefore, the solvent-water, solvent-polymer, and water-polymer interactions have to be taken into account for the determining factors of the characteristic of nanoparticles[44-46]. Also, the possible interaction of loaded drug to the other components occurring during the nanoprecipitation can affect the characteristic of drug-loaded nanoparticles as described earlier in each section.

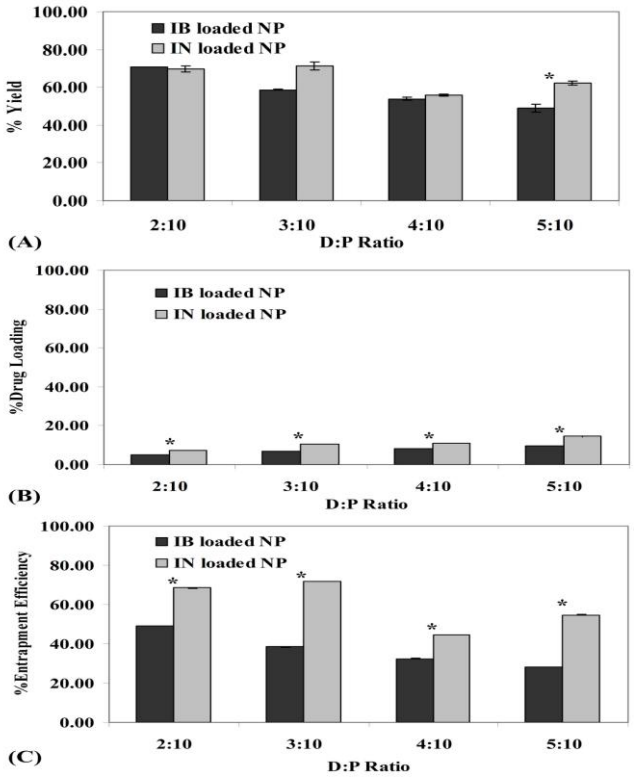

Figure 4. The relationship between drug:PCL polymer ratio (D:P ratio) with % yield, % drug loading, and % entrapment efficiency. *Statistically significant different comparing between the different drug at the same D:P ratio (n=3).

CONCLUSION

PCL polymer synthesized by ROP could be formulated as the nanoparticles using TW80 and SP80 as surfactants and PL188 as a steric stabilizer with the particle size as small as 219 nm. After drug loading, the physicochemical properties of drug-loaded nanoparticles, namely particles size, size distribution, and zeta potential, did greatly depended on the different characteristics of incorporated drug. Besides, the different aqueous solubility of both drugs affected the entrapment efficiency and loading capacity of the PCL nanoparticles. The less soluble drug, IN, was higher entrapped in the hydrophobic core of the PCL nanoparticles as compared to IB. The results showed that the highest %EE and %yield were obtained at drug to PCL polymer ratio of 2:10 and 3:10 for IB and IN, respectively, and the highest loading capacity was attained at 5:10 for both drugs. It can be concluded that the physicochemical property of drug individually affects the characteristics of drug-loaded nanoparticles and should be concerned for the development of drug-loaded nanoparticles.

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