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DECAY TIME DISTRIBUTION ANALYSIS OF Y_t -BASE IN BENZENE-METHANOL MIXTURES

IGNACY GRZYCZYNSKI, WIESLAW WICZK, JOSEPH R. LAKOWICZ[†]

University of Maryland, School of Medicine, Department of Biological Chemistry, 660 West Redwood Street, Baltimore, MD 21201 (U.S.A.)

MICHAEL L. JOHNSON

University of Virginia, Department of Pharmacology, School of Medicine, Charlottesville, VA 22908 (U.S.A.)

Summary

Frequency-domain fluorometry was used to measure intensity decays of synthetic Y_t -base in mixtures of benzene-methanol at 20 °C. Multiexponential analysis shows that the decay of Y_t -base fluorescence in benzene and methanol can be well fitted to a single-exponential model with $\tau = 9.67$ ns and 6.25 ns respectively. In mixtures of benzene-methanol the decays became heterogeneous, and the maximum of heterogeneity observed was in a mixture containing 6% methanol. Since we expected a distribution of Y_t -base solvation states in the solvent mixtures, and because the decay times of Y_t -base are sensitive to solvent, we analyzed the data in terms of decay time distributions. The goodness-of-fit for the unimodal distribution model which has two floating parameters was equivalent to that found using the double exponential model with three floating parameters. The Lorentzian distribution model appears to provide a slightly superior fit relative to the Gaussian distribution model. These results suggest that the intensity decays of solvent-sensitive fluorophores in mixed solvents are described by a distribution of decay times.

Keywords

Frequency-domain fluorometry; fluorescence decay; multiexponential analysis; Lorentzian distribution

1. Introduction

The application of fluorescence spectroscopic methods to biological samples often involves analysis of the intensity and anisotropy decay kinetics. The decay times are of interest because they can represent molecular features of the sample and can give information about the existence of two or more conformations of the biomolecules. They are also usually sensitive to the polarity of the environment surrounding the fluorophore. The intensity decays are generally more complex than a single exponential, and are usually analyzed in terms of a sum of discrete exponential decays (multiexponential model) [1–4]. Recently,

[†]Author to whom correspondence should be addressed.

continuous distributions of decay times were proposed [5–10] as alternative models for the sum of discrete exponentials.

To date relatively few systems have been described for which one can state with confidence that the decay is due to a distribution of decay times. These cases include transient effects in quenching, energy transfer [11] and conformational heterogeneity of proteins [12], but it should be noted that there are alternative models that account for the data from such systems and that these models contain a molecular description of the system. Lifetime distributions have also been proposed to account for the complex decays of single tryptophan proteins [10, 13], but to date there appears to be little statistical evidence to support acceptance of the distribution model. In the present paper we describe a system where one can predict a distribution of decay times, this being the decay of a solvent-sensitive fluorophore 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-1-imidazo-[1,2*a*]-purine (Y_T-base) (Scheme 1) in mixtures of benzene and methanol. Several papers have appeared [14–18] which describe the physicochemical properties of this modified nucleotide. It has been of special interest because of its strong fluorescence which offers a unique tool for probing the conformational properties of t-RNA^{phe} [19, 20], as well as more simple synthetic compounds such as Y_T-(CH₂)_{*n*}-adenine [21–23]. Our data suggest that the intensity decay of Y_T-base in benzene-methanol is in fact described by a distribution of decay times.

2. Theory

Fluorescence intensity decays are usually described as the sum of individual exponentials. The intensity decay following δ -function excitation is described by

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \quad (1)$$

where τ_i is the individual decay time and α_i is the associated pre-exponential factor. The fractional contribution of the i th component to the total emission is

$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \quad (2)$$

where α_i and f_i are normalized values; $\sum \alpha_i = 1$ and $\sum f_i = 1$. We also used an alternative model in which the α_i values are not discrete amplitudes at τ_i but are described by a continuous distribution $\alpha(\tau)$ around a central value $\bar{\tau}$. The intensity decay then contains

components of each lifetime τ with amplitude $\alpha(\tau)$. The component with each individual τ value is given by

$$I(\tau, t) = \alpha(\tau)e^{-t/\tau}$$

(3)

The total decay law is the sum of the individual decays weighted by the amplitudes

$$I(t) = \int_0^{\infty} \alpha(\tau)e^{-t/\tau} d\tau$$

(4)

where $\int \alpha(\tau) d\tau = 1$. The relationship between the two models (eqns. (1) and (4)) can be seen by noting that a Dirac distribution at τ_0 results in a single exponential decay

$$I(t) = \int_0^{\infty} \delta(\tau - \tau_0)e^{-t/\tau} d\tau = e^{-t/\tau_0}$$

(5)

One can expand $\alpha(\tau)$ to include several α -weighted δ -functions, which we refer to as bimodal, trimodal, etc., distributions. Then, application of eqn. (5) yields the multiexponential model.

We selected Gaussian (G) and Lorentzian (L) functions to describe unimodal lifetime distributions. For these functions the $\alpha(\tau)$ values are

$$\alpha_G(\tau) = \frac{1}{\sigma(2\pi)^{1/2}} \exp\left\{-\frac{1}{2}\left(\frac{\tau - \bar{\tau}}{\sigma}\right)^2\right\}$$

(6)

$$\alpha_L(\tau) = \frac{1}{\pi} \frac{\Gamma/2}{(\tau - \bar{\tau})^2 + (\Gamma/2)^2}$$

(7)

where $\bar{\tau}$ is the central value of distribution, σ is the standard deviation of the Gaussian and Γ is the full-width at half-maximum (hw) for the Lorentzian. For a Gaussian the full-width at half-maximum is given by 2.354σ . For ease of interpretation we describe the width of both distributions by the full-width at half-maxima. The use of functional forms for $\alpha(\tau)$ minimizes the number of floating parameters in our fitting algorithms. In the frequency-domain, the frequency response of the emission consists of the frequency-dependent values of the phase angle (ϕ_ω) and the demodulation factor (m_ω), where ω refers to the modulation frequency in rad s^{-1} . These values can be calculated from the sine (N_ω) and cosine (D_ω) transforms of the impulse response function. Using eqn. (4) these transforms are

$$N_\omega J = \int_{t=0}^{\infty} \int_{\tau=0}^{\infty} \alpha(\tau) e^{-t/\tau} d\tau \sin(\omega t) dt$$

(8)

$$D_\omega J = \int_{t=0}^{\infty} \int_{\tau=0}^{\infty} \alpha(\tau) e^{-t/\tau} d\tau \cos(\omega t) dt$$

(9)

$$J = \int_{\tau=0}^{\infty} \alpha(\tau) \tau d\tau$$

(10)

Reversal of the order of integration is possible for well-behaved functions with continuous derivatives, yielding

$$N_{\omega}J = \int_{\tau=0}^{\infty} \frac{\alpha(\tau)\omega\tau^2}{1 + \omega^2\tau^2} d\tau$$

(11)

$$D_{\omega}J = \int_{\tau=0}^{\infty} \frac{\alpha(\tau)\tau}{1 + \omega^2\tau^2} d\tau$$

(12)

For any form of the decay law the phase on modulation values are given by

$$\phi_{\omega} = \arctan(N_{\omega}/D_{\omega})$$

(13)

$$m_{\omega} = (N_{\omega}^2 + D_{\omega}^2)^{1/2}$$

(14)

The measured phase (ϕ_{ω}) and modulation values (m_{ω}) were analyzed by the method of non-linear least squares [24, 25]. Calculated values (c) were obtained using eqns. (8)–(14). The goodness-of-fit was judged by the value of reduced χ_R^2

$$\chi_R^2 = \frac{1}{\nu} \sum_{\omega} \left(\frac{\phi_{\omega} - \phi_{c\omega}}{\delta\phi} \right)^2 + \frac{1}{\nu} \sum_{\omega} \left(\frac{m_{\omega} - m_{c\omega}}{\delta m} \right)^2$$

(15)

where ν is the number of degrees of freedom and $\delta\phi = 0.2^\circ$ and $\delta m = 0.005$ are the uncertainties in the measured phase and modulation values, respectively. These uncertainties

are based on examination of single- and multi-component mixtures [24–26] under a wide variety of experimental conditions.

3. Materials and methods

The frequency-domain data were measured using a 2-GHz harmonic content fluorometer. The excitation wavelength was 310 nm from the frequency-doubled output of an R6G dye laser [26]. Magic angle polarizer orientations were used to eliminate the effects of rotational diffusion. The emission was observed through a Coming 0–52 bandpass filter, which absorbs all the emission and/or scattered light below 340 nm. The Y_I-base was prepared by reacting 3-methylguanine with bromoacetone [27, 28] and was purified by HPLC before the measurements. Benzene and methanol were spectroscopic grade. All measurements were recorded at 20 °C.

4. Results and discussion

Normalized absorption and fluorescence spectra of Y_I-base in benzene and methanol are shown in Fig. 1. The dependence of these spectra on solvent is unusual in that the absorption spectrum shifts to shorter wavelengths (hypsochromic) in the more polar solvent, while the emission spectrum displays the more usual red shift (bathochromic) as the solvent is changed from benzene (non-polar) to methanol (polar). These shifts in Y_I-base spectra can be explained on the basis of solvatochromic theories [29–32]. The dipole moment of the excited state of Y_I-base has a direction which is nearly opposite that of the ground state [33]. Also, the dipole moment changes from 3.5 D in the ground state to 4.3 D in the excited state.

In benzene and methanol the intensity decays are well approximated by the single-exponential model. This is shown in Fig. 2, which shows that the calculated single-exponential curves overlap with the data (top panel). Also, the residuals are randomly distributed (lower panels). The double-exponential analysis (Table 1) shows no decrease in χ_R^2 as compared with the single-exponential model, which indicates the single-exponential model is adequate to account for the data. We note that we can only estimate the standard errors in the data, so that the absolute values of χ_R^2 are not necessarily unity [24]. The goodness-of-fit is best judged by the relative values of χ_R^2 as the model is made more complex. For instance, the value of χ_R^2 for Y_I-base in methanol did not decrease for the one- or two-exponential model (Table 1). Hence $\chi_R^2 = 1.9$ is the noise-limited value of χ_R^2 for this particular set of data.

The intensity decay of Y_I-base becomes more complex in mixtures of benzene and methanol. This is seen from the increase in χ_R^2 found for the single-exponential model (Table 1). The maximum heterogeneity is seen near 6% methanol, where χ_R^2 is elevated about seven-fold relative to the double-exponential model. The changes in the emission of Y_I-base are realized with only a few per cent of methanol in the mixture. By 10% methanol the lifetime and emission become characteristic of that found in 100% methanol (Fig. 3). To distinguish between ground and excited state processes we determined the decay times at various emission wavelengths (Fig. 4). In 100% benzene or 100% methanol the decay time are independent of wavelength. In the mixture the mean decay times increase somewhat with

increasing emission wavelength. If the heterogeneity in the decay is due only to ground state interactions, then we expect the mean decay time to decrease with increasing observation wavelength, owing to an increasing contribution from the red-shifted methanol-like emission spectrum. The increase in decay time indicates that there is some detectable evolution of the emission during the lifetime of the excited state [32, 34]. In the binary solvent there exists a distribution of environments for the fluorophore, owing to the heterogeneous solvent shell. The statistics of such systems have been discussed by other researchers [35–37]. It seems probable that this shell changes dynamically during the lifetime of the excited state, with regard to the number, location and orientation of the methanol molecules.

Since we expected a distribution of Y_t -base solvation states we analyzed our data in terms of lifetime distributions. The results of this analysis, using unimodal Lorentzian and Gaussian distributions of $\alpha(\tau)$ are summarized in Table 2. Both models provide adequate fits to the data, but the Lorentzian model appears to be somewhat superior to the Gaussian model. The values of χ_R^2 are about identical to those found for the double-exponential fit. It should be noted that the distribution model contains only two floating parameters ($\bar{\tau}$ and hw), whereas the double-exponential model has three variable parameters (two τ_i and a_1 , with $a_1 + a_2 = 1.0$). In general we should accept the simplest model which accounts for the data, which in this case is the Lorentzian lifetime distribution.

The largest width in the distributions, and the largest heterogeneity, was found for the mixture containing 6% methanol. The phase and modulation data for this mixture are shown in Fig. 5. The full line shows the best Lorentzian fit and the broken line shows the fit when the half-width was held fixed at 0.01 ns, which corresponds to the single-exponential model. The lower panels show the residuals and it is clear that the data are well fitted by the distribution model, but not when the half-width is fixed at a small value.

The distributions recovered from the analysis are summarized in Fig. 6. These distributions are narrow in the pure solvents, and show increased widths at 6% and 10% methanol. We believe these distributions reflect the heterogeneity of solvation states about the Y_t -base molecules. Wider distributions were recovered from the Gaussian model than from the Lorentzian model. This result is characteristic of the models [11] and not of the samples. The Lorentzian model has significant amplitude at lifetime values distant from the mean lifetime. These amplitudes are suppressed by the fitting algorithm, resulting in apparently narrow distributions near the central value. In contrast, the Gaussian amplitudes decrease rapidly away from the mean, allowing a wider distribution near the center. As this time the data and our knowledge are not adequate to select either model as being a superior representation of the system.

We questioned the uncertainties in the half-widths by examining the χ_R^2 surfaces (Fig. 7). The half-widths were held constant at the value indicated in the X axis, while $\bar{\tau}$ was allowed to vary to minimize χ_R^2 . The broken line indicates the value of χ_R^2 expected 33% of the time owing to random errors, and intersection of the χ_R^2 surface with this line indicates the maximum range of hw values which are connected with the data. There is relatively little uncertainty in the half-widths, typically 0.3 ns. Clearly, the data for Y_t -base in the solvent

mixtures are not consistent with a zero half-width. These half-widths appear to be characteristic of the solvent composition.

5. Conclusion

Frequency-domain measurements of the time-dependent emission of Y_L-base, in mixtures of benzene and methanol, demonstrated the existence of a distribution of decay times. The single-modal distribution models with two variable parameters provided fits to the mixture data which were equivalent to the double-exponential model with three variable parameters. Additional theory and analysis is necessary to link these data to molecular and statistical properties of the solvent-fluorophore interactions.

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References

1. Grinvald A and Steinberg IZ, On the analysis of fluorescence decay kinetics by the method of least-squares, *Anal. Biochem*, 59 (1974) 583–598. [PubMed: 4838786]
2. O'Connor DV, Ware WR and Andre JC, Deconvolution of fluorescence decay curves: a critical comparison of techniques, *J. Phys. Chem*, 83 (1979) 1333–1343.
3. Demas JN, *Excited State Lifetime Measurements*, Academic Press, New York, 1983.
4. O'Connor DV and Phillips D, *Time-correlated Single Photon Counting*, Academic Press, New York, 1984.
5. James DR and Ware WR, A fallacy in the interpretation of fluorescence decay parameters, *Chem. Phys. Lett*, 120 (1985) 455–459.
6. James DR and Ware WR, Distributions of fluorescence lifetimes: consequences for the photophysics of molecules adsorbed on surfaces, *Chem. Phys. Lett*, 120 (1985) 460–465.
7. James DR and Ware WR, Multiexponential fluorescence decay of indole-3-alkanoic acids, *J. Phys. Chem*, 89 (1985) 5450–5458.
8. Alcalá JR, Gratton E and Prendergast FG, Resolvability of fluorescence lifetime distribution using phase fluorometry, *Biophys. J*, 51 (1987) 587–596. [PubMed: 3580485]
9. Alcalá JR, Gratton E and Prendergast FG, Fluorescence lifetime distributions in protein, *Biophys. J*, 51 (1987) 597–604. [PubMed: 3580486]
10. Alcalá JR, Gratton E and Prendergast FG, Interpretation of fluorescence decays in proteins using continuous lifetime distributions, *Biophys. J*, 51 (1987) 925–936. [PubMed: 3607213]
11. Lakowicz JR, Cherek H, Gryczynski I, Joshi N and Johnson ML, Analysis of fluorescence decay kinetics measured in the frequency-domain using distributions of decay times, *Biophys. Chem*, 28 (1987) 35–50. [PubMed: 3689869]
12. Gryczynski I, Eftink M and Lakowicz JR, Conformation heterogeneity in proteins as an origin of heterogeneous fluorescence decays, illustrated by native and denatured ribonuclease T₁, *Biochem. Biophys. Acta*, 954 (1988) 244–252. [PubMed: 3370216]
13. Alcalá JR, Marriott G, Gratton E and Prendergast FG, Fluorescence lifetime distributions in single tryptophan proteins, *Biophys. J*, 49 (1986) 108a (abstr). [PubMed: 19431605]
14. Eisinger J, Feuer B and Yamane T, Luminescence and binding studies in tRNA^{Phe}, *Proc. Natl. Acad. Sci. U.S.A.*, 65 (197) 638–644. [PubMed: 5267144]

15. Evleth EM and Lerner DA, Theoretical analysis of the photophysical properties of E-adenine and the RNA^{Phe} Y-base, *Photochem. Photobiol*, 26 (1977) 103–108. [PubMed: 905355]
16. Paszyc S and Rafalska M, Photochemical properties of the synthetic Y₇-base in aqueous solutions, *Nucleic Acids Res*, 6 (1979) 385–391. [PubMed: 424298]
17. Gryczynski I, Gryczynski Z, Kowski A and Paszyc S, Directions of the electronic transition moments of synthetic Y₁-base, *Photochem. Photobiol*, 39 (1984) 319–322.
18. Gryczynski I, Cherek H, Laczko G and Lakowicz JR, Enhanced resolution of anisotropic rotational diffusion by multi-wavelength frequency-domain fluorometry and global analysis, *Chem. Phys. Lett*, 155 (1987) 193–199.
19. Blumberg WE, Dale RE, Eisinger J and Zuckerman DM, Energy transfer in tRNA^{Phe} (yeast). The solution structure of transfer RNA, *Biopolymers*, 13 (1974) 1607–1620. [PubMed: 4608191]
20. Wells BD and Lakowicz JR, Intensity and anisotropy decays of the Wye base of yeast tRNA^{Phe} as measured by frequency-domain fluorometry, *Biophys. Chem*, 26 (1987) 39–43.
21. Gryczynski I, Kowski A, Paszyc S and Skalski B, Fluorescence quenching of Y₁-base and the model compounds Y₁-(CH₂)_n-adenine by α -bromonaphthalene, *J. Photochem*, 20 (1982) 71–76.22.
22. Gryczynski I, Kowski A, Paszyc S and Skalski B, Rotational motions of synthesized Y₁-base, free or bounded to adenine, in solvents of various viscosities, *Z. Naturforsch. TeilA*, 35 (1980) 1265–1267.
23. Gryczynski I, Kowski A, Paszyc S, Skalski B and Temperek A, Hypochromic effect and electronic excitation energy transfer in Y₁-(CH₂)_n-adenine systems, *J. Photochem*, 30 (1985) 153–165.
24. Lakowicz JR, Gratton E, Laczko G, Cherek H and Limkemann M, Analysis of fluorescence decay kinetics from variable-frequency phase shift and modulation data, *Biophys. J*, 46 (1984) 463–477. [PubMed: 6498264]
25. Gratton E, Lakowicz JR, Maliwal B, Cherek M, Laczko G and Limkemann M, Resolution of mixtures of fluorophores using variable-frequency phase and modulation data, *Biophys. J*, 46 (1984) 473–486.
26. Lakowicz JR, Laczko G and Gryczynski I, A2 GHz frequency-domain fluorometer, *Rev. Sci. Instrum*, 57 (1986) 2499–2506.
27. Kasai H, Goto M, Iheda K, Zama M, Mizuno Y, Takemura S, Sugimoto T and Goto T, Structure of Wye (Y₇-base) and Wyonine (Y₇) from *Torulopsis utilis* phenylalanine transfer ribonucleic acid, *Biochemistry*, 15 (1976) 898–904. [PubMed: 942858]
28. Gryczynski I, Kowski A, Nowaczyk K, Paszyc S and Skalski B, Temperature effect on the luminescence of synthesized Y₁-base in PV A film, *Biochem. Biophys. Res. Commun*, 98 (1981) 1070–1075. [PubMed: 7225126]
29. Lippert E, Spektroskopische Bestimmung des Dipolmomentes Aromatischer Verbindungen im Ersten Angeregten Singulettzustand, *Z. Electrochem*, 61 (1957) 962–975.
30. Bilot L and Kowski A, Zur Theorie des Einflusses von Lösungsmitteln auf die Elektrospektren der Moleküle, *Z. Naturforsch. Teil A*, 18 (1962) 621–627.
31. Bakhshiev NG, Universal molecular interactions and their effect on the position of the electronic spectra of molecules in two component solutions, *Opt. Spectrosc*, 10 (1961) 379–384.
32. Lakowicz JR, *Principles of Fluorescence Spectroscopy*, Plenum, New York, 1983.
33. Gryczynski I, Jung Ch., Kowski A, Paszyc S and Skalski B, Theoretical and experimental investigation on dipole moment of synthesized Y₁-base in the ground and excited states, *Z. Naturforsch. Teil A*, 34 (1979) 172–175.
34. Lakowicz JR and Balter A, Theory of phase-modulation fluorescence spectroscopy for excited state processes, *Biophys. Chem*, 16 (1982) 99–115.
35. Mazurenko JT and Udalsov VS, Spectral relaxation of fluorescence, *Opt. Spectrosc*, 44 (1978) 417–420.
36. Reichardt C, *Solvent Effects in Organic Chemistry*, Verlag Chemie, New York, 1979.
37. Kowski A, Die Solvathülle und ihr Einfluss auf die Fluorezenz, *Chimia*, 28 (1974) 715–723.

Fig. 1. Normalized absorption and fluorescence spectra of Y_t -base in benzene (—) and methanol (---) at 20 °C ($kK = 10^3 \text{ cm}^{-1}$).

Fig. 2.
Frequency-response data. of Y_t -base fluorescence in benzene and methanol. Full lines show the best single-exponential fit.

Fig. 3.
Dependence of the mean lifetime and emission maximum Y_t-base upon the percentage of methanol in benzene.

Fig. 4.
Dependence of mean lifetime of Y_t-base upon the wavelength of observation.

Fig. 5. Phase and modulation data for fluorescence of Y_t -base in mixtures containing 6% methanol. The full line shows the best fit to the Lorentzian distribution model, and the broken line shows the fit with the hw fixed to 0.01 ns.

Fig. 6.
Distribution of lifetimes of Y_t-base fluorescence in benzene-methanol mixtures: top,
Lorentzian unimodal; bottom, Gaussian unimodal.

Fig. 7.

Dependence of χ^2_R on the hw of the Lorentzian lifetime distribution for Y_t-base in mixtures of benzene-methanol. The values of χ^2_R are normalized to unity (χ^2_{RN}) about the minimum values.

Scheme 1.
Structure of Y₁-base.

TABLE 1Multiexponential analysis of the intensity decays of Y_I-base in benzene-methanol mixtures at 20 °C

<i>Per cent methanol</i>	τ_i (ns)	a_i	f_i	χ_R^2
0	9.67	1.0	1.0	1.3
	9.18	0.093	0.079	
	9.82	0.907	0.921	1.3
2	9.23	1.0	1.0	2.0
	0.74	0.023	0.002	
	9.30	0.977	0.998	1.1
4	8.80	1.0	1.0	3.2
	3.67	0.061	0.026	
	9.10	0.939	0.974	1.2
6	8.36	1.0	1.0	8.9
	3.50	0.113	0.048	
	8.92	0.887	0.952	1.2
8	7.84	1.0	1.0	7.5
	3.26	0.102	0.043	
	8.29	0.898	0.957	1.3
10	7.23	1.0	1.0	4.9
	2.99	0.082	0.034	
	7.56	0.918	0.966	1.0
15	6.75	1.0	1.0	3.0
	3.39	0.076	0.038	
	7.00	0.924	0.962	1.3
100	6.25	1.0	1.0	1.9
	5.72	0.408	0.374	
	6.61	0.592	0.626	1.9

TABLE 2

Lifetime distribution analysis of Y_I-base fluorescence decays in benzene-methanol mixtures at 20 °C

<i>Per cent methanol</i>	<i>Lorentzian</i>			<i>Gaussian</i>		
	$\bar{\tau}$ (ns)	hw (ns)	χ_R^2	$\bar{\tau}$ (ns)	hw (ns)	χ_R^2
0	9.67	0.07	1.3	9.67	1.23	1.3
2	9.27	0.64	1.3	9.24	2.76	1.4
4	8.83	1.05	1.1	8.81	3.56	1.3
6	8.38	1.92	1.2	8.36	4.68	1.6
8	7.81	1.52	1.2	7.83	4.13	1.5
10	7.19	1.00	1.1	7.23	3.33	1.3
15	6.71	0.54	1.5	6.74	2.46	1.5
100	6.24	0.11	1.9	6.25	1.05	1.9