1

Introduction

Time flies over us, but leaves its shadow behind.

Nathaniel Hawthorne (1804–1864)

1.1 Time in Chemistry

According to one definition, time is “the indefinite continued progress of existence and events in the past, present, and future regarded as a whole” [1]. For millennia, time has intrigued philosophers and artists. The inevitability of time flow has triggered frustration and hope. While basic chemical knowledge was gathered in antiquity, modern chemistry has been developed since the 17th century. During the first ∼200 years, the notion of time in chemistry was obscure and elusive. It appeared in the spotlight when Peter Waage and Cato Guldberg began to develop the concept of chemical kinetics. Since then, the time dimension was instantly promoted to become an important factor in chemical reactions. In modern chemistry textbooks, potential energy diagrams often represent changes in the energy of reactants along an axis labeled as the reaction path. However, time is the variable that describes the progress of every chemical transition and physical process. Thus, time has always been among the key factors studied in chemical science [2]. Investigating chemical phenomena in relation to time has turned out to be vital for the understanding of fundamental concepts – in particular, reaction kinetics.

Temporal resolution is the ability of a method to discern consecutive transitions in the studied dynamic systems. In the field of analytical chemistry, there exist numerous methods that allow one to measure concentrations of substances in solutions or gaseous mixtures at given time points. However, many conventional methods possess limited temporal resolution. In some cases, samples are obtained from reaction mixtures at specific time points. As a result the temporal characteristics of the studied process can only be described considering the limited frequency of sampling points. The obtained samples can
be regarded as *zero-dimensional*. We live in a *four-dimensional* world that is described by three spatial dimensions. Time is the fourth elusive dimension that describes happenings in the other three dimensions (change of position). Various novel analytical methods have been developed to grasp the 3D nature of chemistry. For example, optical methods are irreplaceable when it comes to 2D and 3D spatial analysis (imaging) of chemical processes [3, 4]. Frequently these methods also provide superior temporal resolutions. Due to numerous technical obstacles, advancement of these multidimensional analysis tools could only happen because of the developments in physics, optics, photonics, and physical chemistry.

Some physical or chemical processes are so fast that their existence can only be verified using highly refined analytical approaches. For example, using infrared (IR) spectroscopy and computational methods, it was possible to confirm the existence of the simplest Criegee intermediate \( \text{(CH}_2\text{OO)} \) which has a lifetime counted in microseconds [5]. Other processes (e.g. radioactive decay of uranium-238 with a half-life of \( \sim 4.5 \times 10^9 \) years) are so slow that their progress cannot easily be observed during a human lifetime. However, most reactions occurring in the biological world are accelerated by biocatalysts (enzymes). Such catalytic processes can be observed on timescales of seconds and minutes. Reaction kinetics encompasses experimental methodology and the associated mathematical treatment aiming to describe the progress of chemical reactions in time. Understanding chemical kinetics can help us to optimize important reactions, so that they can be applied in large-scale synthesis, and used by industry. The kinetic profiles of reactions let us gain fundamental insights on the reaction mechanisms. Similarly, to chemical reactions, there exist other processes which serve chemists every day – distillation and extraction are just two examples. Studying kinetic properties of dynamic processes involving molecules requires the use of appropriate analytical methodology – capable of recording molecular events in the time domain.

Several physical techniques were introduced to chemistry laboratories in order to enable monitoring of chemical and physical processes in time. They include such dissimilar platforms as: fluorescence detection [6], IR spectroscopy [7], diffraction [8, 9], nuclear magnetic resonance (NMR) [10–12] as well as crystallography [13]. Since ultrafast phenomena are relevant to many fundamental studies in physics and chemistry [14], various spectroscopic techniques have been developed which enable investigation of molecular events in the time range from \( 10^{-9} \) to \( 10^{-18} \) s [15, 16]. Ultrafast IR and Raman spectroscopies enable measurements of phenomena which occur on the pico- and femtosecond timescales (1 fs = \( 10^{-15} \) s); corresponding to the elementary steps that affect chemical reactivity, including changes in the electron distribution, molecular structure and translocation of chemical moieties [17]. Pulse fluorometry and phase-modulation fluorometry enable the measurement of fluorescence lifetimes, which typically last over \( 10^{-8} – 10^{-11} \) s [18, 19]. Time-resolved luminescence methods are routinely used in fundamental and applied sciences. For instance, by monitoring the intensity of light emitted following an excitation pulse in the nano- to millisecond range, one can distinguish contributions of de-excitation of individual fluorophores and/or phosphors. This approach enables sensitive detection of labeled molecules (or supramolecular probes) in complex biological samples which possess intrinsic luminescence (e.g., autofluorescence). Lifetime spectroscopy is nowadays almost routinely used in the analysis and imaging of biological specimens [20].

Optical methods have grounded their place in chemistry. They also have intrinsic limitations: the most prominent one is low molecular selectivity. Monitoring unknown substances and identification of unknown analytes, which are frequently present in complex
mixtures, often requires powerful analytical strategies. Two particularly significant ones are NMR and mass spectrometry (MS). These two techniques have dissimilar principles: while MS separates and detects ions in the gas phase, NMR recognizes nuclei based on the characteristic electromagnetic radiation emitted by them at specific conditions in a magnetic field. Due to its versatility in structure elucidation, it is certainly worthwhile studying the principles and applications of NMR in chemistry with the aid of recent monographs (e.g., [21, 22]).

1.2 Mass Spectrometry

MS is one of the main techniques used in chemical analysis nowadays [23–28]. Due to its capabilities in molecular identification and structure elucidation, as well as high sensitivity [29], it has already enabled important discoveries in chemistry and the biosciences. A mass spectrometer consists of three main parts: an ion source, a mass analyzer, and a detector (Figure 1.1). They are supplemented with a number of auxiliary components, including interfaces for sample pre-processing and introduction, ion optic elements for manipulating ion beams, and electronic data acquisition systems.

In the gas phase, it is generally easier to handle and separate ions than neutral molecules. Gas-phase ions are produced in the ion source (see Chapter 2), while the mass analyzer subsequently separates them according to their characteristic mass-to-charge ratios \((m/z)\). There are various types of mass analyzers (see Chapter 3). For example, in a quadrupole mass analyzer, ions pass through a zone between two pairs of metal rods spaced radially along the ion propagation axis. The ratio of alternating and direct current electric fields produced in this section enables selection of ions with a specific \(m/z\) value, and discrimination of other ions. A detector – positioned “at the end” of the quadrupole (on the side opposite to the ion source) – counts the ions which have passed through the quadrupole zone. In order to prevent collisions of gas-phase ions with gas molecules, analyzers and detectors are held under high vacuum. Some ion sources also operate under vacuum while in others the process of ion generation occurs at a higher pressure up to atmospheric pressure.

MS has evolved in the past few decades. There have been several milestone discoveries and inventions (Table 1.1). They encompass fundamental physical phenomena related to ion formation, as well as technical aspects – including the construction of ion sources and mass analyzers. Since the operation of these two components is crucial for the measurements conducted in the time domain, they will be discussed extensively in the following chapters.

![Figure 1.1](image-url) Main components of a mass spectrometer. Reproduced from Kandiah and Urban [29] with permission of The Royal Society of Chemistry
Table 1.1  Selected milestones in MS

<table>
<thead>
<tr>
<th>Year</th>
<th>Discovery/Invention</th>
<th>Credited inventor</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>1897</td>
<td>Electron</td>
<td>J.J. Thomson</td>
<td>[48]</td>
</tr>
<tr>
<td>1918</td>
<td>Electron ionization, first modern mass spectrometer</td>
<td>A. Dempster</td>
<td>[49]</td>
</tr>
<tr>
<td>1919</td>
<td>Accurate determination of the masses of individual atoms</td>
<td>F. Aston</td>
<td>[50]</td>
</tr>
<tr>
<td>1946</td>
<td>Time-of-flight analyzer</td>
<td>W.E. Stephens</td>
<td>[51]</td>
</tr>
<tr>
<td>1953</td>
<td>Ion trap analyzer</td>
<td>W. Paul</td>
<td>[52]</td>
</tr>
<tr>
<td>1957</td>
<td>Time-resolved mass spectrometry of flash photolysis</td>
<td>G.B. Kistiakowsky, P.H. Kydd</td>
<td>[36]</td>
</tr>
<tr>
<td>1960</td>
<td>Quadrupole analyzer</td>
<td>W. Paul</td>
<td>[53]</td>
</tr>
<tr>
<td>1984</td>
<td>Electrospray ionization</td>
<td>J. Fenn</td>
<td>[54]</td>
</tr>
<tr>
<td>1985</td>
<td>Matrix-assisted laser/desorption/ionization</td>
<td>F. Hillenkamp</td>
<td>[55]</td>
</tr>
<tr>
<td>2000</td>
<td>Orbital ion trap</td>
<td>A. Makarov</td>
<td>[56]</td>
</tr>
</tbody>
</table>

A mass spectrum is the primary result of mass spectrometric analysis. The pioneer of MS – Francis Aston – recorded mass spectra of separated ions by exposing a photographic emulsion to the ion stream: the resulting dark bands marked the regions corresponding to the beams with high ion intensities (Figure 1.2a). The positions of these bands along the horizontal axis of such a “radiogram” are related by the $m/z$ of the impinging ions: the darker the band the higher the intensity of the ion beam. Modern mass spectrometers use electronic sensors to measure the intensities of ion fluxes – in space, in time, or in both

![Figure 1.2](image-url)  

(a) Mass spectra obtained by Aston using the early mass spectrometer. Reproduced from Squires [50] with permission of The Royal Society of Chemistry. (b) Mass spectrum of caffeine obtained using a modern ESI-IT mass spectrometer. Courtesy of E.P. Dutkiewicz
intensities are classified into discrete channels. In the course of mass calibration, these channels are related by the \( m/z \). As a result of this operation, a mass spectrum is produced (Figure 1.2b). It can be viewed as a histogram representing ion counts (intensities) in very narrow intervals of the \( m/z \) scale.

### 1.3 Time-resolved Mass Spectrometry

In biochemistry it is often necessary to capture changes in the metabolic composition of samples (e.g., [30, 31]). For instance, animals communicate with each other chemically by releasing signaling molecules called pheromones, which persist in the proximity of an individual for a short period of time [32]. In the domain of exact sciences, it is also important to study temporal changes of chemical systems (reactions) and physical phenomena (e.g., molecular transport). Although many mass spectrometric analyses are often conducted as single-time-point measurements, it is evident from numerous reports published over the past few decades that MS can facilitate the studies of dynamic processes in which concentrations or structures of analyte molecules change over time. In particular, the technique enables structural determination of reactants while preserving temporal resolution [33]. While other techniques – notably, electronic and vibrational spectroscopy – can record time-dependent data, time-resolved mass spectrometry (TRMS) provides orthogonal information (\( m/z \) vs. abundance vs. time) to those of other established techniques. Since short-living reaction intermediates can be detected by various MS methods, this technique is ideal for studies on mechanisms of chemical reactions, chemical kinetics, and biochemical dynamics.

Dynamic phenomena have been investigated with MS for half a century now (e.g., [34–37]). Ions cannot move as fast as photons while mass spectrometers typically operate in the microsecond regime. Therefore, MS cannot equalize temporal resolutions achieved with some optical techniques (which are currently limited by the speed of detection systems rather than the speed of photons). Temporal resolution is a critical limitation when it comes to identifying reaction intermediates and measuring reaction kinetics by MS [38]. As it will be evident later on, the speed of MS is currently limited by the speed of sample processing rather than the speed of ions.

Here we define TRMS as a mass spectrometric approach that allows one to differentiate between two chemical states that can be observed sequentially at two points on the timescale. Such a broad definition encompasses various methods with quite dissimilar temporal resolutions and diverse areas of applications. A large variety of physical and chemical processes can be studied by TRMS: from protein folding, to enzyme kinetics, to mechanisms of organic reaction, extraction, and combustion [33]. Since short-lived reaction intermediates can be detected using several TRMS methods, this platform is ideal for the studies on mechanisms of chemical reactions, chemical kinetics, and biochemical dynamics. Depending on the focus area, temporal resolutions typically range from microseconds to minutes. While many organic reactions occur in hours, they do not require sophisticated setups to follow their kinetics or characterize intermediates (see Chapter 11). On the other hand, some of the studies on protein folding may require temporal resolutions below 1 ms (see Chapter 12).

The need to achieve satisfactory speed of MS analysis encouraged the development of delay-free sampling strategies. Early attempts to demonstrate TRMS date back to
the 1950s, when radicals were recorded for thermally decomposing gases [34, 35], and flash photolysis reactions were captured using customized time-of-flight (TOF) instruments [36] (see also Chapter 4). In some cases, obtaining time-resolved data required construction of complicated experimental systems and solving cumbersome technical problems. Importantly, it is not always necessary to take a lot of effort to assemble complex apparatus since many pieces of hardware are already available commercially, and often minor modifications of these standard instruments are sufficient to carry out TRMS measurements. TRMS encompasses an assortment of methods based on different principles. Some TRMS systems are so dissimilar that the only common feature is the use of a mass spectrometer as the detector. Using customized apparatus, a multitude of physical, chemical and biochemical phenomena can be characterized in the time domain.

1.4 Dynamic Matrices

The concept of \textit{sample} is one of the most widely used in analytical chemistry. According to the International Union of Pure and Applied Chemistry, sample is defined as a portion of material selected from a larger quantity of material [39]. Often we talk about \textit{representative samples} which are supposed to reflect the chemical composition of the sampled medium at the time of sampling. However, many chemical media do not represent a constant chemical composition. Therefore, samples collected at different time points may have different components – qualitatively and quantitatively. For example, the chemical composition of river water may change in the course of rainfall or discharge of effluent from a wastewater treatment plant. Contents of a chemical reactor (reaction vessel) change as the reaction moves on, that is the concentrations of reactants decrease while the concentrations of products increase. If we continuously collect aliquots of media from such systems (river, reactor), and perform chemical analysis, we will find that the composition of the obtained fluid varies qualitatively and quantitatively. Therefore, in some cases it would be helpful to use the term \textit{dynamic sample} (or \textit{dynamic matrix}) to describe chemically unstable media that change with time. The concept of \textit{dynamic sample} is in opposition to \textit{static sample} (or \textit{steady sample}) – which does not change its composition over time. However, in the following discussion we will also deal with cases in which spatial gradients of reaction products are formed downstream from micromixing devices (see Chapter 4). Generation of such gradients enables studies of some fast phenomena (e.g., protein folding measurements, see Chapter 12). Nonetheless, probing analytes from spatial concentration gradients does not rely on time-resolving capabilities of MS. Thus, the composition of an aliquot obtained from a fixed position within a spatial gradient can be referred to as \textit{quasi-static} (or \textit{quasi-steady}).

1.5 Real-time vs. Single-point Measurements

It is necessary to distinguish between two modes of recording temporal information in TRMS:

- \textit{Real-time monitoring} provides the means to record data continuously with a specified temporal resolution. Multiple spectra are recorded at a high frequency (typically, several
spectra per second). Here, the spectral patterns are assumed to reflect the chemical compositions of the investigated systems at given time points. The consecutive spectra are expected to represent different patterns reflecting qualitative and/or quantitative changes in the investigated systems. The time interval between two consecutive analyses (data points) should be as short as possible in order to achieve high temporal resolution of real-time monitoring.

- **Single-point measurements** provide spectral “snapshots” of the investigated systems at selected time points. If the system is dynamic or unstable, it may require quenching before introduction to the ion source. In this case, the time of quenching rather than measurement itself puts the time tag on the molecular composition of the dynamic sample. In some cases, one of the ionization steps (e.g., removal of solvent) may be regarded as quenching.

When investigating highly dynamic systems, the sampling time or the incubation time is an important attribute of MS measurements. Other chemical systems exist in equilibrium, in which case steady samples are obtained, and the sampling time becomes a less important descriptor. As mentioned above, in some designs of TRMS, reaction mixtures are generated continuously in the flow of the reactant stream or droplet plume. In those cases, quasi-steady samples are produced. Thus, single-point measurements of reaction products or intermediates can be conducted following a very short incubation period.

### 1.6 Further Reading

The reference lists included in every chapter contain numerous examples of representative reports on TRMS. For more succinct summaries of TRMS, as well as the coverage of specific topics, readers are also encouraged to consult recent review articles and chapters [33, 40–46]. While some of the following chapters cover important parts of MS workflow, readers interested in more general aspects of the technique are encouraged to consult MS textbooks [23–28]. Those who are specifically interested in the application of MS in the studies of reaction intermediates of chemical reactions, and would like to gain a more extensive overview of that subject, are encouraged to read the book edited by Santos [47].

### References


