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Mechanisms effecting analysis of volatile flavour components by solid-phase microextraction and gas chromatography

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Abstract

Quantitative properties of solid-phase microextraction (SPME) have been studied in order to investigate a simple and reliable method for analysing volatile flavour components in strawberries. Monitoring the chemical composition profile of berries will be of interest for the producers in order to optimise growth and storage conditions. By the use of SPME and capillary gas chromatography selected standard components were quantified with accuracy within ±7% and a linear response were found in all concentration ranges studied, covering three orders of magnitude. Equilibrium constants that describe how various components are distributed between the three phases present, sample, headspace and fibre coating were determined. In the system studied, the majority of analytes remained in the sample. This means that repeated analysis can be performed from a single sample without significantly changing the results. The mass transfers of the flavour components, from the sample and into the fibre, were fitted to a transport model assuming that the rate-controlling step is diffusion within the fibre. The experimental results agreed well with the model for most of the components studied. The response for three of the components (geraniol, linalool and *trans*-2-hexenyl butanoate) did not agree with the model. These components were present in the gas phase in only minute amounts explaining the deviation from the model. Such components will require a long absorption time (longer than 30 min). For quantitative analysis, it is important to use a very precise pre-determined absorption period and well defined sampling conditions. Internal standards can be omitted.

Keywords: Food analysis; Solid-phase microextraction; Fruits; Aroma compounds; Volatile organic compounds

1. Introduction

Solid-phase microextraction (SPME) introduced by Pawliszyn in 1990, involves absorbing the analytes on a fibre coating immersed into the sample (usually the gas phase above the sample) [1,2]. After equilibration the fibre is removed from the sample and the analytes are thermally desorbed in the injector of a gas chromatograph. Since SPME is easy to use, simple, rather inexpensive, and involves no organic solvents, this method is gaining widespread interest. With a reliable method for analysing volatile flavour components, both quantitatively and qualitatively, the analyst possesses a tool to monitor the profile of the flavour components, e.g., in strawberries using different growth conditions and storage environments. The strawberry fruit is a dynamic system whose chemical composition changes constantly due to enzymatic and chemical processes, both during ripening and storage. Components re-

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sponsible for the strawberry flavour constitute a very small part of the berry, and they cover a large range of chemicals like esters, alcohols and carbonyls. Volatiles in wild European strawberries have a very different composition to than that of cultivated species, and there are significant differences between cultivars [3–6]. For qualitative analyses of strawberry volatile flavour, SPME has proven to be a valuable sample preparation technique. SPME reveals all key flavour components in the berries [7–9], and no artefacts due to sample preparation are formed, as seen for the purge and trap method [9].

The purpose of this study was to investigate the quantitative abilities of the SPME method in order to define criteria that can ensure reproducible analyses. It has also been an aim of this work to investigate factors affecting the transport and equilibrium properties of the system.

2. Theory

2.1. Phase equilibrium

During sample preparation by use of SPME, the fibre was immersed in the gas phase above the sample to be analysed. At equilibrium, the amount of any volatile component, i, initially present in the sample will be distributed in the three phases present according the equation:

$$C_{0,i}V_s = C^{\infty}_{f,i}V_f + C^{\infty}_{h,i}V_h + C^{\infty}_{s,i}V_s \quad (1)$$

where $C_{0,i}$ is the initial concentration of the analyte in the sample solution, V_s is the volume of the sample. $C^{\infty}_{f,i}$, $C^{\infty}_{h,i}$ and $C^{\infty}_{s,i}$ are the equilibrium concentrations of analyte, i, in the fibre, headspace and sample, respectively. V_f and V_h are the volumes of the fibre and headspace. At equilibrium, the ratio of the concentration of a component in each of the three phases can be described by the equilibrium constants:

$$K_{fh,i} = C^{\infty}_{f,i}/C^{\infty}_{h,i}$$
$$K_{hs,i} = C^{\infty}_{h,i}/C^{\infty}_{s,i} \quad (2)$$
$$K_{fs,i} = C^{\infty}_{f,i}/C^{\infty}_{s,i}$$

where $K_{fh,i}$, $K_{hs,i}$, $K_{fs,i}$ are the equilibrium constants for component i between the fibre and headspace, between the headspace and sample and between the fibre and sample, respectively. It is assumed that activities can be represented by concentrations. The mass of the analyte absorbed by the fibre coating, $n_i = C^{\infty}_{f,i}V_f$, can be expressed as:

$$n_i = \frac{K_{fs,i}V_f C_{0,i}V_s}{K_{fs,i}V_f + K_{hs,i}V_h + V_s} \quad (3)$$

The fibre may be placed either in the headspace or directly in the sample solution [2]. The three terms in the denominator of Eq. (3) give a measure of the capacity of the fibre ($K_{fs,i}V_f$), headspace ($K_{hs,i}V_h$) and sample (V_s). Because of the small volume of the fibre coating, typically about 10^{-3} ml, analytes are, in most cases, only partially extracted.

2.2. Time dependent effects in mass transfer

Fig. 1 illustrates the concentration profile of a given analyte in a sample–headspace–fibre coating system, some time after the fibre has been immersed into the headspace. It shows how mass (analyte) is transferred from the sample, through the gas phase and into the fibre coating. All scaling is arbitrary, chosen to illustrate the process. The liquid and gas phases are well stirred and, therefore, are assumed to be uniform in concentrations.

Interphase mass transfer between liquid and gas, and gas and fibre surfaces occur by diffusion through thin, stagnant films on the fluid surfaces. The concentration differences across the films are the driving forces for the transport. This model of interphase transport processes is often called the film theory

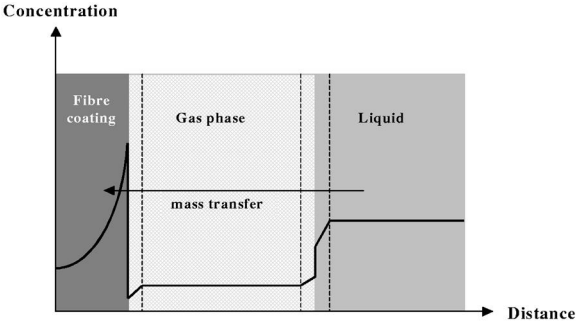

Fig. 1. Concentration profiles in multiphase transport system comprising sample, headspace and fibre coating.

[10]. The steps in concentration at the phase boundaries are defined by the phase equilibrium constants. The mass transfer stops when equilibrium is reached. The distribution of an analyte in the various phases is at that point determined from the total mass, phase volumes and the equilibrium constants.

It is now assumed that the amount of analyte initially present in the sample is large compared to what can be extracted by the headspace and fibre. Furthermore, it is assumed that the mass transfer between sample and headspace is fast compared to the transport in the fibre, and the diffusion coefficient in gas and liquid are larger than in the fibre [2]. It is also assumed that the mass transfer through the gas film surrounding the fibre coating is fast compared to transport in the interior of the fibre coating.

With all the assumptions above, the transport problem is reduced to a system where the fibre is inserted into a perfectly agitated gas of infinite volume. In this system, the concentration of any analyte on the surface of the fibre will be invariant with time. The mass transfer process is entirely determined by what happens inside the fibre coating. Louch et al. [11] described the dynamics of this transport system mathematically. Their starting point is Fick's second law that describes the change in concentration inside a volume element with time:

$$\frac{1}{D_f}\cdot\frac{\partial C}{\partial t}=\frac{1}{r}\cdot\frac{\partial C}{\partial r}+\frac{\partial^2 C}{\partial r^2} \tag{4}$$

where D_f is the diffusion coefficient for the component in question in the fibre coating. Cylindrical co-ordinates are used since the concentration profile along the length axis of the fibre coating (cylinder) is invariant and the concentration does not vary with the radial angle from the centre of the fibre. Further, the mass transport through the end surface of the coating is neglected. The solution of the differential Eq. (4) with the appropriate boundary conditions corresponding to the model will describe the concentration profile as function of time at any position, r, from the centre of the fibre.

Here, one consequence of the solution of the system will be retrieved from the more complete solutions given by Louch et al. [11] and Pawliszyn [2]. This is a graph that shows the amount of any component absorbed at a given time, t (expressed as an average concentration in the fibre coating, C), relative to the amount absorbed at complete equilibrium (C_∞) versus a dimensionless time, $D_f t/(b-a)^2$. The graph is shown in Fig. 2. Numerical values to make the graph were taken from Pawliszyn (Fig. 3.5 in Ref. [2]). The profile shown is valid for any analyte and any type of fibre, as long as the assumptions made for simplifying the system are valid. The figure also shows a fit function to the numerical values of the exact solution. This fit function has the general form:

$$\frac{C}{C_\infty}=1-\exp\left[k_1 D_f t/(b-a)^2\right]^{k_1} \tag{5}$$

where $(b-a)$ is the thickness of the fibre coating. The parameters k_1 and k_2 were estimated in this work to be 5.806 and 0.78, respectively. This gave a simple expression relating absorption in the fibre coating and time.

With basis in Fig. 2, equilibrium criteria for the absorption process may be defined. If, for example, the criteria are set such that a minimum 99% of maximum absorption capacity of the fibre coating is to be satisfied ($C/C_{inf}=0.99$), Fig. 2 shows that the dimensionless time must be 0.8. Solving for the real time in the expression for dimensional time, the result is:

$$t=0.8(b-a)^2/D_f \tag{6}$$

The minimum equilibrium time can, thus, be

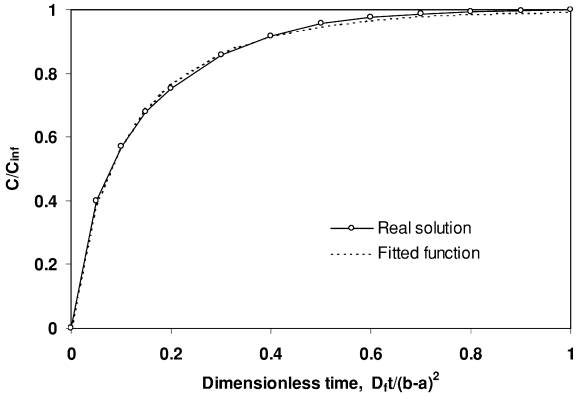

Fig. 2. Mass adsorbed by fibre coating vs. time from a perfectly agitated fluid of infinite volume. The data points for the real solution are from Pawliszyn [2].

calculated if the thickness of the fibre coating and the smallest diffusion coefficients of the components of interest are known. The use of Eq. (6) requires that the transport model used is valid for all components under study. If other transport steps are controlling the absorption rate, other and more complicated equilibrium criteria may be used [2].

3. Experimental

3.1. Standard samples

All chemicals used were of GC and HPLC grade (95–99% purity) from Merck, (Merck Eurolab A/S Oslo, Norway), Fluka, Sigma and Aldrich (Sigma-Aldrich Norway A/S). Standard components were pre-diluted in distilled water–ethanol mixtures. Various amounts were added to a 10-ml glass crimp seal vial with 5 g of ''strawberry syrup''. The syrup was made to simulate a strawberry matrix without any flavour components present [9]. A magnet bar was added to each vial before it was sealed.

3.2. Solid phase microextraction sampling

The SPME device and fibres were purchased from Supelco (Bellefonte, PA, USA). The stationary phase used was polydimethylsiloxane (PDMS) of 100-μm thickness and lot number 246544L. The diameter of the silica fibre core was 100 μm, the thickness of the coating 100 μm and the length 10.6 mm, defining the coating volume as $V_{\text{fibre}} = 0.666$ μl. Samples were equilibrated for 2 h at room temperature. The fibre was inserted through the vial septum and introduced into the headspace. A series of standard samples were analysed using different and predetermined sampling times. A desorption time of 2 min at 240°C was used during splitless injection. The fibre was left in the injector for an additional 10 min to completely remove residues of adsorbed components.

3.3. Direct gas sampling

The sample of interest (40 g) was equilibrated in a sealed glass vial (100 ml) for 1–2 h. From the headspace, 30 ml of gas was aspirated with a gas-tight syringe and injected into the pre-evacuated loop of a gas sampling injection system. The loop was filled with 1 ml of gas at 37°C and 1 atm (1 atm = 101 325 Pa). The loop-content was injected automatically into the column when requested by the GC-data program used for this analysis.

3.4. Direct sampling of liquid phase

Mixtures of various standard components were directly diluted in 96% ethanol. Splitless injection was used and a 1.0-μl liquid sample was injected into the GC system by means of a 10-μl Hamilton syringe as described by Sandra [12].

3.5. Instruments and operating conditions

The gas chromatograph from Hewlett-Packard, 5880A, was equipped with a flame ionisation detection (FID) system. In addition to a split/splitless injection system, the apparatus was equipped with a special device for gas injection. Helium (gas-flow = 1 ml/min) was the carrier gas. The fused-silica column used was 50 m × 0.2 mm I.D., coated with a 0.5-μm non-polar stationary phase, HP PONA, made of cross-linked methyl silicon gum. The column temperature was held at 35°C for 5 min, increased to 90°C at a rate of 3°C/min, further increased to 190°C at a rise of 10°C/min, and kept at 190°C for 20 min. When the GC system was operated using the split mode, the split ratio was set to 1:100. When a splitless injection was performed, the splitless time was set to 2 min. Both the injector and the detector temperatures were 225°C.

4. Results and discussion

4.1. Determination of phase equilibrium constants

Standard solutions were analysed both by SPME and direct gas phase sampling. The concentrations of the various components found in the equilibrium phases were stated and equilibrium constants calculated.

Using the average equilibrium constants given in Table 1, the distribution of the components in a standard mixture between the equilibrium phases was calculated. The volume of fibre was $6.67 \cdot 10^{-7}$ l and

Table 1
Equilibrium constants for the distribution of components between the fibre and liquid sample phases, K_{fs}, and for the distribution of components between the headspace gas and sample liquid phases, K_{hs}

Component number	Component name	Average K_{fs}	Average $K_{hs} \cdot 10^{-6}$
1	Ethyl butanoate	1.2	1200
2	*trans*-2-Hexen-1-ol	0.4	91
3	Ethyl pentanoate	2.5	440
4	Methyl hexanoate	5.0	560
5	3-Methylbutyl propanoate	6.8	330
6	Ethyl hexanoate	13.2	280
7	Linalool	1.5	1
8	*trans*-2-Hexenyl butanoate	32.7	11
9	Geraniol	0.6	–

the volumes of the headspace and liquid sample were 12 and 5 ml, respectively. The results are given in Table 2.

There are several interesting items associated with the results in Table 2. Firstly it is seen that the majority of the masses of all the components resides in the liquid, i.e., in the sample. Even for the component most abundant in the fibre, *trans*-2-hexenyl butanoate, the mass in the fibre coating amounts to only 0.4% of the mass in the sample. For the other components the mass fraction in the coating is considerably less. This means that several parallels can be withdrawn from the sample without changing the results of the analysis significantly. In case a large number of parallels should be needed, the sample volume could be increased compared to the fibre coating volume or a correction could be made for the withdrawal of mass from the sample. Eq. (3) shows that the mass absorbed in the fibre coating is proportional to the amount in the sample. The arguments above are valid for the present components irrespective of the concentrations of the individual components in the sample.

A second item of interest is the affinity of the fibre to the various components seen in relation to their presence in the headspace. As seen in Table 2, linalool, *trans*-2-hexenyl butanoate and geraniol are hardly, or not at all, detectable by injection of the headspace gas. Nevertheless, all three components are present in the fibre-coating phase in easily detectable amounts. This demonstrates one property of SPME, it is the sample–fibre equilibrium that determines the amount absorbed, and the gas–sample equilibrium becomes a less important factor in this context. However, all components must be transported through the gas phase in the present set up, and the gas–liquid sample equilibrium becomes an important issue in that process. When comparing the amounts of the components in the headspace (gas) with the amounts in the fibre coating, it must be kept in mind that the gas phase volume was 12 ml. During direct injection of a gas sample in a GC system, the sample volume is typically 1 ml. Thus, the use of SPME increases the amount injected of all components compared to direct gas injection, with an exception for the volatile ethyl butanoate.

Knowledge of the magnitude of the equilibrium

Table 2
Distribution of components between fibre coating, headspace and liquid sample phase at equilibrium during sampling from a standard mixture

Component	Initial conc.		Mass in phase (μg)		
	C_0 (ug/l)	Liquid start	Fibre	Headspace	Liquid
Ethyl butanoate	48355	241.8	0.048	0.6	241.1
trans-2-Hexen-1-ol	12206	61.0	0.0034	0.01	61.0
Ethyl pentanoate	15710	78.6	0.032	0.08	78.4
Methyl hexanoate	13547	67.7	0.054	0.08	67.6
3-Methylbutyl propanoate	13752	68.8	0.075	0.06	68.6
Ethyl hexanoate	12010	60.1	0.13	0.05	59.9
Linalool	45135	225.7	0.046	ND[a]	225.6
trans-2-Hexenyl butanoate	11619	58.1	0.22	ND	57.9
Geraniol	48238	241.2	0.011	ND	241.2

[a] ND = Not detectable.

constants in the system under study will be a useful tool for experimental design. If repeated analyses are desired, the volume of the phases and the volume of the fibre could be selected at sizes that would ensure insignificant component withdrawals for each analysis.

4.2. Absorption time and equilibration

The kinetics of the absorption process were studied by making a SPME–GC analysis with different absorption times for the fibre in the sample vial. Besides the absorption times, all analytical parameters were kept as constant as possible. Each series of analyses for every standard component should follow a response curve as illustrated in Fig. 2. The quantity C/C_∞ can directly be replaced with the ratio A/A_∞, where A is the area measured for a component at time t and A_∞ is the area obtained at an infinite equilibrium time. In the expression for the dimensionless time, $D_f\ t/(b-a)^2$, the diffusion coefficient in the fibre coating, D_f, is unknown.

Diffusion coefficients of a solute in liquid can be estimated as done by Reid et al. [13]:

$$D = 7.4 \cdot 10^{-8} \cdot \frac{(\phi M_B)^{0.5}}{\mu_B} \cdot \frac{T}{V_A^{0.6}}\ (\mathrm{cm}^2/\mathrm{s}) \qquad (7)$$

where ϕ is a dimensionless association factor for the solvent that varies from 2.6 for the strongly polar fluid water, via 1.9 and 1.5 for, respectively, methanol and ethanol to 1 for unassociated fluids like simple hydrocarbons; M_B is the molecular mass for the solvent polydimethylsiloxane; μ_B is the viscosity of the solvent; V_A is the molar volume of the solute at its normal boiling point and T is the absolute temperature.

From Eq. (7), it is seen that besides the molar volume, V_A, all the other parameters are invariant for the different standard components. Therefore, in the fitting of each series of experimental results to the kinetic model, Eq. (8), there are only two unknown quantities, A_∞ and $(\phi M_B)^{0.5}/\mu_B$. The first quantity will vary for each component and experimental series. The latter quantity is, however, a common quantity for all components and experimental series. By curve-fitting the experimental data, a theoretical

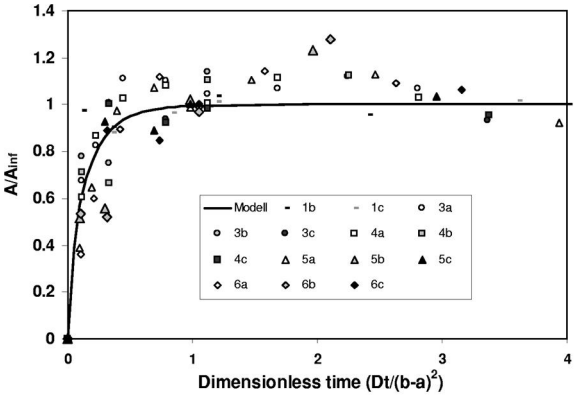

Fig. 3. Relative area response vs. dimensionless time. Experimental results for components 1–6 were used for parameter estimation. Only data points used in the estimation are plotted.

response, A/A_∞, was calculated for all values of the dimensionless time as expressed by Eq. (8):

$$\frac{C}{C_\infty} = \frac{A}{A_\infty} = 1 - \exp\left[-k_1 D_f t/(b-a)^2\right]^{k_2} \qquad (8)$$

Figs. 3 and 4 show the results from the analyses displayed as relative area vs. dimensionless time found by parameter estimation. Eq. (8) was used with values of 5.086 and 0.780 for k_1 and k_2, respectively. With the fibre and chemical systems used in this work, a dimensionless time of 1 correspond to approximately 20 min. The numbered items

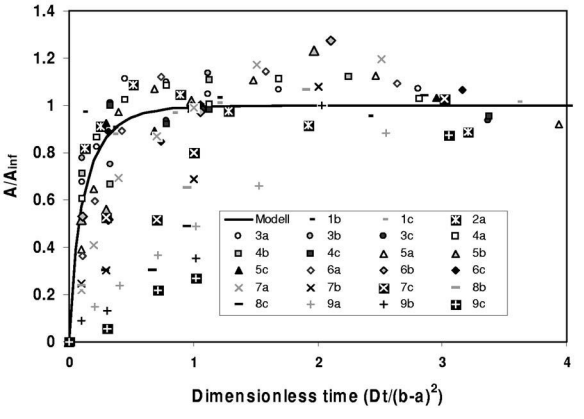

Fig. 4. Relative area response vs. dimensionless time. Experimental results for components 1–6 were used for parameter estimation. All experimental points shown.

in Figs. 3 and 4 correspond to the various standard components listed in Table 1, and the letters *a*, *b* and *c* designates various series of analyses performed on each standard component.

It was seen that the responses of components 7–9 on average were slower than the model response. The parameter estimation procedure was, therefore, repeated, but now only the responses for components 1–6 were used in determination of *dt*. The result is shown in Figs. 3 and 4. The former figure shows only the data points used in the parameter estimation, whereas all data points are included in Fig. 4.

The different responses for components 7–9 compared to the others are clearly evident in Fig. 4. From Table 2, it is seen that these three components, linalool, *trans*-2-hexenyl butanoate and geraniol, are only present in low or no amounts at all in the gas phase. Thus, the transport model employed, assuming that the mass transfer rates through the liquid sample and the gas phase are infinite rapid, is not valid for these components. Due to the low amounts of these components in the headspace, the mass transfer step through the gas will take a finite time and the total mass transport from the sample to the fibre coating takes longer than the components more abundant in the gas phase. As seen in Fig. 4, the data points for linalool, *trans*-2-hexenyl butanoate and geraniol fall under the modelled response. This is the expected behaviour for these three more slowly transported components.

4.3. Repeatability of component area response

A large number of analyses were performed over an extended time period. Single components as well as mixtures of components were used. The area response for the different components was calculated with a repeatability of between 3 and 7% (RSD) for various components. With better controlled experimental procedures with regard to factors, such as temperature control, sample stirring intensity, placement of fibre in the vial and equilibration time, it is believed that there is a potential for further improvement in quantitative reproducibility.

It is necessary completely to desorb the fibre between each analysis to avoid ''carry over'' of components from one analyse to the next. This could be done in the injector or in a separate heated chamber with some flow-through of gas [9].

The linearity of the area response to component concentration was tested using pure standards of variable concentration (covering three orders of magnitude) dissolved in strawberry syrup. The calibration curves exhibited good linearity in the concentration ranges tested. Regret ion coefficients (r^2) values were 0.999, 0.993, 0.998 and 0.983 for *trans*-2-hexen-1-ol, geraniol, linalool and methyl hexanoate, respectively, for calibration curves passing through the origin. Similar experimental series demonstrate that accurate analyses can be made with SPME–GC as long as great care is taken to keep the experimental conditions well defined.

5. Conclusions

In this work quantitative aspects of the method were investigated in connection with the analysis of volatile flavour components in strawberries.

It has been shown that the area response increased linearly with increasing concentration in the ranges tested. The area responses of the various standard components were reproducible within 3–7% of the average values. There is, however, potential for improvement with a well defined analytical procedure. Internal standards will not be necessary.

During SPME sampling, the majority of analytes remained in the sample. This means that repeated analysis might be taken from a single sample without significantly changing the sample composition.

The absorption kinetics showed that diffusion within the fibre was the rate-controlling step in the absorption process for most of the standard components used, except for geraniol, linalool and *trans*-2-hexenyl butanoate. The latter three components were present in the gas phase in only minute amounts and the deviation from the transport model employed could, thus, be expected.

The following aspects regarding the SPME absorption procedure should be taken into account and standardised: the temperature during absorption, size of the gas tight vial, size of the stirring magnet bar and stirring intensity. The absorption time should be strictly defined and kept constant for all analyses, samples as well as standards for calibration. For

analysing strawberries, for example, it may be impractical to wait for complete equilibrium. In such cases, it is of ultimate importance to be using an exact absorption time.

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