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Drug Solubility in Phospholipid Carrier as a Predictive Parameter for Drug Recovery in Microparticles Produced by the Aerosol Solvent Extraction System (ASES) Process

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The solubility of various drugs in a constant ratio of phosphatidylcholine-cholesterol carrier were studied to investigate their influence on drug recovery in drug-lipid microparticles produced by the aerosol solvent extraction system (ASES) process. Solubility of the drugs in such lipid carrier were determined by using differential scanning calorimetry and confirmed by X-ray powder diffraction study. The results showed that drug possessing relatively high solubility in the lipid carrier used could lead to a higher amount of drug recovered in the drug-lipid microparticles produced. However, too high amount of dissolved drug imposed an adverse effect on the solidification of the lipid carrier during ASES processing, which led to partial film formation in the production column and hence a lower yield of microparticles. Such adverse effect was not the case for the drugs with low solubility in the carrier but there was an incomplete recovery of drug in the produced microparticles due to the partial extraction by the supercritical gas instead. The maximum amount of drug recovered in the ASES-prepared microparticles was found to correlate to the solubility of drug in the lipid carrier so that it might be utilized as a predictive parameter for determining the amount of drug to be incorporated into the microparticles.

Keywords drug solubility; phospholipid; microparticles; supercritical carbon dioxide; aerosol solvent extraction system

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INTRODUCTION

The aerosol solvent extraction system (ASES) process is an efficient method of interest currently used for microparticle production in a variety of pharmaceutical applications such as encapsulation of a substance in a biodegradable carrier (Müller & Fischer, 1989; Bleich et al., 1993; Young et al., 1999; Thiering et al., 2001; Sze Tu et al., 2002), preparation of polymeric microspheres (Bodmeier et al., 1995; Reverchon, 1999), micronization of drug substances and biopolymers (Steckel et al., 1997; Kerč et al., 1999; Reverchon, 1999; Thiering et al., 2001; Sze Tu et al., 2002), and preparation of liposomes in a dry form (Magnan et al., 2000; Badens et al., 2001; Kunastitchai et al., 2006), etc. This technique possesses a pronounced advantage in that the organic solvent(s) can be completely eliminated by using supercritical carbon dioxide ($scCO_2$) as an anti-solvent with a relatively short period of drying (Bleich & Müller, 1996; Ruchatz et al., 1997; Thiering et al., 2001). Moreover, it can provide solid microparticles instantaneously in a single step of production after the organic liquid has been extracted from the sprayed droplets.

To apply this technique for a given purpose, several studies demonstrated that the properties of substance(s) sprayed into the $scCO_2$ including polarity, partition coefficient, and their solubility in supercritical fluid(s) were the key parameters determining particle formation and drug extraction by the supercritical gas at a given condition of the process, resulting in different yields and percentages of drug recovered (Bleich et al., 1993; Steckel et al., 1997; Reverchon & Della Porta, 1999; Engwicht et al., 2000; Lucien & Foster, 2000). Bleich et al. (1993) and Engwicht et al. (2000) found that an absence

of particle formation of some biodegradable polymers prepared by the ASES could not be avoided by an alteration of the processing conditions but by some modifications of the critical properties of the polymers used. Reverchon and Della Porta (1999) demonstrated that choice of solvent was a key factor for successful precipitation/micronization of antibiotics. Steckel et al. (1997) showed that steroids with a high value of log P could readily be extracted by the supercritical gas, leading to a film formation at the column wall and thus to a reduction of the yield. However, those previous investigations were carried out for microparticle production of single-component system. In the case of multi-component system like liposome preparation, the crucial key parameter determining product characteristics could not simply be identified in practice. This is due to the fact that the characteristics of the ASES-products obtained are basically determined by a combination of factors involving hydrodynamic, mass transfer and thermodynamic aspects (Lucien & Foster, 2000) that make it be extremely complex. Any interactions between solutes of such multi-component system during ASES-processing must essentially be taken into consideration, especially, regarding their influences on the solubility of each other in CO_2. Accordingly, the solubility analysis of single component in $scCO_2$ would not practically be estimated and used for predicting such complex system. Moreover, the other variables such as type of other excipients in preparation, solvent power of CO_2, etc., can also influence the product characteristics somehow. On the other hand, the solubility analysis of the drug in the lipid carrier used could however be considered as a more applicable study for a critical parameter determining product characteristics, particularly drug recovery in the ASES-prepared phospholipid microparticles. The key parameter obtained would certainly be valuable as a guideline for different lipid types or compositions in preparing dry and reconstitutable liposomes by the ASES process. Hence, it is of interest to examine the influence of solubility of various drugs in a constant ratio of phosphatidylcholine-cholesterol carrier on the amount of drug recovered in drug-lipid microparticles produced by the ASES process. The model drugs chosen for this study were diazepam (DZP), miconazole base (MCZ), chloramphenicol (CRP), and betamethasone valerate (Beta-val).

MATERIALS

Diazepam (DZP) (Synopharm, Hamburg, Germany), miconazole base (MCZ) (GUFIC Biosciences, Gujarat, India), chloramphenicol (CRP) (Mainland, Frankfurt-M, Germany), and betamethasone valerate (Beta-val) (Roussel Uclaf, Paris, France) were used as model drugs in this study. Phosphatidylcholine (PC) (Phospholipon® 90H; Rhône-Poulenc Rorer, Köln, Germany) and cholesterol (Chol) (H. Erhard Wagner GmbH, Bremen, Germany) were used as components of the lipid carrier. All reagents used –methylene chloride, methanol, and acetonitrile– were of analytical grade (Merck KG, Darmstadt, Germany). The utilized carbon dioxide was of high quality (99.97%) (Kohlensäurewerk Hannover EG, Laatzen, Germany).

METHODS

Preparation of Drug-Lipid Mixture

The drug-lipid mixtures of all drugs (DZP, MCZ, CRP, Beta-val) at 10–100% (w/w) concentrations in lipid carrier (8:2 (w/w)-PC:Chol) were prepared based on a solvent casting method by mixing the drug with the lipids and then dissolving in 8:2 (w/w)-methylene chloride:methanol mixture. The drug-lipid solutions were cast onto the petri-dishes and the solvent was allowed to evaporate at room temperature for 1–2 hr. The obtained films were then placed in a hot air oven at 40–45°C for 24 hr to completely remove the residual solvent. Dried films were ground into powders and kept in a desiccator until further analysis.

Production of Dry Microparticles by the ASES Process

The detail of ASES process for microparticle production has previously been described (Bleich et al., 1993). In brief, the spraying solutions containing various percentage amount of each drug (10, 19 and 38%, w/w, based on total solutes) at a constant ratio of lipid carrier (8:2 (w/w)-PC:Chol) were prepared by dissolving in a solvent mixture of 8:2 (w/w) methylene chloride:methanol. The total concentrations of spraying solutions were used at the optimal range of 8–15% (w/w). These solutions were sprayed by using an HPLC pump (Gynkotek, Munich, Germany) through a nozzle (Schlick GmbH, Coburg, Germany) with a diameter of 0.4 mm and a spraying angle of 15° into supercritical carbon dioxide ($scCO_2$) filled in a high pressure vessel. Regarding the structural phospholipid used (Phospholipon® 90H), temperature and pressure were kept constant at optimized conditions of 35°C and 8.0 MPa, respectively, throughout the extraction process (Kunastitchai et al., 2006). For DZP and MCZ preparations, a carbon dioxide continuous-current was employed at a flow rate of 6 kg hr^{-1} with a solution feed rate of 6 mL min^{-1}. For CRP and Beta-val formulations, a static carbon dioxide phase was applied to increase the yield of microparticles with the same rate of spraying (6 mL min^{-1}) but lower carbon dioxide flow rate (4 kg hr^{-1}) in the drying stage. This is due to the fact that the produced microparticles were too fine to remain in the column and tended to be lost with the circulating $scCO_2$ much more than the former cases. The particle formation under the optimal extraction conditions should normally occur instantaneously when the organic solvents are miscible with the $scCO_2$ and extracted. In static mode, the carbon dioxide pump was switched off during spraying the solution and in the first hour after the spraying process to allow the produced microparticles to sediment. This sedimentation phase does not influence the particle formation, but improves the yield of

product (Steckel et al., 1997). Subsequently, the precipitated particles were dried by washing with continuous flow of $scCO_2$ to diminish the remaining organic solvents. This drying step was carried out within 3–4 hr in which the organic solvents were drained out of the separator periodically. The expanded carbon dioxide was then condensed and returned into the CO_2-storage vessel. At the end of the process, after the supercritical gas was discharged from the high-pressure column, solid microparticles were finally collected from the precipitation column and stored in a cool-dry place before further investigations.

Physicochemical Characterization

Thermal Behavior

The thermal behavior of drugs in the drug-lipid mixtures was examined by using a differential scanning calorimeter (DSC7, Perkin-Elmer, Norwalk). The temperature was scanned from 30 to 200°C at a heating rate of 10°C min^{-1}. Nitrogen was utilized as a carrier gas with a flow rate of 20 mL min^{-1}. The enthalpies of melting of all drugs at various concentrations in the drug-lipid mixtures were determined and analyzed for their solubilities in the lipid carrier.

X-Ray Powder Diffraction (XRPD) Patterns

X-ray powder diffraction patterns were obtained in transmission mode using an X-ray diffractometer with a rotating Cu anode (Stoe and Cie GmbH, Darmstadt, Germany) operating at 1200 W. The Cu Kα1 radiation was generated at 30 mA and 40 kV and monochromatized by a graphite crystal. The powder was packed into a rotating sample holder between two films (PETP). Diffraction patterns recorded the X-ray intensity as a function of 2-theta angle covering from 5.0° to 50.0°.

Yield

The yield was evaluated concerning microparticle formation of the lipid mixture (liposome preparation) at standard operating conditions by weighing the microparticles recovered in the production column and calculating the percentage of yield with respect to the initial amount of total solutes being added into the ASES processing system. Film formation at the column wall was classified as an unsuccessful precipitation (no microparticles given in the precipitation container).

Particle Size and Morphology (Scanning Electron Microscopy; SEM)

Particle size and morphology of the microparticles were observed under a scanning electron microscope (Model Philips XL20, Philips, Eindhoven, Netherlands). Samples were fixed on an aluminium stub with conductive double-sided adhesive tape (Leit-Tabs, Plano GmbH, Wetzlar, Germany) and coated with gold in an argon atmosphere (50 Pa) at 50 mA for 50 s (Sputter Coater, Bal-Tec AG, Liechtenstein).

Drug Content in Microparticles

The drug content of the produced microparticles was analyzed by a validated high-performance liquid chromatography (HPLC) with a UV-detector at suitable wavelength for each drug (230 nm for MCZ, 254 nm for DZP and Beta-val, and 280 nm for CRP). The HPLC system consisted of a high precision pump (Model 300 Gynkotek, Munich, Germany), an autosampler (Kontron HPLC Autosampler 360) and a detector (Kontron HPLC Detector 430, Kontron Instruments, Milano, Italy). The columns used were C8 column (BDS Hypersil 5 μm; 250 × 4.6 mm; Thermohypersil, PA) for MCZ analysis and RP-18 column (5 μm; 150 × 4.6 mm; Merck, Darmstadt, Germany) for DZP, Beta-val, and CRP analysis. A 10-mg sample was accurately weighed and dissolved in an appropriate amount of methanol. A mixture of 2.5% w/v of ammonium acetate solution, acetonitrile, and methanol at 2:3:5 ratio by volume was utilized as a mobile phase for MCZ. For DZP and Beta-val analysis, a mixture of acetonitrile and water at 60:40 ratio by volume was used. For determination of CRP content, a mixture of methanol and water at 45:55 ratio by volume was the mobile phase. Sample solutions of 80 μL were injected at the mobile-phase flow rate of 1.3, 1.8, 2.0, and 1.2 mL min^{-1} for MCZ, DZP, Beta-val, and CRP, respectively.

RESULTS AND DISCUSSION

Solubility of Drug in Lipid Carrier

The enthalpies of melting of several drugs, i.e., DZP, MCZ, CRP, and Beta-val, at various concentrations in lipid carrier (8:2 (w/w)-PC:Chol) determined by DSC are shown in Table 1. Determination of solubility of drugs in such lipid carrier could be accomplished by extrapolation of the linear plot of percentage amount of drug in the lipid carrier versus the enthalpy of melting to zero heat of melting (Figure 1), which indicated the maximum amount of drug existing in the form of solid solution within the lipid carrier (Theeuwes et al., 1974; Jenquin & McGinity, 1994). The solubilities of DZP, MCZ, CRP, and Beta-val in lipid carrier were found to be 11.8, 20.4, 36.4, and 72.0% (w/w), respectively, as given in Table 2. Such estimated values of drug solubility in the lipid carrier were precisely confirmed by their corresponding X-ray patterns (Figures 2–5), in which no main peak of the drugs could be detected at the concentrations below their solubilities. For DZP, the main peaks could usually be observed at 26.3º, 26.8º, 27.1º, 27.6º, 29.5º, 29.9º, 30.6º, and 31.0º 2θ. For MCZ, the main peaks were 25.5º, 26.0º, 26.4º, 26.7º, and 27.4º 2θ. The major peaks of CRP could be visualized at 24.4º, 26.1º, 26.5º, 26.8º, 27.3º, and 27.7º 2θ, and for Beta-val at 19.7º, 24.2º, a innd 24.3º 2θ. All X-ray patterns at concentrations below drug solubilities in such lipid carrier were simply similar to that of the drug-free lipid carrier (8:2 (w/w)-PC:Chol).

TABLE 1
Observed Endothermic Heat of Melting of Several Drugs at Various Concentrations in Lipid Carrier (8:2 (w/w)-PC:Chol)

Drug Substance	Concentration of Drug in Lipid Carrier (%, w/w)	Observed Endotherm ($J\ g^{-1}$)
DZP	10	0.065
	20	4.313
	33	16.789
MCZ	20	0.902
	33	18.715
	43	39.445
CRP	33	0.873
	50	9.011
	60	21.508
	67	33.338
Beta-val	85	23.572
	90	29.194
	100	48.409

TABLE 2
Comparison of Solubility of Various Drugs in Lipid Carrier (8:2 (w/w)-PC:Chol) and Percentage Amount of Drug Recovered in ASES-prepared Dry Microparticles

Drug Substances	Drug Solubility in Lipid Carrier (%, w/w)	Percentage Amount of Drug Initially Added into the System (%, w/w)	Percentage Amount of Drug Recovered in Microparticles (%, w/w)
DZP	11.8	10	1.6
		19	2.8
		38	12.5
MCZ	20.4	10	3.4
		19	5.5
		38	19.1
CRP	36.4	10	10.3
		19	18.2
		38	36.0
Beta-val	72.0	10	10.5
		19	20.9
		38	38.3

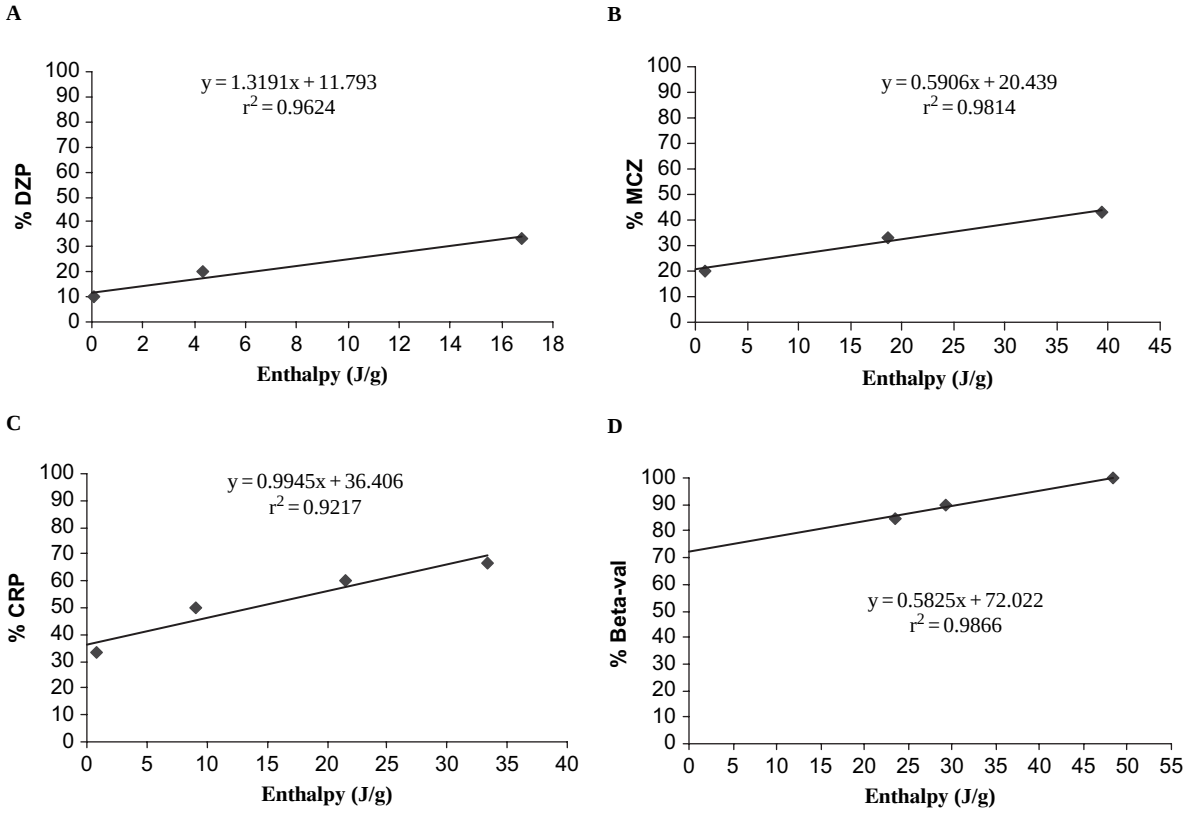

FIGURE 1. Determination of solubility of various drugs in lipid carrier (8:2 (w/w)-PC:Chol) by extrapolation of the plot of percentage amount of drug versus enthalpy of melting to zero value: (A) DZP, (B) MCZ, (C) CRP, (D) Beta-val.

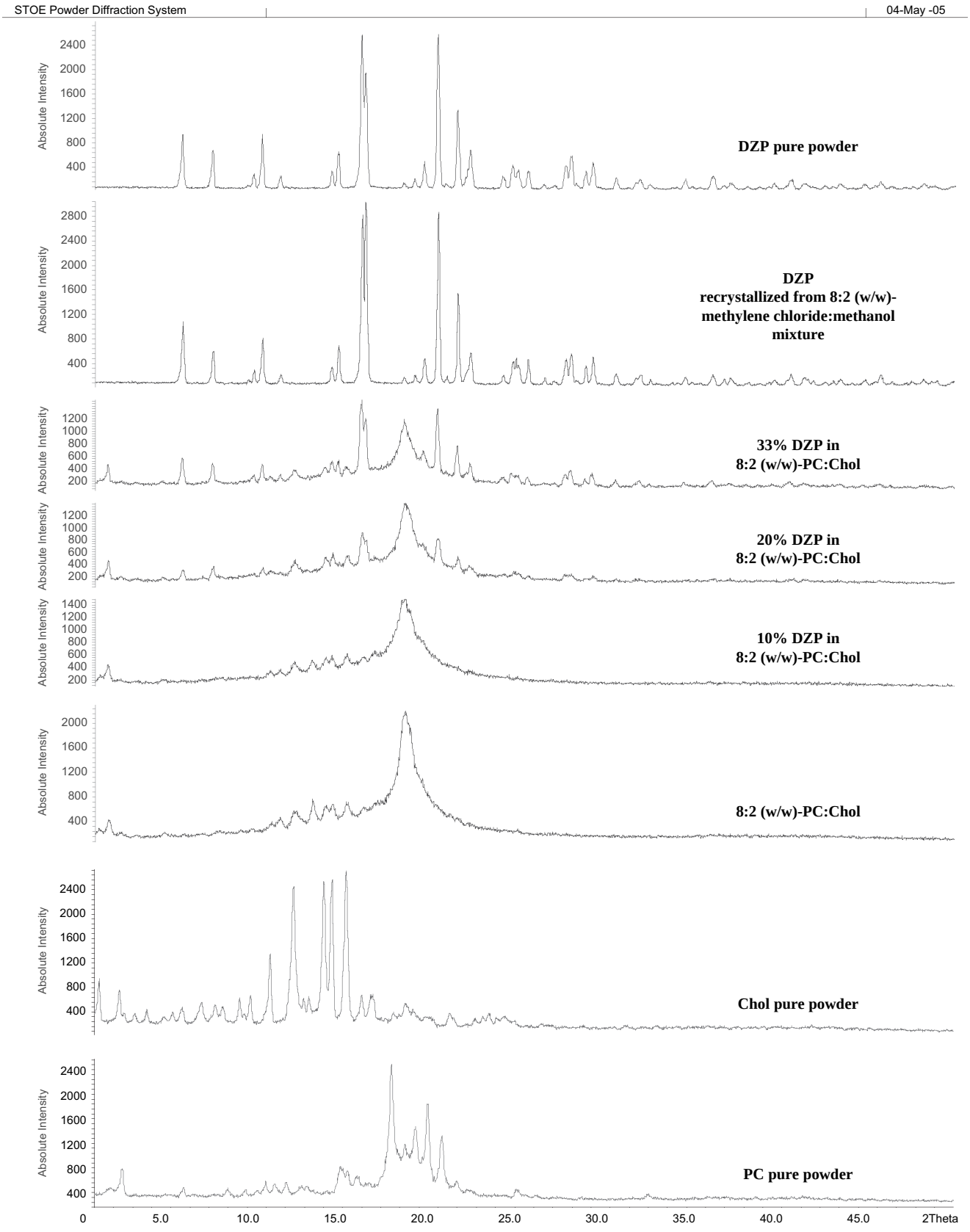

FIGURE 2. XRPD patterns of pure compounds, lipid carrier, and DZP-lipid mixtures at various DZP concentrations.

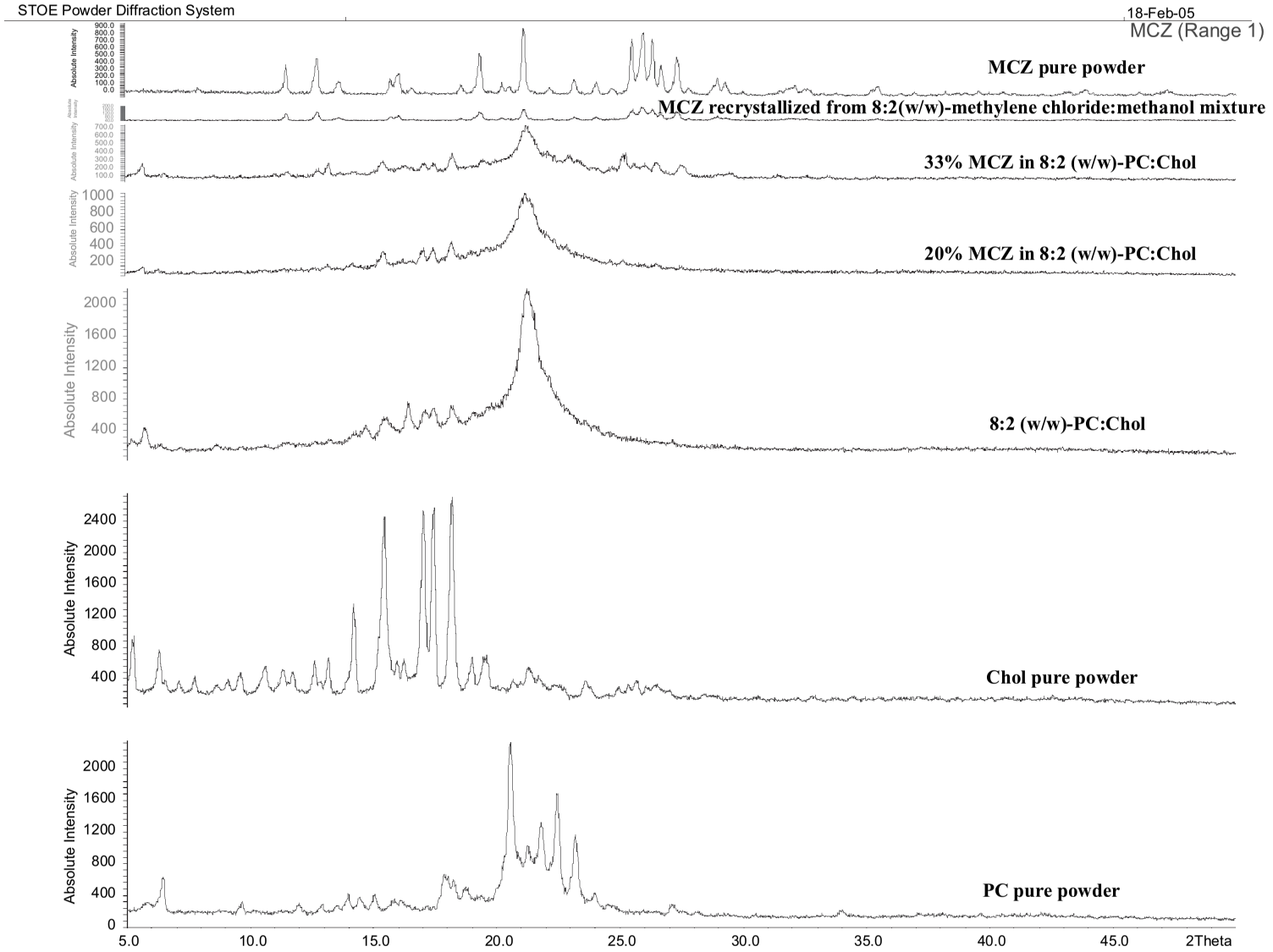

FIGURE 3. XRPD patterns of pure compounds, lipid carrier, and MCZ-lipid mixtures at various MCZ concentrations.

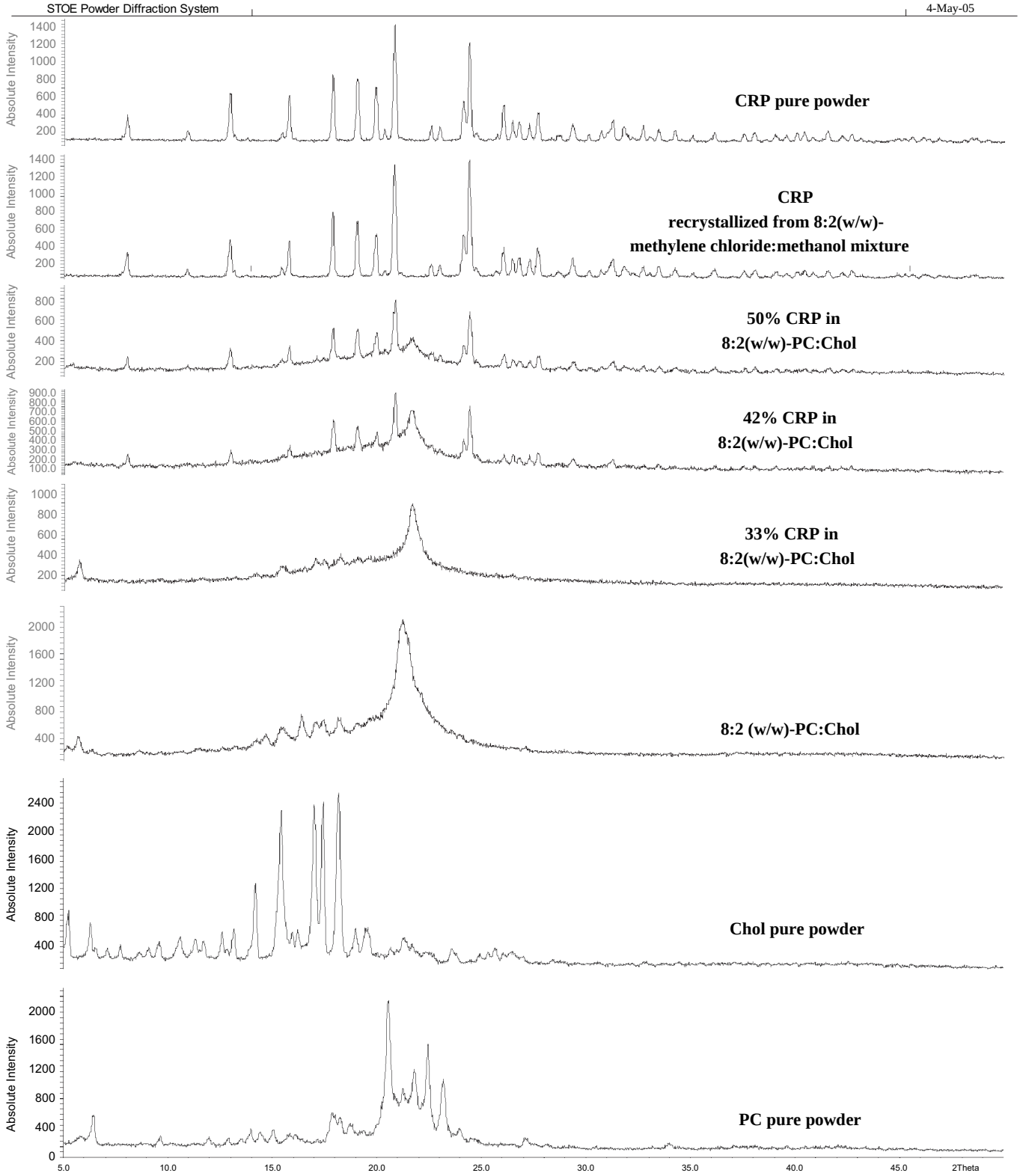

FIGURE 4. XRPD patterns of pure compounds, lipid carrier, and CRP-lipid mixtures at various CRP concentrations.

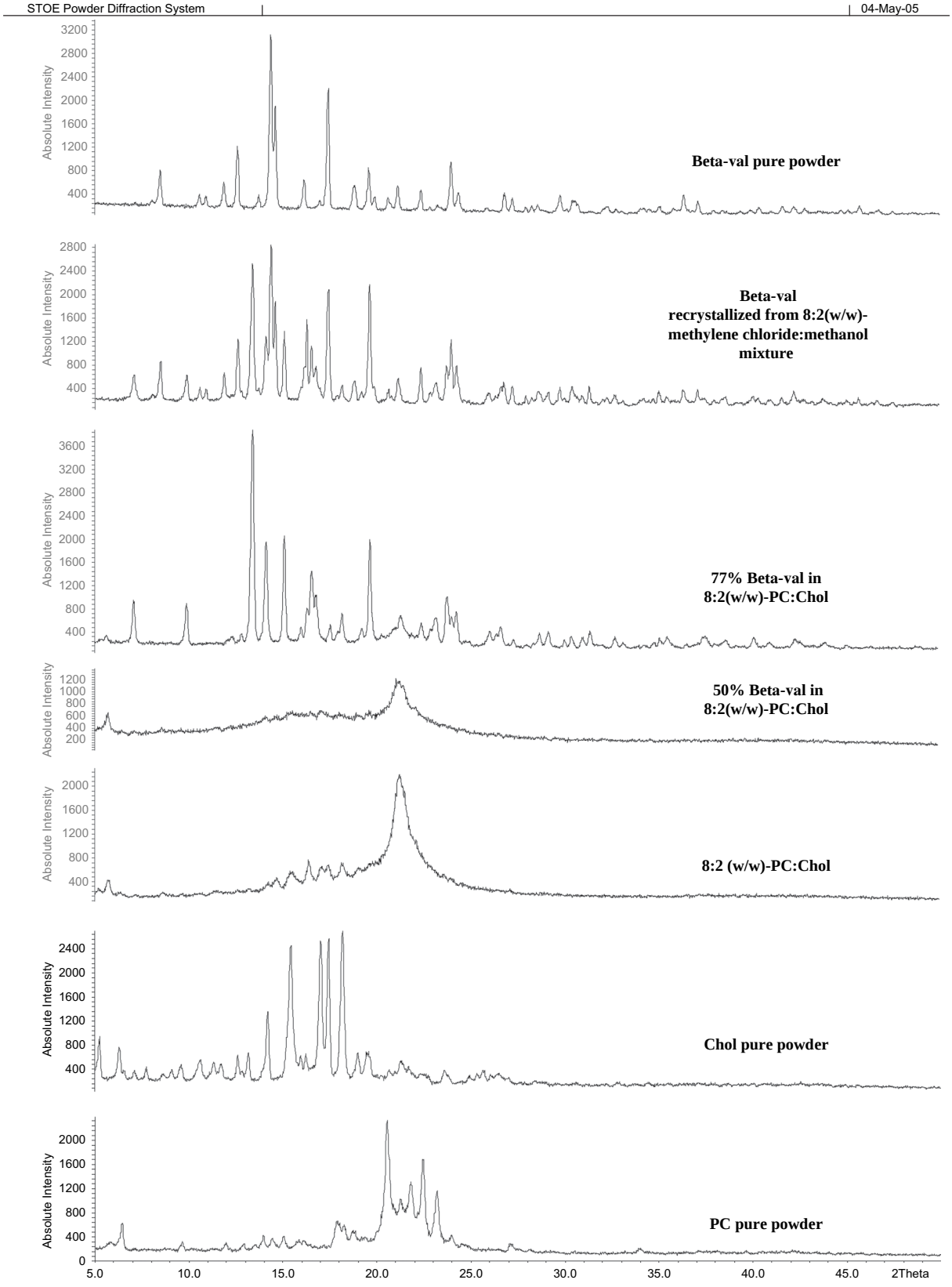

FIGURE 5. XRPD patterns of pure compounds, lipid carrier, and Beta-val-lipid mixtures at various Beta-val concentrations.

Effect of Solubility of Drug in Lipid Carrier on the Amount of Drug Recovered

An incomplete recovery of drug the produced microparticles has basically been recognized as an indication of the drug loss due to partial extraction by the $scCO_2$ during particle formation. This was obviously found to be the case for the systems containing drugs with low solubility in lipid carrier such as DZP and MCZ (Table 2). Although this incidence was not observed in the systems containing drugs with high solubility in lipid carrier such as CRP and Beta-val, a significant amount of drugs detected in the organic solvents recovered from the ASES process (data not shown) suggested the possibility of partial extraction by the $scCO_2$. Moreover, a dry lipid film was also found in the separator for collecting the organic solvents during the process, indicating that the lipid components would partially be extracted by the $scCO_2$ as well. The slightly higher values of the percentage amount of drug recovered being observed in the microparticles containing Beta-val could therefore be attributed to partial extraction of both drug and lipid components. Nevertheless, the effects of these partial extractions seemed to be at the same degree so that the percentage of Beta-val recovered was only slightly higher than the initial amount of drug added into the system.

Close examination of Table 2 revealed that there appeared to be a correlation of solubility of the drug in the lipid carrier and the percentage amount of drug recovered in the produced microparticles in that the latter could be increased with the increasing of the former. In the case of drugs with lower solubility in the lipid carrier (DZP and MCZ), the percentage amount of drug recovered in the microparticles could be increased by increasing the drug concentration in the spraying solution. For drugs with higher solubility (CRP and Beta-val), the amount of drug recovered appeared to have approximately the same value as the initial amount of drug added, as long as the desired amount of drug in the microparticles was still within its solubility. However, too high amount of dissolved drug conversely exhibited a negative effect on the solidification of the lipid carrier during ASES processing as observed in the preparations of 19% (w/w) CRP and 19% (w/w) Beta-val. Those preparations led to a coalescence of microparticles produced and partial film formation in the production column, thus to a lower yield of microparticles being collected (Figure 6). Such behavior could be attributed to the possible reduction in phase transition temperature of the phospholipid used due to large amount of the drug being incorporated into the system. Our previous investigations showed that production conditions above phase transition temperature of the structural lipid used brought about partial film formation adhering to the column wall and thus lowering the collected yield (Kunastitchai et al., 2006).

The XRPD patterns of ASES-prepared microparticles of various drugs illustrated in Figures 7–10 revealed that the physical states of the drugs in microparticles were quite similar to those in their corresponding drug-lipid mixtures at concentrations below their solubilities as shown in Figures 2–5. Such findings implied that the formation of the ASES-prepared microparticles would be a result of the co-precipitation of the drug and the lipid carrier with an optimum concentration of drug solubilized in such a carrier system. An excessive amount of drug being incorporated was not found to recrystallize in the ASES-prepared microparticles, but was rather either extracted/solubilized into the $scCO_2$ or induced a coalescence of microparticles being formed. No particle formation of drug-lipid microparticles was observed in preparations of 38% (w/w) CRP and 38% (w/w) Beta-val. Figure 11 shows the successful precipitation of drug-lipid microparticles prepared by the ASES process. At this standard operating conditions, spherical and nonporous microparticles associated in aggregates varying from a few microns to 100 μm were produced. Apart from the proper operating conditions for ASES, it should be remarked that the particle formation of drug-lipid microparticles could be satisfactorily achieved when the suitable concentration of drug is added into a structural lipid carrier.

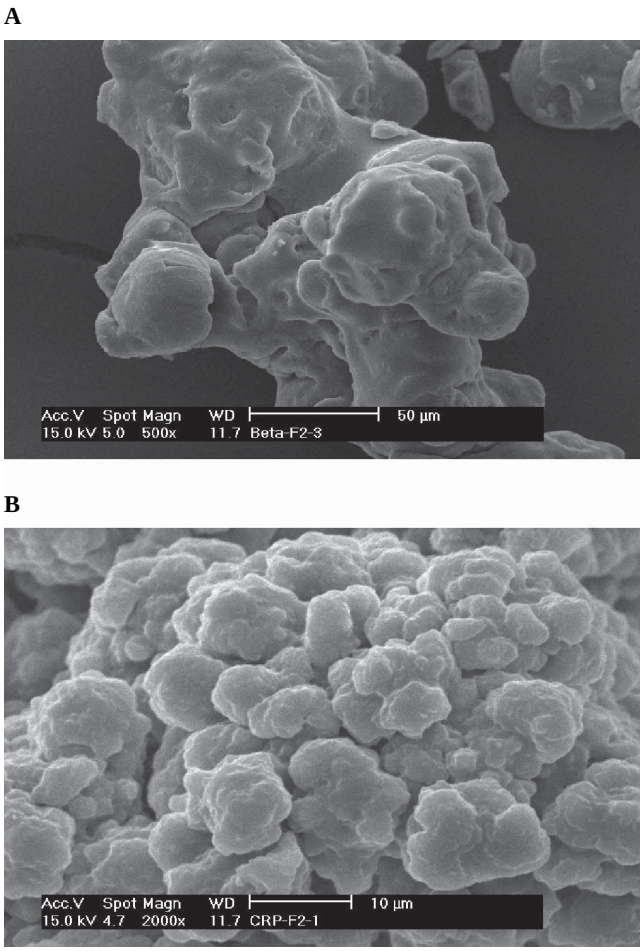

FIGURE 6. SEM photomicrographs of ASES-prepared microparticles bringing about coalescence and film formation at higher drug concentration in lipid carrier (8:2 (w/w)-PC:Chol): (A) 19% (w/w) Beta-val, (B) 19% (w/w) CRP.

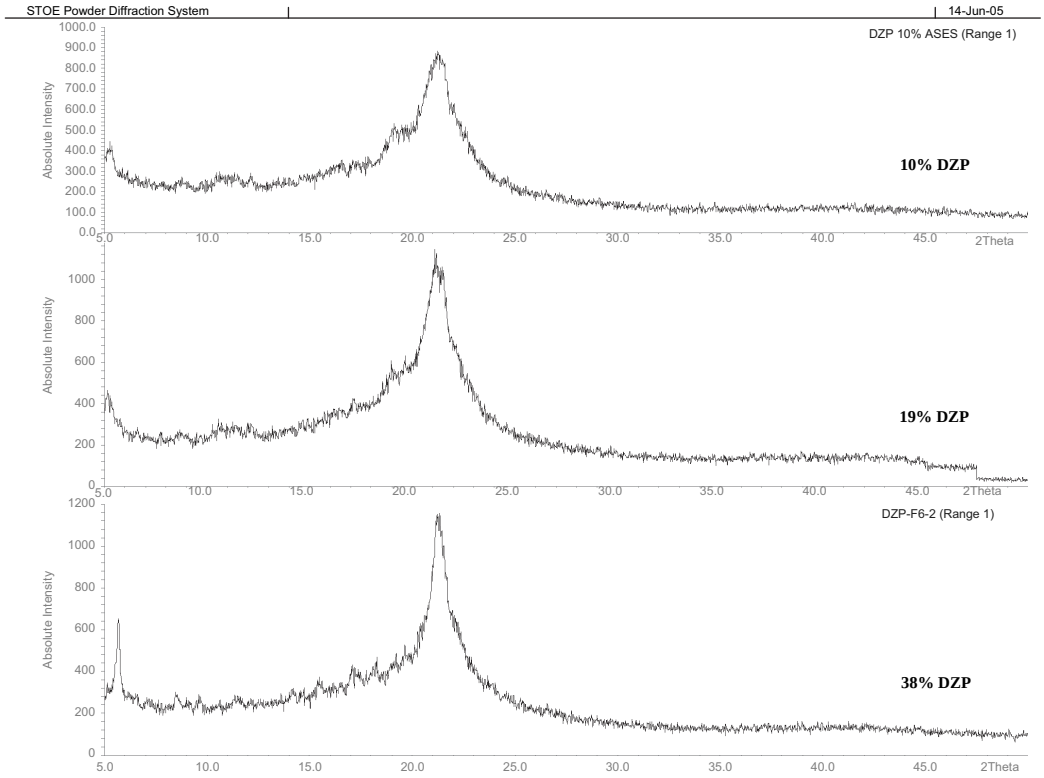

FIGURE 7. XRPD patterns of ASES-prepared DZP microparticles at various DZP concentrations.

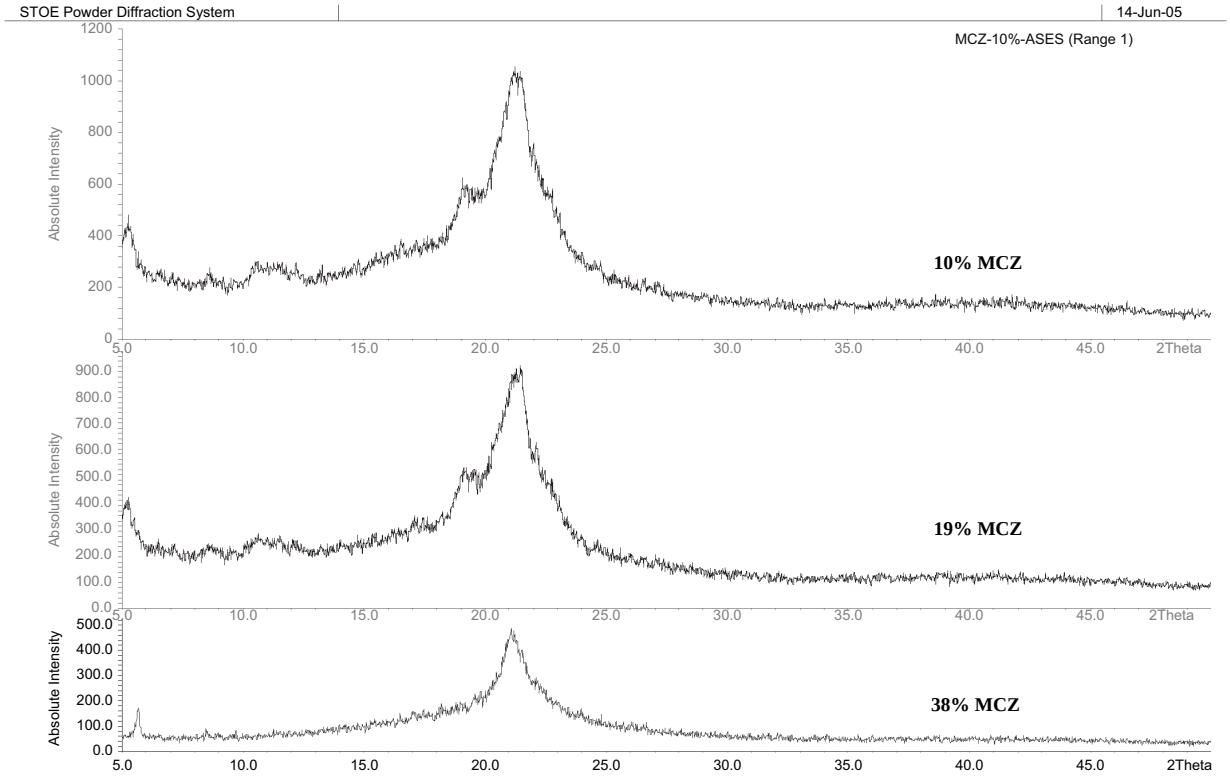

FIGURE 8. XRPD patterns of ASES-prepared MCZ microparticles at various MCZ concentrations.

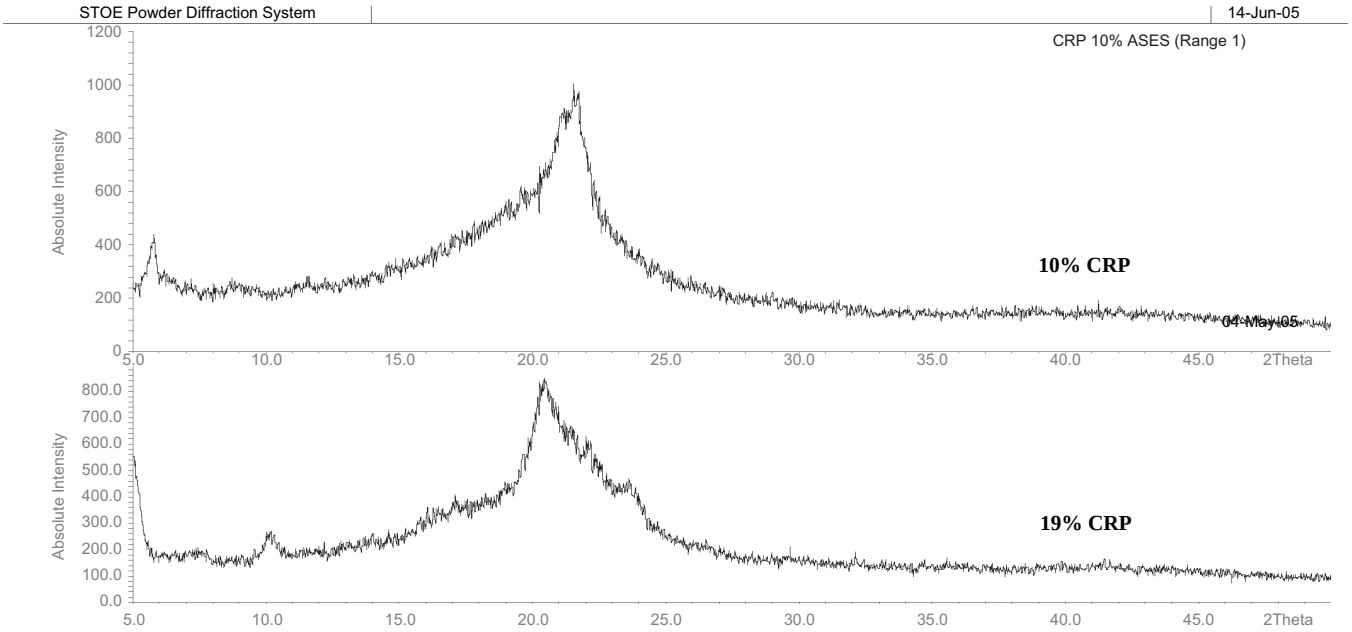

FIGURE 9. XRPD patterns of ASES-prepared CRP microparticles at various CRP concentrations.

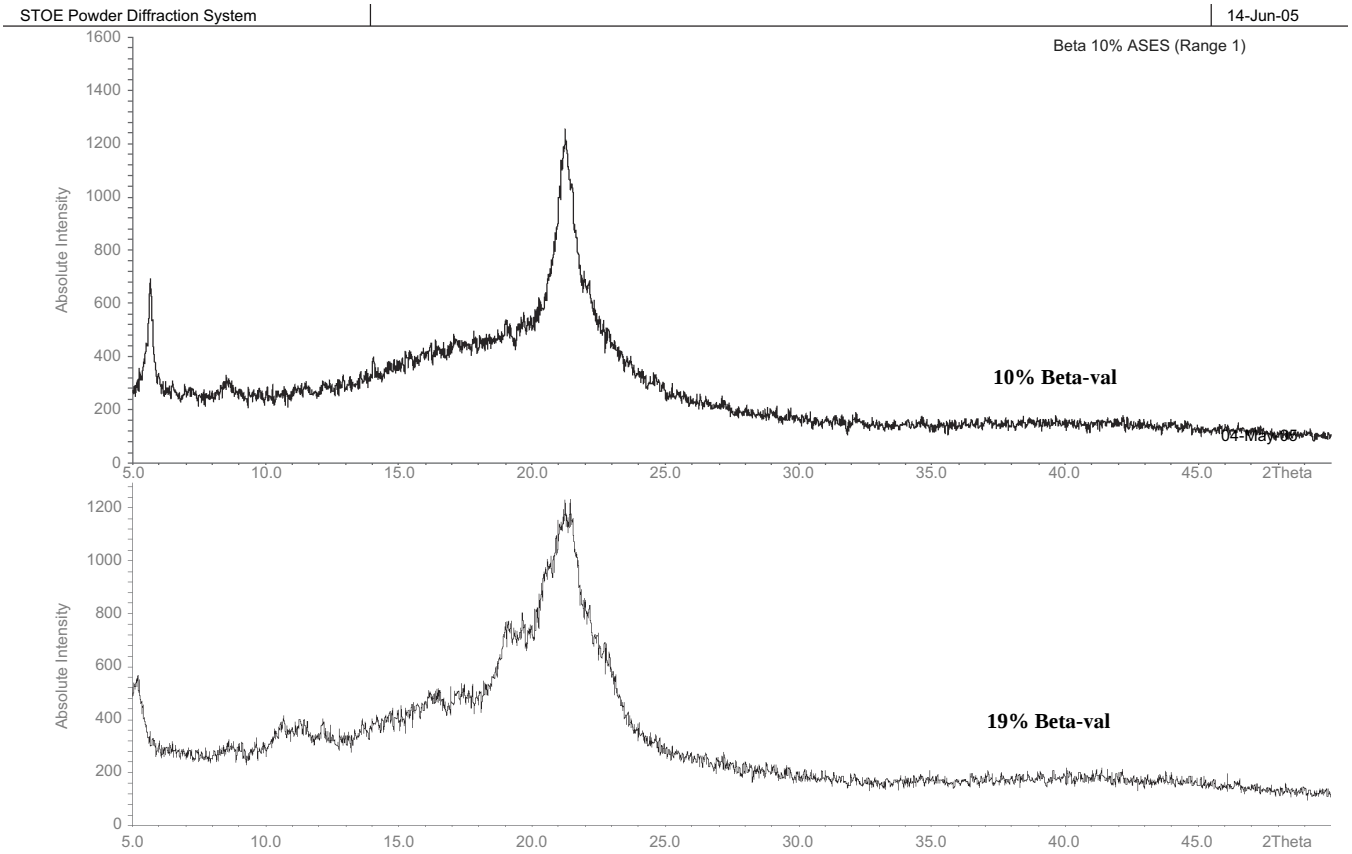

FIGURE 10. XRPD patterns of ASES-prepared Beta-val microparticles at various Beta-val concentrations.

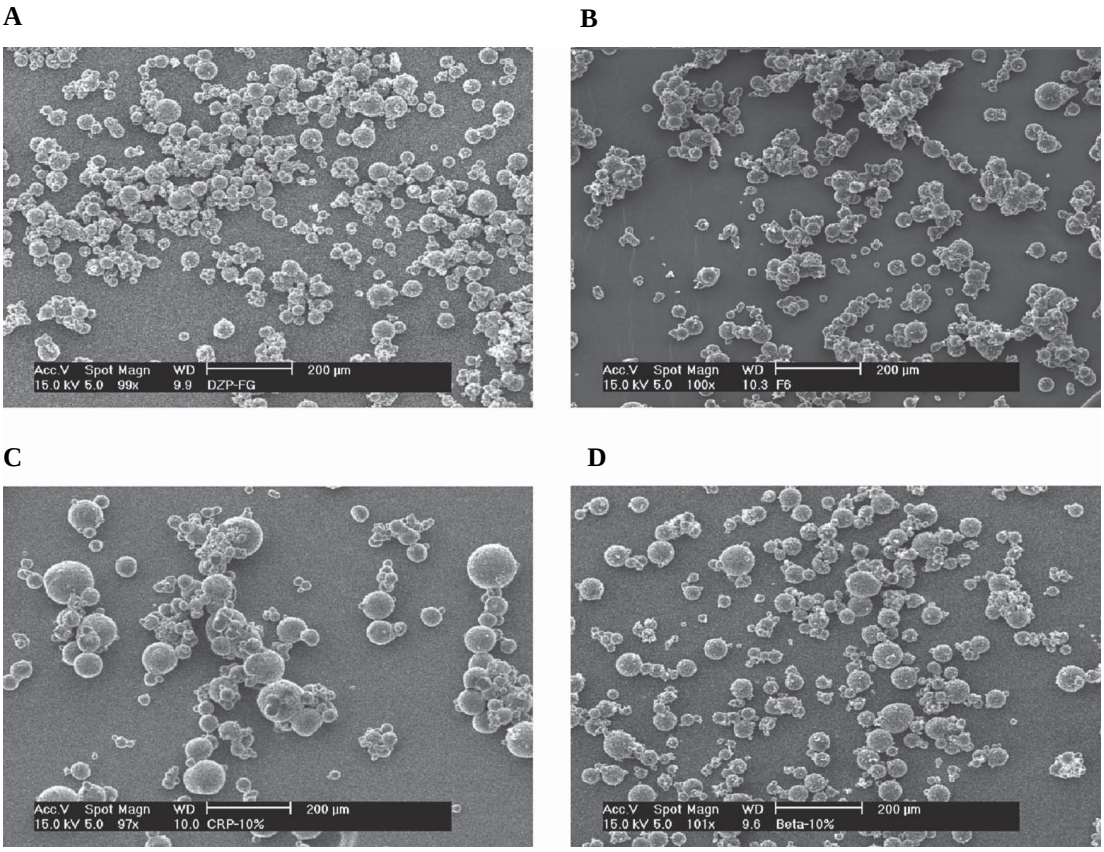

FIGURE 11. SEM photomicrographs of ASES-prepared microparticles of various drugs: (A) 38% (w/w) DZP-, (B) 38% (w/w) MCZ-, (C) 10% (w/w) CRP-, (D) 10% (w/w) Beta-val—in 8:2 (w/w)-PC:Chol.

CONCLUSION

The drug solubility in the phosphatidylcholine-cholesterol carrier seemed to be the promising parameter to predict the amount of drug recovered in the drug-lipid microparticles produced by the ASES process. An exceedingly high concentration of drug solubilized in the lipid carrier led to the formation of film instead of particles, resulting in a lower yield of the produced microparticles. On the other hand, drug with low solubility in the carrier tended to be partially extracted by the $scCO_2$ to a greater extent, leading to a lower drug recovery. The maximum amount of drug recovered in the produced microparticles can be estimated from the solubility of the drug in the lipid carrier used.

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