Characterization of the Salt and Free Base of Active Pharmaceutical Ingredients Based on NMR Relaxometry Measured by Time Domain NMR

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NMR relaxometry measurement by time domain NMR (TD-NMR) is a promising technique for characterizing the properties of active pharmaceutical ingredients (APIs). This study is dedicated to identifying the salt and free base of APIs by NMR relaxometry measured by the TD-NMR technique. Procaine (PC) and tetracaine (TC) were selected as model APIs to be tested. By using conventional methods including powder X-ray diffraction and differential scanning calorimetry, this study first confirmed that the salt and free base of the tested APIs differ from each other in their crystalline form. Subsequently, measurements of $T_1$ and $T_2$ relaxation were performed on the tested APIs using TD-NMR. The results demonstrated that these NMR relaxometry measurements have sufficient capacity to distinguish the difference between the free base and salt of the tested APIs. Furthermore, quantification of the composition of the binary powder blends consisting of salt and free bases was conducted by analyzing the acquired $T_1$ and $T_2$ relaxation curves. The analysis of the $T_1$ relaxation curves provided a partly acceptable estimation: a good estimation of the composition was observed from PC powders, whereas for TC powders the estimation accuracy changed with the free base content in the binary blends. For the analysis on $T_2$ relaxation curves, a precise estimation of the composition was observed from all the samples. From these findings, the NMR relaxometry measurement by TD-NMR, in particular the $T_2$ relaxation measurement, is effective for evaluating the properties of APIs having different crystalline forms.

Key words NMR relaxometry; time domain NMR; $T_1$ relaxation curve; $T_2$ relaxation curve; active pharmaceutical ingredient; crystalline form

Introduction

Time domain NMR (TD-NMR) is a low-field (20 MHz) benchtop 1H-NMR instrument dedicated to NMR relaxometry investigations. Although it omits the acquisition of NMR spectra, TD-NMR enables rapid and easy measurement of the $T_1$ and $T_2$ relaxation times irrespective of the physical state of the sample; the measurement is applicable to both liquid and solid samples. The spin–lattice relaxation time, $T_1$, is the time constant with which the transverse polarization of the spin ensemble returns to equilibrium by rearranging with the external magnetic field, whereas the spin–spin relaxation time, $T_2$, reflects the fact that the transverse relaxation is the time constant with which the transverse polarization of the spin ensemble returns to its zero equilibrium value after excitation by a radiofrequency (RF) pulse.1–3 These NMR parameters reflect the molecular mobility of the substances. To date, TD-NMR has been used in various fields of research and industry to investigate the physicochemical properties of samples in terms of molecular mobility. The applications have also been expanded into the fields of chemistry,4,5 food,6–8 and plants.9,10

The number of pharmaceutical studies applying TD-NMR has increased of late.1,2,11,12 These studies showed that TD-NMR has a significant advantage in evaluating pharmaceutical properties, including high reproducibility, cost-effectiveness, and ease of use. In our view, TD-NMR is useful for characterizing properties of active pharmaceutical ingredients (APIs). In the pharmaceutical industry, a new analytical method for API properties would be in much demand for all stages, from early drug development to manufacturing. Schumacher et al. examined the hydration states of model APIs (i.e., caffeine and theophylline) by TD-NMR.13 They extracted an NMR signal that was specific for the water in the hydrates from the $T_1$ and $T_2$ relaxation curves and then generated reliable calibration curves to quantify the proportion of hydrate/anhydrate mixtures. Stueber and Jehle proposed a novel and efficient quantification method for solid mixtures using TD-NMR.2 This method is based on the mathematical fitting of the acquired $T_1$ relaxation curves. By testing binary powder blends consisting of various APIs, the authors confirmed that the components in test blends were precisely quantified by TD-NMR. Furthermore, they show the possibility of performing differentiation of API crystalline polymorphism from the analysis of $T_1$ relaxation curves of two anhydrous polymorphs (forms I and II) of a hepatitis C virus drug candidate. The test binary blend consisted of 63.2 and 36.8% of forms I and II, respectively. Consequently, they successfully estimated the composition of the binary polymorph bend within a 1% error. We also investigated the crystalline state of APIs by using TD-NMR.14-16

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In previous studies, we demonstrated that the $T_1$ and $T_2$ relaxation curves measured by TD-NMR could be applied to identify differences between amorphous and crystalline APIs: amorphous APIs showed shorter $T_1$ and longer $T_2$ than crystalline APIs because of the higher molecular mobility.

The present study investigated the NMR relaxometry of the salt and free base of APIs by using TD-NMR. Procaine (PC) and tetracaine (TC) were selected as the model APIs to be tested. From the appearance of the powders, we speculated that the salt and free base were in crystalline forms that differed from each other. By considering the previous report by Stueber and Jehle, there is a significant prospect that the different crystalline forms can be distinguished by $T_1$ relaxation curves. However, to the best of our knowledge, there is no technical study using the $T_2$ relaxation measurement for the investigation of different crystalline forms. The main reason for that is the fact that the difference between the $T_2$ relaxation curves is much smaller than that between the $T_1$ relaxation curves. Nevertheless, developing a $T_2$ relaxation-based method would be a significant advantage. One reason is that the measurement time for the $T_2$ relaxation time is much shorter than that for $T_1$. After developing the $T_2$ relaxation-based method, TD-NMR will be a valuable method for evaluating larger numbers of samples within a short period. This will improve the practicability of the NMR relaxometry measurement by TD-NMR for API characterization, leading to significant progress in the analytical methodology for pharmaceuticals including the process analytical technology (PAT) used in the pharmaceutical industry.

The present study first characterized the crystalline forms of salt and free base of APIs by conventional methods including powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC). After that, the $T_1$ and $T_2$ relaxation curves were acquired by TD-NMR. In addition to single powders of salt and free base of APIs, this study tested binary powder blends mixing salt and free base at designated weight ratios. Eventually, this study showed that both $T_1$ and $T_2$ relaxation curves are applicable to evaluate properties of APIs having different crystalline forms.

### Experimental

**Materials**  
PC (minimum purity 98.0%), TC (minimum purity 98.0%) and tetracaine hydrochloride (TC-HCl) (minimum purity 98.0%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Procaine hydrochloride (PC-HCl) (minimum purity 99.0%) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

**Preparation of Free Base/Salt Powder Blends**  
To prepare binary blends of free base/salt of PCs and TCs, the free bases (PC or TC) were mixed with their salt (PC-HCl or TC-HCl) at the weight ratios of 20:80, 40:60, 60:40, and 80:20.

**PXRD Measurement**  
PXRD patterns of all samples were obtained using D8 DISCOVER (Bruker BioSpin Corp., Billerica, MA, U.S.A.) with Cu-Kα radiation ($\lambda = 0.154\text{nm}$). The count scanning rate was $2^\circ$/min and the scanning angle $2\theta$ was from 5 to 50°.

**DSC Measurements**  
The DSC measurements were conducted with a EXSTER DSC 7020 instrument (SII Nanotechnology Inc., Chiba, Japan). Measurements were taken in a nitrogen atmosphere, with heating scans at a rate of 5°C/min. The samples were placed in an aluminum pan (Hitachi High-Tech Science Corp., Tokyo, Japan). The transition temperatures were determined as the peaks of the endothermic transition peaks.

**TD-NMR Measurement**  
The $^1H$ $T_1$ and $^1H$ $T_2$ sample relaxation curves were measured by TD-NMR using a Bruker minispec mq20 apparatus (Bruker Biospin GmbH, Rheinstetten, Germany) at a $^1H$ frequency of 20 MHz at 25°C. The solid-echo sequence was used for the measurement. The recycle delays ranged from 0.01 to 20 s. The other acquisition parameters were as follows: number of both scans and dummy scans was 8, data points (number of recycle delay) were 50. After acquisition of $T_2$ relaxation curves with different recycle delays, a saturation recovery curve of the $T_1$ relaxation was obtained to calculate the $T_1$ relaxation time. The calculation of $T_1$ relaxation time was performed using the TD-NMR Analyze software (Bruker Biospin GmbH, Rheinstetten, Germany) according to Eq. (1):

$$I(t) = I_0 \left(1 - \exp\left(\frac{-t}{T_1}\right)\right), \tag{1}$$

where $t$ is the recycle delay, which is the time delay interval used during the NMR relaxation measurement, and $I_0$ are the $T_1$ relaxation time, signal intensities at time $t$, and equilibrium, respectively. Eq. (2) is a mathematical transformation of Eq. (1) for linearizing.

$$\ln\left(1 - \frac{I(t)}{I_0}\right) = -\frac{t}{T_1}. \tag{2}$$

The $T_2$ relaxation time was also calculated from the $T_2$ relaxation curves acquired with a 20 s recycle delay using the TD-NMR Analyze software. The $T_2$ relaxation behaviors were calculated according to Gaussian curve fitting (Eq. 3).

$$I(t) = I_0 \exp\left(-\frac{1}{2}\left(\frac{t}{T_2}\right)^2\right), \tag{3}$$

where $T_2$ is relaxation time, $t$ is the acquisition time, and $I_0$ and $I_0$ are the signal intensities at times $t$ and 0.

### Results and Discussion

This study first characterized the crystalline state of free base and salt of tested APIs by conventional methods. The PXRD patterns of the free base and salt of the model APIs are presented in Fig. 1. All the samples showed numerous distinctive peaks indicating their high crystallinity. In the case of the PXRD pattern of PCs, in addition to the common peaks ranging from 10 to 30°, the PC (free base) showed significant peaks at 7.1, 34.8, and 35.9°, whereas the PC-HCl (salt) showed the peaks at 40.7 and 47.8°. This indicates that the free base and salt have different crystalline forms. For TCs, the peaks for TC (free base) were 8.5, 12.7, 15.8, 17.0, 19.2, 21.9, 23.2, 23.6, and 25.4°, whereas those for TC·HCl (salt) were 6.8, 13.5, 14.1, 14.7, 18.9, 20.2, 20.9, 24.5, 25.5, 27.0, 27.5, 28.0, 29.0 and 48.5°. According to the previous studies, the TC·HCl exists as Form I. From these findings, it was confirmed that the salt and free base of both APIs exist as different crystalline forms.

This study further measured DSC curves of the samples
(Fig. 2). It was clear that the endothermic peak temperatures were different between salt and free base. Overall, the peaks of the salts were higher than those of the free bases. Namely, the endothermic peak temperatures for PC·HCl and PC were 157 and 61 °C. TC·HCl showed two major endothermic peaks at 142 and 151 °C and one exothermic peak immediately after the initial endothermic peak. Based on the relevant reports, each peak is characterized as follows. The initial endothermic peak represents the melting of the metastable form (Form 1); then, the following endothermic peak expresses the phase transition into the stable form (Form 3), and then the higher endothermic peak indicates the melting of the stable form. In contrast to TC·HCl, TC showed a sole peak at 43 °C. Substances with lower molecular mobility are supposed to show higher melting points, indicating that the molecular mobility of salts is more restricted than that of free bases. The salt forms have ionic interactions, and for molecular mobility such interactions require a higher energy to break.

In the next phase of this study, NMR relaxometry measurements were conducted by using TD-NMR. In addition to the single powders of salt and free base of the tested APIs, binary blends were tested by mixing these powders at the designated weight ratios. The $T_1$ relaxation curves were acquired by the saturation recovery (Figs. 3a, b). Namely, after measurement of the $T_2$ relaxation curves with different recycle delays by solid-echo sequence, the initial NMR signals of the curves were plotted as a function of the recycle delay, resulting in the generation of $T_1$ relaxation curves. Furthermore, the resulting $T_1$ relaxation curves were transformed by Eq. (2) (Figs. 3c, d) to make it easier to understand the $T_1$ relaxation times: the inverse of the slope corresponds to $T_1$. From the $T_1$ relaxation curves, $T_1$ relaxation times were calculated (Table 1 and Figs. 3e, f). For both PC and TC powders, the $T_1$ values of the salt APIs were significantly longer than those of the free base APIs ($p < 0.01$): the $T_1$ relaxation times for PC·HCl and PC were $1.733 \pm 0.005$ and $0.144 \pm 0.000$ s, while those for TC·HCl and TC were $0.993 \pm 0.002$ and $0.260 \pm 0.002$ s. The net magnetization shown in the $T_1$ relaxation curves of salts did not reach equilibrium; thus, the $T_1$ relaxation times obtained in this study are likely to be shorter than the actual values. The difference between the salt and free base observed from PC powders appeared to be much larger than that from TC powders: the percentages of $T_1$ relaxation times of free base to those of salt were about 8.3 and 26.1% for PC and TC powders, respectively. Moreover, the relationship between NMR relaxometry ($T_1$ and $T_2$ relaxation times) and molecular mobility is fully understood. As far as the solid state substances are concerned, a longer $T_1$ and a shorter $T_2$ relaxation time indicate that the substance has a lower molecular mobility. Based on this knowledge, for the tested APIs, the
salts were regarded as having lower molecular mobility than the free bases. The DSC measurements pointed to the same conclusion because the melting points of the salts were higher than those of the free bases.

With regard to the binary blends consisting of free base and salt of the tested APIs, the $T_1$ relaxation times decreased steadily with increasing proportion of free bases (Figs. 3e, f). In addition, a number of $T_1$ relaxation curves showed biphasic behaviors (Figs. 3c, d). In particular, clear inflection points were found in Fig. 3c: for example, the $T_1$ relaxation curve of the 80% PC-containing blend (PC80/20) showed that the NMR signal rapidly decreased within 0.5 s and then the remaining signal gradually decreased. Based on our previous study, each phase corresponds to the $T_1$ relaxation behaviors of components in the binary blends. To further discuss this matter, we carried out a binary analysis by the following Eq. (4).

$$
\ln \left( 1 - \frac{I(t)}{I_0} \right) = \ln \left[ \frac{P_{\text{short}} \exp \left( -\frac{t}{T_1(\text{short})} \right)}{+(1-P_{\text{short}}) \exp \left( -\frac{t}{T_1(\text{long})} \right)} \right],
$$

where $T_1(\text{short})$ and $T_1(\text{long})$ are the $T_1$ relaxation times of components having fast or slow relaxation rates: in this case, they correspond to $T_1$ relaxation times of the free base and salt, respectively. $P_{\text{short}}$ is the proportion of protons corresponding to $T_1(\text{short})$. The free base contents in the binary powder blends can be estimated from the $P_{\text{short}}$. A detailed explanation on this is given in the relevant article.

The parameters approximated by Eq. (4) for the $T_1$ relaxation curves are listed in Table 1. Regarding the PC powders, both the $T_1(\text{short})$ and $T_1(\text{long})$ values were close to the $T_1$ relaxation times of the single powders of the free base and salt: $T_1(\text{short})$ ranged from 0.122 to 0.135 s, whereas $T_1(\text{long})$ ranged from 1.528 to 1.946 s. Furthermore, the free base contents in the powder blends were estimated from $P_{\text{short}}$. As shown in Table 1, the estimated values were acceptable: the estimated free base contents of PC20/80, PC40/60, PC60/40, and PC80/20 were 26.30 ± 0.19, 46.36 ± 0.95, 64.11 ± 3.02 and 79.26 ± 1.72%, respectively. By contrast, in the case of the TC binary blends, the estimation accuracy for the free base content worsened with increasing the salt content in the powder blends. Namely, acceptable free base contents were estimated from the powder blends with a lower free base content: the estimated values of TC20/80 and TC40/60 were 24.07 ± 0.16 and 37.47 ± 0.30%, whereas from TC60/40 and TC80/20: 49.40 ± 0.46 and 50.89 ± 1.10% the estimation was poor. For the parameters approximated by Eq. (4), although the data deviation was small, the values of $T_1(\text{short})$ ranging from 0.118 to 0.139 s were far apart from the $T_1$ relaxation time of the single powder of the salt, 0.993 s. By contrast, $T_1(\text{long})$ substantially changed from 0.954 to 0.535 s with increasing the free base content. In particular, TC60/40 and TC80/20 showed a markedly shorter $T_1(\text{long})$, 0.763 and 0.535 s, than the $T_1$ relaxation time of the single powder of the salt, 0.993 s. From
these results, the poor estimation is likely to be due to the significant difference in $T_1$ (long).

Binary analysis may well lead to a lower approximation accuracy under the condition that the $T_1$ relaxation times of components are too close to each other. Stueber and Jehle estimated the composition of the binary powder blends consisting of different APIs by the same analysis. They reported that the estimation accuracy was significantly affected by the difference in the $T_1$ relaxation times between the two components. For example, in the case of the binary blends consisting of ibuprofen and indomethacin (IMC), the composition was estimated precisely because their $T_1$ relaxation times were distinct from each other: 630 ms and 3750 ms, respectively. By contrast, the authors found a poorer estimation from the binary blends consisting of ibuprofen and itraconazole because the $T_1$ relaxation time of itraconazole, 720 ms, was close to that of ibuprofen. Considering this limitation, the results observed from this study seem to be quite reasonable, because the difference in $T_1$ relaxation times between PC and PC·HCl was larger than that between TC and TC·HCl. For further information, Stueber and Jehle demonstrated that the poor estimation observed from the binary blends consisting of ibuprofen and itraconazole could be improved by modifying acquisition parameters including the scan number and data points. Thus, there is a good possibility that the poor estimation observed from the TC binary blends can be improved by a similar procedure.

Subsequently, $T_2$ relaxation measurements were conducted in this study. The $T_2$ relaxation curves of the tested API powders are shown in Figs. 4a, b. Regarding the single free base and salt of the tested APIs, there were slight but significant differences in the $T_2$ relaxation curves between them ($p < 0.01$): The $T_2$ relaxation times for PC were 9.30 ± 0.01 and 8.22 ± 0.01 μs for the free base and salt, respectively, whereas the values for TC were 9.77 ± 0.01 and 8.48 ± 0.02 μs for the free base and salt, respectively (Table 2). As mentioned earlier, a solid compound with lower molecular mobility is supposed to show longer $T_1$ and shorter $T_2$ relaxation times.

Table 1. Curve Fitting Analysis of $T_1$ Relaxation Curves of Powder Blends

| API       | Sample name | Free base content (%) | $T_1$ (s) | Bi-exponential fitting analysis$^a$ | Estimated free base content (%) |
|-----------|-------------|-----------------------|----------|------------------------------------|----------------------------------|
| Procaine  | PC·HCl (salt) | 0                     | 1.733 ± 0.005        | —                | —                                |
| PC (free base) | 100          | 0.144 ± 0.000         | —                | —                                |
| PC20/80   | 20          | 0.965 ± 0.001         | 0.122 ± 0.002      | 1.946 ± 0.012      | 26.30 ± 0.19                     |
| PC40/60   | 40          | 0.486 ± 0.010         | 0.134 ± 0.002      | 1.884 ± 0.025      | 46.36 ± 0.95                     |
| PC60/40   | 60          | 0.278 ± 0.024         | 0.135 ± 0.001      | 1.792 ± 0.046      | 64.11 ± 3.02                     |
| PC80/20   | 80          | 0.193 ± 0.004         | 0.134 ± 0.003      | 1.528 ± 0.078      | 79.26 ± 1.72                     |
| Tetracaine| TC·HCl (salt) | 0                     | 0.993 ± 0.002        | —                | —                                |
| TC (free base) | 100          | 0.260 ± 0.002         | —                | —                                |
| TC20/80   | 20          | 0.832 ± 0.003         | 0.118 ± 0.003      | 0.954 ± 0.002      | 24.07 ± 0.16                     |
| TC40/60   | 40          | 0.670 ± 0.001         | 0.137 ± 0.002      | 0.893 ± 0.006      | 37.47 ± 0.30                     |
| TC60/40   | 60          | 0.492 ± 0.009         | 0.139 ± 0.005      | 0.763 ± 0.017      | 49.40 ± 0.46                     |
| TC80/20   | 80          | 0.357 ± 0.001         | 0.128 ± 0.002      | 0.535 ± 0.010      | 50.89 ± 1.10                     |

$^a$ Calculated according to Eq. (4). Each value represents the mean ± standard deviation ($n = 3$).

Fig. 4. $T_2$ Relaxation of Powder Blends Consisting of Salt and Free Base of Procaine (a and c) and Tetracaine (b and d) at Designated Weight Ratio

The $T_2$ relaxation curves (a and b) were acquired, and then $T_2$ relaxation times (c and d) were calculated.
tested APIs had lower molecular mobilities than free bases. Measurement led to the conclusion that the free bases of the binary blend can be performed by the following equation. For the binary powder blends, the $T_2$ relaxation times steadily shortened with increasing the free base content (Table 2 and Figs. 4c, d): the values for the PC binary blends ranged from 8.43 to 9.07 $\mu$s, whereas those of the TC blends ranged from 8.70 to 9.44 $\mu$s, respectively. Considering this result, it was expected that a precise estimation of the composition of the binary blend can be performed by the following equation.

$$\frac{1}{T_2} = \frac{1 - P_{long}}{T_2(long)} + \frac{P_{long}}{T_2(short)}, \quad (5)$$

where $T_2$ is the overall $T_2$ relaxation time, and $T_2$ (short) and $T_2$ (long) are the $T_2$ relaxation times of the protons of each component. (In this study, the $T_2$ relaxation times of salt and free base of the tested APIs correspond to $T_2$ (short) and $T_2$ (long), respectively.) $P_{long}$ is the proton fraction having $T_2$ (long). Based on the $P_{long}$ values, the free base contents in the binary blends could be estimated. We have already demonstrated this analysis by testing two different binary blends consisting of IMC (a model API) and polyvinylpyrrolidone (PVP) (a model additive) and consisting of amorphous and crystalline IMC. Table 2 shows the free base contents estimated from the $T_2$ relaxation curves by using Eq. (5). Appropriate estimations were obtained not only from PC powders but also from TC powders. For PC powders, the estimated free base contents of PC20/80, PC40/60, PC60/40, and PC80/20 were 21.87 ± 3.45, 46.20 ± 1.60, 63.95 ± 4.39 and 80.83 ± 1.15, respectively. This precise estimation was achieved despite the very small difference in the $T_2$ relaxation times between salt and free base: the percentages of salt to free base for PC and TC were about 88.4 and 86.8%, respectively.

This study investigated the applicability of the NMR relaxometry measurement by TD-NMR for characterization of the salt and free base of the tested APIs. They have different crystalline forms. Both $T_1$ and $T_2$ relaxation curves were proved to have sufficient capacity to distinguish between them. In particular, a precise estimation of the binary powder blend component was achieved by the $T_2$ relaxation method. In addition to the precise estimation, the $T_2$ relaxation method has several advantages as an analytical method for API property characterization. One such advantage is that the measurement time is very short. In the present study, the $T_2$ relaxation measurement was completed in only 160 s, which was much shorter than the measurement time of $T_1$ relaxation, which was 30 min. We believe that the measurement time could be shortened further by modifying the acquisition parameters including echo time, scan point, recycle delay, etc. Moreover, we have demonstrated that the same $T_2$ relaxation curve can be acquired from sample powders as long as their composition is the same, even though their mixed state is changed. Namely, our previous studies compared the $T_1$ and $T_2$ relaxation curves of a physical mixture (PM) and solid dispersion (SD) composed of IMC and PVP in the same composition. The mixed SD state was substantially different from that of PM: SD has a homogeneous structure produced by uniformly mixing with IMC with PVP, but IMC and PVP were separately distributed in PD in a microspatial perspective. As a result of the experiments, the $T_1$ relaxation curves changed substantially: PMs showed biphasic curves, whereas SDs showed monophasic relaxation curves. By contrast, the $T_2$ relaxation curves between SDs and PMs were completely consistent with each other. This characteristic might become an advantage for API property characterization because it contributes to good reproducibility of the measurements. These days, various analytical techniques have been employed to evaluate the crystalline state of APIs. They include PXRD, near-IR (NIR) and Raman spectroscopy. PXRD is the most conventional technique for this purpose; however, it is inferior to the other methods in terms of detection sensitivity of crystalline state. NIR and Raman can identify polymorphs of API with high accuracy. In addition, they are capable of nondestructive and rapid measurements. We think these advantages are similar to those of NMR relaxometry measurements by TD-NMR. When TD-NMR and NIR are compared, TD-NMR can be regarded as bulk measurement method; NMR signals are generally derived from entire samples, while NIR measurements tend to emphasize surface information. By considering these things, the NMR relaxometry by TD-NMR, in particular the $T_2$ relaxation measurement, has a strong potential to be applied in practical pharmaceutical industry. The TD-NMR methods could be ap-

| API          | Sample name      | Free base content (%) | $T_2$ ($\mu$s) | Estimated free base content (%) |
|--------------|------------------|-----------------------|----------------|---------------------------------|
| Procaine     | PC·HCl (salt)    | 0                     | 8.22 ± 0.01    | —                               |
|              | PC (free base)   | 100                   | 9.30 ± 0.01    | —                               |
|              | PC20/80          | 20                    | 8.43 ± 0.03    | 21.87 ± 3.45                    |
|              | PC40/60          | 40                    | 8.68 ± 0.02    | 46.20 ± 1.60                    |
|              | PC60/40          | 60                    | 8.88 ± 0.05    | 63.95 ± 4.39                    |
|              | PC80/20          | 80                    | 9.07 ± 0.01    | 80.83 ± 1.15                    |
| Tetracaine   | TC·HCl (salt)    | 0                     | 8.48 ± 0.02    | —                               |
|              | TC (free base)   | 100                   | 9.77 ± 0.01    | —                               |
|              | TC20/80          | 20                    | 8.70 ± 0.00    | 18.65 ± 0.07                    |
|              | TC40/60          | 40                    | 8.96 ± 0.02    | 40.20 ± 1.57                    |
|              | TC60/40          | 60                    | 9.20 ± 0.01    | 59.00 ± 0.58                    |
|              | TC80/20          | 80                    | 9.44 ± 0.01    | 77.19 ± 0.94                    |

$^a$ Calculated according to Eq. (5). Each value represents the mean ± standard deviation (n = 3).
plied in many different settings including as a PAT tool and an analytical method to evaluate crystalline polymorphs and so on.

Conclusion

This is the first technical report comparing NMR relaxometry between the free base and salt of APIs by using TD-NMR. This work proved that the $T_1$ and $T_2$ relaxation curves had sufficient capacity to distinguish between the free base and the salt of the test APIs, which differ in their crystalline forms. Subsequently, this study estimated the composition of binary powder blends from the acquired relaxation curves. Eventually, a more precise estimation of the components was achieved by analyzing the $T_2$ relaxation curves. This study provides valuable information for further progress in analytical methods in pharmaceutical science.

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