Application Note

Academic

Phase Diagram of the Ouzo Effect Using Temperature-Controlled UV-Vis Spectroscopy

Simultaneous temperature ramping experiments using the Agilent Cary 3500 UV-Vis and the multizone feature of the Multicell sampling module

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Introduction

A phase diagram is a fundamental concept in chemistry. It shows how the physical state of a substance changes as a function of two variables, such as temperature and pressure or temperature and concentration. Phase diagrams are constructed by plotting curves that show the conditions under which different phases of matter coexist in equilibrium. The lines on a phase diagram represent phase boundaries, such as the boundary between a solid phase and a liquid phase or between a liquid phase and a gas phase. These boundaries indicate the conditions at which phase transitions occur. Therefore, changing the appropriate variable in a particular phase diagram to move a value from one side of that barrier to the other indicates the conditions needed for a phase transition. This process can be time-consuming, as multiple experiments must be carried out to establish a comprehensive phase diagram.

A solubility chart, which is considered a related figure to a phase diagram, outlines how one or more substances combine with another substance. A solubility chart reports solubility (solutes forming solutions with solvents based on concentration and temperature) and miscibility (substances mixing to form a homogenous solution without precipitation). The charts are based on favorable or unfavorable interactions between the chemicals being studied.

Solubility charts and phase diagrams reveal information about how chemicals physically interact with each other and the environment. These studies are therefore essential to understanding the principles of chemistry.

As UV-Vis spectroscopy has been shown to be suitable for the characterization of turbid samples[1], it was used to study the ouzo effect. The ouzo effect describes a type of emulsification that occurs between water and the terpenes of alcoholic beverages such as Greek ouzo or French pastis. For example, a well-known brand of pastis contains a highly hydrophobic terpene, anethole (Figure 1), which is responsible for the anise flavor of the liqueur. In its neat form, the liqueur has a 45% (v/v) ethanol concentration. At this ethanol alcohol level, the anethole molecules are fully soluble in the water/alcohol mixture.

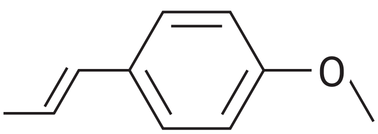

Figure 1. Chemical structure of anethole—a highly hydrophobic terpene that is present in some pastis liqueurs.

Pastis is commonly served with water and ice, which decreases the alcohol level through dilution and lowers the temperature of the mixture. As a result, the anethole molecules precipitate into the aqueous fraction, turning the liqueur from clear amber to cloudy yellow (Figure 2). The formation mechanism of this phenomenon is not completely understood, but the near instantaneous change fascinates chemists and non-chemists alike.[2]

Figure 2. Preparation of a typical pastis drink, illustrating the ouzo effect. Combining neat pastis (left), water (middle left), and ice cubes (middle right) lead to a cloudy yellowish drink (right).

In this work, the phase transition in different water/ethanol mixtures was assessed by measuring the extent of light scattering created by the anethole suspension in the pastis mixture. As demonstrated previously, the Agilent Cary 3500 UV-Vis spectrophotometer has unique capabilities for characterizing turbid samples thanks to its unique optical design.[1]

Cary 3500 UV-Vis spectrophotometer

The Cary 3500 UV-Vis spectrophotometer is a fully interchangeable modular system that uses a xenon (Xe) flash lamp as a source and a double, out-of-plane Littrow monochromator. The spectral bandwidth (SBW) of the monochromator can be varied from 0.1 to 4.0 nm. Optic fiber technology is used to deliver the source signal directly to all cuvettes simultaneously.

When the Agilent Compact sampling module is used with the common Cary 3500 UV-Vis engine, the analyst has access to two cuvette positions, while eight cuvettes are accessible with the Agilent Multicell sampling module.

Both the Compact Peltier and Multicell Peltier sampling modules use an air-cooled Peltier temperature control system that can attain and hold a set temperature between –5 and 110 °C. The system is also capable of temperature ramping at rates ranging from 0.1 to 40 °C/min.

The unique Multizone feature of the Multicell sampling module enables four temperature-controlled experiments (four sample and reference pairs) to be carried out simultaneously in separate zones. This multizone capability significantly improves the productivity of building multipoint phase diagrams.

The Cary 3500 with Multicell Peltier sampling module was used in this study to perform temperature ramping studies of four different alcohol/water mixtures simultaneously. For each set of experiments, the temperature was cycled from 55 to –2.5 °C, under constant agitation, using eight cuvettes (four sample cuvettes and four reference cells) in the sampling module. The results show how the Cary 3500 Multicell Peltier UV-Vis system can be used to build an eight-point solubility phase diagram of the ouzo effect in a time-efficient way.

Experimental

Sample preparation

A total of eight dilutions of the pastis sample were prepared directly into standard open-top 10 mm quartz cuvettes fitted with a star-shaped stir-bar and lid. The concentrations of the samples were 45.0 (neat), 44.0, 42.5, 40.0, 37.5, 35.0, 32.5, and 30.0% (v/v) alcohol. For consistency, each cuvette was filled with a total volume of 2.00 mL of solution.

Reference cells containing corresponding dilutions of ethanol with de-ionized (DI) water were prepared in optically matched quartz cuvettes that were also fitted with a stir bar and lid. The use of optically matched quartz cuvette pairs eliminated the need for zeroing or baselining before starting the measurements. All sample cuvettes were fitted with a temperature probe, which was set to a fixed height so that the tip was completely submerged in solution (Figure 4).

Instrumentation

The Cary 3500 UV-Vis spectrophotometer equipped with a Multicell Peltier sampling module was controlled using Agilent Cary UV Workstation software.

Each cell of the Multicell Peltier module is paired with its own detector and each cuvette can be equipped with a stirrer and standalone temperature probe. So, in this study, all four sample cells were fitted with a temperature probe to monitor, measure, and control the temperature of each zone (comprising a sample and reference), based on the actual temperature reading of the sample cuvette.

Wavelength scans of key mixtures/components

Before temperature-controlled experiments at the various alcohol levels, a UV-Vis spectrum of the neat pastis liqueur, a diluted pastis solution made up to 45% (v/v) alcohol with ethanol, and a diluted pastis solution at 30% (v/v) alcohol were collected using the settings given in Table 1. The corresponding ethanol solution in DI water was used in the reference cell.

Table 1. Agilent 3500 UV-Vis parameters used for the acquisition of UV-Vis spectra of the neat and diluted pastis sample at 25 °C.

Parameter	Setting
Wavelength Range	200 to 1,100 nm
Data Interval	1.00 nm
Averaging Time	0.02 s
Spectral Bandwidth	4.0 nm
Stirring Speed	800 rpm
Temperature	25.0 °C

Investigation of the solubility temperature

The temperature ramping parameters used to measure the solubility temperature for each pastis mixture are summarized in Table 2. For all experiments, a starting point of 25.0 °C was selected. Some mixtures were found to be already insoluble at room temperature. Therefore, stage 1 of the temperature ramping program raised the temperature of the cuvette to 55 °C to ensure that any remaining particles in suspension were fully solubilized. Stage 2 then collected the actual data from 55 to –5 °C in absorbance mode. For all measurements, the temperature probe fitted in the sample cell of each zone was used for the feedback loop of the temperature controller. More details on the selection of these parameters are discussed in the next section.

Table 2. Agilent 3500 UV-Vis parameters for each stage of the temperature program.

	Stage 1 (Heating)	Stage 2 (Cooling)
Wavelength	750.0 nm	750.0 nm
Averaging Time	1.0 s	1.0 s
Spectral Bandwidth	4.0 nm	4.0 nm
Stirring Speed	400 rpm	400 rpm
Start Temperature	25.0 °C	55.0 °C
End Temperature	55.0 °C	–5.0 °C
Hold Time	10.0 min	0 min
Data Interval	1.0 °C	1.0 °C
Rate	5.0 °C/min	1.0 °C/min

The Cary 3500 Multicell Peltier sampling module is equipped with a purge line to prevent condensation on the outer walls of the cuvette when carrying out experiments at temperatures under the dewpoint. Since the study of the ouzo solubility phase diagram required collecting absorbance measurements below 0 °C, the freezing point of water, nitrogen (N_2) was used as a purge gas at a flow rate of 10 L/min.

Results and discussion

Identification of the wavelength range

The characteristic amber color of neat pastis at 45% (v/v) alcohol is caused by chromophores associated with the formulation of the specific liqueur that was used in this study. Therefore, to establish the suitable wavelength range for the phase transition measurements, a few initial control scans were conducted.

The neat pastis (blue curve in Figure 3) strongly absorbs at wavelengths below 500 nm up to the maximum of the photometric range of the Cary 3500 equipped with a Multicell Peltier module, i.e., about 4.5 absorbance units.

To visualize the chromophores associated with the pastis liqueur, a diluted pastis solution was prepared while keeping the ethanol concentration constant to prevent the anethole from precipitating. The yellow curve in Figure 3 shows two distinct peaks at 244 and 297 nm, as well as tailing up to 460 nm, likely from the edge of an unresolved peak. This tailing feature likely extends to 600 nm in the neat pastis liqueur spectrum (blue curve). It can be concluded from these measurements that there are no significant chromophores that contribute to the measurement above 600 nm. The green curve in Figure 3 of a lower alcohol percentage mixture (30%) is representative of a cloudy pastis solution at room temperature.

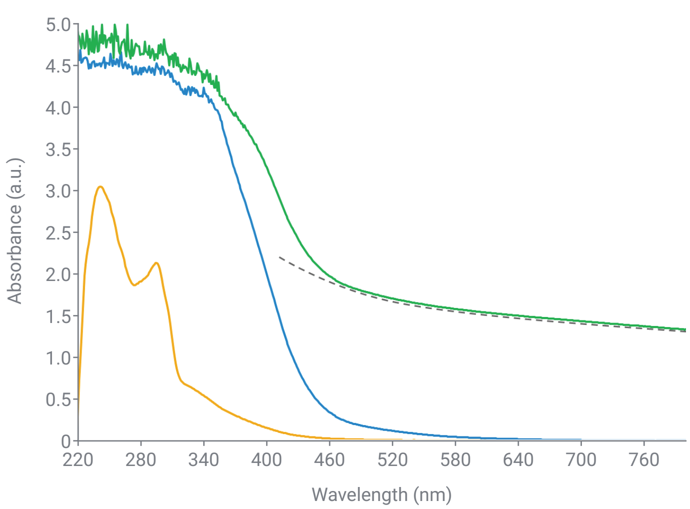

Figure 3. UV-Vis spectrum of undiluted pastis (blue), 10-fold diluted pastis in 45% (v/v) ethanol (yellow), and cloudy pastis solution at 30% (v/v) alcohol (green). The light scattering contribution to the green curve is highlighted by the gray dashed line.

In addition to the chromophore features in the 200 to 600 nm range, an additional contribution can be observed > 600 nm, as indicated by the gray dashed line in Figure 3. This slow decaying signal that offset the signal across the entire spectral range is associated with the light scattering caused by undissolved particles in suspension.

A wavelength of 750.0 nm was selected for the determination of the phase transition temperatures. The apparent absorbance signal at 750.0 nm is solely a function of light scattering and is free of any contribution from chromophores.

Optimizing temperature conditions and cooling rates

The lower percentage alcohol mixtures in this investigation had transition temperatures close to ambient temperature. A typical solubility curve for a low percentage alcohol sample is shown in Figure 4. At the starting temperature of 25.0 °C, the apparent absorbance at 750 nm is significant due to the light scattering caused by undissolved anethole in suspension. The initial heating of the samples to 55.0 °C (stage 1 in Table 2) brings the apparent absorbance down to ~0 as any precipitated anethole redissolves in the ethanol fraction of the pastis solution. The heating rate is irrelevant at this stage since the objective is to dissolve any particles that could lead to light scattering. The samples are held at the upper temperature for 10 minutes to allow any slow dissolution processes to reach completion.

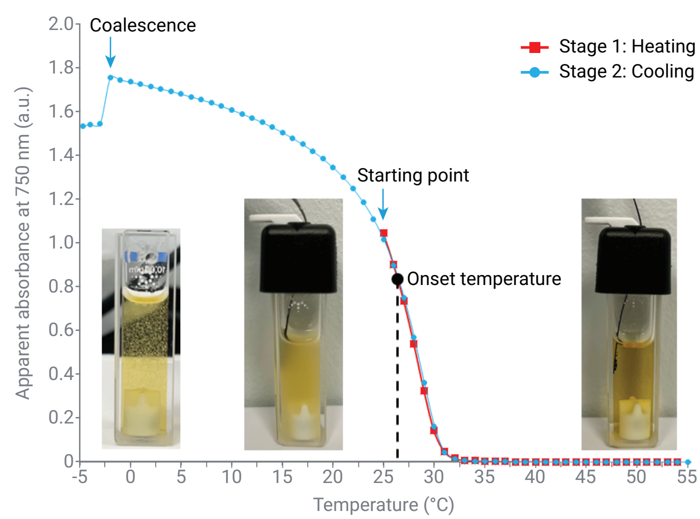

Figure 4. A typical curve associated with a phase transition of a pastis sample at 30% (v/v) alcohol percentage. During stage 1 (■), the samples were heated to 55 °C under constant stirring, producing a clear solution as illustrated by the cuvette on the right. During stage 2 (●), the sample was slowly cooled to –5 °C, producing a cloudy solution shown by the middle cuvette. An additional phase transition occurred near the freezing point of water, as illustrated by the cuvette on the left.

During the cooling phase (stage 2 in Table 2), the temperature in each zone was slowly brought to –5.0 °C at a rate of 1.0 °C/min. Preliminary testing at a faster cooling rate (>2 °C/min) revealed that the anethole phase transition was not reaching equilibrium despite the efficient stirring of the solution. The kinetics of the phase transition were too slow at the faster cooling rate, resulting in a hysteresis cooling/heating curve (results not shown).

Reproducible results were obtained at a cooling rate of 1.0 °C/min or slower. When the temperature conditions are satisfied, the apparent absorbance sharply increases before reaching a plateau. This tipping point is referred to as the onset temperature. The amount of light scattering increases as more anethole precipitates, and there is some anethole precipitation in the aqueous fraction of even slightly dilute pastis samples.

For some solutions, a sudden apparent absorbance drop was observed below –3 °C. This drop is shown in Figure 4. Some solutions began to destabilize and phase-separate below –3 °C, as confirmed from visual observation of the cuvettes, despite the continuous thorough stirring of the solution. This phenomenon has been observed previously and was explained by coalescence (anethole droplets combining into larger droplets), flocculation (anethole droplets assembling without combining), sedimentation (separation due to density differences), and Ostwald ripening (increase in anethole droplet size).[2] Each of these processes causes absorbance to decrease significantly, as the uniform cloud of precipitated anethole collapses. This low temperature phase transition was found not to be reversible, i.e., a hysteresis could be observed upon reheating of the solution. Following these observations, temperatures lower than –2.5 °C were omitted for in the solubility phase diagram as they did not impact the onset of the initial precipitation.

Effects of alcohol % level on precipitation temperature

The experiments were repeated using freshly prepared solutions at different alcohol percentage levels (neat to 30.0% v/v). The cooling curves for the different samples can be seen in Figure 5. The results show that even the slightest addition of water to the liqueur causes a shift in the onset temperature at which the anethole precipitates. There is a clear trend towards a higher precipitation temperature as the percentage of ethanol in the solution decreases.

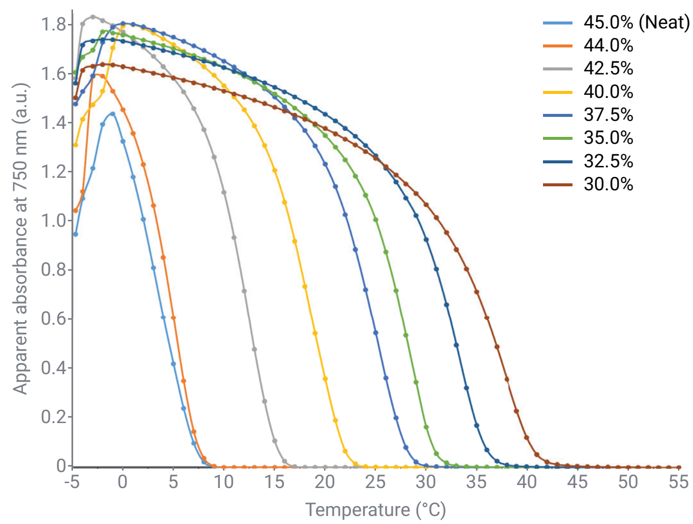

Figure 5. UV-Vis cooling curves (stage 2) of pastis solutions at 45.0, 44.0, 42.5, 40.0, 37.5, 35.0, 32.5, and 30.0% (v/v) alcohol concentration.

The experiment was limited to 30% (v/v) alcohol, as samples with lower alcohol concentrations would require lowering temperatures close to the boiling point of ethanol/water to initially solubilize the mixture.

Determination of phase temperatures

The first derivative method was applied to accurately determine the phase temperature for each of the curves shown in Figure 5. Average temperatures were used to construct the anethole solubility phase diagram shown in Figure 6. The error bars correspond to the standard deviation from the three replicates values.

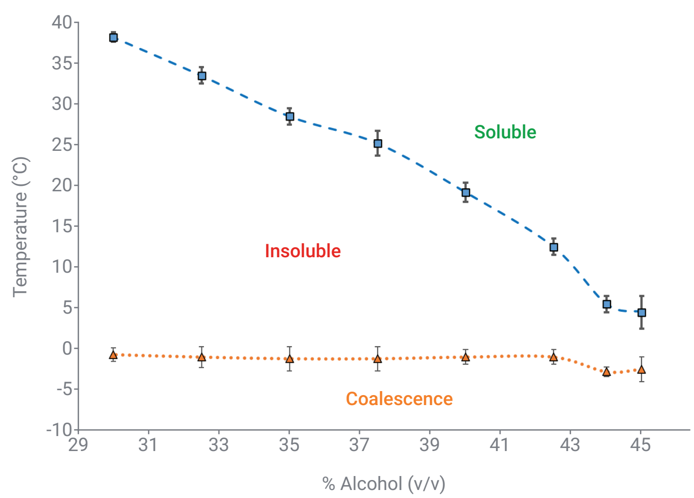

Figure 6. Solubility phase diagram of anethole in a pastis liqueur. Each data point corresponds to the average temperature value, and the error bars relate to the standard deviation of three replicates.

UV-Vis spectroscopy may not be the most obvious or suitable tool to use to investigate the phase separation that occurs near the freezing point of water. However, the temperature at which it occurs could also be estimated using the first derivative method. This additional phase transition appears to be independent of the alcohol level in the solution, as shown by the orange dotted line in Figure 6. Since the transition sits very close to 0 °C, it could be speculated that it is associated with pure water domains within the emulsion.

Conclusion

The Agilent Cary 3500 UV-Vis spectrophotometer equipped with an Agilent Multicell Peltier sampling module was used to build an eight-point phase diagram of anethole in a pastis liqueur.

The multizone feature of the Peltier temperature-controlled Multicell facilitated four temperature ramping experiments to be run in parallel, comprising cuvettes containing a sample and reference pairing. This capability enabled a comprehensive solubility phase diagram to be established for pastis in two runs of 73 minutes using the Cary 3500 UV-Vis—much quicker than the time needed to build a phase diagram using other techniques.

To prevent sedimentation during measurements, each of the eight cuvettes in the Multicell was fitted with an independent stirrer. Constant stirring also ensured the uniformity of temperature across all cuvettes, which is critical for temperature ramping experiments. The combination of the air-cooled Peltier system and nitrogen gas purge feature ensured that temperature set points significantly above ambient and below the water freezing point were achieved with no condensation.

The study has demonstrated the suitability and efficiency of the easy-to-use Cary 3500 UV-Vis system to establish phase diagrams of complex samples and to provide insights into the ouzo effect.

References

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Further information

- Cary 3500 Multicell UV-Vis Spectrophotometer
- Cary 3500 Compact UV-Vis Spectrophotometer
- Cary UV Workstation Software
- UV-Vis Spectroscopy & Spectrophotometer FAQ

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