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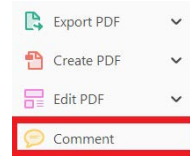
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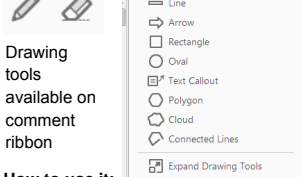
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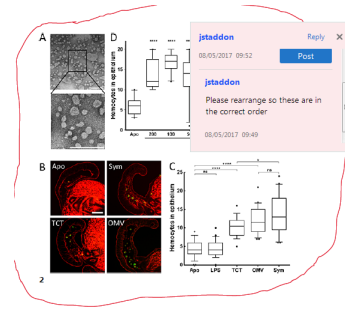
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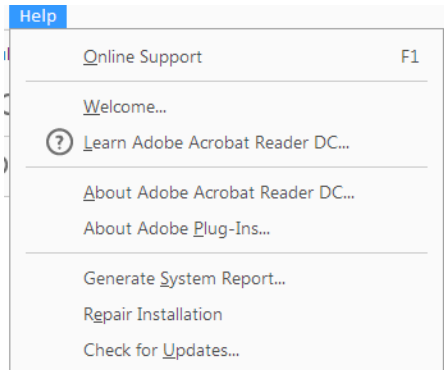
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RESEARCH ARTICLE

Illustration of inversion-recovery and Carr-Purcell-Meiboom-Gill sequences by the determination of ethanol content in alcoholic beverages

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Abstract

In Nuclear Magnetic Resonance (NMR) education, the introduction of the relaxation phenomenon and the relaxation times (T_1 and T_2) is an important and compulsory step, as is the description of the Carr-Purcell-Meiboom-Gill (CPMG) and inversion-recovery (IR) measurement sequences. Indeed those sequences are still used nowadays for, respectively, the measurement of T_2 and T_1 but also in Magnetic Resonance Imaging (MRI) and NMR spectroscopy. Practical works with the students, performed for example with water, allow to illustrate this part of the teaching. In this work we propose an alternative and funny way to introduce these important topics. With a few microliters of a concentrated Gd^{3+} solution, a few milliliters of an alcoholic beverage and a low resolution and low field NMR device, it is possible, thanks to the relaxation phenomenon and using CPMG and IR sequences, to measure the alcohol content of the beverage provided that the alcohol proton exchange with water protons is taken into account. First the method is validated with synthetic water-ethanol mixtures, then it is used to study nine different alcoholic beverages. The correlation of the ethanol volume fractions determined by NMR with the actual ethanol content of the beverages is rather good, especially for the method based on T_2 relaxation, with a correlation coefficient $r^2 = 0.994$. However, it seems that the method developed in this work always underestimates the ethanol volume fraction at high ethanol content for a reason which remains to be found.

KEYWORDS

alcohol content, Carr-Purcell-Meiboom-Gill, inversion-recovery, nuclear magnetic resonance, relaxation time, T_1, T_2

1 | INTRODUCTION

In the development of Nuclear Magnetic Resonance (NMR), the understanding of the relaxation phenomenon and the measurement of the relaxation times has played a crucial role.[1-4] Indeed, since they describe the dynamics of return to thermal equilibrium of the nuclear magnetic moments, relaxation times also have a major effect on the measured signal. NMR relaxation governs very practical aspects of the two main applications of NMR, namely NMR spectroscopy and Magnetic Resonance Imaging (MRI). For example, if the longitudinal relaxation time (T_1) of water protons were about 1 hour, MRI would be so slow that it would not be suited for medical applications. Moreover, the contrast in MRI is in part due to the differences of the relaxation times of hydrogen protons belonging to

different tissues. Similarly, if the transverse relaxation time (T_2) of protons were extremely fast (like is the electron relaxation time), the detection of the NMR signal would have been even more challenging than what it actually is. In spectroscopy, relaxation is also important. For example, the peak width—which is an essential feature of the spectrum—depends on the transverse relaxation time.

The first sequences of pulsed NMR were devoted to the measurement of the relaxation times. T_2* is the transverse relaxation time in the absence of refocusing pulses, it governs the evolution of the signal obtained after a simple 90° pulse, called a free induction decay (FID). T_2* being very sensitive to instrumental magnetic field inhomogeneities, the use of refocusing pulses has been proposed by Hahn[5] in 1950. Successive improvements of the spin echo sequence by Carr and Purcell[6] and then by Meiboom and Gill[7] led to the well-known Carr-Purcell-Meiboom-Gill (CPMG) sequence (Figure S1). For the measurement of T_1, the inversion recovery (IR) sequence (Figure S2) was only proposed in 1968 by Vold et al.[8] All those sequences are still the basis of many spectroscopy and MRI experiments used nowadays.[9,10]

It is therefore not surprising that the introduction of relaxation phenomenon appears in the first chapters of many NMR textbooks, together with the description of the sequences devoted to the measurement of T_1 and T_2, as the IR and the CPMG sequences.

The corresponding practical work on a spectrometer or a low resolution NMR relaxometer is often devoted to the measurement of water protons T_1 and T_2. The first thing students notice is that pure water has quite slow dynamics, which can make experiments boring. In this article, we propose a simple experiment that can be easily performed by the students with low resolution NMR devices and which illustrates the relaxation phenomena. It allows to introduce T_1 and T_2 measurement sequences, the principle of MRI paramagnetic contrast agents, and even to observe a consequence of the alcohol proton exchange with water protons. The only chemicals needed are an alcoholic beverage, like vodka, whiskey or even wine, and a few microliters of a concentrated (~100 mmol/L) Gd^{3+} aqueous solution. Contrary to the NMR spectroscopic techniques which were developed to quantify ethanol in alcoholic beverages,[11-17] the proposed experiment does not require a homogeneous magnetic field and can be performed with low resolution devices.

2 | MATERIALS AND METHODS

2.1 | Principle of the experiment

It was previously shown that solutions made of a mixture of methanol and water had a peculiar NMR behavior when in presence of paramagnetic ions.[18] Indeed, the proton relaxation induced by the paramagnetic ion—which is perfectly monoexponetial in pure aqueous solution—presents a marked biexponential relaxation behavior in water-methanol solutions. The two fractions of protons associated to this biexponential evolution of nuclear magnetization correspond to the "OH" protons of water and methanol for the fast relaxing fraction and to the "CH" protons of methanol for the slowly relaxing fraction. This behavior can be explained by the fact that water protons have a preferential access to the paramagnetic ion because the first coordination sphere of the ion is constituted of water molecules. This results in fast relaxation for those protons. The methanol molecules are kept apart from this sphere, and their relaxation is slower. Interestingly, because of the alcohol proton exchange with water protons,[19] the fast relaxing fraction corresponds to all the "OH" protons (water + methanol), since the relaxation "information" is shared with the alcohol proton of methanol. In the original paper, this has been used to evaluate the contributions of inner sphere and outer sphere mechanisms to the relaxation of different types of MRI paramagnetic contrast agents. Inner sphere relaxation is due to the interaction of hydrogen proton spins belonging to the water molecules of the first coordination shell with the electronic magnetic moment of the paramagnetic ion. Outer sphere relaxation is caused by the dipolar interaction of hydrogen protons belonging to water molecules diffusing around the paramagnetic ion with its electronic magnetic moment.[20] This rather fundamental study can find an exotic and prosaic application: determining the alcohol content of different alcoholic beverages. Indeed, what works with water-methanol mixtures also works with water-ethanol mixtures. Adding a small amount of a concentrated paramagnetic ion (Gd^{3+} for example) solution to a water-ethanol mixture will also result in a biexponential relaxation whose fast and slow relaxing fractions of protons will, respectively, correspond to OH protons of water and methanol, and CH protons of ethanol, respectively. Those fractions are proportional to the molar fractions of the OH and CH protons, which allows for calculation of the molar fractions of water and ethanol. Indeed, if the total number of moles of protons is N and if f_{CH} is the fraction of CH protons in the mixture, we know that the number of ethanol moles in the mixture is simply $Nf_{CH}/5$ (since there are 5 CH protons per ethanol molecule). This corresponds to a mass of ethanol equal to $m_{eth} = (Nf_{CH}/5)$ 46.1, where 46.1 is the molar mass of ethanol in g/mole. The number of moles of water in the mixture is $(N(1 - f_{CH} - f_{CH}/5))/2 = (N(1 - 6f_{CH}/5))/2$ corresponding to a mass of $m_{wat} = ((N(1 - 6f_{CH}/5))/2)18$. The mass fraction of ethanol can be obtained using Equation (1):

$$f_{\text{mass}}^{\text{ethanol}} = \frac{46.1}{46.1 + 9(\frac{5 - 6f_{\text{CH}}}{f_{\text{CH}}})} \quad (1)$$

The volume fraction of ethanol $f_{\text{volume}}^{\text{ethanol}}$ is eventually determined. For example, for 1 g of solution at 20°C, the volume of ethanol in cm^3 is given by ($f_{\text{mass}}^{\text{ethanol}}/0.789$), where 0.789 is the density of ethanol at 20°C, while the total volume of the solution is $1/d_{\text{mixture}}$ where d_{mixture} is the density of the water-methanol mixture. The volume fraction of ethanol in the solution is thus given by Equation (2):

$$f_{\text{volume}}^{\text{ethanol}} = \frac{f_{\text{mass}}^{\text{ethanol}}}{0.789} d_{\text{mixture}} \quad (2)$$

This calculation is not as simple as it seems because of the noticeable contraction of water-ethanol solutions, due to the breaking of hydrogen bonds of water caused by ethanol. As a consequence, the density of the water-ethanol mixture cannot be calculated as the weighted mean of water and ethanol densities. One should use the corrected values for the density of water-ethanol mixtures which can be found in reference tables.[21] This correction can lead to relative differences of 5% for the volume fraction.

2.2 | Preparation of the samples

Deionized water and absolute ethanol were used for the preparation of the synthetic water-ethanol solutions. Samples with different mass fractions of ethanol were prepared simply by mixing two known masses of ethanol and water. Using mass fractions is more logical since the NMR method naturally provides a molar fraction which can be converted to a mass with a simple calculation. The alcoholic beverages (3 vodkas, 1 gin, 1 triple sec, 2 white wines, 1 liquor and 1 slivovitz) were kindly provided by the authors and their colleagues. A few milliliters of a 100 mmol/L Gd^{3+} solution were prepared with GdCl$_3$·6H$_2$O (Sigma Aldrich G7532). For the NMR measurements, 10 μL of the concentrated Gd^{3+} solution was added to 990 μL of the water-ethanol mixture (synthetic or alcoholic beverage). 350 μL of this solution were used for the measurements on our device. The amount of sample will have to be adapted depending on the NMR relaxometer which is used. The decrease in ethanol fraction caused by the addition of 10 μL of water was subsequently corrected. The use of alcoholic beverages in the laboratory could be problematic, but alternatives exist. Some disinfecting solutions could be used since they are constituted of a 70% mixture of water and ethanol. However, these samples will probably have to be diluted before the measurement in order to be in the same range of ethanol fraction as the alcoholic beverages.

2.3 | NMR measurements

The NMR experiments were carried out at 20°C on a homemade relaxometer using a permanent magnet producing a field of 0.47 T (which corresponds to a proton Larmor frequency of 20 MHz) coupled to a lapNMR console from Tecmag and a Tomco RF power amplifier. However, commercial benchtop NMR devices can be used, as for example those proposed by Bruker® (Minispec), NMR-Design® (Spintrack), Magritek® (Spinsolve), Nanalysis® (NMReady), Thermo-Fisher® (Picospin). CPMG sequences with an echo train covering a time interval of ~3 T_2 of the slow relaxing fraction were used with an interecho time comprised between 1 and 3 ms depending on the experimental conditions. The T_1 experiments were carried out using the IR sequence. The last delay of the IR curve was chosen as 3 T_1 of the slow relaxing fraction. The repetition time was taken as 5 T_1 of the slow relaxing fraction. Only the even echoes were considered for the fitting of the data which allowed the determination of OH and CH fractions, since in a CPMG sequence only the even echoes are effectively occurring in the transverse plane. The fitting of the curves was performed with Sigma-Plot 11.0.

2.4 | Conventional ethanol content determination

The ethanol content of alcoholic beverages is usually determined through a measurement of density by pycnometry, hydrostatic balance or electronic densimetry.[22] Liquid chromatography and infrared spectrometry can also be used. The Beer Alcolyzer (Anton Paar) used in this work combines electronic density measurement with infrared absorption to determine the alcohol content. Some of the samples had to be diluted with distilled water before the measurement. This technique was used as the gold standard, providing the "real" ethanol content, since it is recognized by different official organizations as the European Brewery Convention (method 9-2-6) and the Central European Commission for brewing Analysis.

2.5 | Pedagogical aspect

This experiment has been used during the practical works of a course of NMR for 15 years, with students in their first year of master of physics (in Belgium). We think it could also be suited for students in biology or chemistry. Depending on the universities, such a course can appear in Master or Bachelor degree. The students must understand some important concepts before the experiment: fundamentals of NMR, relaxation, CPMG and IR sequences and the principle of MRI contrast agents. After the lab session, the students will be more familiar two important NMR

sequences but also with the effects of relaxation on the signal and one example of relaxation mechanism. This was useful for our students, especially for those who began a PhD after their master studies.

From our experience, the main difficulties encountered by the students were (a) the understanding of the two separated relaxation mechanisms for CH and OH protons (b) performing biexponential fittings of the relaxation curves and (c) the conversion of the molar fraction into volume fraction. Therefore, time must be spent on the explanation of the relaxation induced by paramagnetic ions, with a sketch of inner and outer sphere relaxation. In order to save time, the fittings of the relaxation curves must be done with the teacher during the lab session, with a dedicated software and the biexponential fitting equation already implemented. But we think it is crucial to perform both mono and biexponential fittings (as in Figure 1) in order to show the students that a simple exponential fitting is not satisfactory. The conversion of molar fraction to volume fraction will just necessitate some reminder. Finally, we should mention that we always compared the ethanol volume fraction obtained by NMR with the one provided on the bottle, so there is no need to use a specific alcohol measurement equipment.

3 | RESULTS

3.1 | Proof of concept with synthetic water-ethanol solutions

Figure 1A shows a typical curve of return to equilibrium of the transverse magnetization during a CPMG sequence for a synthetic water-ethanol mixture containing Gd^{3+}. Figure 1B shows, for the same sample, the curve of return to equilibrium of longitudinal magnetization obtained with the IR sequence. The relaxation is logically faster than what is observed for pure water or ethanol; because of the presence of the paramagnetic Gd^{3+} ion. Both curves exhibit a clear biexponential behavior. The corresponding mass fraction of ethanol can be calculated from the fraction of the NMR signal corresponding to the slow relaxing population of protons f_{CH} thanks to Equation (1). The obtained fractions are compared with actual ethanol mass fractions of the prepared solutions in Figure 2.

3.2 | Alcoholic beverages

The relaxation curves of a Gd^{3+} containing vodka sample are presented in Figure 3. Both transverse and longitudinal relaxation dynamics are biexponential as what was previously observed for synthetic water-ethanol mixtures. The volume fractions of ethanol calculated from the biexponential fitting of T_2 and T_1 data are respectively compared in Figure 4A,B with the ethanol volume fractions measured by the Alcolyzer apparatus, for the different alcoholic beverages used in this study.
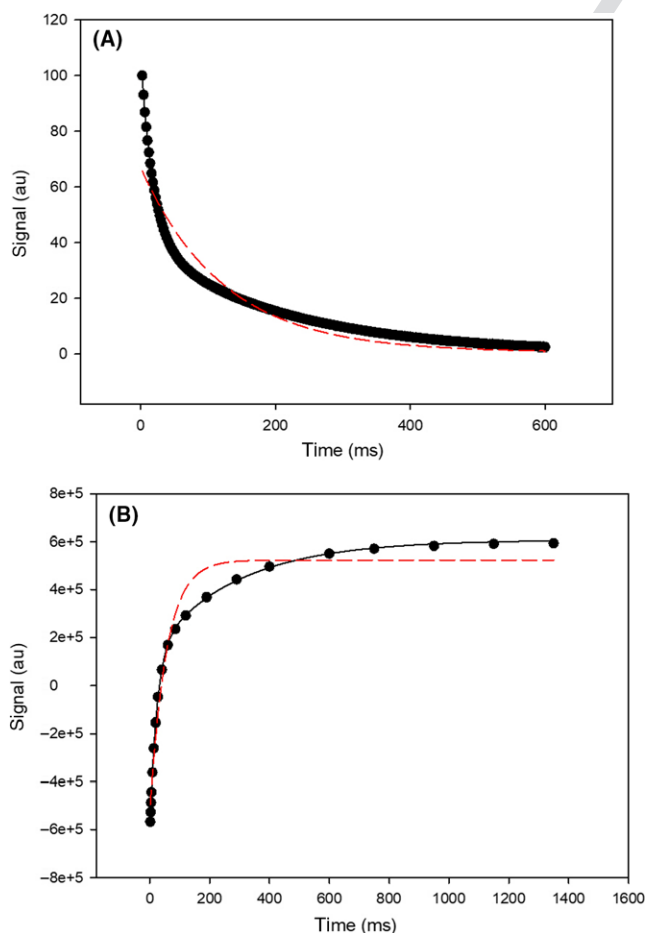

FIGURE 1 A, CPMG curve of a synthetic water-ethanol (43.8% weight) mixture containing Gd^{3+}; B, IR curve of a synthetic water-ethanol mixture (43.8% weight) containing Gd^{3+}
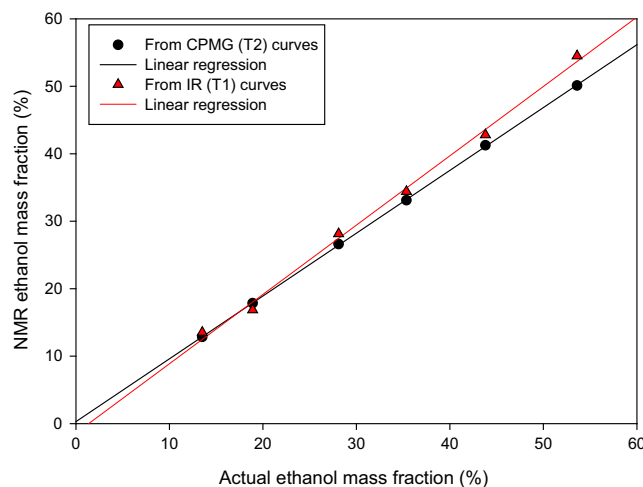
FIGURE 2 Correlation between the mass fractions obtained by NMR relaxometry and the actual mass fractions of synthetic water-ethanol mixtures
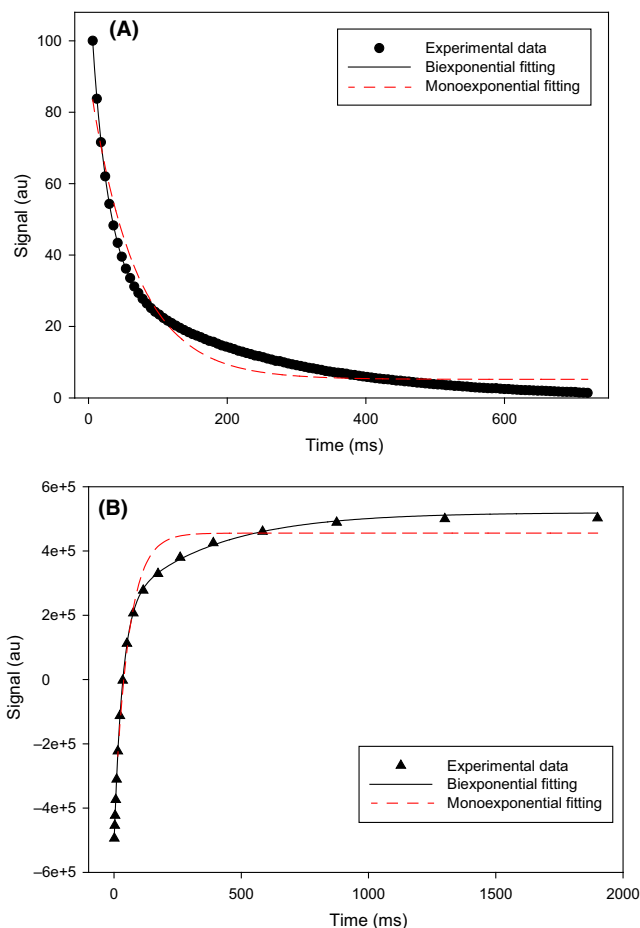
FIGURE 3 A, CPMG curve of a vodka sample (40.7% vol) containing Gd^{3+}; B, IR curve of a vodka sample (40.7% vol) containing Gd^{3+}

FIGURE 4 A, Comparison of the ethanol volume fraction obtained by NMR with CPMG curves and by the alcolyzer for different alcoholic beverages; B, Comparison of the ethanol volume fraction obtained by NMR with IR curves and by the alcolyzer for different alcoholic beverages

4 | DISCUSSION

4.1 | Synthetic water-ethanol solutions

The correlation is excellent for T_2 CPMG curves ($r^2 = 0.9999$) even if the NMR mass fractions are always smaller than the actual mass fractions since the slope of the relationship is 0.936 instead of 1 for T_2 curves. When using T_1 IR curves, the correlation coefficient is also very good ($r^2 = 0.992$). In both cases, the intercept of the linear regression is almost zero which is consistent.

4.2 | Alcoholic beverages

For T_2, the agreement with the real ethanol fraction of the alcoholic beverage is fine even if at high ethanol content, the NMR technique clearly underestimates the ethanol content of the beverage. A linear regression of the data of Figure 4A provides an excellent correlation coefficient ($r^2 = 0.994$) with a slope of 0.856 and an intercept of 4.56%. For volume fractions obtained with T_1 NMR

(Figure 4B), the agreement with the real volume fraction is not so good since the linear regression of the data provides a correlation coefficient $r^2 = 0.972$ with a slope of 0.821 and an intercept of 5.35%. Interestingly the T_1 method also underestimates the real ethanol volume fraction for high volume fractions. In light of the correlation coefficients, the T_2 method thus seems more efficient than the T_1 method to determine the actual ethanol volume fraction. The linear regression of T_1 volume fractions versus T_2 volume fractions also provide interesting information. The value of the slope is 1.02 with an intercept of 0.7% and a correlation coefficient of 0.974. The slope is really close from 1 and the intercept is almost zero proving the coherence between the T_1 and T_2 NMR methods.

It should be noted that the intercepts of both linear regressions are non-negligible and the slopes are significantly smaller than 1, which remains unexplained. This could be due to the complex nature of the liquid, which

does not only contain ethanol and water but also other organic molecules. However, when looking at the NMR spectra of gin and vodka presented in the SI of Hill et al,[13] this possibility has to be ruled out. Indeed, the fraction of molecules other than water and ethanol seems almost negligible while the NMR method largely underestimates the ethanol volume fraction for gin and vodka. Another possible reason could be the presence of water-rich ethanol hydrates and ethanol clusters in alcoholic beverages. These may be present in different amounts and have different access to the Gd^{3+} ion which could maybe influence the results provided by our method. This could also be the reason of the underestimation, by the NMR method, of the real ethanol content in synthetic water-ethanol solutions.

5 | CONCLUSIONS

The experiments presented in this article allow to introduce different important NMR concepts in an original way, for example during a session of practical work. Transverse and longitudinal relaxation, T_1 and T_2 measurements sequences, paramagnetic contrast agents and proton exchange are nicely illustrated. It only requires a few microliters of a concentrated Gd^{3+} solution, a few milliliters of vodka and a low resolution time-domain NMR machine using a permanent magnet.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Substitute character or substitute part of one or more word(s)	/ through letter or ⊢———⊣ through characters	new character / or new characters /
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Change to capitals	≡ under matter to be changed	≡
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Change to bold type	⌇ under matter to be changed	⌇
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Start new paragraph	⌐	⌐
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Transpose	⊔⊓	⊔⊓
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