Thermochimica Acta 655 (2017) 137–144

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Microscale calorimetric device for determining reaction parameters

Masoud Modaresifar, Gregory J. Kowalski*

Department of Mechanical and Industrial, Faculty of Mechanical Engineering, Northeastern University, Boston, USA

ARTICLE INFO

Keywords:
Calorimeter
Enthalpy of reaction
Nanohole array sensor
Extraordinary optical transmission

ABSTRACT

Two microscale calorimeter designs based on nanohole array sensors are described. These devices use significantly less compound, are significantly smaller and require less time to perform calorimetric experiments which make them attractive to the drug discovery process. They also have the potential for high throughput screening which would provide key information earlier in the drug discovery process and for multiplexing experiments. The described device is a departure from the calorimeters, ITC and DSC, currently being used. The devices tested were used to measure the thermodynamic property, Enthalpy of reaction or dilution, using the observed change in the extraordinary optical transmission, EOT, through a nanohole array sensor. Experiments that confirm that the observed EOT changes are related to the thermal effects of the reaction are provided. Preliminary results indicate that the device can determine the concentration changes in a water-ethanol dilution experiment and the Enthalpy of dilution to within 1.3%. A relative comparison of the enthalpy of reaction at different reactant concentrations for the co-flow configuration of the device are within 5% of those predicted using literature values. Means of processing the observed EOT to eliminate unwanted fringe behaviors are discussed and illustrate the close agreement between thermistor and EOT thermal responses.

1. Introduction

Calorimetry is utilized to detect the energy released in chemical reactions and it has an extensive application in pharmaceutical industries and drug development research. Today, the application of calorimetry is increasing in cell biology, food research and pharmaceutical areas because it provides crucial data to determine thermodynamic binding parameters, Enthalpy of Reaction, Gibbs Free Energy changes and equilibrium constants [1]. Calorimetry provides important information about molecule interactions. Early use of Calorimetric data could be added to decision making in pioneer discovery [2]. Researchers in pharmaceutical industry, biophysics, cell biology and food research examine their respective systems using calorimetry. For biologists to have a good understanding of biochemical process, the structure of the compounds alone is not sufficient. They need quantifications of the change of states and the thermodynamic parameter measurements provide them with these results [2].

Ladbury et al. [2] has conducted a study for analyzing the various HIV protease inhibitors. According to their study which starts from 1995 to 2006, protein ligand interactions are driven by thermodynamics. In this research the best in class approved drugs for each year is presented with their corresponding enthalpy of reaction, Gibbs free energy changes and entropy changes. Over the time of the study the HIV drugs improved. The improved drug therapies corresponded to classes of drugs with larger changes in the enthalpy of reaction and decreased changes in entropy. If these characteristics could have been identified earlier, it is likely that quicker progress on the drugs and the disease could have been obtained at a lower cost. While not guaranteeing lower cost and shorter drug development time, earlier use calorimetry data would increase the probability of making better decisions of successful drug therapies.

Fig. 1 shows schematically the pharmaceutical development process. The process starts with a large number of compounds that undergo a high throughput testing process. After these large numbers of tests were conducted over a period of time, calorimetry is used very late in the process to identify the thermodynamic parameters before proceeding to the phase 1, 2 and 3 of the testing process (the upper line in Fig. 1). The reason for the late introduction of calorimetry is that it is time consuming and requires a large mass of expensive compounds. The benefits of the microcalorimeter that will be presented are its shorter operating and cleaning time and its reduction of compound usage. It also has the potential to be multiplexed for use in a high throughput process. These characteristics suggest that calorimetry could be moved earlier in the drug development process (the lower line in Fig. 1) and would allow better decisions to reduce the number of potential candidates and shorten the drug discovery process.

* Corresponding author.
E-mail address: gkowal@coe.neu.edu (G.J. Kowalski).

http://dx.doi.org/10.1016/j.tca.2017.05.023
Received 20 February 2017; Received in revised form 2 May 2017; Accepted 31 May 2017
Available online 03 June 2017

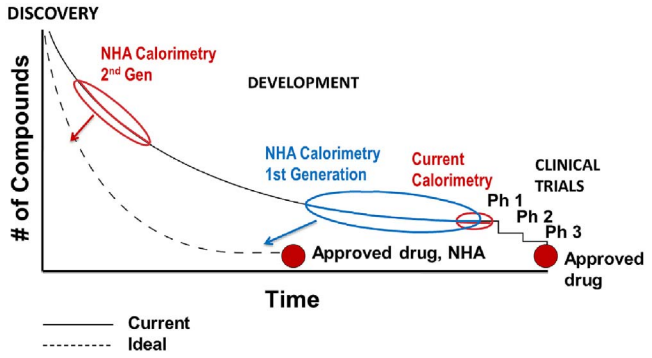

Fig. 1. Pharmaceutical development overview.

1.1. Calorimeter overview

A direct measurement of thermodynamic parameters is needed to assist in the drug discovery process. These measurements are obtained by high sensitive microcalorimetric instrumentation [3]. In biochemical and biological applications the binding affinity is influenced by the Gibbs free energy which is related to the changes in enthalpy and entropy [4]. Gibbs free energy is related to the number of binding sites in protein ligand interactions.

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

Calorimetry utilizes the direct measurement of the heat of the interaction, either a heat transfer rate or change in power to investigate the extent of binding between molecules during the titration of one component into another [1]. During this process the concentration of each compound is known and the equilibrium binding constant, K_b can be determined ($K_b = 1/K_d$, K_d: equilibrium constant). If the enthalpy and binding constant are known, the change in the Gibbs free energy would be [1]:

$$\Delta G = -RT \ln K_b \quad (2)$$

Where, R is the gas constant, T is the temperature of experiment and K_b is the binding constant.

The thermodynamic parameters ΔG, ΔH are measures of the energy associated with going from the free to the bound state at a given temperature. The change in entropy, ΔS, term is related to how well the drug and protein will interact. Many pharmaceutical experiences show the optimization of enthalpy is more difficult due to the forces that contribute to the binding [4]. Moreover, its improvement does not always result in a better affinity. On the other hand binding entropy is easier to optimize [4].

There are two widely used calorimetry techniques; Differential Scanning Calorimetry (DSC) and Isothermal Titration Calorimetry (ITC).

DSC is the most direct experimental technique to resolve the energy of conformational interactions of biological transition. It determines the thermodynamic mechanism by measuring the temperature dependence of the partial heat capacity [3]. The DSC instrument is equipped with two cells (cell volume 0.5 ml–1 ml). The sample cell, which contains the solute, and the reference cell that has an equal amount of solvent are place into a shield. The system is heated quasi-adiabatically at a constant rate. The heat capacity of the two cells is different because of the changes in the sample cell as the temperature changes. To maintain a constant temperature between the cells, $\Delta T = 0$; a differential power is required. This differential power is monitored continuously as a function of temperature and is directly proportional to heat capacity difference between the two cells [3,5]. This heat capacity versus temperature data can be used to obtain the calorimetric transition enthalpy.

ITC is the most direct method to measure the energy exchange during a reaction [3]. An ITC Instrument consists of a reference and a sample cell. The volume of the sample cell is between 0.2–1.4 ml in commercial instruments [3]. Both cells contain one of the compounds and are kept at the same temperature in an adiabatic jacket. The second reacting compound is injected into the sample cell and the energy associated with binding produces a change in temperature. The binding can be exothermic or endothermic. A change in power in the sample cell is needed to maintain both cells at the same temperature. The change in power is recorded for each injection. These changes in power are creating peaks in an energy released versus time plot. After several injections into the sample cell, the available binding sites of the component that were initially available will gradually saturate and smaller peaks appear in the plot as time evolves. The change in the molar enthalpy of the interaction can be determined by integrating the raw data peaks with respect to time. Since the total concentrations of the components of the interaction at any given point in the titration are known, a plot of ΔH against the molar ratio is constructed. The equilibrium constant is determined using this plot.

The application of the ITC and DSC calorimetry has been investigated by many authors [3,6,7–11].

1.2. Microscale calorimeter

This study presents a different approach for calorimetry [12–16]. A unique calorimetry design using Nanohole Arrays (NHA) in a co flow micro channel to determine the change in concentration and energy released in a chemical reaction. Microchannels have appealing features in drug discovery which make them an ideal means for biological systems [17,18]. NHA sensors have a resolution of 9.4(10^{-8}) index of refraction units [19] which combined with the (dn/dT) parameter for water (94(10^{-6}) index of units/K) provides an estimate of the accuracy of the temperature measurement to be 1 mK. The literature also states that at an incident wavelength of 632 nm the surface plasmon effect is limited to 100 nm above the NHA sensor. This sensitivity and small sensor volume illustrate the sensitivity and resolution of the NHA sensors. Their time response is on the order of speed of light. This new approach decreases the amount of compound used and reduces the time because we have very small volume. The device has the potential for multiplexing and moving calorimetry earlier in the drug discovery process.

Fig. 2 shows schematic of the microscale calorimeter with its co flow microchannel and an exploded view of a NHA site. Compounds A and B enter a laminar microfluid channel and begin to react. The floor of this micro channel is a NHA sensor chip. The chip is a metallic gold layer 100 nm thick that is deposited onto a silicate substrate. NHAs are arrays of 150 nm diameter apertures that are microfabricated in a pattern across the device.

When light is incident on the chip, the amount of light transmitted through NHAs exceeds that predicted by diffraction theory by orders of magnitude. This phenomenon is called Extraordinary Optical Transmission, EOT [20]. This phenomenon is attributed to the surface Plasmon effect which is sensitive to change in dielectric constant of the medium above the chip. A CCD camera is used to detect the intensities

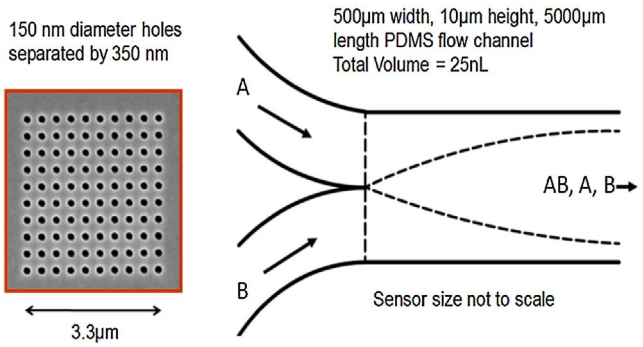

Fig. 2. A NHA site (left) and schematic of a co flow microchannel (right) [12].