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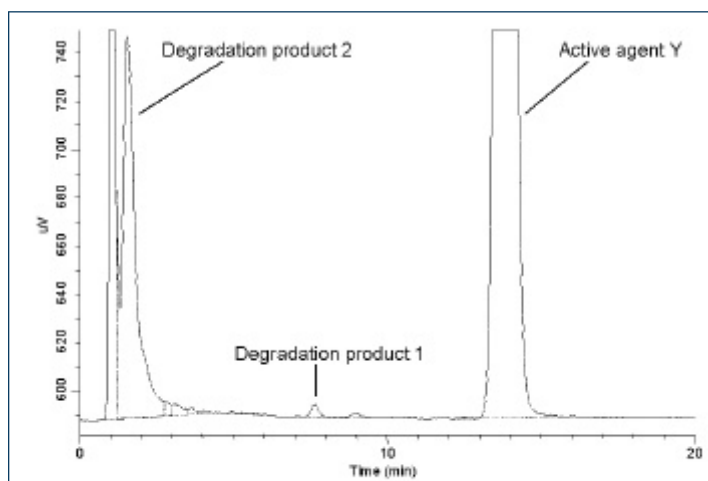


Figure 3. Pharmaceutical Formulation 2 after dispersion using ultrasonic probe (8 minutes, 4.6 volts)

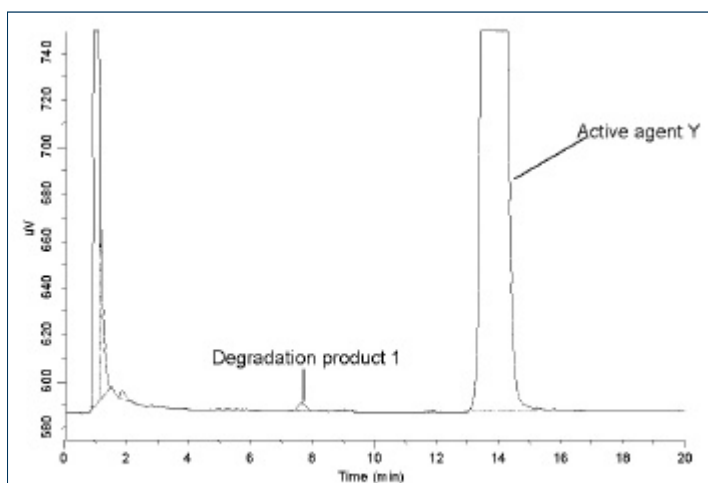


Figure 4. Pharmaceutical Formulation 2 after dispersion using an ultrasonic bath (30 minutes)

## Conclusions

The sonication probe fitted to the Zymark Prelude sonication workstation is an effective means of dissolving most samples. The power of the system, however, does mean that under some conditions sample degradation can occur. The level of control afforded by the Prelude software does allow degradation to be minimized to a large extent. In some instances, however, degradation of active agents may remain a problem. In these cases, an alternative means of dissolving samples may be required.

A further observation is the increase in the temperature of the sample solution during sonication. This can lead to loss of solvent due to evaporation and consequent inaccuracies in sample preparation.

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## Automated Sonication and Sample Degradation; A Study of Two Pharmaceutical Formulations

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### Introduction

Sample preparation, whether for HPLC analysis or a UV assay, is a process that lends itself to automation. A number of automated sample preparation systems are available on the market, but all share one thing in common, that is, the problem of getting a sample into solution. One company, Zymark Corporation (Hopkinton, Massachusetts, USA), has addressed this problem in the design of their Prelude<sup>®</sup> automated sample preparation Workstation. This has been designed to accommodate a sonication workstation in one of its optional configurations.

The sonication workstation is equipped with a high power sonication probe that can quickly and effectively disperse almost any sample. The probe has a 130 Watt power rating and operates at 40kHz<sup>1</sup>. The Prelude software allows a good degree of control over the sonication probe. It is possible to control the power of the probe, the length of each sonication step, and to monitor the temperature of the sample solution. The position of the probe can be altered within the sample tube in order to achieve maximum effect.

## Sonication

Sonication is a very effective method for getting materials into solution. The effects of ultrasound arise from acoustic cavitation<sup>2</sup>. This is the formation, expansion and sudden collapse of bubbles in ultrasonically irradiated liquids. Studies have shown that the temperature of the gaseous phase within the collapsing cavity can reach as high as 5000K, while the liquid phase immediately surrounding the cavity can reach temperatures of up to 1900K<sup>2</sup>. In aqueous samples, the extreme conditions generated during the collapse of cavitations can lead to dissociation of water molecules and the formation of OH<sup>-</sup> radicals that may then go on to react with the compounds being dissolved<sup>3</sup>. This may in turn lead to sample degradation.

The use of sonication to degrade organic pollutants in water has been reported<sup>3</sup>. Similarly, there are examples of sample degradation occurring during sample solubilization by sonication<sup>4,5</sup>. This raises two questions:

1. Can the use of a sonication probe during the automated sample preparation of analytical samples lead to sample degradation?
2. Does the Prelude software allow sufficient control of the sonication probe to prevent degradation occurring?

## The Automated Sample Preparation Of Two Pharmaceutical Formulations - A Case Study

The effects of sonication on the stability of the active agents found in two different pharmaceutical formulations have been studied. The formulations used during this study were chosen because it was thought that they might be susceptible to some degradation. However, they were not untypical of the type of material that an automated sample preparation system would be expected to work with. In addition, the manual sample preparation processes did involve the use of an ultrasonic bath to dissolve the samples. Both formulations were tablets.

### Pharmaceutical Formulation 1

#### Experimental

Pharmaceutical formulation 1 was dissolved in a mixture of acetonitrile and phosphate buffer using either an ultrasonic probe or ultrasonic bath. The active agent in formulation 1 was known to degrade when exposed to prolonged sonication. The dispersed solutions were filtered through a 0.45mm nylon filter and the resultant filtrates analyzed by HPLC for active agent content and the formation of degradation products. The sonication conditions and results obtained are presented in Table 1.

#### Results

Sonication Time (minutes)	Sonication Setting (V)	% Recovery(Active Agent)	% Deg Product A
8	4.6	100	0.65
5	4.6	100	0.25
3	4.6	100	0.12
10	2.3	100	0.15
5	2.3	100	0.12
10	1.0	100	0.13
30 x 5 second bursts	2.3	100	0.12
10	Bath	100	0.12

Table 1 - Effects of sonication on Formulation 1

#### Discussion

As expected, prolonged sonication of formulation 1 at higher power level resulted in increased degradation of the active agent to give degradation product A (see Figure 1). Using the Prelude software, however, it was found to be an easy process to change the sonication conditions to achieve solubilisation of the active agent with degradation product levels comparable to those obtained when using an ultrasonic bath (see Figure 2). At full power (4.6V), 100% recovery of the active agent was obtained after only 3 minutes sonication without significantly increasing the level of degradation. Under these conditions, though, the temperature of the sample solution increased from 29°C to 51°C. This in turn resulted in some loss of solvent from the sample solution and a consequent increase in the concentration of the sample. The optimum conditions were found to be a series of 30 x 5 second bursts of sonication with a 20 second pause between each burst at a setting of 2.3V. The temperature of the sample did increase from 29°C to 34°C but there was no significant loss of solvent.

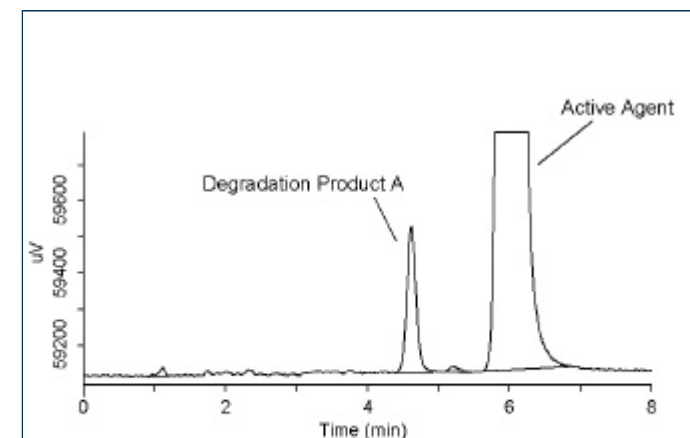


Figure 1. Pharmaceutical Formulation 1 after dispersion using ultrasonic probe (8 minutes, 4.6 volts)

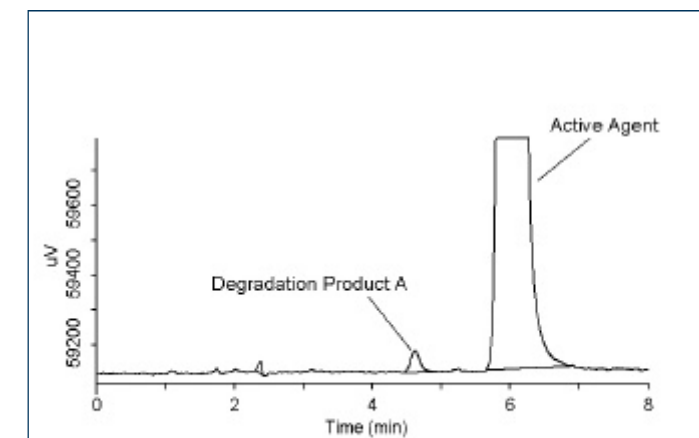


Figure 2. Pharmaceutical Formulation 1 after dispersion using ultrasonic bath (10 minutes)

### Pharmaceutical Formulation 2

#### Experimental

Pharmaceutical formulation 2 contained two active agents, X and Y, within a single tablet. Active agent X was present at 20 times the concentration of active agent Y. Formulation 2 was dissolved in a mixture of acetonitrile, water and tri-fluoroacetic acid using either an ultrasonic probe or ultrasonic bath. The dispersed solutions were filtered through a 0.45µm nylon filter. The resultant filtrates were analyzed using an HPLC method designed to monitor for the degradation products of active agent Y. The sonication conditions and results obtained are presented in Table 2.

#### Results

Sonication Time (minutes)	Sonication Setting (V)	% Recovery (Active Agent Y)	% Deg Product 1	% Deg Product 2
30	1	100	0.32	8.50
8	4.6	100	0.13	7.10
5	4.6	100	0.12	3.20
3	4.6	100	0.09	0.08
10	2.3	94	0.12	6.60
5	2.3	88	0.10	1.99
5	1	77	0.10	2.40
30	Bath	98	0.09	0.00

Table 2 - Effects of sonication on Formulation 2

#### Discussion

The sonication probe was found to be an excellent means of getting active agent Y into solution. Two degradation products were formed in the process, however, one of them at a high level with respect to active agent Y (see Figure 3). Further investigation revealed that neither of these degradation products was related to active agent Y but both were actually degradation products of active agent X. They were therefore present at very low levels with respect to active agent X. Controlling their formation was still important, though, since they could potentially interfere with the determination of degradation products of active agent Y as they had similar elution times. Although degradation product 1 had been found in samples prepared using an ultrasonic bath, degradation product 2 had not been observed before (see Figure 4).

By increasing the power of the sonication probe and reducing the length of time over which sonication took place it was possible to control the formation of degradation product 1. This approach, however, was not as successful in controlling the formation of degradation product 2. Shortening the sonication time and lowering the probe power did reduce the formation of degradation product 2, but also resulted in decreased recoveries of active agent Y. The optimum conditions for the preparation of formulation 2 samples using the sonic probe was found to be 3 minutes at full power. Even under these conditions, however, degradation product 2 was still present.