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Simultaneously measuring concentrations of a model drug and a model excipient in solution using ultrasonic spectrometry

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Abstract

A newly commercialized high-resolution ultrasonic spectrometer was evaluated for simultaneously measuring concentrations of a model excipient (hypromellose acetate succinate polymer, HPMCAS, CAS No. 71138-97-1) and a model drug (Fenofibrate, CAS No. 49562-28-9) in acetone solution. It was demonstrated that the measurements of both velocity and attenuation had sufficient accuracy and precision. The velocity was found to be directly proportional to concentrations of both HPMCAS polymer and Fenofibrate in solution. The attenuation was found to be directly proportional to concentration of HPMCAS polymer in solution. By establishing linear relationships of measured velocity and attenuation to the concentrations of HPMCAS polymer and the Fenofibrate in a series of standard solutions, it was feasible to simultaneously analyze concentrations of both HPMCAS polymer and Fenofibrate in a test solution. However, it was found that both temperature and moisture had significant influence on the measurement. While the change in velocity was inversely proportional to the change in temperature, the change in velocity was directly proportional to the change in moisture content in solutions.

Keywords: Ultrasonic spectrometry, Ultrasonic spectroscopy, Ultrasonic velocity, Ultrasonic attenuation, Hypromellose acetate succinate, Fenofibrate

1. Introduction

In some pharmaceutical manufacturing processes such as spray- or freeze-drying, a drug and one or more excipients are dissolved in a suitable solvent first. The solution is then spray- or freeze-dried to form solid dispersion for further or final manufacturing process. The quality control is usually performed on the finished product after drying. Since both spray- or freeze-drying processes are expensive as compared to the dissolution of drug and excipient(s) into a solvent. It would be desirable to check concentrations of both the drug and the excipient(s) prior to drying to ensure correct potency can be reached afterwards. Furthermore, remedial action can be taken in dissolution process for any deviation in concentration of either drug or excipient(s) or both, whereas any remedial action after drying process is either prohibitively expensive or nearly impossible. The current common analytical practice at dissolution stage is taking samples and doing off-line analyses. Off-line measurement is time-consuming. As a result, usually only drug concentration is monitored by HPLC analysis. To increase business efficiency and to ensure final product quality, it would be desirable to monitor concentrations of both the drug and the excipient(s) in the solution. In this paper, a newly commercialized high-resolution ultrasonic spectrometer was evaluated for simultaneously measuring concentrations of a model excipient (hypromellose acetate succinate polymer, HPMCAS, CAS No. 71138-97-1) and a model drug (Fenofibrate, CAS No. 49562-28-9) in acetone solution. Ultrasonic spectrometry was chosen for the technical evaluation based on the following reasons: (1) ultrasonic spectrometry has the potential to simultaneously monitor concentrations of both the drug and the polymeric excipient in a solution; (2) it offers fast and non-destructive analysis; (3) more importantly, the ultrasonic spectrometry has the potential to be adapted into an on-line analytical method

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for process analytical technology (PAT) application. PAT application aims at better understanding, monitoring, and controlling of the manufacturing process, which is consistent with current FDA's philosophy on pharmaceutical quality system—quality cannot be tested into products; it should be built in or should be by design.

Ultrasonic spectroscopy is a spectroscopic technique for material analysis utilizing high-frequency acoustical (ultrasonic) waves—waves with a frequency greater than 100 kHz [1–3]. Ultrasonic spectrometry (often named spectroscopy) in liquids has increasingly become an important tool for basic and applied research in many scientific fields such as physics, physical chemistry, material sciences, biology, and medicine [4–7]. The two major parameters measured in ultrasonic spectrometry are the velocity and the attenuation of the waves. Ultrasonic velocity is determined by the density and the elasticity of the medium it travels through. It is very sensitive to intermolecular interactions and composition of the sample. Ultrasonic attenuation is determined by the energy losses in the ultrasonic wave propagating through the sample and is proportional to the high frequency viscosity. It allows the analysis of the microstructure of samples (particle sizing in suspensions and emulsions, for example), kinetics of fast chemical reactions, and structural characteristics of gel networks.

2. Experimental methods and materials

2.1. Sample information

Acetone (HPLC grade) was purchased from JT Baker (Phillipsburg, NJ). Fenofibrate (Catalogue No. F6020) was purchased from Sigma–Aldrich (Milwaukee, WI). Hypromellose acetate succinate polymer (HPMCAS, MG grade) was purchased from Shin Etsu Chemical Co. Ltd. (Tokyo, Japan). All solutions were made in the laboratory by accurate weighing of the required amount of Fenofibrate, HPMCAS polymer, and acetone into glass vials. The solutions were capped and stirred for at least 5 h (in most cases overnight to ensure proper dissolution). All concentrations were expressed in percentage on mass basis.

2.2. Experimental details

The ultrasonic parameters (velocity, attenuation) were measured by a high-resolution ultrasonic spectrometer (HR-US 101) made by Ultrasonic Scientific Ltd. (Dublin, Ireland). This instrument allows high-resolution measurement of both the velocity and the attenuation of acoustic waves propagating through fluids at high ultrasonic frequencies (4–14 MHz) [8]. It provides fast and non-destructive analysis of a wide spectrum of properties of materials in fluids. Measurements were done at multiple frequencies (near 5.0, 7.6, and 11.4 MHz) at 25 °C except in temperature variation experiments, in which temperature was changed to either 20 or 30 °C. Temperature of both the sample and reference cells was controlled with an accuracy of ±0.01 K. Prior to each experiment, the test solution was transferred into the sample cell via a positive pressure pipette. Acetone was also filled to the reference cell via a plastic pipette. Care was taken to prevent generating air bubbles inside the cell during the transfer process. Both cells were tightly capped and allowed to reach thermal equilibrium at set temperature for at least 20 min prior to a measurement. After each experiment, the sample cell was thoroughly cleaned by rinsing with acetone several times and air-dried.

3. Results and discussion

A series of experiments have been performed to evaluate applicability of this technique for simultaneously measuring concentrations of both HPMCAS polymer and Fenofibrate in acetone solution. The measured values of velocity and attenuation from the sample cell were directly used for analysis. The measured value of acetone velocity in the reference cell only served as system suitability check. Manufacturer recommended a procedure for calculating sample velocity value by adding the established literature value of acetone velocity to the difference of respective velocity values between the sample and reference cells. In this way, any difference caused by temperature fluctuation would be compensated for high-resolution measurement. We chose not to follow the procedure for two reasons: First, our intention was to evaluate this technique for possible on-line application as a process analytical technology (PAT). For on-line PAT application, it would be desirable that a single sample cell is used. Secondly, our experiments have proven that the results from a single sample cell measurement with accurate temperature control had sufficient accuracy and precision as demonstrated later in this section.

3.1. Measurement accuracy and precision

According to the manufacturer, the instrument has an absolute accuracy of ±1 m/s and a precision of ±0.1 m/s for velocity measurement. The velocity values for water has been extensively measured and critically reviewed [9–12]. To assess the accuracy of the ultrasonic measurement, pure water was filled into the reference cell and its velocity was measured at 25 °C. The mean values of five replicate measurements were 1496.711 ± 0.005 and 1496.681 ± 0.006 m/s, respectively, measured at 2 days with fresh water filled in the reference cell each day. The reported literature value is 1496.687 ± 0.015 m/s [9]. Since all solutions were prepared in acetone solution, all measurements were performed with acetone filled in the reference cell. Although measurement of the reference cell was not directly used in the calculation, measurement of acetone velocity from the reference cell could be used as a system suitability check for the instrument. Experiments were then performed to determine velocity measurement accuracy and precision for acetone. The

Table 1
Assessment of velocity measurement accuracy and precision for acetone

Sample no.	5.1 MHz		7.6 MHz		11.6 MHz	
	Reference[a]	Sample[b]	Reference[a]	Sample[b]	Reference[a]	Sample[b]
Acetone 1	1167.38	1167.39	1167.38	1167.40	1167.33	1167.40
Acetone 2	1167.15	1167.30	1167.14	1167.21	1167.02	1167.27
Acetone 3	1167.09	1167.24	1167.12	1167.25	1167.09	1167.30
Acetone 4	1167.11	1167.21	1167.14	1167.24	1167.02	1167.30
Acetone 5	1167.21	1167.24	1167.24	1167.27	1167.16	1167.21
Mean	1167.19	1167.28	1167.20	1167.27	1167.12	1167.30
S.D.[c]	0.12	0.07	0.11	0.08	0.13	0.07
CI (95.0%)[d]	0.15	0.09	0.14	0.09	0.16	0.08

[a] Velocity (m/s) measured in the reference cell at 25 °C.
[b] Velocity (m/s) measured in the sample cell at 25 °C.
[c] S.D. stands for standard deviation.
[d] 95% confidence interval for the mean.

results were shown in Table 1. The half-width of the 95% confidence intervals for the measured mean velocities from the sample and reference cells were estimated to be $\leq$0.16 and $\leq$0.09 m/s, respectively. The values of measured acetone velocity varied between 1161 and 1166.5 m/s at 25 °C in literature [13–15] and generally lower than the value we consistently got (1167.4 m/s at 25 °C). As discussed later in this section, it was found that moisture had a significant impact on the measured velocity value in acetone solution. The water content in acetone used in our experiments was 0.2% and the purity of acetone was 99.8%. The water contents in acetone used in literature were unknown, but the purity used in Ref. [15] was labelled as >99% only. The lower value of velocity might be caused by the lower purity of acetone used. Nevertheless, velocity measurement for acetone in the reference cell was determined to be very reproducible and was suitable for system suitability check purpose. For test solutions, the reproducibility for velocity and attenuation measurement was demonstrated by the experimental results for six individually prepared solutions of Fenofibrate and HPMCAS polymer shown in Table 2.

3.2. Linearity assessment

Measurements were performed to assess the relationship of changes in velocity and attenuation in response to changes

Table 2
Assessment of measurement precision for test solutions

Sample no.	Velocity (m/s)			Fenofibrate (%, w/w)	Polymer (%, w/w)
	5.0 MHz	7.6 MHz	11.4 MHz		
1	1184.47	1184.55	1184.89	12.003	4.000
2	1184.65	1184.72	1185.00	12.002	4.000
3	1184.59	1184.69	1184.96	12.002	4.000
4	1184.78	1184.85	1185.12	12.002	4.000
5	1184.75	1184.81	1185.15	12.002	4.000
6	1184.67	1184.74	1185.05	12.002	4.000
Mean	1184.65	1184.72	1185.03		
S.D.[a]	0.11	0.11	0.099		
CI (mean, 95%)[b]	±0.12	±0.11	±0.10		

Sample no.	Attenuation (m^{-1})			Fenofibrate (%, w/w)	Polymer (%, w/w)
	5.0 MHz	7.6 MHz	11.4 MHz		
1	12.08	19.09	42.25	12.003	4.000
2	12.26	19.39	40.78	12.002	4.000
3	11.92	19.25	39.76	12.002	4.000
4	12.46	19.70	40.51	12.002	4.000
5	12.37	19.42	42.81	12.002	4.000
6	12.40	19.58	41.83	12.002	4.000
Mean	12.25	19.40	41.32		
S.D.[a]	0.21	0.22	1.16		
CI (mean, 95 %)[b]	±0.22	±0.23	±1.22		

[a] S.D. stands for standard deviation.
[b] 95% confidence interval for the mean.

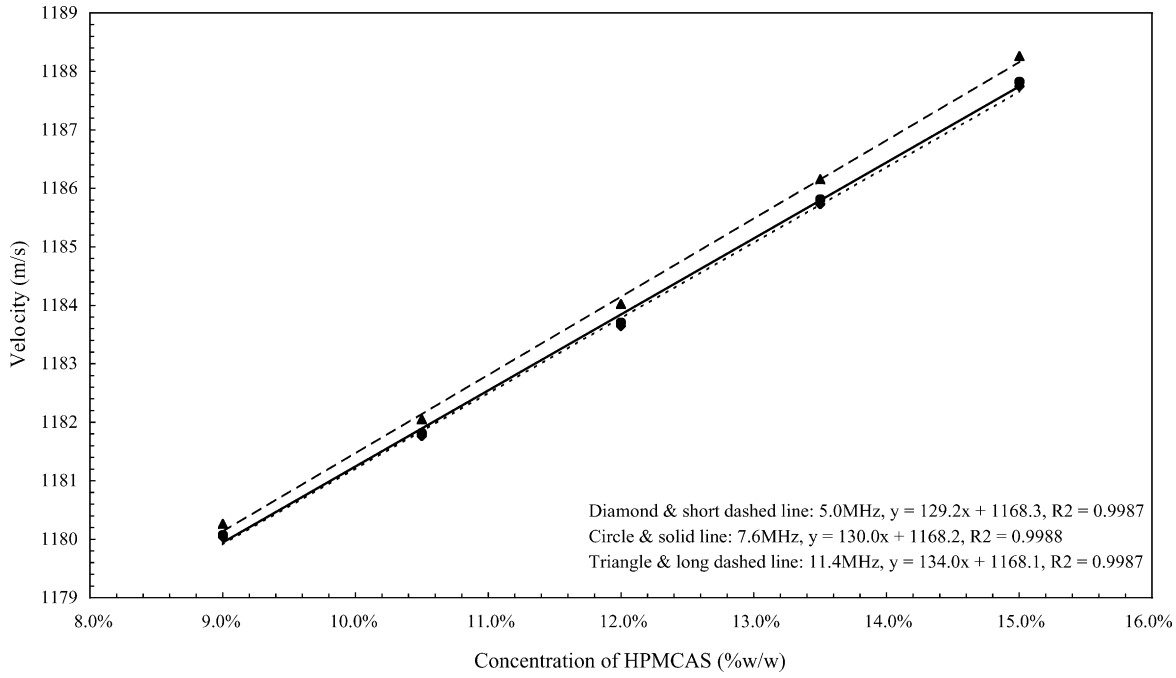

Fig. 1. Plot of changes in measured velocity vs. changes in HPMCAS polymer concentration in solutions with 4% Fenofibrate.

in concentration of either HPMCAS polymer or Fenofibrate in solutions. The results were shown in Figs. 1–4. For all velocity values measured in the three frequencies, there was linear relationship of increasing velocity in response to increasing HPMCAS polymer concentration in solutions of 4% Fenofibrate (see Fig. 1). Similarly, there was also a linear relationship between the change in velocity and the change in Fenofibrate concentration in solutions of 12% HPMCAS polymer (see Fig. 2). There was a linear relationship of increasing attenuation in response to increasing HPMCAS polymer concentration in solutions of 4% Finofibrate (see Fig. 3). Furthermore, the slopes became progressively larger as the measuring frequency got higher. It was also evident that the attenuation was a strong function of measuring frequency with a larger attenuation at higher frequency. This was an expected behaviour of long polymer chains dissolved in solution. For Fenofibrate, there was no significant linear correlation between the measured attenuation and the Fenofibrate concentration (see Fig. 4). For small molecule such as Fenofibrate, attenuation is usually much smaller as compared

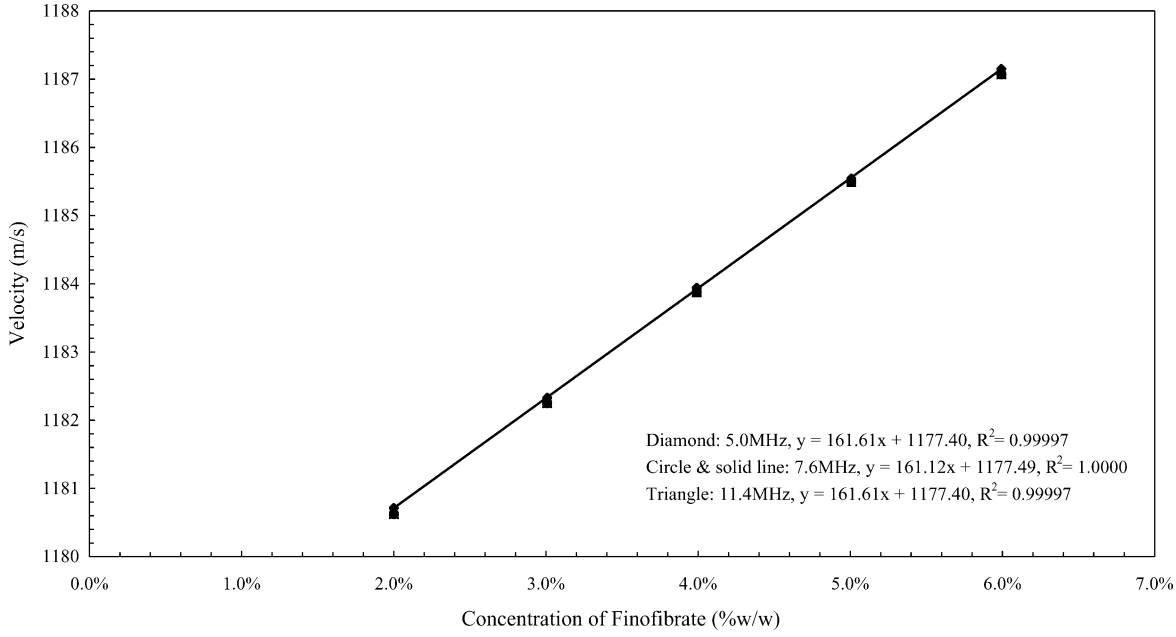

Fig. 2. Plot of changes in measured velocity vs. changes in Fenofibrate concentration in solutions with 12% HPMCAS polymer.

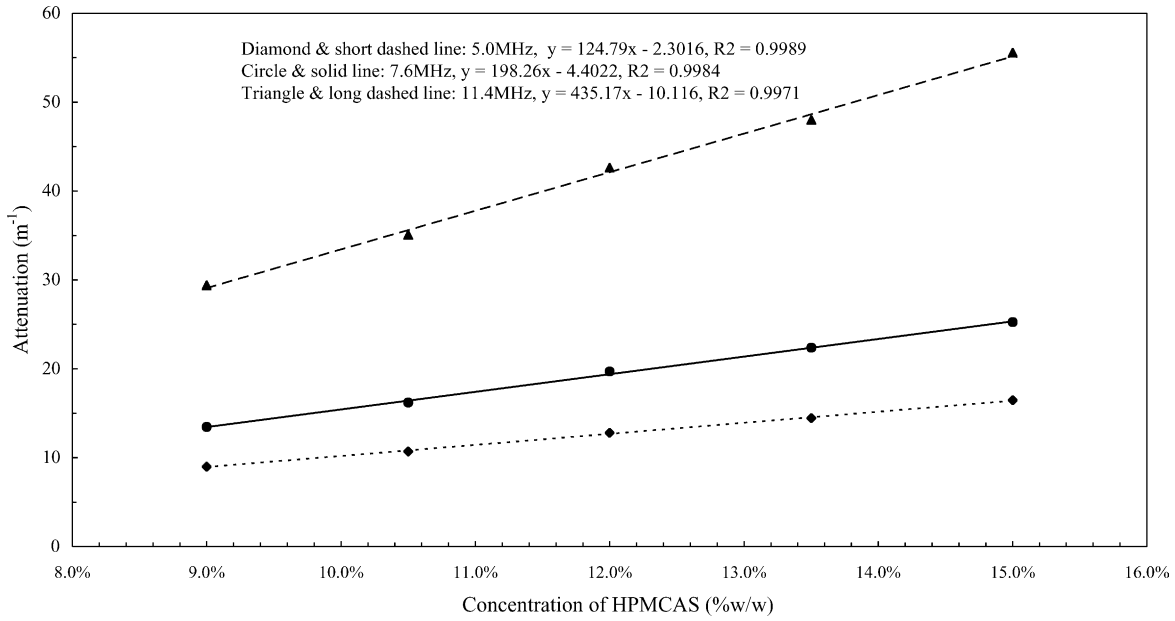

Fig. 3. Plot of changes in measured attenuation vs. changes in HPMCAS polymer concentration in solutions with 4% Fenofibrate.

to that for polymeric chains, unless some kinds of aggregation or colloid are formed in solution.

3.3. Temperature effect

It was known that velocity of fluids in general has large temperature dependence [1]. This is confirmed by our experimental measurements for solutions of 4% Fenofibrate, 12% HPMCAS polymer, and the solution of 4% Fenofibrate and 12% HPMCAS polymer (all dissolved in acetone), as well as acetone. The results were summarized in Table 3. The change in velocity in response to change in temperature was linear in all cases and the slopes were approximately the same. The large values of the slopes indicated that temperature control was very important for accurate measurement of velocity in test solutions.

3.4. Moisture effect

Since there was always moisture present in the HPMCAS polymer and the amount varied depending on the packaging and external storage environment, it was decided that a mois-

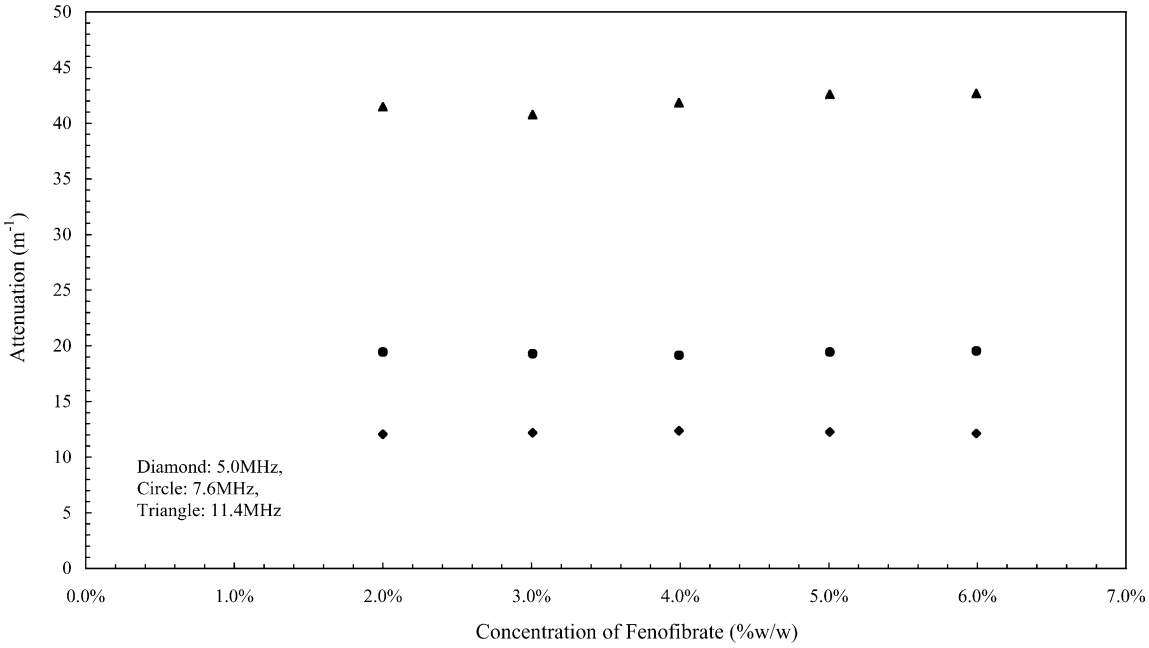

Fig. 4. Plot of changes in measured attenuation vs. changes in Fenofibrate concentration in solutions with 12% HPMCAS polymer.

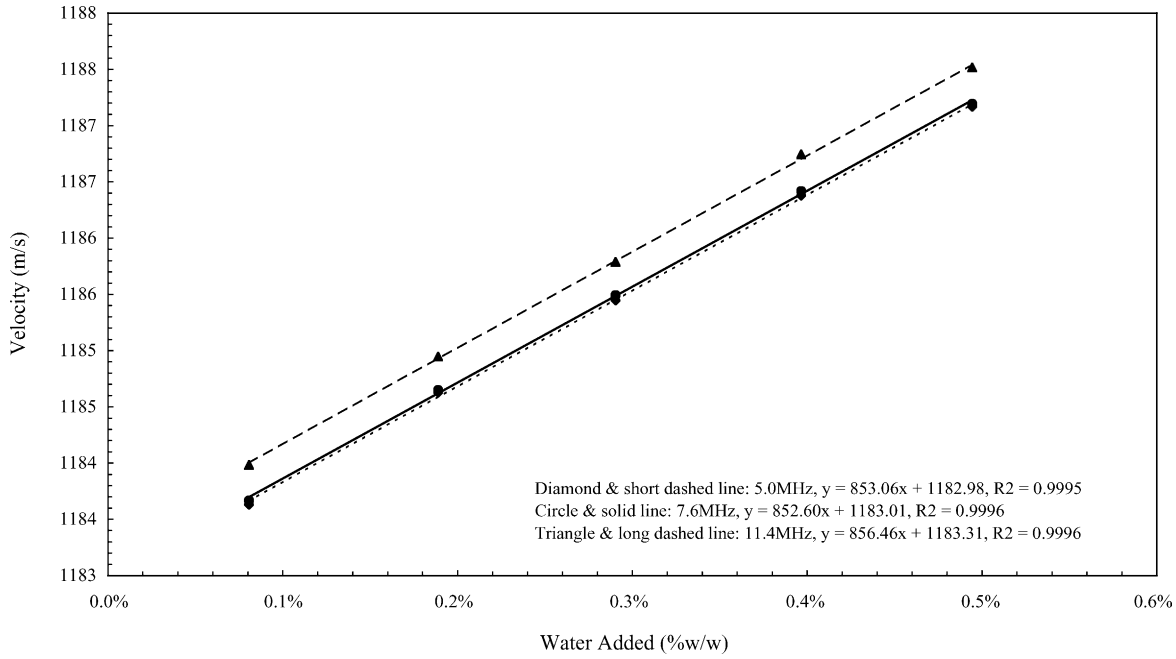

Fig. 5. Plot of changes in measured velocity vs. amount of added water in a test solution of 12% HPMCAS polymer and 4.0% Fenofibrate.

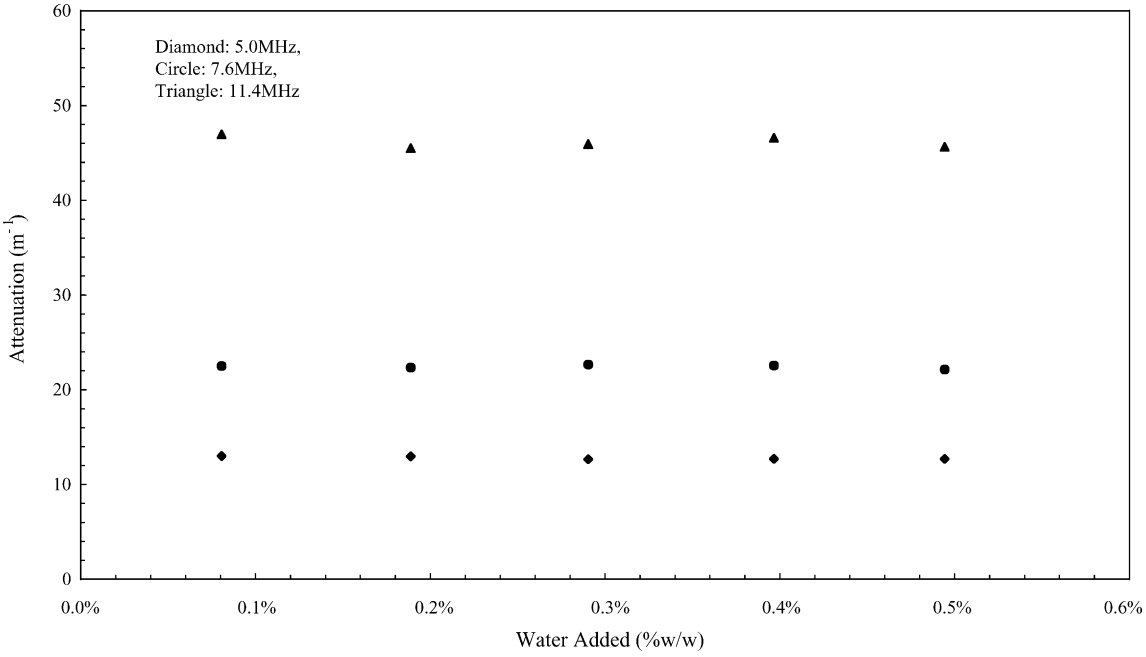

Fig. 6. Plot of changes in measured attenuation vs. amount of added water in a test solution of 12% HPMCAS polymer and 4.0% Fenofibrate.

ture influence study should be performed. The samples were prepared by adding water to a stock solution as follows: first, a solution of 4% Fenofibrate and 12% HPMCAS polymer was prepared as stock solution; then, a measured amount of water was added to a measured amount of the stock solution in a glass vial to make five samples with different water content (0.1, 0.2, 0.3, 0.4, and 0.5%, w/w). The results were shown in Fig. 5. Noted that the *x*-axis corresponded to the added amount of water, so the result for 0% water corresponded to

Table 3
Summary of results for temperature dependence assessment

Linearity for temperature effect (20–30 °C)	Velocity (m/s) at 7.6 MHz		
	Slope	Intercept	R^2
Acetone	−4.312	1275.2	1.0000
4% drug (Fenofibrate)	−4.282	1279.2	1.0000
12% polymer (HPMCAS)	−4.296	1284.2	0.9999
Test solution (4% drug + 12% polymer)	−4.254	1289.5	0.9999

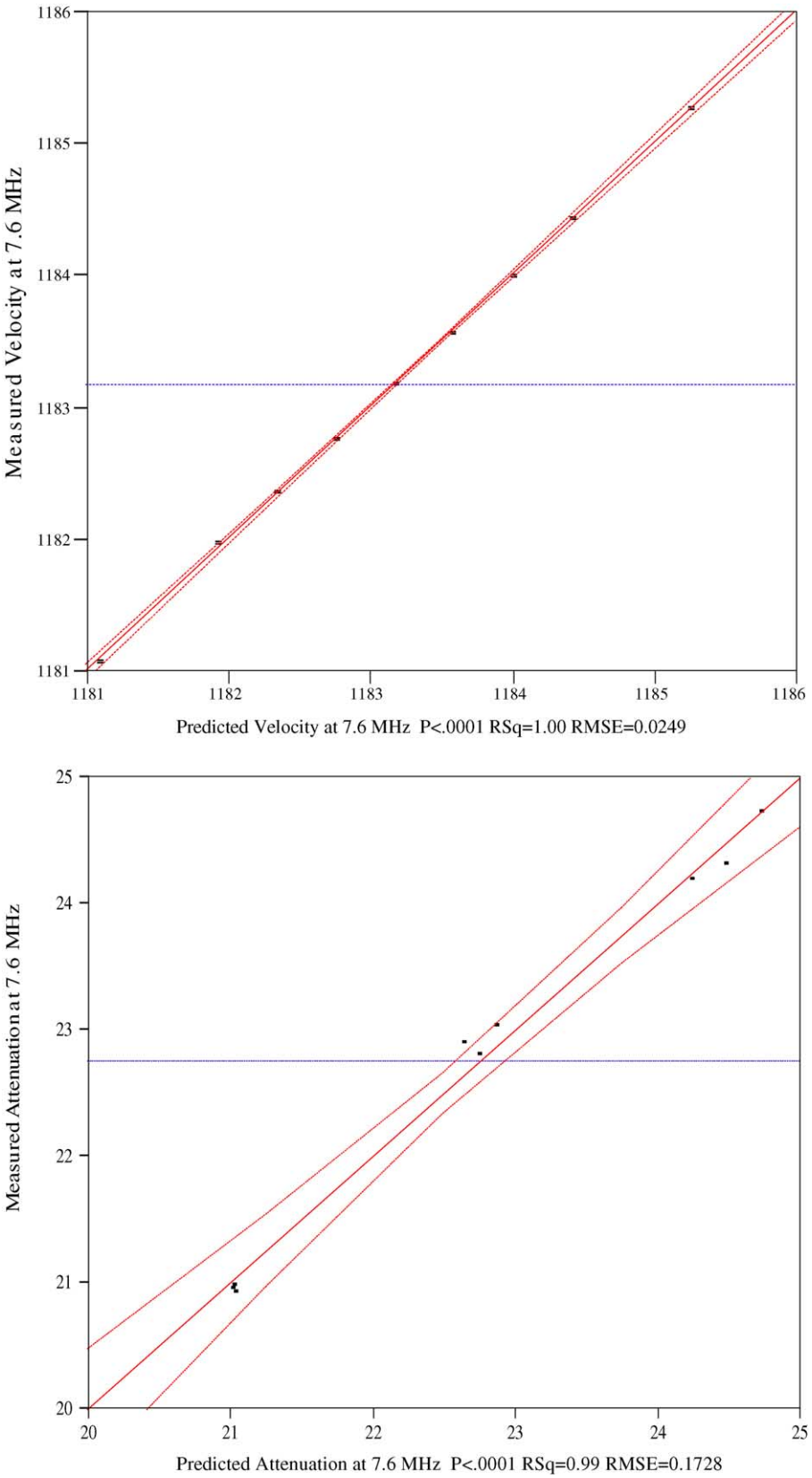

Fig. 7. Examples of regression analysis of DOE runs using data at 7.6 MHz. In both plots, symbols represented data points and the two outside lines represented 95% confidence limits. The middle lines in each plot represented theoretical best fitting.

Table 4
Summary of design of experiment results

Sample no.	Fenofibrate (%)	HPMCAS (%)	Velocity (m/s)			Attenuation (m^{-1})		
			5.0 MHz	7.6 MHz	11.4 MHz	5.0 MHz	7.6 MHz	11.4 MHz
1	3.500	11.002	1181.05	1181.08	1181.42	11.90	20.95	46.77
2	4.000	11.002	1181.94	1181.98	1182.22	11.98	20.97	42.15
3	4.500	11.002	1182.77	1182.76	1183.00	12.26	20.93	41.69
4	3.500	12.001	1182.30	1182.35	1182.64	13.17	23.04	47.60
5	4.000	12.000	1183.11	1183.17	1183.49	13.08	22.80	48.30
6	4.500	12.000	1183.94	1184.00	1184.27	13.11	22.90	46.20
7	3.500	13.001	1183.52	1183.57	1183.83	14.75	24.73	49.18
8	4.000	13.000	1184.38	1184.44	1184.72	14.22	24.31	49.28
9	4.500	13.000	1185.27	1185.27	1185.64	14.39	24.20	51.04

that of the stock solution, which inevitably should have some moisture present already prior to water adding. Surprisingly, moisture had a significant influence on the measured velocity. For all velocity values measured in the three frequencies, there was linear relationship of increasing velocity in response to increasing water content in solutions. However, addition of water had an insignificant impact on the attenuation as shown in Fig. 6. The water content of acetone solvent was 0.2% from the certificate of analysis. The Fenofibrate drug substance contained <0.1% water. Water content in the HPMCAS polymer was measured to be 2.1% by Karl–Fisher titration. However, HPMCAS polymer is hygroscopic and is capable of picking up to 6.3% water at the condition of 25 °C and 75% relative humidity. These results demonstrated the need to accurately measure and control moisture in test solution for accurate and reliable determination of the polymer and Fenofibrate concentrations by ultrasonic spectrometer.

3.5. Results from design of experiments and ANOVA analyses

A statistical design of experiments was used to assess viability of simultaneous determination of both HPMCAS polymer and Fenofibrate concentrations in test solution by the ultrasonic spectrometry. Nine runs were designed to probe two factors (concentrations of HPMCAS polymer and Fenofibrate) with three levels (high, normal, and low) for each factor. The temperature was controlled at 25 °C and all samples were prepared in the same condition at the same day to minimize moisture influence. The experimental results from these runs were summarized in Table 4. Statistical analyses were performed on the data of velocity and attenuation separately. As shown in Fig. 7 for data measured at 7.6 MHz, statistical analyses were able to build linear models for velocity and attenuation, respectively, shown as follows:

$$\text{velocity (m/s)} = 167.87C_{\text{Drug}} + 124.35C_{\text{Polymer}} + 1161.54; \quad R^2 = 0.9998 \tag{1}$$

$$\text{attenuation } (m^{-1}) = -23.27C_{\text{Drug}} + 173.08C_{\text{Polymer}} + 2.92; \quad R^2 = 0.9918 \tag{2}$$

Using the two equations, both HPMCAS polymer and Fenofibrate concentrations in a test solution could be determined from a single ultrasonic measurement of velocity and attenuation. ANOVA analyses were performed on all sets of data in Table 4. As an example, the calculations for the data at 7.6 MHz were summarized in Table 5. For the velocity analysis, concentrations of both HPMCAS polymer and Fenofibrate turned out to be statistically important factors, while interaction of HPMCAS polymer with Fenofibrate was statistically insignificant in influencing velocity measurement. It was also demonstrated that the estimated parameters in Eq. (1) had little variability from one set of data to another, as the variances in velocity measurement were very small. For

Table 5
Summary of ANOVA calculations for data (7.6 MHz) in Table 4

Source	N_{parm}	DF	Sum of squares	F-ratio	Prob > F
Effect tests for velocity data measured at 7.6 MHz					
HPMCAS	1	1	9.2778	14939.7228	<0.0001
Fenofibrate	1	1	4.2269	6806.43813	<0.0001
HPMCAS*Finofibrate	1	1	0.0001	0.19484312	0.6774
Effect tests for attenuation data measure at 7.6 MHz					
HPMCAS	1	1	17.9747	602.0021	<0.0001
Fenofibrate	1	1	0.0812	2.7195	0.1600
HPMCAS*Finofibrate	1	1	0.0640	2.1438	0.2030

the attenuation analysis, only the concentration of HPMCAS polymer was a statistically significant factor, while concentration of Fenofibrate had only a minor impact on the value of attenuation. However, it was found that the estimated parameters in Eq. (2) had larger variability from one set of data to another, as the variances in attenuation measurement were larger as compared to those for velocity measurement. Multi frequencies were used in the experiments, but practically single measurement at one frequency was sufficient to determine concentrations of both HPMCAS and Fenofibrate in a test solution.

4. Conclusions

It was found that ultrasonic spectrometer gave accurate and precise measurement of both velocity and attenuation of acoustic waves in acetone solutions of a model drug (Fenofibrate) and a model excipient (HPMCAS polymer). By establishing linear relationships of measured velocity and attenuation to the concentrations of HPMCAS polymer and the Fenofibrate in a series of standard solutions, it was feasible to simultaneously analyze both concentrations of HPMCAS polymer and Fenofibrate in a test solution. However, it was found that both temperature and moisture had significant influence on the measurement. While the change in velocity was inversely proportional to the change in temperature, the change in velocity was directly proportional to the change in moisture content in the solution.

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