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Research paper

Solubilization of an amphiphilic drug by poly(ethylene oxide)-*block*-poly(ester) micelles

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Abstract

The purpose of this study was to investigate the solubilization of an amphiphilic drug, i.e, amiodarone (AMI) in methoxy poly(ethylene oxide)-*block*-poly(ester) micelles of different core structure. The effect of core-forming block structure as well as molecular weight, applied drug to polymer ratios and assembly condition on AMI solubilization; stability of the solubilized formulation upon dilution in phosphate buffer and the hemolytic activity of solubilized AMI against rat red blood cells were assessed and compared to those parameters for the commercial intravenous formulation of AMI. In general, polymeric micelles of different core structure were found to be more efficient in retaining their AMI content upon dilution than surfactant micelles in the commercial formulation of AMI for injection. Micelles with a poly(ε-caprolactone) (PCL) core were more efficient than poly(D,L-lactide) and poly(L-lactide) cores in the solubilization and stabilization of encapsulated AMI within the carrier. Encapsulation of AMI by methoxy poly(ethylene oxide)-*block*- poly(ε-caprolactone) (MePEO-*b*-PCL) micelles having higher PCL chains increased the level of AMI solubilization and decreased its hemolytic activity. Compared to O/W emulsion, application of solvent evaporation method led to higher encapsulation efficiency and lower hemolytic activity for AMI in micelles. An increase in the level of AMI added to the co-solvent evaporation process led to an increase in the solubilized AMI levels, but made the formulation more hemolytic. In conclusion, PEO-*b*-PCL micelles, particularly those with longer PCL chains, were found to be efficient carriers in encapsulating amphiphilic AMI, retaining encapsulated AMI within the carrier and reducing its hemolytic activity.

Keywords: Amiodarone; Polymeric micelles; Solubilization; Amphiphilic drug; Hemolysis

1. Introduction

Amiodarone (AMI) is an antianginal and antiarrhythmic drug widely used in the treatment of ischemic heart disease. Amiodarone in its commercial intravenous formulation, i.e., Cordarone®, is solubilized with the aid of a low molecular weight surfactant, polysorbate 80, and a co-solvent, benzyl alcohol [1]. Dilution of this formulation in blood after intravenous injection leads to the precipitation of AMI, phlebitis and pain at the site of injection [2]. The level of polysorbate and benzyl alcohol used in this formulation is already above the recommended doses of these solubilizing agents for intravenous injection and cannot be increased to compensate for the blood diluting effect [3]. Besides, solubilizing agents used in the commercial injectable formulation of AMI add to complications associated with AMI administration in clinic. Neither polysorbate 80 nor benzyl alcohol are inert ingredients. Both have negative inotropic effects and cause vasodilatation and result in a decrease in arterial blood pressure [4–6]. Several studies report that rapid infusion of Cordarone® results in a significant decrease in systolic blood pressure that may lead to cardiovascular collapse in patients that are already hypotensive.

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The search for an alternative formulation for the intravenous administration of AMI has been the subject of several studies [4,6–11]. To date, several strategies such as pH modification [6,7], formation of microemulsions [9,10], and encapsulation by lipid nanocarriers [11] have been examined to obtain an alternative water soluble and safe formulation for the intravenous administration of AMI. Elimination of polysorbate 80 and benzyl alcohol has made these alternative formulation strategies successful in reducing the hypotensive effects of injectable AMI. The possibility of AMI precipitation after injection is not completely accounted for, however.

The objective of this study was to assess the potential of polymeric micelles as solubilizing vehicles for the formulation of injectable AMI. In recent years, polymeric micelles have been the focus of much interest as alternative vehicles for the solubilization of poorly water-soluble molecules rendering clear advantages over current solubilizing agents in drug delivery [12–14]. For instance, surfactant micelles may be diluted below their critical micellar concentration (CMC) after intravenous administration, fall apart and lose their drug content, rapidly. The rapid loss of drug from surfactant micelles leads to the precipitation of the solubilized drug immediately after administration. In comparison to low molecular weight surfactant micelles, polymeric micelles have shown higher thermodynamic and kinetic stabilities. Therefore, polymeric micelles are expected to withstand the diluting effect of blood, stay in a micellar form and even act as a circulating depot drug delivery system after intravenous administration.

Efficient solubilization of hydrophobic drugs in different polymeric micellar structures is well-documented in the literature [13–21]. However, limited information on the solubilization of amphiphilic molecules like AMI (Fig. 1) in polymeric micelles exists [22–24]. In the present study, solubilization of AMI by different methoxy poly(ethylene oxide)-*block*-poly(ester) micelles was investigated. The effect of assembly conditions (i.e., core-forming block structure and molecular weight, loading method and applied drug to polymer concentration ratios) on the level of AMI solubilization, precipitation upon dilution, and hemolytic activity is described.

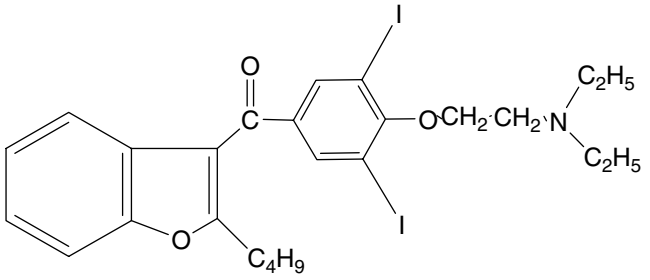

Fig. 1. Chemical structure of AMI.

2. Materials and methods

2.1. Materials

Amiodarone hydrochloride, ε-caprolactone, D,L-lactide, methoxy poly(ethylene oxide) (average molecular weight of 5000 g/mol), cyclosporine A were purchased from Sigma (St. Louis, MO, USA). Methoxy poly(ethylene oxide)-*block*-poly(L-lactide) (MePEO-*b*-PLLA) was from Polymer Source Inc., Dorval, Quebec, Canada. Stannous octoate (96%) and biphenyl (99.5%) were obtained from Aldrich (Milwaukee, WI, USA). Methanol, acetonitrile, and chloroform (all HPLC grade) were supplied by Fisher Scientific (Nepean, Ontario, Canada). Amiodarone HCL for injection was provided by Norme Sabex (Boucherville, Quebec, Canada). Sodium chloride injection 0.9% was obtained from Abbott Laboratories (Montreal, Canada). Other chemicals used were of analytical grade.

2.2. Methods

2.2.1. Preparation of AMI incorporated polymeric micelles and their characterization

Methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (MePEO-*b*-PCL) co-polymers were synthesized by ring opening polymerization of ε-caprolactone using methoxy polyethylene oxide (M. wt of 5000 g/mol), stannous octoate (0.5% w/w) and ε-caprolactone (different ratios) as described before [18]. Methoxy poly(ethylene oxide)-*block*-poly(D,L-lactide) (MePEO-*b*-PDLA) was prepared through ring opening polymerization of D,L-lactide under similar condition. Prepared block co-polymers were characterized for their number average molecular weight by ^{1}H NMR (AM-300 MHz). A nomenclature of 5000–5000, 5000–13,000 and 5000–24,000 in which the left number corresponds to the theoretical molecular weight of the shell forming block (MePEO) and the right number corresponds to the molecular weight of the core forming block (PCL, PDLA or PLLA) is used throughout the manuscript to distinguish between different block co-polymers.

Assembly of block co-polymers and drug loading in polymeric micelles was accomplished through co-solvent evaporation [25]. An O/W emulsion method was also used for the assembly and AMI loading in 5000–13,000 MePEO-*b*-PCL micelles. In the co-solvent evaporation method, block co-polymer (20 mg) and AMI (4–10 mg) were dissolved in 1 mL of acetone. Double distilled water (2 mL) was added in a dropwise manner (50 μL every 10 s) to this solution while stirring. The solution was left stirring at room temperature for 4 h. The remaining of the organic solvent was removed by evaporation under vacuum at room temperature. Prepared micelles were centrifuged (11,600 × *g*, 5 min) to remove any precipitates.

For the O/W emulsion method, polymer (20 mg) and drug (4 mg) were dissolved in 0.3 mL of chloroform. This solution was poured into 2 mL of distilled water under vigorous stirring at once. The solution was left stirring at

room temperature overnight for chloroform to be evaporated. Prepared micelles were centrifuged ($11{,}600 \times g$, 5 min) to remove any precipitates.

The level of incorporated AMI in MePEO-*b*-PCL micelles was defined by HPLC. An aliquot of the micellar solution in water was diluted in acetonitrile: methanol: phosphate buffer (28:49:23, pH 6.7) to disrupt the micellar structure. After dilution encapsulated levels of AMI were measured using reverse-phase HPLC. The HPLC instrument consisted of a ChemMate pump and an auto-sampler. An LC_1 column (Supleco) was equilibrated with a mobile phase of acetonitrile:methanol:phosphate buffer (28:49:23, pH of 3.1 adjusted with phosphoric acid) at a flow rate of 1 mL/min. The column was heated at 65 °C using an Eppendorf CH-30 column heater. AMI concentrations were estimated by UV detection at 244 nm (Waters, model 481) after injection of 100-μL samples. Amiodarone loading and encapsulation efficiency were calculated from the following equations:

$$\text{AMI loading (M/M)} = \frac{\text{amount of loaded AMI in mole}}{\text{amount of added polymer in mole}}$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{amount of loaded AMI in mg}}{\text{amount of added AMI in mg}} \times 100$$

As control, free AMI (4 and 10 mg) was dissolved in acetone (2 mL) in the absence of block co-polymer. Water was then added dropwise to this solution and acetone was evaporated (following an identical procedure for AMI encapsulation in polymeric micelles). The final solution was centrifuged ($11{,}600 \times g$) to remove any precipitate and the level of AMI dissolved in the supernatant was measured by HPLC as mentioned above.

Mean diameter and polydispersity of self-assembled structures in aqueous media were defined by light scattering (3000 HSA Zetasizer Malvern, Zeta-PlusTM zeta potential analyzer, Malvern Instrument Ltd., UK).

2.2.2. Assessing the stability of AMI solubilized in different vehicles upon dilution in phosphate-buffered saline (PBS)

Free AMI (solubilized in water by the mentioned procedure), polymeric micellar and commercial formulation of AMI were diluted 2-fold with PBS (pH 7.4) and incubated at 37 °C for different time periods up to 6 h. Separate samples were prepared for each time point. One sample was taken at 5, 15, 30 min, 1, 2, and 6 h and centrifuged at $11{,}600 \times g$ for 6 min to separate the precipitate. The theoretical concentration of AMI for all original samples before dilution and incubation was either 2 mg/mL except for one sample, which contained an original AMI concentration of 5 mg/mL in 5000–13,000 MePEO-*b*-PCL micelles. A solution of polymeric micellar AMI for each sample without dilution was also incubated under the same condition, as control. An aliquot of supernatant was diluted with mobile phase (acetonitrile:phosphate buffer:methanol at a ratio of 28:23:49) and the amount of AMI in the supernatant was determined by HPLC as explained in the previous section. Each experiment was conducted in triplicate. The percentage of precipitated AMI was calculated by the following equation and plotted versus time.

$$\text{Precipitated AMI (\%)} = \frac{\text{original AMI added (mg)} - \text{AMI remained in the supernatant (mg)}}{\text{original AMI added (mg)}} \times 100$$

2.2.3. Assessing the hemolytic activity of AMI formulations against rat red blood cells (RBCs)

Blood was collected from Sprague–Dawley rats (250–350 g) by cardiac puncture under anesthesia and centrifuged. Supernatant and buffy coat were removed. RBCs were washed and diluted with isotonic PBS, pH 7.4. The proper dilution factor was estimated from the UV/VIS absorbance of hemoglobin at 576 nm in the supernatant after RBCs were lysed by 100 μg/ml of amphotericin B in Fungizone®. Free AMI was also solubilized in water with the aid of acetone using an identical process to the co-solvent evaporation of encapsulation in the absence of block co-polymer. Different MePEO-*b*-PCL micellar formulations of AMI, commercial formulation of AMI for intravenous injection, and free AMI dissolved in water by the aid of acetone were incubated with diluted RBCs at 37 °C for 3 h at different AMI concentrations and placed in ice afterwards to stop hemolysis [26]. The unlysed RBCs were removed by centrifugation ($11{,}600 \times g$ for 30 s), and the supernatant was analyzed for hemoglobin by UV/VIS spectroscopy at 576 nm. The percent of hemolysis was determined using the following equation:

$$\text{Hemolysis (\%)} = \frac{(\text{Abs} - \text{Abs0})}{(\text{Abs100} - \text{Abs0})} \times 100$$

where Abs, Abs_0, and Abs_{100} are the absorbances for the sample, control with no drug and control in the presence of hemolytic dose of amphotericin B as part of Fungizone®, respectively.

2.2.4. Statistical analysis

Compiled data were presented as means ± SD. Where feasible, the data were analyzed for statistical significance by unpaired Students' *t* test. The level of significance was set at $P \leqslant 0.05$.

3. Results

3.1. Solubilization of AMI by polymeric micelles

With block co-polymers having PCL, PDLA, and PLLA as their hydrophobic block (at similar molecular weight of 4000–5000 g/mol), solubilized AMI to polymer molar ratios were found to be 2.42, 1.22, and 1.00 M/M, respectively. Polymeric micelles bearing a PCL core were more efficient in the solubilization of AMI than PDLA and

PLLA core structures ($P < 0.05$), but the difference in solubilized drug levels between MePEO-*b*-PDLA and MePEO-*b*-PLLA micelles was not significant ($P > 0.05$). Moreover, with an increase in the molecular weight of the core forming block, AMI solubilized levels in MePEO-*b*-PCL micelles were increased ($P < 0.05$). In the presence of MePEO-*b*-PCL having 5000, 13,000, and 24,000 g/mol of PCL, the water solubilized AMI levels were increased from 1.65 to 1.74, and 1.85 mg/mL reflecting a drug/polymer molar ratio of 2.42, 4.59, and 7.87, respectively (Table 1). Drug incorporated MePEO-*b*-PCL micelles also became larger as the length of the PCL chain was increased. With 5000, 13,000, and 24,000 g/mol of PCL, micelles with an average diameter of 45.2, 74.5, and 95.2 nm were formed after AMI encapsulation, respectively. The size of empty MePEO-*b*-PCL micelles with 5000, 13,000, and 24,000 g/mol PCL molecular weight was 54.1, 74.9, and 87.1 nm, respectively.

An increase in the level of AMI added to the co-solvent evaporation process led to an increase in the solubilized AMI levels. In the presence of 5000–13,000 MePEO-*b*-PCL (10 mg/mL), the increase in the initial concentration of AMI from 2 to 5 mg/mL resulted in a raise in the level of water solubilized AMI from 1.74 to 4.23 mg/mL, corresponding to 4.59 and 11.2 drug to polymer molar ratios (Table 2). The encapsulation efficiency of AMI in MePEO-*b*-PCL micelles was similar (around 85%) for all AMI initial levels. Using an identical loading procedure in the absence of the block co-polymer, with 2 or 5 mg/mL of initial AMI, solubilized AMI levels reached 0.672 and 2.05 mg/mL of water, respectively.

Application of the co-solvent evaporation method led to the formation of smaller polymeric micelles and higher AMI encapsulation. The average diameter of AMI loaded 5000–13,000 MePEO-*b*-PCL micelles prepared by O/W emulsion was 127 nm compared to an average diameter of 74.5 nm for AMI loaded micelles prepared through a co-solvent evaporation method (Table 3). At identical AMI (2 mg/mL) and polymer concentrations (10 mg/mL), AMI solubility in water reached a level of 1.4 and 1.7 mg/mL in the presence of 5000–13,000 MePEO-*b*-PCL micelles using O/W emulsion and co-solvent evaporation methods, respectively.

3.2. Stability of polymeric micellar AMI upon dilution in PBS

In contrast to surfactant micelles present in the commercial formulation of AMI, polymeric micelles were able to retain most of their AMI content in a solubilized form after dilution with PBS. Around 100% of AMI content was precipitated from solubilized free AMI sample and commercial injectable formulation of AMI within 1 and 2 h after incubation with PBS. In contrast, only 41.5% of solubilized AMI was precipitated from the 5000–13,000 MePEO-*b*-PCL micelles after 1 h incubation in PBS (Fig. 2). This level reached 43.6 and 46.5% of the added AMI content after 2 and 6 h of incubation, respectively (Fig. 2). Polymeric micellar AMI incubated at 37 °C without dilution with PBS as control retained 100% of its AMI content.

At higher AMI content (initial AMI concentration of 5 mg/mL) 28.8% of initial AMI was precipitated upon dilution and incubation of polymeric micelles in 37 °C PBS for 2 h (Fig. 3). After 6 h incubation, this level reached 31.7% of the initial AMI level. For comparison, for free AMI solubilized under identical condition in the absence of polymer, 93.3% and 100% of applied AMI levels were

Table 1
Characteristics of AMI loaded MePEO-*b*-poly(ester) micelles having different core structures

Core forming block	Rounded molecular weight of the core-forming block[a] (g/mol)	Drug loading ± SD (M/M)	Encapsulation efficiency ± SD (%)	Micellar Size (nm)	Polydispersity	AMI precipitated after incubation in PBS ± SD (%)	
						1 h	6 h
PLLA	5000	1.00 ± 0.26	37.5 ± 9.67	32.7 ± 8.63[b]	0.83	80.4 ± 0.27	80.8 ± 0.27
PDLA	4000	1.22 ± 0.17	44.0 ± 4.76	68.1 ± 2.97	0.52	75.3 ± 0.73	76.8 ± 4.61
PCL	5000	2.42 ± 0.18	82.6 ± 0.13	45.2 ± 6.70	0.35	44.1 ± 7.00	48.7 ± 0.33
PCL	13,000	4.59 ± 0.02	86.8 ± 0.19	74.5 ± 3.00	0.07	41.5 ± 0.52	46.5 ± 0.88
PCL	24,000	7.87 ± 0.17	92.4 ± 0.20	95.2 ± 9.20	0.12	45.7 ± 8.04	50.2 ± 1.42

[a] Based on ^{1}H NMR. The molecular weight of PEO block was 5000 g/mol for all block co-polymers.
[b] Secondary peak was observed at approximately 400 nm (peak population <20% total population).

Table 2
The effect of initial drug levels on the characteristics of AMI loaded in 5000–13,000 MePEO-*b*-PCL micelles through the co-solvent evaporation method

Added AMI concentration (mg/mL)	Added polymer concentration (mg/mL)	Drug loading ± SD (M/M)	Encapsulation efficiency ± SD (%)	Micellar size ± SD (nm)	Polydispersity
2	10	4.59	86.8 ± 0.19	74.5 ± 2.95	0.07
3	10	6.49	82.2 ± 0.13	71.9 ± 8.83	0.07
4	10	9.03	85.5 ± 0.15	72.9 ± 2.26	0.08
5	10	11.2	84.6 ± 0.15	86.5 ± 3.11	0.05

Table 3
The effect of loading process on the encapsulation of AMI in 5000–13,000 MePEO-*b*-PCL micelles

Method of preparation	Drug loading ± SD (M/M)	Encapsulation efficiency ± SD (%)	Micellar size ± SD (nm)	Polydispersity
Co-solvent evaporation	4.59 ± 0.02	86.8 ± 0.19	74.5 ± 2.95	0.07
O/W emulsion	3.64 ± 0.13	67.0 ± 0.50	127 ± 6.01	0.27

precipitated out of solution after 2 and 6 h incubation at 37 °C in PBS, respectively.

An increase in the molecular weight of the core forming block did not affect the precipitation profile of MePEO-*b*-PCL micellar formulations of AMI upon dilution in PBS. With MePEO-*b*-PCL micelles having 5000, 13,000, and 24,000 g/mol PCL as the core forming block 44.1, 41.6 and 45.7% of applied AMI was precipitated after 1 h of incubation in PBS, respectively. This level reached 48.7, 46.5, and 50.2% within 6 h of incubation, respectively (Table 1). When AMI was encapsulated in MePEO-*b*-PDLLA and MePEO-*b*-PLLA micelles, 75.3 and 80.4% of the encapsulated drug was precipitated after 1 h of incubation in PBS, respectively. The level of precipitated AMI remained similar for these polymeric micelles after 6 h of incubation (Table 1).

3.3. Hemolytic activity of polymeric micellar AMI

The commercial formulation of AMI for intravenous injection caused >90% hemolysis against rat RBCs at the AMI concentration of 30 μg/mL. At a similar concentration, AMI encapsulated in 5000–13,000 MePEO-*b*-PCL micelles by co-solvent evaporation and O/W emulsion techniques caused 3 and 43% hemolysis towards rat RBCs, respectively (Fig. 4). Interestingly, free AMI solubilized with the solvent evaporation method in the absence of block co-polymer showed a similar hemolytic activity to

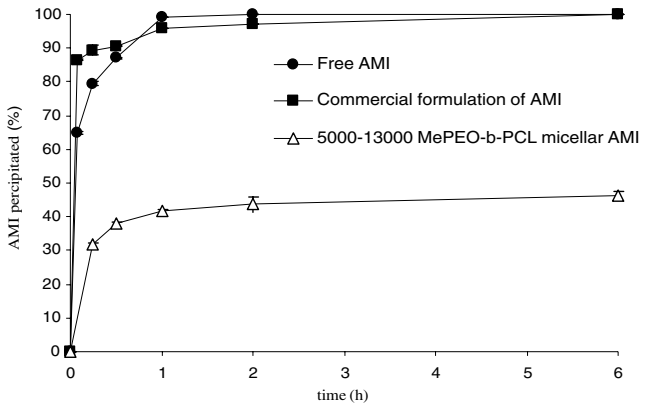

Fig. 2. The stability of different solubilized forms of AMI upon two times dilution and incubation with PBS (pH 7.4) at 37 °C. Each point represents average ± SD ($n = 3$).

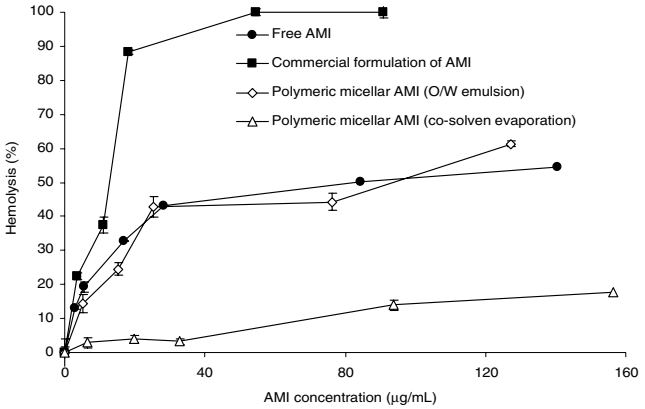

Fig. 4. The effect of loading process and solubilization vehicle on the hemolytic activity of AMI. Each point represents average ± SD ($n = 3$).

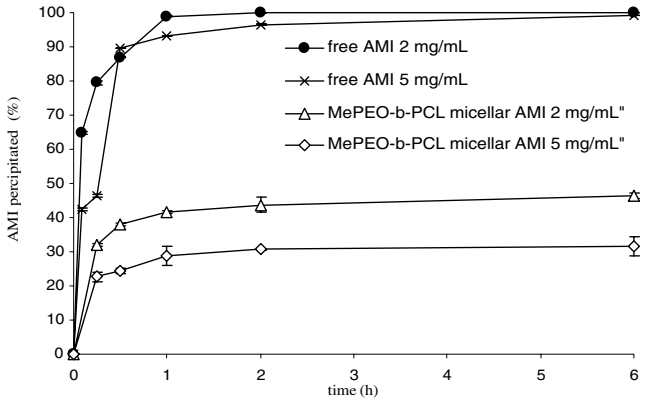

Fig. 3. The effect of initial AMI level on the stability of AMI incorporated in PEO-*b*-PCL micelles (5000–13,000) upon two times dilution and incubation with PBS (pH 7.4) at 37 °C. Each point represents average ± SD ($n = 3$).

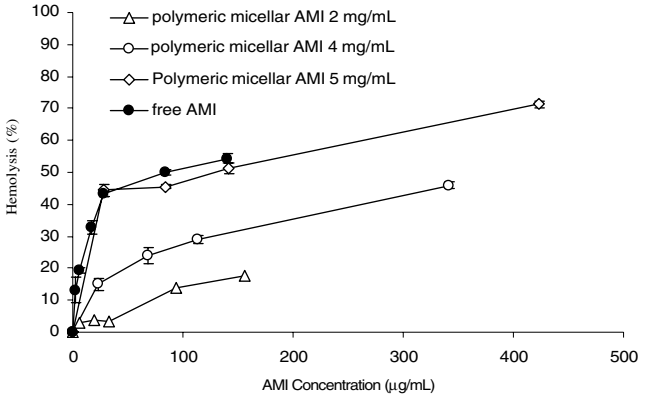

Fig. 5. The effect of AMI initial drug levels on the hemolytic activity of AMI incorporated in MePEO-*b*-PCL (5000–13,000) micelles by the co-solvent evaporation method. Each point represents average ± SD ($n = 3$). Concentration of free AMI was 2 mg/mL.

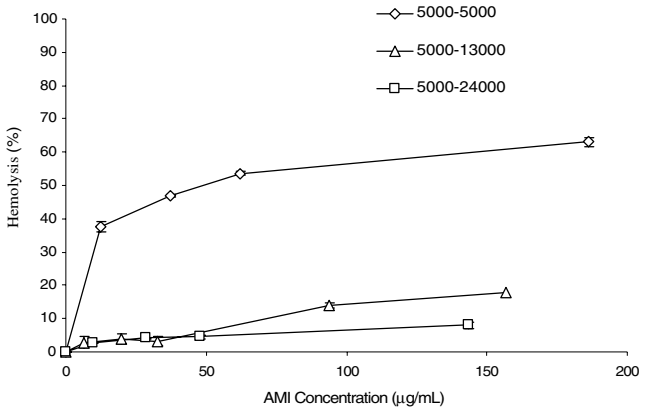

Fig. 6. The effect of PCL molecular weight on the hemolytic activity of AMI incorporated in MePEO-*b*-PCL micelles by a co-solvent evaporation method. Each point represents average ± SD ($n = 3$).

AMI solubilized in the presence of MePEO-*b*-PCL by an O/W emulsion method (Fig. 4).

An increase in the hemolytic activity of AMI loaded MePEO-*b*-PCL micelles was observed when the initial level of AMI applied in the co-solvent evaporation process was raised (Fig. 5). A rise in the molecular weight of the PCL block from 5000 to 13,000 and 24,000 g/mol, on the other hand, led to a decrease in the hemolytic activity of MePEO-*b*-PCL micellar AMI (Fig. 6). A difference in the hemolytic activity of polymeric micellar AMI between block co-polymers with 13,000 and 24,000 g/mol PCL was only observed at AMI concentrations $\geqslant 50$ μg/mL. At $\geqslant 100$ μg/mL AMI level, around 60, 14, and ~6% hemolysis was observed for AMI incorporated in MePEO-*b*-PCL micelles having 5000, 13,000, and 24,000 g/mol PCL chains, respectively.

4. Discussion

The potential of polymeric micelles for the solubilization of an amphiphilic drug has been investigated in this study. In this context, solubilization of AMI in polymeric micelles with poly(ester) core structures of various polarities was assessed. The hydrophobic core of PCL was found to be more efficient than relatively polar cores of PDLA and PLLA in the solubilization of AMI (Table 1). Consistent with this observation, a further increase in the hydrophobicity of the micellar core, achieved through an increase in the molecular weight of the PCL block, was found to enhance the molar ratio of loaded AMI to polymer (Table 1). On the other hand, no significant difference between the solubilization of AMI in polymeric micelles with amorphous core of PDLA in comparison to crystalline core of PLLA was observed (Table 1).

AMI reached aqueous solubilized levels of 1.74–4.23 mg/mL in the presence of MePEO-*b*-PCL co-polymers, when its initial level in the encapsulation process was raised from 2 to 5 mg/mL (Table 2). At 5 mg/mL, in the absence of block co-polymer, AMI produced a supersaturated solution and dissolved at a level of 2.05 mg/mL (three times higher than water solubility of AMI). Hence, the increase in AMI solubilization in MePEO-*b*-PCL micelles as a function of initial AMI concentration added to the encapsulation process (Table 2) might have resulted from either supersaturation of aqueous medium outside micelles with solubilized AMI and/or solubilization of AMI by polymeric micelles in a presaturation phase. To account for the possibility of either case and investigate the degree as well as strength of AMI association with polymeric micelles, stability of encapsulated AMI after two times dilution in PBS (pH 7.4) was assessed and compared to the same parameter for commercial formulation of AMI. The design of this study was based on an observation by Ward et al. who reported a minimum AMI solubility of ~0.0025 mg/mL in a mixture of Sorensen's buffer (pH 7.4) and commercial formulation of AMI at a volume ratio of 50:50 or above [1]. In the present study, the precipitation of AMI from its commercial formulation was followed after 50:50 dilution with PBS (pH 7.4) for different time periods to determine the required time to reach equilibrium. Most of solubilized AMI (87%) was found to precipitate out of its commercial formulation after two times dilution in PBS within 5 min. This level was slightly increased within the first hour and reached plateau afterwards. The concentration of polysorbate in the commercial formulation of AMI is expected to be still above CMC in this solution after two times dilution with PBS; therefore, the rapid precipitation of AMI could not have resulted from micellar dissociation. Instead, it may reflect weak association of AMI with polysorbate micelles and/or salting out of solubilized AMI from its benzyl alcohol (co-solvent) solution in the presence of high salt concentrations and physiological pH (which is above the p*K*a of basic AMI) in PBS. Interestingly, the rate and extent of AMI precipitation from its commercial vehicle upon dilution in PBS was similar to that of free supersaturated AMI solution (Fig. 2).

In contrast to commercial formulation and supersaturated solution of AMI that lost their drug content rapidly upon dilution in PBS, polymeric micelles, especially those with a PCL core, retained their encapsulated AMI effectively. MePEO-*b*-PCL micelles were able to retain >50% of the solubilized AMI after two times dilution in PBS (pH 7.4), while 100% of AMI from its supersaturated solution was precipitated (Table 1 and Figs. 2 and 3). The precipitation profile after dilution was similar for micelles with different PCL molecular weights. However, polymeric micelles with PDLLA and PLAA cores only retained 20 and 24% of their drug content after 1 h incubation in PBS. Moreover, the rate and extent of precipitation for free AMI upon dilution with PBS was independent from the solubilized drug levels as samples prepared with 2 and 5 mg/mL of initial AMI showed similar precipitation profiles (Fig. 3). In case of polymeric micelles, the solubility profile upon dilution was dependent on the initial AMI levels, however (Fig. 3).

In the next step, the hemolytic activity of solubilized AMI in MePEO-*b*-PCL micelles was compared to this factor for commercial formulation of AMI. Amiodarone is an amphiphilic molecule. It is likely that the hydrophobic portion of AMI (benzofuran–phenyl ketone) inserts into the lipid bilayer of the RBC membrane and the hydrophilic portion (protonated amino group) remains in the region of the polar head groups of the phospholipids (Fig. 1). Localization of the amphiphilic molecule in the cell membrane may perturb cell membrane and eventually lead to cell lyses. Involvement of oxidative mechanisms in the damaging effects of AMI on RBC membranes has also been reported [26].

Overall, AMI encapsulated in MePEO-*b*-PCL micelles was shown to be less hemolytic than commercial formulation of AMI (Fig. 4). Compared to MePEO-*b*-PCL micelles having PCL blocks of 5000 g/mol, polymeric micelles with 13,000 and 24,000 g/mol PCL showed a reduced hemolytic activity for incorporated AMI (Fig. 6). Empty MePEO-*b*-PCL micelles having different PCL block lengths were not hemolytic (data not shown). This is consistent with previous observations by different groups on the hemolytic activity of MePEO-*b*-PCL block co-polymers and micelles [27].

Hemolytic activity of AMI was increased as the initial level of AMI added to the MePEO-*b*-PCL micelles was raised, however (Fig. 5). Under this condition, more solubilized AMI will be available outside of micelles that can interact with cell membrane of RBCs and cause cell lyses. In fact the hemolytic activity of encapsulated AMI at 5 mg/mL initial AMI levels was comparable to the hemolytic activity of solubilized free AMI prepared at AMI concentration of 2 mg/mL. This is consistent with previous observations on the effect of an increase in drug/polymer loading that has enhanced the hemolytic activity of another amphiphilic drug, amphotericin B, loaded in a different polymeric micellar delivery system [24].

Finally, the co-solvent evaporation method was proved to be a better choice for AMI encapsulation since application of the O/W emulsion method resulted in a larger size for prepared polymeric micelles; lower AMI loaded levels (Table 3); and increased hemolytic activity for encapsulated AMI (Fig. 4).

The results of previous studies by Eisenberg et al. on the solubilization of model amphiphilic dyes in polystyrene-*b*-poly(acrylic acid) micelles demonstrated the degree of solubilization for amphiphilic molecules to be dependent on the interfacial area and affinity of solubilizate for the micellar interface [22]. The results suggest the core/cornea interface to be a possible site for the localization of amphiphilic molecules in polymeric micelles. In this study the solubility of AMI in polymeric micelles as well as degree of interaction between polymeric micelles and encapsulated AMI were found to be independent from the crystallinity of the core structure but dependent on its hydrophobicity. This may provide an indirect evidence for the solubilization of AMI in micellar core/shell interface rather than the micellar core, at least, for MePEO-*b*-PDLLA and MePEO-*b*-PLLA micelles. The higher AMI/polymer molar loading ratios for block co-polymers with longer PCL chains in this study may also be attributed to an increase in the size and thus in interfacial area (possible solubilization site for amphiphilic drugs in polymeric micelles) in micelles formed from those block co-polymers. Although, the superiority of 5000–5000 PEO-*b*-PCL micelles to MePEO-*b*-PDLA micelles, despite smaller diameter, highlights the importance of core structure rather than micellar size for efficient and stabilized encapsulation of AMI.

The capability of MePEO-*b*-PCL micelles in retaining >50% of solubilized AMI, provided evidence for strong interaction between the encapsulated AMI and MePEO-*b*-PCL micelles. This points to the superiority of MePEO-*b*-PCL micellar carriers over surfactant micelles as solubilizing agents for AMI. The precipitation of part of solubilized AMI from polymeric micellar solution after dilution may partly be attributed to the presence of supersaturated AMI outside micelles. However, the difference between the level of precipitation for various polymeric micellar systems (40% precipitation for MePEO-*b*-PCL versus 75–80% precipitation for MePEO-*b*-PDLA and MePEO-*b*-PLLA) clearly implies a role for the micellar core polarity in the strength of AMI/micelle interaction. The lower hemolytic activity of polymeric micellar AMI may reflect a lower rate of drug release from MePEO-*b*-PCL micelles with longer PCL chains, which is consistent with previous observations of this study that revealed stronger interaction between encapsulated AMI and polymeric micelles with more hydrophobic core structures.

5. Conclusions

Among different MePEO-*b*-poly(ester)s, MePEO-*b*-PCL micelles, especially those formed from block co-polymers of loner PCL chains by a co-solvent evaporation method, can effectively increase the water solubility of AMI, limit AMI precipitation upon dilution in physiological media and reduce its hemolytic activity. The results of this study revealed a superiority for more hydrophobic core structures for solubilization and controlled delivery of amphiphilic AMI.

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