Preparation, Characterization and Evaluation of Coenzyme Q10 Binary Solid Dispersions for Enhanced Solubility and Dissolution

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Phase solubility behavior of coenzyme Q10 (CoQ10) at 25 °C in various molar solutions of poloxamer 188 (P188) in water was observed and their binary solid dispersions (BSD) at different weight ratios were prepared by a simple, rapid, cost effective, uncomplicated and potentially scalable low temperature melting method. BSDs were characterized by scanning electron microscopy (SEM) and differential scanning calorimetry (DSC), and evaluated for improved solubility at 25 °C and 37 °C and *in-vitro* release of CoQ10 at 37 °C in distilled water. Solubility of CoQ10 increased with increasing concentrations of P188 in water. Gibbs free energy (ΔG_{tr}°) values were all negative indicating the spontaneous nature of CoQ10 solubilization and decreased with increasing concentration of P188 demonstrating that the reaction conditions became more favorable as the concentration of P188 increased. DSC and SEM analysis indicated that the homogeneity of dispersion was not at the molecular level. However, BSDs exhibited a remarkably improved aqueous solubility and dissolution of CoQ10.

Key words　coenzyme Q10; poloxamer 188; binary solid dispersion; dissolution; solubility

Coenzyme Q10 (CoQ10) (Fig. 1), a yellow crystalline powder with a melting point of about 50 °C is a lipid soluble vitamin like substance that inhabits inside of the inner mitochondrial membrane where it functions as an integral part of electron transport of oxidative phosphorylation.[1] It is used as a nutritional supplement, antioxidant and in the treatment of cardiovascular disorders such as angina pectoris, hypertension, and congestive heart failure. It is practically insoluble in water and poorly absorbed (T_{max} 5—10 h) from the gastrointestinal tract due to its high molecular weight and poor water solubility thereby presenting a challenge in the development of a formulation for oral administration.[2] Many approaches for formulating CoQ10 have been reported. Oil based or powder filled capsules and tablet formulations are currently available on the market as nutritional supplements.[3,4] However, dissolution and oral bioavailability of these formulations differ widely.[5] Other reported formulation strategies include a solubilized system with soy lecithin,[6] a micellar solution of CoQ10 with polyoxyethylene (60) hydrogenated castor oil,[7] lipid microspheres prepared as a soybean oil emulsified with yolk phospholipids,[8] a redispersible dry emulsion,[9] the complexation of CoQ10 with cyclodextrins,[10] self-emulsifying drug delivery systems,[3] and a solubilized form of CoQ10 in a blend of polysorbate 80 and medium chain triglycerides.[11] However, dissolution profiles are not reported for most of these formulations either due to their oily nature and poor aqueous solubility or due to the absence of a suitable dissolution medium. Further, these approaches were tedious, time consuming and costly. Thus,
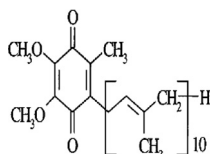
there is a great need for an efficient, easy, quick, and cost-effective method to improve the solubility and dissolution of CoQ10.

Dispersion of poorly water soluble drug in an inert hydrophilic carrier matrix at solid state either by melting or solvent or solvent-melting method leads to products known as solid dispersions (SD)[12,13] that have tremendous potential for improving drug solubility[14,15] because of the drug solubilizing or co-solvent effect of hydrophilic carrier, better wettability and dispersibility of drug by the carrier material, and the formation of amorphous forms of drug and carriers.[12—15] However, the SDs prepared by high temperature melting, solvent or solvent-melting method *etc.* are problematic because 1. The high melting temperatures could chemically decompose drugs and carriers,[12] 2. The hardening of melts could lead to difficulties in pulverization for subsequent formulation into an appropriate dosage form, 3. It is difficult to identify a common solvent to dissolve hydrophobic drug and hydrophilic carrier, 4. The large volumes of solvents and heating are necessary to enable complete dissolution of both components. 5. Vacuum drying,[16,17] spray drying,[18—21] spraying on sugar beads using a fluidized bed-coating system,[22] lyophilization[23] *etc.* used for the removal of organic solvents from SDs further make it tedious, costly and most reports did not address how much residual solvents were present in SDs when different solvents, carriers, or drying techniques were used.

Hence, the objective of this work was to provide a process, the low temperature melting method, which does not have the disadvantages of prior art and makes it possible to prepare a Poloxamer 188 (P188)/CoQ10 binary solid dispersion (BSD) in a rapid, cost effective and uncomplicated manner to improve the water solubility and dissolution of CoQ10.

MATERIALS AND METHODS

Materials　CoQ10 was gifted by Dong Wha Pharmaceu-

Fig. 1.　Structure of Coenzyme Q10

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tical Industrial Co., Ltd. (DWH), Seoul, South Korea, and P188 was purchased from BASF Aktiengesellschaft (Ludwigshafen, Germany). All other chemicals were of reagent grade and used without further purification.

Selection of a Hydrophilic Carrier Polymer for CoQ10 Required amount of CoQ10 and polyvinylpyrrolidone K-30 (PVPK30), hydroxyl propyl cellulose, polyethylene glycol 4000 and P188 (CoQ10 : Polymer 1 : 30 w/w ratio) were mixed for 5 min in a glass container to get a homogeneous mixture and sieved through a 40-mesh screen. Exactly weighed 150 mg each of these physical mixtures was added to 10 ml distilled water in a screw-capped test tube, wrapped with aluminium foil, vortexed for 2 min and shaken in dark at 25±0.1 °C or 37±0.1 °C in a temperature controlled water bath (Shaking water bath KMC 12055 WI) at 150 rpm. After 24 h, samples were filtered (0.45 μm, Whatman syringe filter), suitably diluted with distilled water and analyzed by HPLC.

Phase Solubility Study of CoQ10 0.5, 1.5, 3, 6, 8, and 10 mM solutions of P188 were prepared in water and to 10 ml of each of these solutions in a screw-cap test tube, 10 mg CoQ10 was added, screw-capped, wrapped with aluminium foil, vortexed for 2 min and shaken in dark at 25±0.1 °C in a temperature controlled water bath (Shaking water bath KMC 12055 WI) at 150 rpm. After 48 h, samples were filtered (0.45 μm, Whatman syringe filter), suitably diluted with corresponding P188 solutions and analyzed by HPLC.

Preparation of Binary Solid Dispersions, and Determination of Drug Content and Percent Yield BSDs were prepared by low temperature melting method at 1 : 1, 1 : 5, 1 : 7, and 1 : 10 weight ratios of CoQ10 : P188. Required amount of materials were mixed in a mortar and pestle (under yellow light) to obtain a homogeneous physical mixture, sieved through 40-mesh screen and transferred into a locally designed micro-ointment glass vessel which was wrapped with aluminium foil to prevent the photodegradation of CoQ10. Hot water (70—80 °C) was continuously circulated through a circulating water bath and the resulting molten mixture was magnetically stirred at 700 rpm. After 10—15 min, the mixture was cooled rapidly by circulating ice water (<4 °C) for about 1 h and the solidified BSDs were then pulverized in a mortar and pestle, sieved through a 40-mesh screen (Chung Gye Sang Gong Sa, Seoul, South Korea) and stored in a screw-cap vial at 4 °C until further use.

BSDs equivalent to 5 mg of CoQ10 were accurately weighed (Mettler-Toledo), dissolved in a suitable quantity of absolute alcohol, filtered (0.45 μm, Whatman syringe filter), suitably diluted with absolute alcohol and analyzed by HPLC. The percent yield of each formulation was calculated according to the total recoverable final weight of BSD and the total weight of CoQ10 and P188 used.

Differential Scanning Calorimetry The differential scanning calorimetry (DSC) measurements were performed on a DSC-6100 (Seiko Instruments, Japan) differential scanning calorimeter with a thermal analyzer. About 2 mg of CoQ10, physical mixtures or BSDs was placed in sealed aluminum pans, before heating under nitrogen flow (25 ml/min) at a scanning rate of 5 °C/min from 0 to 100 °C. An empty aluminum pan was used as reference.

Scanning Electron Microscopy The surface morphol-

ogy of CoQ10, P188, physical mixtures and BSDs were examined by using scanning electron microscope (S-4100, Hitachi, Japan). The powders were fixed on a brass stub using double sided adhesive tape and made electrically conductive by coating in a vacuum (6 Pa) with platinum (6 nm/min) using Hitachi Ion Sputter (E-1030) for 240 s at 15 mA. The pictures were taken at an excitation voltage of 15 kV and a magnification of 200, 300, or 500×.

Determination of Solubility Solubility of CoQ10 from BSDs was measured at 25 °C and 37 °C. CoQ10, physical mixtures or BSDs equivalent to 25 mg of CoQ10 was added to 10 ml distilled water in screw capped test tubes that were wrapped with aluminium foil, vortexed for 2 min and shaken in dark at 25±0.1 °C or 37±0.1 °C in a temperature-controlled water-bath (Shaking water bath KMC 12055 WI). After 24 h, samples were filtered (0.45 μm, Whatman syringe filter), suitably diluted with distilled water of corresponding temperatures and analyzed by HPLC.

Stability of CoQ10 during Dissolution Test and Dissolution Studies 900 ml pure CoQ10 solution in water : absolute alcohol mixture (99 : 1% v/v) was placed in the beaker of the dissolution test apparatus (Shinseang Instrument Co., South Korea) that was covered with alumunium foil, warmed to 37±0.5 °C and rotated at the paddle speed of 50 rpm. At appropriate time intervals, small aliquot of samples were withdrawn, filtered (0.45 μm, Whatman syringe filter), and analyzed by HPLC for remaining CoQ10. Considering the initial concentration of CoQ10 as 100%, the remaining percentage of CoQ10 was determined as a function of time.

Dissolution studies of CoQ10, physical mixtures and BSDs (equivalent to 10 mg CoQ10) were performed using US Pharmacopeia (USP) model digital tablet dissolution test apparatus (Shinseang Instrument Co., South Korea) at a paddle rotation speed of 50 rpm in 900 ml distilled water at 37±0.5 °C. At the specified times, 0.5 ml samples were withdrawn, filtered (0.45 μm, Whatman syringe filter), and assayed for CoQ10 content by HPLC. 0.5 ml of fresh medium, which was warmed to 37±0.5 °C, was replaced into the dissolution medium after each sampling to maintain sink condition throughout the test.

Drug Analysis The concentration of CoQ10 was analyzed by Jasco P987 HPLC system equipped with a Jasco UV detector (UV-975), using Borwin program. HPLC separation was performed with 50 μl injection volume on a reverse-phase C 18 column (Inertsil GL Science column. 5 μm particle size, 4.6×150 mm). The mobile phase was methanol : ethanol 7 : 3 v/v and the eluent was monitored at 275 nm at a constant flow rate of 1.2 ml/min.[24]

RESULTS AND DISCUSSION

Results In solubility study of CoQ10 1 : 30 w/w physical mixtures with different hydrophilic polymers (Fig. 2), the highest solubility (13.73 μg/ml) was observed for CoQ10 : P188 physical mixture at 37 °C and the lowest for CoQ10 : PVPK30 physical mixtures (0.25 μg/ml) in the order of PVPK30<polyethylene glycol 4000<hydroxy propyl cellulose<P188 at 25 °C<P188 at 37 °C. In phase solubility study, the increase in the apparent solubility of CoQ10 in water at 25 °C in the presence of P188 was linear with respect to the concentration of P188. CoQ10 solubility in-
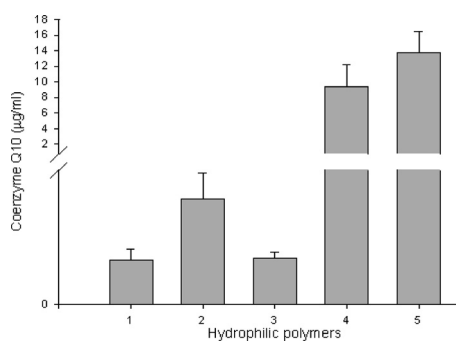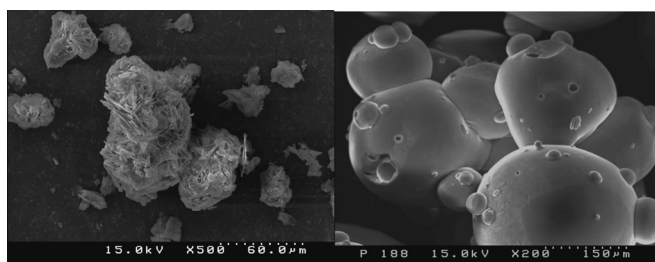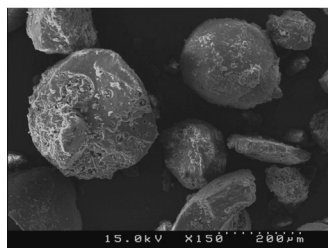
Fig. 2.   Solubility Profiles of Coenzyme Q10 from Its 1 : 30 w/w Physical Mixtures with Different Hydrophilic Polymers at 25 °C and 37 °C

1. Polyvinylpyrrolidone K30, 2. hydroxypropyl cellulose, 3. polyethylene glycol 4000, 4. poloxamer 188 at 25 °C, and 5. poloxamer 188 at 37 °C. Data are expressed as mean±S.D. (n=3).

A

B

C

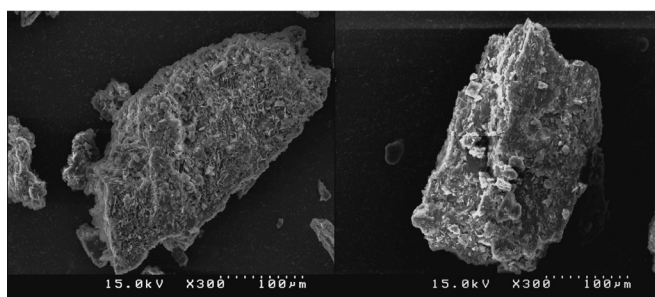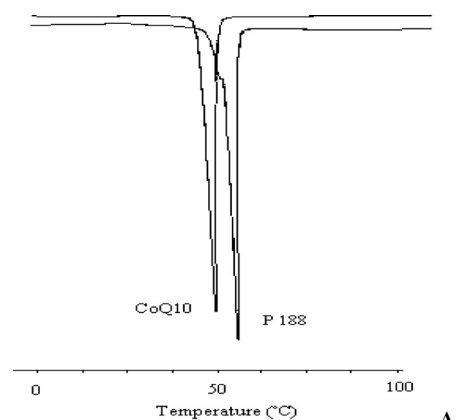D.   E.
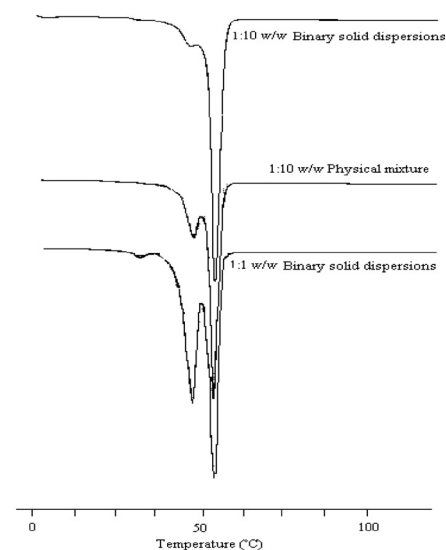
Fig. 3.   Scanning Electron Micrographs

(A) Coenzyme Q10, (B) poloxamer 188, (C) coenzyme Q10 : poloxamer 188 1 : 10 w/w physical mixtures, (D) coenzyme Q10 : poloxamer 188 1 : 1 w/w binary solid dispersions, and (E) coenzyme Q10 : poloxamer 188 1 : 10 w/w binary solid dispersions.

A.

B.

Fig. 4.   Differential Scanning Calorimetric Thermograms

(A) Coenzyme Q10 and poloxamer 188, (B) coenzyme Q10 : poloxamer 188 1 : 1 w/w binary solid dispersions, coenzyme Q10 : poloxamer 188 1 : 10 w/w physical mixtures, and coenzyme Q10 : poloxamer 188 1 : 10 w/w binary solid dispersions.

creased from 3.984 μg/ml in 0.5 mM P188 solution to 38.27 μg/ml in 10 mM P188 solution (Figure not shown). Assay of CoQ10 in all BSDs was almost 100% and the percentage yield was greater than 97% (data not shown).

In scanning electron micrographs (Fig. 3), CoQ10 ap-

peared as irregular shaped crystalline mass with rough surface having projections (A), P188 is presented as smooth-surfaced spherical particles (B). Physical mixtures had a porous rough surface without the projections of CoQ10 and some P188 spheres had few CoQ10 particles adhered on their surface (C). Similarly, 1 : 1 w/w BSD appeared as a rough and porous surfaced mass with a relatively smoother surface than CoQ10 but still having few projections (D). However, 1 : 10 w/w BSD appeared as relatively more uniform and homogeneously mixed mass having relatively more smooth surface without projections (E). In DSC thermograms (Fig. 4), CoQ10 and P188 (A) showed apparent endothermic peak at 50.16 °C and 55.99 °C, respectively corresponding to their individual melting points. Other thermograms showed no major shift in the peak positions and as seen from the figure, major peaks for the physical mixture and pure drug were retained in BSDs. A sharp decrease in the intensity of peak with 1 : 10 w/w BSD and physical mixtures than 1 : 1 w/w BSD was observed. The melting peak of P188 in BSD was observed at slightly lower temperatures (between 55.12—55.31 °C) than that of pure P188.

Water solubility of CoQ10 (Fig. 5) increased with an increment in the amount of P188 in BSDs and was 3.57,
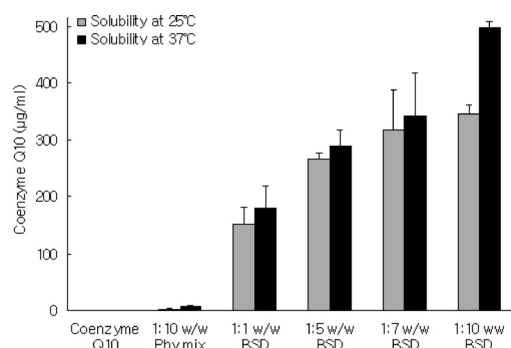
Fig. 5. Solubility Profiles of Coenzyme Q10 : Poloxamer 188 Binary Solid Dispersions at 25 °C and 37 °C
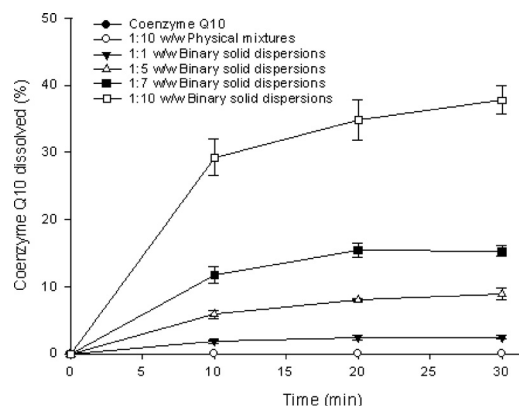
Data are expressed as mean±S.D. (*n*=3).

Fig. 6. Dissolution Profiles of Coenzyme Q10 : Poloxamer 188 Binary Solid Dispersions in Distilled Water at 37 °C

Data are expressed as mean±S.D. (*n*=3).

152.04, and 345.82 μg/ml at 25 °C, and 6.80, 180.07, and 498.62 μg/ml at 37 °C, respectively for 1 : 10 w/w physical mixture, 1 : 1 w/w BSD and 1 : 10 w/w BSDs. CoQ10 stability study in simulated dissolution tests showed that it was stable for 18 h (figure not shown). In dissolution studies, pure CoQ10 and CoQ10 : P188 1 : 10 w/w physical mixtures could not be detected in the dissolution medium till the end of the test (lower limit of detection 20 ng/ml) but the dissolution of CoQ10 was considerably enhanced from BSDs. At the end of 30 min, approximately 37.73% CoQ10 was released from 1 : 10 w/w BSD (Fig. 6).

Discussion As the sole aim of finding the solubility of CoQ10 from its physical mixtures was to select a polymer (among various hydrophilic polymers) that have relatively higher solubility enhancing effect, the CoQ10 : polymer ratio was empirically decided to be high enough (1 : 30 w/w) because the CoQ10 was poorly soluble in water and an extensive review of earlier works on this compound did not reveal sufficient reports on its solubility in presence of these polymers. In addition to its highest solubility enhancing effect, P188 was selected for its additional advantages of having low melting point (50—56 °C), non ionic surfactant properties and oral safety. Solubility at 37 °C was determined only for the most efficient polymer (CoQ10 : P188 1 : 30 w/w physical mixtures) to predict CoQ10 solubility in physiological temperature. In phase solubility study, a systematic linear increase in CoQ10 solubility with an increasing concentration

Table 1. Thermodynamic Parameters of the Solubility Process of Coenzyme Q10 in Aqueous Solutions of Poloxamer 188 at 25 °C

Poloxamer 188 (mM)	ΔG_{tr}° (kJ/mol)
0.5	−15.58
1.5	−18.90
3.0	−20.61
6.0	−21.86
8.0	−22.44
10.0	−22.91

Data are expressed as mean±S.D. (*n*=3).

of P188 in water might be due to the improved wettability and dissolution of CoQ10 particles in water by P188. An indication of the process of transfer of CoQ10 from pure water to the aqueous solutions of P188 was obtained from the values of Gibbs free energy change. The Gibbs free energy of transfer (ΔG_{tr}°) of CoQ10 from pure water to the aqueous solutions of P188 was calculated using Eq. 1. Where So/Ss is the ratio of molar solubility of CoQ10 in aqueous solutions of P188 to that of the pure water. The obtained values of Gibbs free energy are presented in Table 1. ΔG_{tr}° values were all negative indicating the spontaneous nature of CoQ10 solubilization and they decreased with an increment in P188 concentration demonstrating that the reaction became more favorable as the concentration of P188 increased.

$$\Delta G_{tr}^{\circ} = -2.303RT \log(So/Ss) \tag{1}$$

Although the CoQ10 solubility and dissolution increased with an increment in P188 content in BSDs, BSDs composed of CoQ10 : P188 more than 1 : 10 weight ratios were not prepared and tested because of their volume and feasibility of the final product or dosage form as the usual dose of CoQ10 is 50—300 mg. Cooled masses of BSDs were easily breakable into free flowing granules of any desired sizes. This could be significantly important in the industrial application of this method as the pulverization of cooled mass of solid dispersions was the most serious problem encountered in earlier methods. Moreover, this method was highly feasible because of the lower melting point of both CoQ10 (*ca.* 50 °C) and P188 (*ca.* 55—57 °C). It also avoided most of the disadvantages of previously reported solid dispersion techniques in case of CoQ10 and replaced traditional method of melting in the frying pan, beaker *etc.* where the temperature and shearing rate were difficult to control. It is likely that the industrial scale up of this method would be reproducible, controllable and can be done in any ointment formulation plant. The major advantage of this method is its very short duration of preparation (about 1—2 h). Probability of thermal degradation of CoQ10 during BSD preparation was evaluated by determining CoQ10 content in BSD and comparing it with the amount originally used. Assay of CoQ10 in all BSD was almost 100% indicating that the method used had no detrimental effect on the thermal stability of CoQ10. Thus, this method could be a more rational approach to enhance the solubility and dissolution of CoQ10.

In DSC study, the retention of peaks for the physical mixture and CoQ10 in BSDs reflects that there was no major change in the physicochemical characteristics of the drug and a decrease in the intensity of the peaks with 1 : 10 w/w BSDs and physical mixtures than 1 : 1 w/w BSDs was only due to a

lower loading of drug per unit weight of 1 : 10 w/w BSDs or physical mixtures.The decrease in the intensity of the peaks with 1 : 10 w/w BSDs than 1 : 10 w/w physical mixtures might be due to some molecular interaction between CoQ10 and P188. However, such interactions could not be explained. Absence of peak shifting indicates the solubility of CoQ10 in P188, but the presence of CoQ10 peak in 1 : 10 w/w BSDs suggests that the CoQ10 was not completely soluble in the liquid phase with varying amounts of P188 up to 10% w/w. This is further supported by the fact that the melting peak of P188 in BSDs was observed at slightly lower temperatures than that of pure P188, indicating a partial miscibility of CoQ10 in P188. Taken together, the DSC study demonstrated that the CoQ10 BSDs, composed of higher concentration of P188 might be more than simple physical mixtures of their individual components. Although the surface morphology of BSDs was different than that of pure CoQ10, it still had some surface characteristics resembling to CoQ10. SEM results matched with DSC findings. The DSC thermogram of 1 : 1 w/w BSDs had prominent CoQ10 peak along with P188 peak. This phenomenon was reflected in SEM picture where the 1 : 1 w/w BSDs had projections characteristics of CoQ10. Similarly further reduction in the intensity of DSC thermograms in 1 : 10 w/w physical mixtures and BSDs are correlated to corresponding SEM pictures with relatively more uniform and homogeneously mixed mass having relatively smoother surface with minimal projections of CoQ10. DSC and SEM analysis indicated that the homogeneity of dispersion was not at the molecular level.

Increase in CoQ10 solubility with the increment of temperature might be due to the temperature aided increase in solubility, low melting point of CoQ10, and a favorable interaction of P188 in BSDs with water at higher temperature. In CoQ10 stability study in simulated dissolution test conditions, 1% v/v absolute alcohol was used to affect CoQ10 solution, test was performed under yellow light and the apparatus was covered by aluminum foil to minimize any possible photodegradation. CoQ10 stability suggested that it was sufficiently protected to perform the dissolution test. Most of the dissolution studies concerning CoQ10 and other poorly water-soluble drugs have been performed in dissolution mediums that are different from those normally used for water soluble drugs *e.g.* incorporation of a small amount of surfactants or acids *etc.* in the dissolution medium. The use of exogenous surfactants in the dissolution medium may accelerate the *in vitro* dissolution of poorly water-soluble drugs by their wetting, micellar solubilization, and/or deflocculation properties. Hence, the dissolution of the same formulation may be very low in pure water. This problem has been previously reported by some authors. Cheng-Hsuan Hsu *et al.*, 2003, could not detect CoQ10 release over 7 d when the nanoparticles were suspended in water, presumably due to the low aqueous solubility of CoQ10 from nanoparticles. Therefore, he used a 5% Tween 80 solution as the medium for further study. However, after 150 h, only 15% of CoQ10 was released from nanoparticle in the dissolution media containing 5% Tween 80.[25] Thus, the conclusion of increased dissolution of CoQ10 from improved formulations cannot be justified until a control dissolution in water is carried and compared. As the aim was to enhance CoQ10 dissolution in an immediate release manner, dissolution test was performed

for 30 min in distilled water. The possible mechanisms of increased dissolution as proposed by Ford, 1986 include: a solubilization effect of P188, improved wettability and dispersibility of CoQ10 from BSDs, dissolution of CoQ10 in P188 solution in distilled water and combinations of these mechanisms.[13]

CONCLUSION

Solubility and *in vitro* dissolution of CoQ10 in water were remarkably improved by formulating its binary solid dispersions with P188 by low temperature melting method that did not have the disadvantages of the prior art and which makes it possible to prepare a binary solid dispersion in a rapid, cost effective and uncomplicated manner. Further, this method could be easily scaled up for commercial purpose. The faster release of free CoQ10 is thought to be beneficial since, once being administered orally, the quickly released CoQ10 may be passively partitioned into the gastrointestinal tract tissues quickly (because of the favorable concentration gradient) resulting into a rapid onset of action and an improved bioavailability. Thus, the BSDs of CoQ10 with P188 could provide an alternative solid formulation to the existing dosage forms.

REFERENCES

 1) Folkers K., Wolaniuk A., Vadhanavikit S., Sakmato N., Takemura K., Baker L., Richardson P. C., "Biomedical and Clinical Aspects of Coenzyme Q," Vol. 5, ed. by Folkers K., Yamamura Y., Elsevier, Amsterdam, 1986, pp. 375—391.
 2) Greenberg S., Fishman W. H., *J. Clin. Pharmacol.*, **30**, 590—608 (1990).
 3) Kommurur T. R., Gurley B., Khan M. A., Reddy I. K., *Int. J. Pharm.*, **212**, 233—246 (2001).
 4) Weis M., Mortensen S. A., Rassing M. R., Moller-Sonnergaard J., Poulsen G., Rasmussen S. N., *Mol. Aspects Med.*, **15** (Suppl.), S273—S280 (91994).
 5) Kishi H., Kanamori N., Nishii S., Hiraoka E., Okamato,T., Kishi T., "Biomedical and Clinical Aspects of Coenzyme Q," ed. by Folkers K., Yamamura Y., Elsevier, Amsterdam, 1984, pp. 131—142.
 6) Takada M., Yuzuriha T., Katayama K.,Yamato C., Koyama N., *J. Nutr. Sci. Vitaminol.*, **31**, 115—120 (1985).
 7) Kimura A., Yamaguchi H., Watanabe K., Hayashi M., Awazu S., *J. Pharmacol.*, **38**, 659—662 (1986).
 8) Ozawa Y., Mizushima Y., Koyama I., *Drug Res.*, **36**, 689—690 (1986).
 9) Takeuchi H., Sasaki H., Niwa T., Hino T., Kawashima Y., Uesugi K., Ozawa H., *Int. J. Pharm.*, **86**, 25—33 (1992).
10) Lutka A., Pawlaczyk J., *Acta Pol. Pharm.*, **52**, 379—386 (1995).
11) Chopra R. K., Goldman R., Sinatra S. T., Bhagavan H. N., *Int. J. Vit. Nutr. Res.*, **68**, 109—113 (1998).
12) Chiou W. L., Riegelman S., *J. Pharm. Sci.*, **60**, 1281—1302 (1971).
13) Ford J. L., *Pharm. Acta Helv.*, **1986**, 69—88 (1986).
14) Leuner C., Dressman J., *Eur. J. Pharm. Biopharm.*, **50**, 47—60 (2000).
15) Damian F., Blaton N., Naesens L., Balzarini J., Kinget R., Augustinjns P., Mooter G. V., *Eur. J. Pharm. Sci.*, **10**, 311—322 (2000).
16) Fernandez M., Rodriguez I. C., Margarit M. V., Cerezo A., *Int. J. Pharm.*, **84**, 197—202 (1992).
17) Singla A. K., Vijan T., *Drug Dev. Ind. Pharm.*, **16**, 875—882 (1990).
18) Corrigan O. I., Holohan E. M., *J. Pharm. Pharmacol.*, **36**, 217—221 (1984).

19) Takahashi Y., Tsukuda T., Izumi C., Ikemoto K., Kokubun K., Yagi N., Takada M., *Chem. Pharm. Bull.*, **36**, 2708—2710 (1988).

20) Jachowicz R., Nurnberg, E., Hoppe R., *Int. J. Pharm.*, **99**, 321—325 (1994).

21) Kai T., Akiyama Y., Nomura S., Sato M., *Chem. Pharm. Bull.*, **44**, 568—571 (1996).

22) Ho H. O., Shu H. L., Tsai T., Sheu M. T., *Int. J. Pharm.*, **139**, 223—229 (1996).

23) Betageri G. V., Makarla K. R., *Int. J. Pharm.*, **126**, 155—160 (1995).

24) Rousseau G., Varin F., *J. Chromatogr. Sci.*, **36**, 247—252 (1998).

25) Hsu C. H., Cui Z., Russell J. M., Michael J., *AAPS Pharm. Sci. Tech.*, **4**, Article 32 (2003).