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Dichloromethane Evaporative Behavior during the Solidifying Process of Ovalbumin-Loaded Poly (DL Lactic-co-Glycolic Acid) Microparticles

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ABSTRACT

The purpose of this study is to develop an analytical method for determining the evaporative behavior of dichloromethane (DCM) during the solidifying process of poly(DL lactic-co-glycolic acid) (PLGA) in the preparation of ovalbumin-loaded microparticle (OVA-MP) with double emulsion solvent extraction method. The time courses of DCM levels in the external phase and total mixture were determined by a gas chromatography (GC) method combined a headspace sampler. Samples were spiked with an internal standard carbon tetrachloride, prior to mixing with a large volume of chloroform. The retention times of carbon tetrachloride, DCM and chloroform were 2.6, 2.8 and 3.3 min, respectively. The analytical method had a minimum quantitative concentration of DCM 7.3 mM and good linearity from 7.5 to 75 mM with coefficient of variations 1.2~3.9%. Four formulations containing NaCl (0~5%) and urea, an osmotic agent to adjust osmolarity at 1240 mOsm/kg, were investigated. The disappearance of DCM levels in total mixtures of four formulations were described as a function of time by zero-order (0~15 min) and first-order (after 15 min) kinetics. During the initial 15 min, these formulations had almost identical zero-order rate constants, 2.30~2.63 mmole/min. After 15 min, formulation with 5% NaCl (F4) showed a significant lower value of rate constant (0.193 min^{-1}) in comparison with other formulations using the lower amounts of NaCl ($0.288\text{--}0.367 \text{ min}^{-1}$). It indicated that the surface characteristic of the F4 OVA-MP was different from other formulations by forming a crust-like structure to inhibit the efflux of DCM from the inner layer. In conclusion, we established a rapid and convenient GC approach without the need for sample pre-treatment for determining DCM levels during the solidifying process of microparticles. The analytical method could be applied to further assess the relationship between DCM level and formulation factors of drug-loaded microparticles.

Key words: PLGA, gas chromatography, salt effect, dichloromethane evaporation

INTRODUCTION

Poly(DL-lactic-co-glycolic acid) (PLGA), a biodegradable polymer, can be degraded to endogenous substances such as lactic acid and glycolic acid after applied in the body. PLGA has been extensively investigated in controlled release delivery systems of therapeutic proteins and vaccine antigens^(1,2). The approach of double emulsion (water-in-oil-in-water, w/o/w) combined with solvent evaporation method is widely used to encapsulate drugs in PLGA matrices for preparing drug-loaded microparticles. Because PLGA is almost insoluble in water, the copolymer is frequently dissolved in dichloromethane (DCM) prior to mixing with drug aqueous solution. Afterwards, the two immiscible liquids are homogenized to obtain a primary emulsion, and then a large volume of external aqueous phase was added into the primary emulsion to form a multiple emulsion. While the DCM of the w/o/w emulsion was evaporated under a gentle stirring rate, the combined drug was encapsulated in the polymeric matrix to obtain a

drug-loaded microparticle during the solidifying process of the polymeric dispersion^(3,4). However, only few reports examined the relationship between DCM levels and formulation factors in preparing drug-loaded microparticles during DCM evaporation. The DCM evaporation time, ranging from 3 to 24 hr, was usually decided by the investigator's experience^(4,5). DCM is a controlled organic solvent which may cause severe syndromes after inhalation, oral administration or skin contact. The solvent significantly increases the incidence of lung and liver tumors as well as benign mammary tumors in tested mice⁽⁶⁾. Thus, a short DCM evaporation time may not completely remove the solvent, whereas DCM residue in drug-loaded microparticle may be too high and possibly harmful for human use. However, in addition to wasting time, the long evaporation preparing process may cause drug degradation especially for long-term exposing protein therapeutic agents in liquid condition⁽⁷⁾. Thus, it is important to establish a practical analytical method for measuring DCM evaporation behavior during the preparation of PLGA microparticle.

In this study, we developed a gas chromatography (GC) method with a headspace sampler for determining

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DCM levels in PLGA multiple emulsions as a function of time during the solidifying process of PLGA microparticle preparation. Four formulations, combined with various sodium chloride levels (0~5%) and an osmotic agent urea, were investigated using ovalbumin (OVA) as a model protein drug to prepare OVA-load microparticles (OVA-MPs). The purpose of this study was to develop a GC analytical approach for investigating the DCM evaporative behaviors during the solvent evaporation stage in preparing OVA-MPs. The relationship between DCM levels and formulation factors was further discussed.

MATERIALS AND METHODS

I. Materials

Ovalbumin (OVA, grade V), polyvinylpyrrolidone (PVP, MW 40,000), sodium chloride, and urea were provided by Sigma (St. Louis, MO, USA). Poly(DL-lactic-co-glycolic acid) (PLGA) with a ratio of 50/50 (Resomer RG503, intrinsic viscosity 0.4 dl/g in chloroform) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Dichloromethane (DCM), chloroform and carbon tetrachloride were purchased from BDH (Poole, UK).

II. Preparing Microparticles

OVA was encapsulated in PLGA microparticle by modifying the reported methods using w/o/w solvent evaporation technique^(3,4,8). Components of the four formulations are shown in table 1. The preparation method is briefly stated as follows. One milliliter of OVA aqueous solution (30 mg/mL) was added to 5 mL of DCM containing 300 mg of PLGA. The mixture was homogenized at 8,000 rpm for 1.5 min to obtain a primary emulsion. Then, the emulsion was mixed with 30 mL of aqueous continuous phase containing PVP 5% w/v as an emulsion stabilizer combined with various concentrations of sodium chloride (0, 3 and 5% w/v, depending on the protocol) and urea as a tonic agent to adjust osmolarity to 1240 mOsmol/Kg. The resulting mixtures were homogenized at 8,000 rpm for 2.5 min to obtain multiple emulsions. The w/o/w multiple

emulsions were controlled at 25°C in a water-jacketed beaker and stirred with a magnetic stirrer (800 rpm) for 18 hr to evaporate DCM. The microparticles were washed and lyophilized. Four OVA-MPs were obtained at the end.

III. Morphology of OVA-MPs

A gold sputtering technique was used for preparing OVA-MPs samples. Morphologies of OVA-MPs samples were characterized by scanning electronic microscope (SEM; Jeol 5400, Tokyo, Japan).

IV. Sampling Method of Dichloromethane

While evaporating DCM from the prepared w/o/w multiple emulsion, samples were withdrawn from the multiple dispersion system to evaluate the DCM evaporative behavior. The w/o/w multiple emulsion was kept at 25°C and stirred at 800 rpm for 1 hr. At 0, 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 min, 0.5 mL and 0.2 mL of total mixtures were sampled. The 0.2-mL sample was sealed in a GC analytical vial to determine the DCM level. The 0.5-mL sample was centrifuged, then, 0.2 mL of supernatant was collected and sealed in a GC analytical vial to determine the DCM level in the external phase medium.

V. Determining Dichloromethane by Gas Chromatography

Samples were spiked with an internal standard, carbon tetrachloride, and determined by a GC system (GC-17A, Shimadzu, Tokyo, Japan) using headspace sampling method. Internal standard solution was freshly prepared by mixing 1 mL of carbon tetrachloride and 50 mL of chloroform. A 0.2 mL aliquot of sample was mixed with 0.5 mL of the internal standard solution. The mixture was sealed in a GC analytical vial with teflon septum and aluminum cap. In the analysis, nitrogen gas was used as a carrier with a flow rate of 1 mL/min. The GC system was equipped with a headspace sampler (HSS-4A, Shimadzu, Tokyo, Japan), a polyethylene glycol column (SGE, 25 m × 0.22 mm), and a flame ionization detector connected with a computer. The operating temperatures were set as the following: column oven: 80°C; injection port: 250°C; detector: 200°C; vial heater: 80°C; and syringe: 80°C. The analytical time was set at 6 min with a pre-heat time of 4 min. Both high and low DCM calibration curves were constructed by plotting the peak height ratio of DCM and internal standard (carbon tetrachloride) versus DCM concentration for calculating the DCM contents of samples.

VI. Effect of Chloroform Volume on Dichloromethane Assay

In the analysis of DCM by the GC method, four different volumes of chloroform were also used to evaluate the effect of chloroform volume. Three components, 0.2 mL of water, 10 μL of internal standard (carbon tetrachloride), and various volumes of chloroform (0, 0.1, 0.5, and 1

Table 1. Components of PLGA formulations and DCM evaporation rate constants

Formulations	External phase (H ₂ O, 30 mL)			DCM evaporation rate constants	
	Urea (%)	PVP (%)	NaCl (%)	Zero-order	First-order
				R _{0-15 min} (mmole/min)	K _{15-30 min} (min ⁻¹)
F1	0	5	0	2.63 ± 0.25	0.351 ± 0.076*
F2	10.3	5	0	2.48 ± 0.17	0.288 ± 0.059*
F3	4.1	5	3	2.30 ± 0.12	0.367 ± 0.050**
F4	0	5	5	2.41 ± 0.64	0.193 ± 0.021

^aInner phase: 30 mg of OVA in 1 mL of water; organic phase: 300 mg of PLGA in 5 mL of DCM. **p* < 0.05; ***p* < 0.01; in comparison with F4, by *t*-test (Mean ± SD, *n* = 3).

mL), were mixed with different amounts of DCM and sealed in a GC analytical vial with teflon septum and aluminum cap. The GC analytical condition was the same as described above.

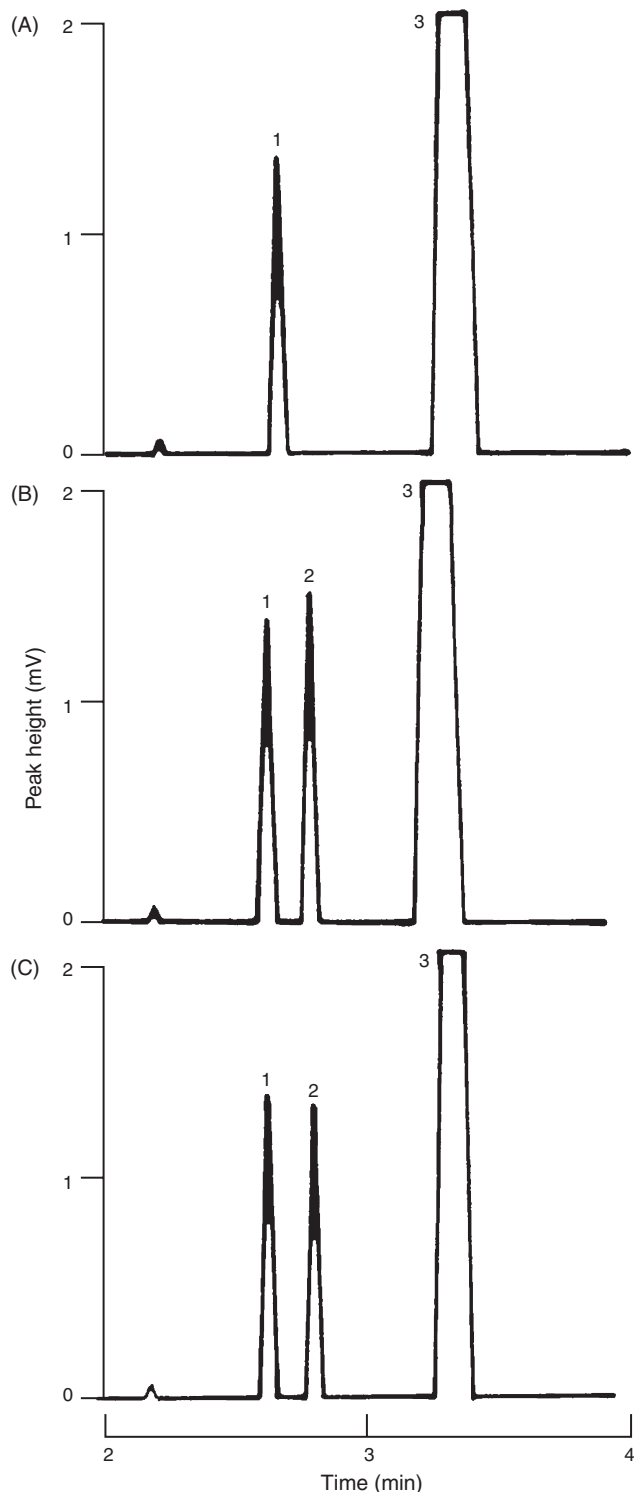


Figure 1. GC chromatograms of DCM by spiking DCM or PLGA dispersion into 0.2 mL of aqueous solution. (A): blank. (B): spiked 300 mM DCM. (C): w/o/w PLGA dispersion sample. Key: peak 1: carbon tetrachloride as internal standard; peak 2: DCM; and peak 3: chloroform.

VII. DCM Evaporative Characteristics

The time course of DCM content in the total mixture was described as a function of time by zero-order (0~15 min) and first-order (after 15 min) kinetics during DCM evaporation stage. The zero-order rate constant was derived from the slope by plotting the total content of DCM versus time. The first-order rate constant was obtained by multiplying the slope by 2.303, having plotted the logarithm of total content of DCM versus time. These two rate constants were used to characterize the DCM evaporative behaviors for different formulations.

RESULTS

I. Effect of Chloroform Volume

In this study, DCM levels were analyzed by the established GC method, in which carbon tetrachloride was used as an internal standard combined with chloroform to modify the evaporation of DCM and carbon tetrachloride during head space sampling in the GC analysis. Figure 1 shows the GC chromatograms of carbon tetrachloride, DCM, and chloroform. The retention times of carbon tetrachloride, DCM, and chloroform were 2.6, 2.8 and 3.3 min, respectively. GC chromatograms of standard solutions containing 0 and 300 mM DCM are shown in Figure 1-A and B, respectively. Figure 1-C is the GC chromatogram of formulation F4 total mixture sample at 25 min during DCM evaporative stage. There was no interference in DCM peak for the total mixture sample with PLGA polymer. Figure 2 and 3 show the profiles of the peak height of DCM and the peak height ratio of DCM/carbon tetrachloride with different volumes of chloroform versus DCM concentration, respectively. All plots are slightly curvature ranging from 7.5 mM to 3000 mM. The peak heights of DCM decreased as chloroform increased (Figure 2), whereas the peak height ratios of DCM/carbon tetrachloride increased

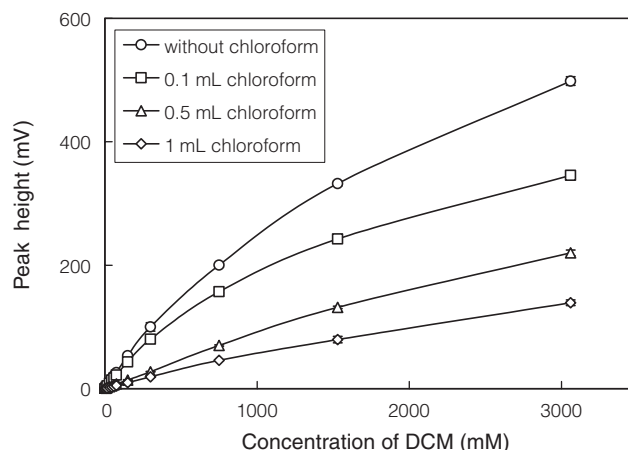


Figure 2. Profiles of DCM peak height versus DCM concentration determined by GC method for combining various volumes of chloroform.

(Figure 3). The linearities of four plots were evaluated by two linear regressions for both low (7.5–75 mM) and high DCM concentration range (75–1500 mM). The slope differences between low and high concentrations were -43.1%, -50.1%, -12.3%, and -16.6% for combining with 0, 0.1, 0.5, and 1.0 mL of chloroform, respectively (Table 2). The deviation of slopes for 0.5, and 1.0 mL chloroform was much less than that of 0, and 0.1 mL chloroform. These results demonstrated that the linearity of DCM calibration curve was significantly improved by spiking 0.5 mL or higher volume of chloroform.

II. Analytical Method of Gas Chromatography

To plot the peak height ratio of DCM and carbon tetrachloride (internal standard) versus the concentration of DCM by spiking 0.5 mL of chloroform, a slightly curved line was obtained (Figure 3). In order to precisely determine the DCM concentration of samples, two calibration curves were established including low DCM concentration range from 7.5 to 75 mM and high range from 75 to 1500 mM. Two linear equations were obtained as follows:

$$\text{Low range: } Y = 0.00725 X + 0.00505 \quad (r = 0.999, p < 0.001, n = 6) \quad (1)$$

$$\text{High range: } Y = 0.00636 X + 0.077 \quad (r = 0.999, p < 0.05, n = 5) \quad (2)$$

Precision and accuracy of intra-day analysis are shown in Table 3. The analytical results of DCM determination demonstrated the established GC method yielded acceptable precision and accuracy. The coefficients of variation were from 0.49% to 3.85%, and the relative errors were from -3.93% to 3.09%. Figure 4 shows the scatter plot of intra-day analyses to represent the deviations estimated from low

Table 2. Differences of fitted linear slopes between low DCM concentration range (7.5~75 mM) and high DCM concentration range (75~1500 mM) for combining various spiked chloroform volumes in the GC analyses

DCM concentration range	Spiked chloroform volume (mL)			
	0	0.1	0.5	1.0
Low (7.5 ~ 75 mM)				
Intercept	0.00098	-0.00315	0.00505	0.00393
Slope	0.00188	0.00470	0.00725	0.00781
r ²	0.999	0.996	0.999	0.985
High (75 ~ 1500 mM)				
Intercept	0.158	0.328	0.077	0.279
Slope	0.00107	0.00235	0.00636	0.00726
r ²	0.979	0.991	0.999	0.997
^a Difference of slope (%)	-43.1	-50.1	-12.3	-16.6

^aDifference of slope (%) = (slope_{high range} - slope_{low range}) / slope_{low range} × 100

Table 3. Coefficient of variation and error of intra-day analyses in the established analytical method of dichloromethane

Spiked DCM concentration (mM)	Peak height ratio (mean ± SD, n = 3)	CV (%)	Fitted concentration (mM)	Error (%)
7.5	0.058 ± 0.001	1.21	7.37 ^a	-1.73
15	0.114 ± 0.003	2.50	14.99 ^a	-0.05
30	0.219 ± 0.003	1.35	29.55 ^a	-1.49
45	0.335 ± 0.013	3.85	45.55 ^a	1.23
60	0.447 ± 0.008	1.85	60.95 ^a	1.59
75	0.54 ± 0.02	3.60	74.15 ^a , 73.22 ^b	-1.13 ^a , -2.37 ^b
150	1.02 ± 0.01	0.67	148.93 ^b	-0.71
300	1.91 ± 0.02	0.49	286.34 ^b	-3.93
750	4.99 ± 0.08	1.65	773.19 ^b	3.09
1500	9.56 ± 0.31	3.29	1490.58 ^b	-0.63

^aCalculated from low range (7.5~75 mM): Y = 0.00725 X + 0.00505.

^bCalculated from high range (75~1500 mM): Y = 0.00636 X + 0.077.

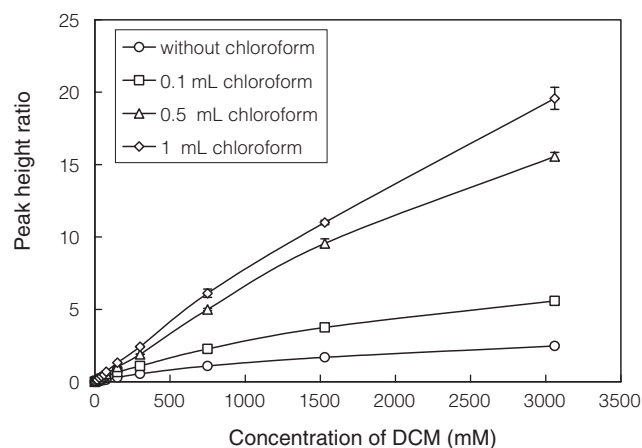


Figure 3. Profiles of DCM peak height ratio (DCM/carbon tetrachloride) versus DCM concentration determined by GC method for combining various volumes of chloroform.

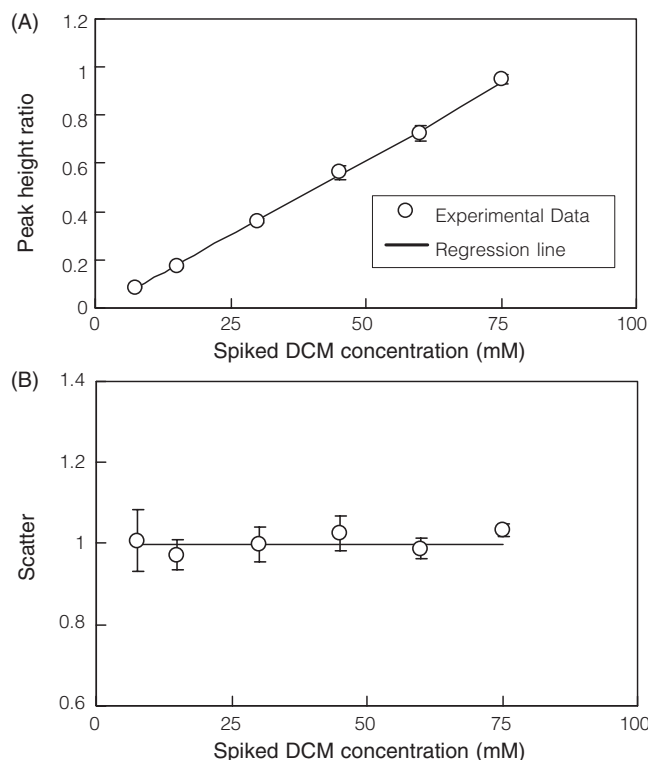


Figure 4. Calibration curve (A) and scatter plot (B) for low concentration range of DCM (7.5~75 mM).

range standard curve. Most fitted data were consistent in the spiked concentrations with values around 1.0 in the scatter plot.

III. Morphology of Ovalbumin-Loaded Microparticle

Figure 5 shows the SEM micrographs of four formulations. F1 formulation, without sodium chloride and urea in the external aqueous phase, was used as a control. F1 had a smooth surface characteristic. F2, without sodium chloride but containing urea for adjusting osmolarity, had a similar feature as F1 except for a small portion of microparticles with some pores on their surfaces. As for F3, containing 3% sodium chloride and urea for adjusting osmolarity, irregular microparticles were obtained and associated with wrinkle surface. F4, containing 5% sodium chloride but without urea, the microparticles were highly indented surface and deep crack. As the results, the higher the sodium chloride content in the external aqueous phase, the more variety of surface characteristics of OVA-MPs were fabricated.

IV. DCM Evaporative Characteristics

Figure 6 shows the GC chromatograms of F4, as an example to represent the time course of DCM level in the total mixture during the solvent evaporation stage. DCM levels in the total mixture were below detecting limit for the samples of 35~60 min. The DCM concentrations in the external phase seemed to maintain a constant value (0.20 ± 0.02 M) in the initial 15 min for four formulations. The DCM levels could be converted to total contents of DCM in four investigated multiple emulsions. Figure 7 shows the total contents of DCM in multiple emulsions fitted by zero-order (0~15 min) and first-order (after 15 min) kinetics during DCM evaporation stage. These rate constants are listed in table 1. During the initial 15 min, four formulations had almost identical rate constants, 2.30~2.63 mmole/min. After 15 min, F4 showed a significant lower value of first-order rate constant, 0.193 min^{-1} , in comparison with other formulations, $0.288\text{--}0.367 \text{ min}^{-1}$. It indicated that high sodium chloride level affects the DCM evaporative behavior in preparing OVA-MPs.

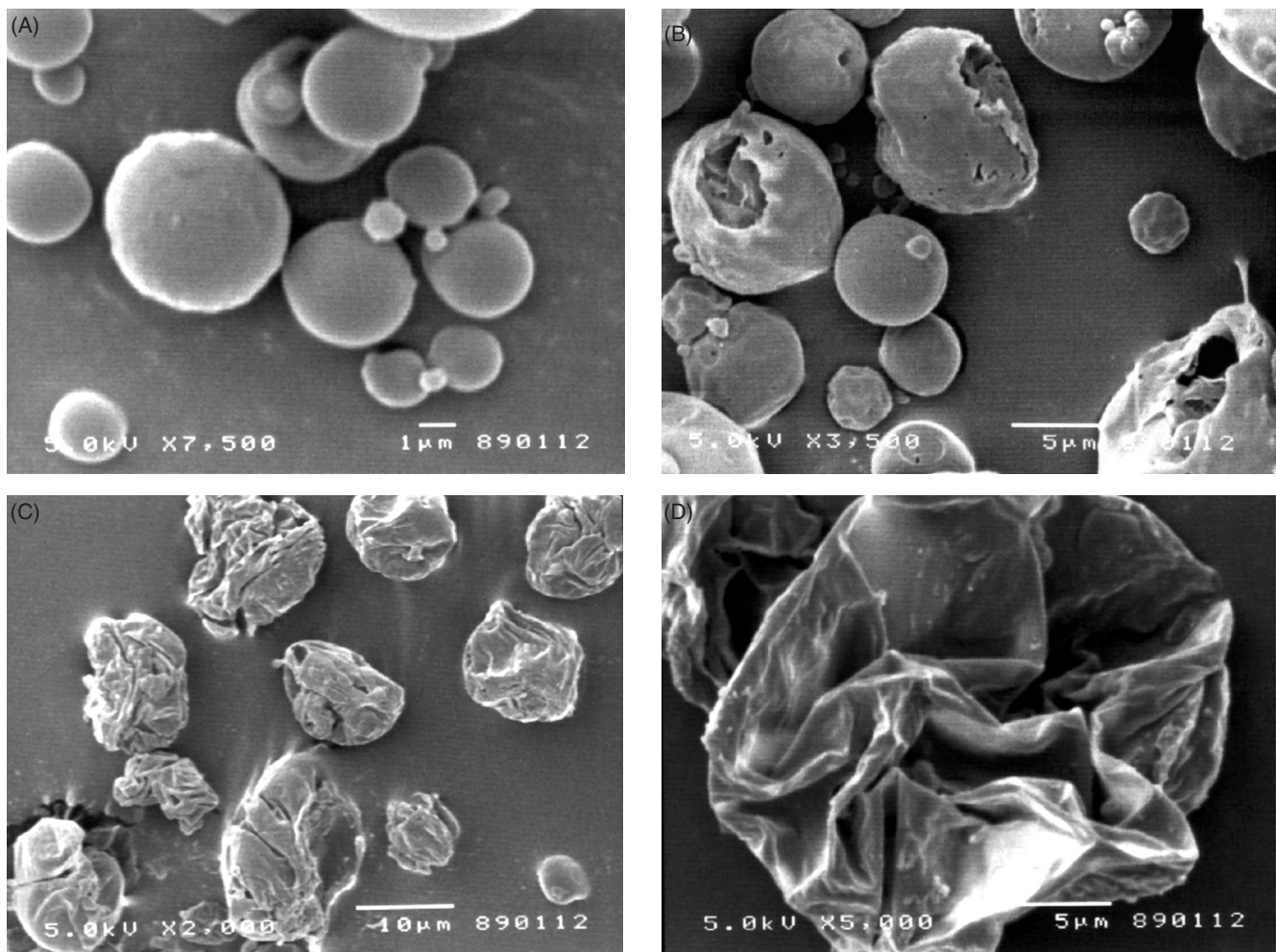


Figure 5. SEM photographs of OVA-loaded PLGA microparticles. Four formulations combined with various NaCl contents in the external phase. (A) F1, without NaCl and urea, (B) F2, 0% NaCl and 10.3% urea, (C) F3, 3% NaCl and 4.1% urea, (D) F4, 5% NaCl without urea.

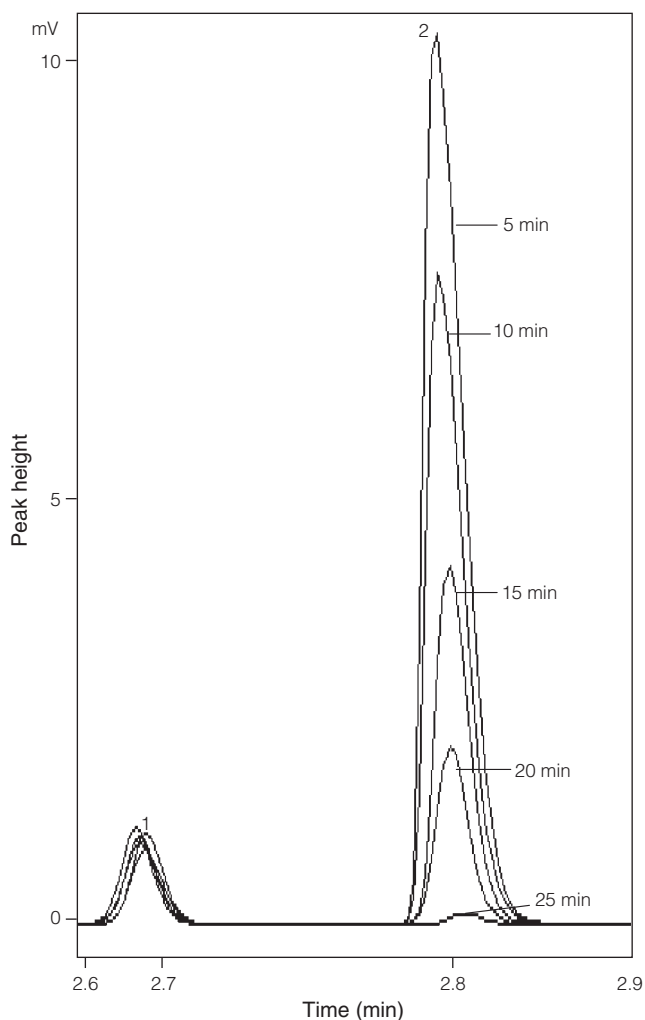


Figure 6. Typical GC chromatograms of DCM samples. Samples were collected at different time points from the w/o/w multiple emulsion of F1 formulation during the solvent evaporation stage at 25°C. Key: peak 1: carbon tetrachloride; and peak 2: DCM.

DISCUSSION

I. Dichloromethane Levels and Analytical Method

Although GC associated with a headspace sampling method has been developed more than three decades for determining volatile organic solvent in samples. But until now only few studies report the analytical method for assessing microparticle fabrication^(9,10). In this report, we established a simple and convenient GC analytical method for determining DCM level during the solidifying process of microparticle. DCM, chloroform and carbon tetrachloride, are 2 Cl, 3 Cl, and 4 Cl substitutions of methane, respectively. DCM was the analytical target, carbon tetrachloride was as an internal standard for improving precision and accuracy of the analysis, and chloroform was used as a solvent to dissolve PLGA matrix. The retention times of carbon tetrachloride, DCM, and chloroform were 2.6, 2.8 and 3.3 min, respectively. The sequence of three

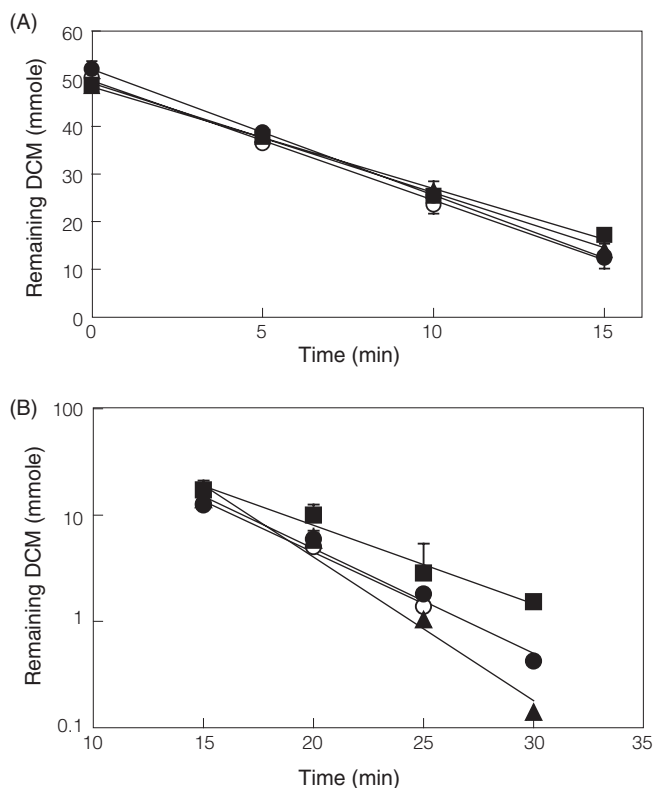


Figure 7. Remaining DCM in 30 mL of the w/o/w multiple emulsions during the solvent evaporation stage. (A) 0~15 min, zero-order kinetics; (B) 15~30 min, first-order kinetics. Key: (○) F1, without NaCl and urea, (●) F2, 0% NaCl and 10.3% urea, (▲) F3, 3% NaCl and 4.1% urea, (■) F4, 5% NaCl without urea.

chloromethane derivatives in retention times could not follow the Cl substitution numbers. In this study, PEG (polyethylene glycol) coated column was used. The stationary phase of PEG has higher polarity. The dipole momentum of carbon tetrachloride is 0 which had the shortest retention time among three analogues. In comparing DCM and chloroform, the dipole momentum of DCM (1.5 D) is larger than that of chloroform (1.1 D). DCM possibly had higher interaction with PEG than chloroform in liquid state. However, DCM had a lower normal boiling temperature (39.8°C vs. 61.2°C) and a similar molar heat of evaporation (6835 cal/mole) of chloroform (7281 cal/mole)⁽¹¹⁾. At 80°C column condition, DCM had higher vapor pressure than chloroform (3.5 vs. 1.8 atm, estimated from the integration of Clausius-Clapeyron equation), which might more significantly reduce the interaction between DCM and PEG to obtain shorter retention time than chloroform. Although three analogues had different retention times, but the differences were less than one min in the GC analysis to significantly reduce the analytical time.

By using the headspace sampler, 5 mL of organic vapor was sampled in the GC analysis. As the chloroform volume of the injected sample increased, the peak height of DCM decreased (Figure 2), but the peak height ratio of

DCM and carbon tetrachloride increased (Figure 3). The decreases of DCM peak heights were resulted from the reduction of DCM molar fraction in vapor phase after increasing the volume of chloroform in the sample. The peak height of carbon tetrachloride was reduced more in comparison with DCM, the reason might be explained as the following. The normal boiling temperature (T_b) of a liquid is the temperature of its vapor pressure equal to 1 atm, the T_b is elevated under a higher pressure condition. In the study, the GC analysis vial has a volume of 23 mL to contain approximately 0.94 mmole gas at 1 atm, and 25°C (using ideal gas law, $PV = nRT$). After combining a large volume of chloroform (0.1, 0.5, 1 mL of chloroform are about equal to 1.2, 6.2, and 12.4 mmole), the total gas pressure was markedly increased at the sampling temperature (preheat at 80°C). The T_b s of DCM, chloroform, and carbon tetrachloride are 39.8, 61.2, and 76.7°C, respectively. The preheat temperature of the sample was much higher than the boiling points of DCM and chloroform, but close to the normal T_b of carbon tetrachloride. Thus, the evaporation of carbon tetrachloride was much reduced by the elevated T_b due to the higher pressure produced from volatile organic solvents in the sealed vial at higher temperature. However, DCM and chloroform were not much affected.

There were three advantages for including chloroform in the GC analytical method. First, the relative large volume of chloroform (such as 0.5 mL) could improve the linearity of the calibration curve in the analysis. Second, PLGA dispersion was swelled in chloroform, DCM was easily extracted in one step without further treatment and directly analyzed by GC with headspace sampler. Third, due to the DCM in the analyzed sample was significantly diluted in a higher boiling temperature solvent (chloroform) to avoid evaporation by reducing the molar fraction during the sample preparing process at room temperature. But the decrease of DCM peak height also attenuated the detected sensitivity and increased the analytical variation in low concentration samples by combining 1 mL of chloroform. For improvement in analytical precision, carbon tetrachloride was used as an internal standard and prepared as 2% in chloroform (combining 0.5 mL of chloroform) to analyze DCM in our studies.

For precisely determining the DCM concentration, two calibration curves were established including low range from 7.5 to 75 mM and high range from 75 to 1500 mM. Good precision and accuracy were demonstrated with CV and error both less than 4%.

II. DCM Evaporative Characteristics

In F1~F4, the DCM evaporation followed a zero-order kinetic in the initial 15 min by decreasing the DCM amount from 50 to 10 mmole (Figure 7-A). After 15 min, the decline of DCM followed an exponential kinetic pattern (Figure 7-B). The DCM saturated concentration in the external phase had been determined as 0.22 ± 0.01 M ($n =$

3, at 25°C)⁽⁴⁾ to be 6.6 mmole of DCM in 30 mL of aqueous solution.

During the initial 15 min in the external phase, the DCM levels were approximately 0.20 M for all four formulations, close to the saturated concentration of DCM. The results suggested that the DCM evaporation rates depended on the concentrations of DCM in the external phase. However, after 15 min, the PLGA dispersions were gradually solidified as the DCM levels decreased, DCM evaporations followed the first-order kinetic instead of the initial zero-order kinetic thereafter. These rate constants might predominately depend on the diffusion process of DCM from the core crossing through the polymeric matrix of the embryonic microparticle. Different formulations could produce different dispersions with their own surface characteristics, as well as change the efflux rates of DCM. F4 showed a markedly smaller value of first-order rate constant. High sodium chloride content of the external phase plays an important role to precipitate PLGA in the interface and form a crust-like structure for further decreasing the efflux rate of DCM. The residual amounts of DCM in the prepared OVA-loaded microparticles could be estimated from the first-order rate constants. The limit of DCM content of finished product is less than 600 ppm (0.06% or 9.2 mM) in USP specification⁽¹²⁾. The established GC method had a quantitative limit (LOQ) of 7.3 mM ($LOQ = 10 \times$ standard deviation of intercept/slope)⁽¹³⁾. The lowest concentration of calibration curve was 7.5 mM which was close to LOQ with CV and error, 1.21% and -1.73%, respectively. Our results showed that the evaporation periods of OVA-MPs were 30 min for F1 and F3, and 35~40 min for F4 to meet the limit of DCM in the requirement of USP.

CONCLUSIONS

In this study, we established a GC analytical method for determining DCM content during the evaporation stage of preparing PLGA microparticles. In the GC analytical method, 0.5 mL of chloroform could be used as an organic solvent to improve analytical results. The analytical method was simple and rapid without the need for sample pretreatment. The GC method has a low quantitative limit value of 7.3 mM. The GC analytical method was also successfully applied for studying the DCM evaporative kinetics during PLGA microparticle preparation process and DCM residue evaluation.

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