

Accurate Intensities of Broad NMR Lines from Composite Pulse Experiments

N. Hedin and I. Furó

Division of Physical Chemistry, Department of Chemistry, Royal Institute of Technology, SE-100 44 Stockholm, Sweden

Received December 7, 1998; revised June 23, 1999

Accurate determination of integral intensities of broad lines is difficult when spin relaxation during the applied pulses cannot be neglected and/or when ringing of the tank circuit interferes with the signal. Here we present an extension of the analytical solution of the generalized Bloch equations (G. A. Morris and P. B. Chilvers, *J. Magn. Reson. A* 107, 236 (1994)), which is then used to evaluate the signal intensity obtained in a composite pulse experiment designed to cancel ringing effects. Comparing intensities of broad and narrow ^{81}Br spectral lines tests and proves the accuracy of this approach. © 2000 Academic Press

Key Words: ringing; composite pulse; relaxation; Bloch equations; ^{81}Br NMR.

INTRODUCTION

Although the integral intensity, compared to chemical shift and spin relaxation times, is usually a less important spectral parameter of NMR lines, there are several applications that require an accurate estimate of this intensity. One obvious situation demanding accurate intensity is when NMR is used as an analytical tool for measuring concentration. This is particularly difficult when comparing samples or lines with very different linewidths (I), either within the same or in different samples. A field of particular interest is the NMR of various $I > 1$ metal and halogen ions in biological tissues (2) and in complex fluids (3). In those systems, the presence or absence of an undetected broad component in the NMR spectrum contains information, through spin relaxation, about the microstructure of the material. Our direct motivation is also connected to complex fluids where one cannot correctly interpret spin relaxation data of $I > 1$ nuclei without first establishing whether the measured spectrum corresponds to the full expected intensity.

Measuring broad NMR lines ideally requires a short RF pulse, a short ring-down delay, and a fast digitizer. Starting with the easiest requirement, fast digitizers (with \geq MHz bandwidth) are routinely available on many of the spectrometers one can purchase today. If short RF pulses are a requirement, it is best to use solenoid probes to which large RF power can be transmitted without arcing. Often, however, only routine high-resolution probes are available whose saddle coils

cannot be fed with too high RF power. Such probes are the preferred ones for many complex fluid systems where it is often difficult to enclose a sensitive sample horizontally in a tight, chemically inert, and nondamaging way.

Nevertheless, the most severe problem is with ringing (4), either electroacoustic or purely electronic. Although the ringing problem can often be reduced by a careful construction of the probe, this is usually cumbersome and, moreover, may suppress ringing less than required. A more suitable solution is to use composite pulses as in Ref. (5) where three successive 90° pulses were applied to obtain an FID signal. In that composite pulse each individual part creates a ringing signal that, in contrast to the NMR signal, is to a first approximation independent of the other pulses. Thus, the ringing signal can be cancelled via an appropriate phase cycle (5, 6). The performance of this composite pulse is demonstrated in Fig. 1, where spectra with a broad ^{81}Br line, recorded in a conventional saddle-coil high-resolution probe, are presented. Obviously, the single-pulse spectrum (Fig. 1a) is rendered useless for determining its intensity by ringing while the one recorded by the composite pulse (Fig. 1b) is free of ringing and has the potential to be explored for measuring its intensity. In this particular case, a component of the ringing signal and the FID have roughly the same decay rates, as illustrated in Fig. 1c. Hence, simply increasing the acquisition delay cannot cancel the systematic errors in the intensity measurements.

On the other hand, a composite pulse may be long enough for the magnetization to relax significantly during the pulse itself, which would then provide a low apparent FID amplitude. To obtain an accurate intensity estimate, this effect has to be accounted for via correctly extrapolating the magnetization back to beginning of the pulse.

THEORETICAL CONSIDERATIONS

Extrapolating the magnetization to zero time, that is, calculating the initial equilibrium magnetization from the measured FID response, should be done by solving the generalized Bloch equations (GBE) (7), which is simplest for a single line. This approach is particularly transparent if one uses the recently published analytical solution to the GBE (8, 9). There, the

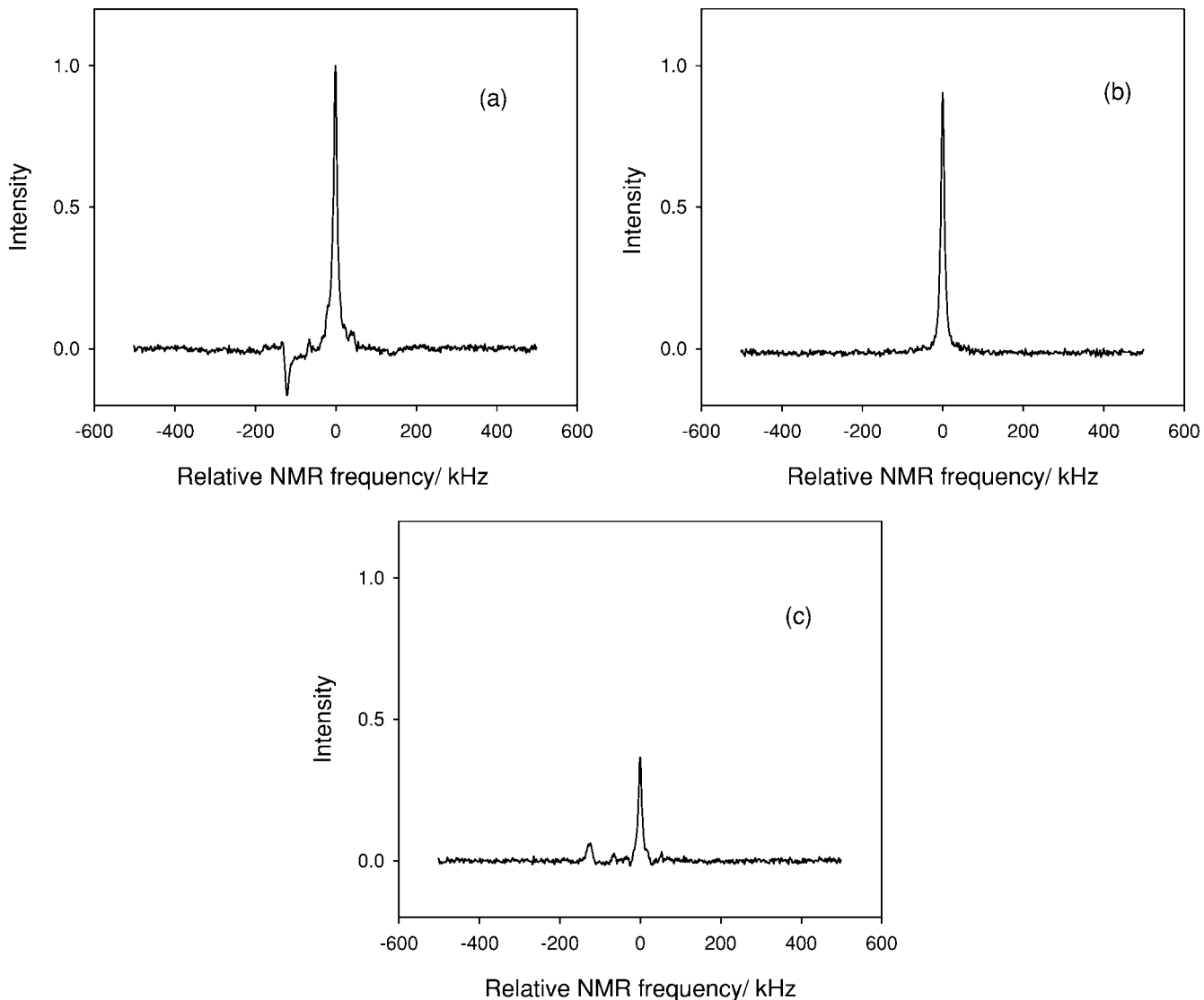


FIG. 1. ^{81}Br NMR spectra of 50 mM NaBr-ethanol solution, acquired at 54 MHz with a single 9.5- μs -long 90° pulse followed by 35- μs acquisition delay (a), with a composite pulse consisting of three 9.5- μs 90° pulses ((5), see text) followed by 14- μs acquisition delay (b) and with a single 9.5- μs -long 90° pulse followed by 65- μs acquisition delay (c). The intensities are normalized to the maximum peak amplitude in (a).

effect of a single RF pulse of zero (assigned as x) phase has been provided for arbitrary relaxation rates, frequency offset, and initial magnetization as

$$\bar{M} = G_x(\bar{M}_0), \quad [1]$$

where \bar{M}_0 and \bar{M} are the initial and final magnetizations, respectively, and G_x denotes the solution (the full formal solution is recalled in the Appendix).

The three-pulse composite excitation scheme (5), where the phases of the individual pulses vary as simplest $\phi_1 = x, x, -x, -x$, $\phi_2 = x, x, x, x$, $\phi_3 = y, -y, y, -y$, $\phi(\text{receiver}) = y, -y, -y, y$, requires adapting this solution to other phases. Since the relaxation has a full rotational symmetry in the xy

plane, the solution for an arbitrary phase can be easily produced as

$$\bar{M} = \exp(-i\phi)G_x(\exp(i\phi)\bar{M}_0), \quad [2]$$

where ϕ is the phase difference to x (in the complex xy plane). What remains then is to apply this expression consecutively along the composite sequence; in other words, each single pulse provides the initial conditions for the next one, and the initial condition for the first pulse is the thermal equilibrium. (It should be noted that the result of nonnegligible relaxation during the RF pulses is that the effects of pulse combinations such as $90_x 90_x 90_{-x}$ and $90_x 90_{-x} 90_x$ are not identical any more.) The results from the various phase combinations of the

phase cycle are then added. The final outcome provides the initial intensity and phase of the transverse magnetization, which then decays solely under the effect of transverse relaxation.

When implemented in practice (see below), it is important to proceed with the full complex FID instead, for example, of the decay recorded in one of the spectrometer “channels.” While this latter approach is not leading to any systematic error when applied to an on-resonance signal, off-resonance results in a covariance between the frequency offset and the transverse relaxation rate. Attention should also be paid to the effect of digitization. Although modern NMR spectrometers usually apply simultaneous digitization to the real and imaginary components, some older machines may provide sequentially digitized points instead. Moreover, applying an analogue (as we chose to do) filter to the incoming FID signal renders the digitized points to correspond close to the end of the individual time windows. Any error due to this latter complication can be made negligible by choosing a fast enough digitization rate.

EXPERIMENTAL TEST

The performance of the method presented above has been first tested by comparing the ^{81}Br NMR signals of equimolar (115 mM) solutions of NaBr (Mallinckrodt, 99.5%) in water (H_2O , deionized), methanol (Merck, 99.8%), and ethanol (Kemetyl, 99.5%). These solutions were selected since they provide a factor-30 variation in ^{81}Br linewidth and, moreover, the relaxation of ^{81}Br , caused by $\sim\text{ps}$ hydration dynamics, is in the extreme narrowing limit. Equal volumes of the prepared solutions were filled into 5-mm NMR tubes, up to the level of ≈ 1.5 cm. The added masses were measured and then the added volumes were recalculated from the known densities of the solvents (the added salt had a very small effect on the densities of the three liquids). The differences of the calculated volumes were within 2%; the measured signal intensities were normalized by the respective calculated volumes.

We found that it is particularly important to insert the samples as softly as possible into the NMR probe. When using the conventional air-lift system of the spectrometer, sometimes the samples must have been slightly shaken at landing in the probehead (as witnessed by eventual small droplets on the tube wall over the liquid surface). The reproducibility and the accuracy of our intensity estimates have improved markedly after having implemented a modified sample holder rotor that has been manually lowered on a rope into the magnet.

^{81}Br FIDs were acquired at 54 MHz on a Bruker DMX200 spectrometer using a 90° pulse length of $9.5 \mu\text{s}$ and a delay of $1 \mu\text{s}$ between the pulses in the composite pulse. The corresponding RF field strength is $\omega_1 = \gamma B_1 = 1.65 \times 10^5 \text{ s}^{-1}$. The probe tuning and matching required slight readjustment for the different samples; this is a consequence of different (at most by about a factor of 6) sample conductivities. Since the pulse lengths are difficult to be accurately calibrated on the

TABLE 1
 ^{81}Br FID Amplitudes after the Composite Pulse and a Short Acquisition Delay, $I(\text{comp})$, and the Calculated Equilibrium Magnetizations $I(0)$ for the Different Solutions of NaBr

Solvent	R_2 (10^3 s^{-1})	$I(\text{comp})$ (au)	$I(0)$
H_2O	1.23 ± 0.01	27.98 ± 0.05	1 (normalized)
Methanol	13.3 ± 0.16	20.85 ± 0.16	1.013 ± 0.010
Ethanol	34.8 ± 0.6	12.06 ± 0.25	0.99 ± 0.02

Note. First, the exponential FID signal is extrapolated to the end of the composite pulse by making use of the fitted transverse relaxation rate R_2 of the FID. Thereafter, the extrapolated intensity is further normalized by the relaxation-dependent reduction factor, calculated for the composite pulse as described in the text, to yield $I(0)$. The intensities obtained in the methanol and ethanol solutions are normalized to the experimental $I(0)$ of the water sample. The given errors correspond to $\pm 2 \sigma$ estimates of the experimental random error. The systematic errors are estimated to ± 1 –2% (see text).

^{81}Br lines, we used the much narrower ^{23}Na resonances of the same samples to establish that the amplitude of the B_1 field (and, reciprocally, the receptivity of the spectrometer (I_0 , I_1)) is only marginally ($\sim 1\%$) influenced by this tuning–matching adjustment. The spectral width was set to 1 MHz and an acquisition delay of $17 \mu\text{s}$ was inserted after the composite pulse to cancel a minute surviving fraction of the original ringing. Scans (16–128 k) were collected for the various samples and the measured intensities were normalized to an equal number of scans.

In measuring the true integral intensities of the three lines we proceeded as follows. First, the complex FIDs were fitted to a single exponential decay, which provided us with R_2 (inhomogeneous broadening was found negligible by ^{23}Na NMR), the frequency offset, and the amplitude of the signal immediately after the composite sequence, $I(\text{comp})$. Second, the experimental relaxation rate R_2 (and the frequency offset) was inserted (see Appendix) into the sum representing the effect of all phase combinations in the composite sequence, calculated via Eq. [2]. This yielded the correction factor by which the extrapolated intensity had to be multiplied to provide the true intensity $I(0)$. The numerical results are presented in Table 1; the obtained true integral intensities agree within the error that propagated from the errors of the fitted FID intensities and the relaxation rates.

To investigate eventual systematic error we have also measured the ^{23}Na intensities in the same samples and on the same instrument. The ^{23}Na spectra are two to three orders of magnitude narrower than the ^{81}Br spectra and therefore the correction factors due to the application of the composite pulse are minute. Moreover, it was also possible to compare the intensities obtained from spectra from a single-pulse and from composite pulse experiments. The only difference between the ^{23}Na and the ^{81}Br experiments was that for the ^{23}Na FIDs the inhomogeneous broadening of the line is not negligible to the transverse relaxation rate. On the other hand, the baselines of

the obtained sodium spectra were flat and, therefore, the apparent intensity was determined from spectral integrals. Within $\pm 1\%$, we obtained identical ^{23}Na intensities for the three investigated samples.

One particular finding from the ^{23}Na experiments is that the intensities determined from a one-pulse and composite pulse FIDs do not coincide for the same sample. This is an obvious consequence of the inhomogeneity of the B_1 field, often significant for saddle-coil probes (11). To test this point, we have modeled the B_1 inhomogeneity distribution as a symmetrical three-step function, where the width of the field distribution was fitted to provide the experimentally observed intensity difference between ^{23}Na lines produced by 90° and 450° pulses ($\sim 25\%$). Within this model, we could completely reproduce the observed small ($\sim 6\%$) differences between the intensities obtained from single-pulse and composite pulse FIDs. Most importantly, however, the *relative* intensities of different samples obtained from the composite pulse FIDs are influenced only weakly by B_1 inhomogeneity. For the ^{23}Na spectra, this effect is completely negligible. For the ^{81}Br lines of the methanol and ethanol samples, where the relaxation during the composite pulse is not negligible, the intensities obtained by our procedure outlined above are somewhat ($\sim 1\%$) influenced by the B_1 inhomogeneity. In other words, our systematic errors are smaller than our present random errors, displayed in Table 1.

As a second test, we have applied the method to check whether all ^{81}Br signal is detectable in a micellar solution of the surfactant CTAB (hexadecyltrimethylammonium bromide, Merck, 99%, prepared in an aqueous solution of 115 mM). The temperature was set to 30°C to prevent precipitation of CTAB crystals. In contrast to the previous salt solutions, ^{81}Br spin-relaxation in the CTAB solution is out of extreme narrowing (12). Here, the Br^- ions are collected in the close vicinity of the oppositely charged surface of the micellar aggregates formed by alkyltrimethylammonium molecules. The correlation function describing the fluctuation of the quadrupole coupling constant decays in two steps (3, 13), one fast decay followed by a slow one. While fast ($\sim 1\text{--}10$ ps) dynamics of the hydration shell still averages out most of the instantaneous coupling (14), a small residual part becomes averaged out by the much slower diffusion of the ions over the micellar surface. (In fact, studying this latter process provided the original motivation to this project.) A slight complication arises from the fact that ^{81}Br has a spin $I = \frac{3}{2}$ and, hence, both the longitudinal and the transverse magnetization decays are double exponential in the nonextreme narrowing case (15, 16). However, in the case of a weak frequency dispersion of the relaxation rates (as anticipated and measured in the investigated micellar solution), these decays can be well approximated as single exponential (17, 18). The effective relaxation rates R_1^{eff} and R_2^{eff} are simply the averages of the two respective decay constants weighed by the relative amplitudes of the two components. The close experimental values, $R_2^{\text{eff}} = (25.6 \pm 0.3) \times 10^3 \text{ s}^{-1}$ and $R_1^{\text{eff}} =$

$(24.0 \pm 0.7) \times 10^3 \text{ s}^{-1}$, support the validity of this approximation. The obtained $I(0)$ for CTAB, normalized as the values in Table 1, is 0.95 ± 0.02 . Thus, there is no undetected broad component in the ^{81}Br spectrum of CTAB micelles. (This result, in turn, confirms that the frequency dispersion of the ^{81}Br relaxation in this system is, indeed, weak (12).)

CONCLUSION

Measuring the integral intensity of broad lines may be difficult because composite pulses, which are often indispensable in reducing the ringing, are themselves relatively long. Thus, relaxation during the RF pulses may significantly reduce the detected intensity. Here we have shown that this can be conveniently corrected for by using the analytical solution of the Bloch equations. The success of this approach is convincingly demonstrated on a signal, which is approximately halved during the composite pulse excitation.

In some cases, one could also try to estimate the true intensity $I(0)$ by detecting the FID after a single short ($20\text{--}30^\circ$) RF pulse and a long acquisition delay at the cost of drastically increased experimental time. In that approach, the short pulse would allow one to neglect the relaxation effects during the pulse and the long delay would cancel the eventual ringing effects. For our broadest lines (the NaBr-ethanol and CTAB-water solutions), however, this could not have helped. The ringing signal, which remains in Fig. 1c both at the ^{81}Br frequency and slightly off-resonance, has approximately the same decay rate as the NMR signal. Therefore, any additional acquisition delay would not change their relative amplitudes and would therefore not allow us to accurately (better than $10\text{--}20\%$) measure the true intensity of the NMR signal. Of course, the lower the Br concentration the less accurate the intensity obtained in this way becomes.

APPENDIX

To aid the reader, in this Appendix we recapitulate the results of Refs. (8, 9). The Bloch equations in the rotating frame and with a B_1 magnetic field in the direction of the x -axis can be written as

$$\frac{d\bar{M}}{d\tau} = \bar{B}\bar{M} + \alpha \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix}, \quad [3]$$

$$\bar{B} = \begin{bmatrix} -\beta & -\delta & 0 \\ \delta & -\beta & -1 \\ 0 & 1 & -\alpha \end{bmatrix}; \quad [4]$$

the nuclear magnetization \bar{M} is normalized with the equilibrium nuclear magnetization and the time variable, the frequency offset (in angular frequency units) and the relaxation rates are all normalized with the angular frequency of nutation

$\omega_1 = \gamma B_1$ as $\tau = \omega_1 t$, $\delta = \Delta\omega/\omega_1$, and $\alpha = R_1/\omega_1$, $\beta = R_2/\omega_1$. Hence, all involved quantities and parameters are dimensionless. In general, the initial condition (which includes the case of a nonequilibrium spin system) is

$$\bar{M}_0 = \begin{bmatrix} u_0 \\ v_0 \\ m_0 \end{bmatrix}. \quad [5]$$

The analytical solution for the time evolution of the nuclear magnetization contains three transient and one steady-state term as

$$\begin{aligned} \bar{M}_\tau &= G_x(\bar{M}_0) \\ &= (\bar{A}\bar{M}_0 + \bar{C}_1)e^{-a\tau} \\ &\quad + (\bar{M}_0 - \bar{A}\bar{M}_0 - \bar{C}_1 - \bar{C}_2)e^{-b\tau}\cos s\tau \\ &\quad + ((a-b)(\bar{A}\bar{M}_0 + \bar{C}_1) + b(\bar{M}_0 - \bar{C}_2) \\ &\quad + \bar{B}\bar{M}_0 + \bar{C}_3)\frac{e^{-b\tau}\sin s\tau}{s} + \bar{C}_2, \end{aligned} \quad [6]$$

with

$$\bar{A} = a \begin{bmatrix} 1 + (\alpha - a)(\beta - a) & & \\ \delta(\alpha - a) & & \\ \delta & & \\ -\delta(\alpha - a) & \delta & \\ (\alpha - a)(\beta - a) & -(\beta - a) & \\ (\beta - a) & ((\beta - a)^2 + \delta^2) & \end{bmatrix} \kappa^{-1}, \quad [7]$$

and with some auxiliary variables introduced as

$$\begin{aligned} \theta &= \alpha - \beta, \quad a = \frac{1}{3}(\alpha + 2\beta) - (\epsilon_+ - \epsilon_-), \\ b &= \frac{1}{3}(\alpha + 2\beta) + \frac{1}{2}(\epsilon_+ + \epsilon_-), \\ \epsilon_\pm &= \sqrt[3]{\frac{\zeta \pm \sqrt{\zeta^2 + 4\eta^3}}{54}}, \quad s = \frac{\sqrt{3}}{2}(\epsilon_+ - \epsilon_-), \\ \zeta &= \theta(9 - 2\theta^2 - 18\delta^2), \\ \eta &= 3(1 + \delta^2) - \theta^2, \quad \kappa = a((b - a)^2 + s^2). \end{aligned} \quad [8]$$

The coefficients \bar{C}_i in Eq. [6] are

$$\begin{aligned} \bar{C}_1 &= \alpha \begin{bmatrix} -\delta \\ \beta - a \\ -((\beta - a)^2 + \delta^2) \end{bmatrix} \kappa^{-1}, \\ \bar{C}_2 &= \frac{\alpha}{\alpha(\beta^2 + \delta^2) + \beta} \begin{bmatrix} \delta \\ -\beta \\ \beta^2 + \delta^2 \end{bmatrix}, \quad \bar{C}_3 = \begin{bmatrix} 0 \\ 0 \\ \alpha \end{bmatrix}. \end{aligned} \quad [9]$$

In our experimental situations (see above) the applied B_1 field is strong enough to nutate the decaying magnetization, represented as oscillating terms in Eq. [6] appearing under the condition $(\zeta^2 + 4\eta^3) > 0$. While it is straightforward to program the presented formulas in any mathematics software, the corresponding MATLAB (Mathworks, Inc.) functions can be requested from the authors.

ACKNOWLEDGMENTS

The Swedish Natural Science Research Council (NFR) has supported this work. N. H. thanks the Ernst Johnsson Foundation for a scholarship.

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