

A New Method for Monitoring the Contamination of Glycerol with Diethylene Glycol

M. T. S. R. GOMES, M. I. S. VERÍSSIMO AND J. A. B. P. OLIVEIRA

Department of Chemistry, University of Aveiro, 3810 Aveiro, Portugal

Abstract

A quartz crystal microbalance has been used to check the purity of glycerol.

The experimental procedure involves no derivatization or other preliminary step except determination of water content. The protocol consists of the direct reading of the frequency of a quartz crystal with one face in contact with an aqueous solution of the glycerol to be tested.

The method is inexpensive, simple, rapid, and highly sensitive; diethylene glycol contamination at the 3.61% level could be detected.

In 1996, in Haiti, at least 59 children died of acute kidney failure caused by contaminated paracetamol syrup; it is not the first time that such an incident has been reported with fatal results (Allanson & Charvill 1997). The incident reported was traced to glycerol (Allanson & Charvill 1997; Blecher & Mercill 1998) containing up to 26% diethylene glycol (Allanson & Charvill 1997).

Pharmaceutical manufacturers should confirm the identity of the starting materials, to ensure that an incorrectly labelled rogue material cannot go undetected, and to guarantee its purity.

The similarity between glycerol and diethylene glycol makes contamination particularly difficult to detect. Ph. Eur./BP identification tests are based either on infrared spectroscopy or on a combination of chemical reactions and refractive index (Allanson & Charvill 1997). However, none of these methods is simple to use, as they all need precise control of the experiments. In fact, neither chemical reactions nor refractive index alone differentiate adequately between glycerol contamination with diethylene glycol, and the refractive index must be determined under carefully controlled conditions. The infrared spectra for glycerol and diethylene glycol are very similar; it is essential to record the spectrum of the material under optimized conditions, with regard to both the film thickness and the instrument set-up conditions, and to compare it with the spectrum of a reference glycerol sample,

acquired at the same time (Allanson & Charvill 1997). A recent study (Davies et al 1998) used Fourier transform infrared spectroscopy (FTIR) then spectra manipulation to produce the first-order derivative. The authors claimed to distinguish clearly, with this technique, contamination of glycerol with 30% of diethylene glycol, although contamination with 10% diethylene glycol or less might be confused with background noise (Davies et al 1998).

It can be concluded that there is a need for a simple, reliable, fast, and inexpensive method for detecting and quantifying glycerol contamination. Since 1982 it has been known that the frequency of a piezoelectric crystal in contact with an organic liquid depends on the density and viscosity of the liquid (Nomura & Okuhara 1982) and so any impurity in a sample of glycerol would lead to a change in the resonance frequency of the crystal in contact with it. The quartz-crystal microbalance (QCM) is highly sensitive and inexpensive; this makes it a promising technique for evaluating glycerol purity. A quartz crystal with gold electrodes was used to detect glycerol contamination and to quantify diethylene glycol.

Materials and Methods

Reagents

Glycerol for standards was anhydrous extra pure DAB, Ph. Eur., BP, USP, E422, JP 98.0–100.5% (Merck) and diethylene glycol was p.a. (Fluka).

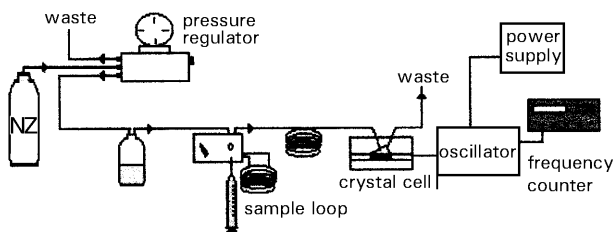


Figure 1. Schematic diagram of the equipment used.

The glycerol sample to be analysed was bought in bulk.

Apparatus

A schematic diagram of the flow injection analysis (FIA) methodology used is presented in Figure 1. A pressure regulator (Omnifit 3101) forces the water carrier flow (Milli-Q) from a bottle to the crystal cell. The sample was injected into the water carrier through an injection valve (Omnifit 1106) with a 0.5-mL sample loop. A 4-m coil separates the injection valve from the crystal cell (International Crystal Manufacturing; ICM) to create a time lag between sample signals and pressure pulses. The bottle and tubes were immersed in a thermostatic bath set to a temperature close to room temperature (23°C) to prevent slight temperature variations during the day, because these would affect both density and viscosity. All tubes were 0.8 mm i.d. Teflon.

The piezoelectric crystals were 9 MHz, polished AT-cut HC-6/U with gold electrodes (ICM). Oscillator and power supply were both home-made, and the frequency was monitored with a frequency counter (Leader LF826).

Procedure

The cell housing the crystal is shown in Figure 2. The 70- μ L chamber above the crystal was specially designed to reduce the possibility of air bubbles remaining on the crystal after filling from the dry state and to enable air bubbles in the liquid phase to

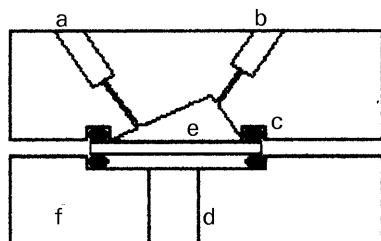


Figure 2. Piezoelectric crystal cell: a liquid entrance; b liquid exit; c O-rings; d chamber for batch analysis, dry and exposed to air; e piezoelectric quartz crystal; f acrylic body of cell.

pass out without sticking to the crystal. Care needs to be taken at the beginning of the experimental work to displace all air bubbles from the tubes, and mainly from the crystal surface in contact with the flowing liquid.

The frequency of the crystal is highly dependent on the flow rate of the liquid in contact with the crystal, and must be kept constant during the experiments; this is easily achieved by regulating the N₂ pressure by means of the pressure-regulator unit. The optimum flow rate, that which results in the greatest stability of the crystal frequency under a constant stream of water, was found to be 7 mL min⁻¹, corresponding to an N₂ pressure of 0.2 bar.

Milli-Q water flowed continuously through the crystal cell, while the frequency of the crystal was monitored. The sample to be analysed (0.5 mL) was then injected into the flow and the observed frequency decrease was recorded. Different sample loops (0.5, 1, 2 and 5 mL) were initially tested and it was found that the highest measurement precision was achieved with the smallest volume. All the samples were prepared by weighing precise amounts of the pure compounds, or mixtures, with Milli-Q water.

Results and Discussion

The analytical signals consisted of the frequency shifts observed after injection of 0.5 mL of the sample into the water flow. This decrease in frequency was observed as soon as the sample reached the crystal (1.5 min (approx.) after injection) and was observed for a few seconds, after which an inflection, corresponding to a return to the initial frequency of the crystal, was observed. The analytical signal was the difference between the constant frequency of the crystal in flowing water and the frequency at the inflection point. The return to the initial frequency of the crystal was slower than the initial frequency decrease. A second injection could be made only after complete recovery of the crystal frequency, and the waiting time was proportional to the magnitude of the observed frequency shift. No recovery took longer than 2 min.

Figure 3 shows the difference between the frequencies of the crystal in contact with different solutions and with water. Different frequency shifts were obtained with solutions containing equal concentration of different compounds. These results show that it is possible to use the frequency reading of a quartz crystal to test for glycerol purity. However, to guarantee that a particular

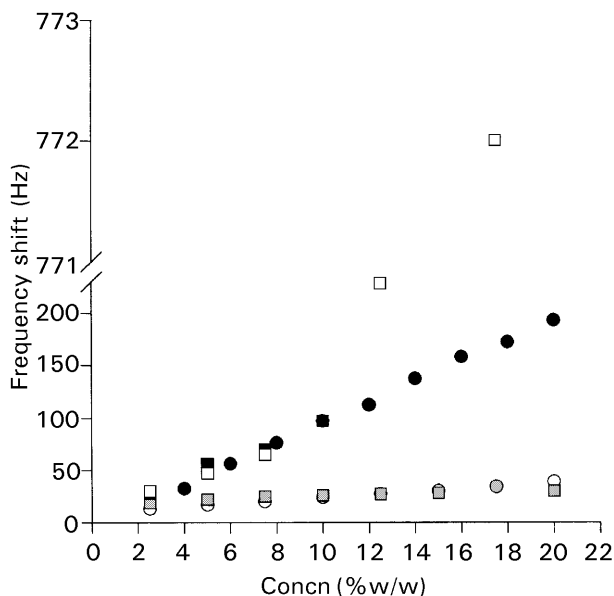


Figure 3. Frequency shifts observed with aqueous solutions of different compounds: ○ ethanol, □ methanol, ■ glucose, ● glycerol, □ acetaldehyde.

contaminant is detected, it is necessary that a previous study has shown a clear distinction between the frequency of the crystal in contact with a glycerol solution with the contaminant deliberately added, and the frequency of the same crystal in contact with a solution of pure glycerol.

Figure 4 shows the observed frequency decreases when aqueous solutions of different concentrations of pure glycerol or diethylene glycol were injected. Solutions of higher concentration than those displayed impaired crystal oscillation. Glycerol induced a decrease in the oscillation frequency of

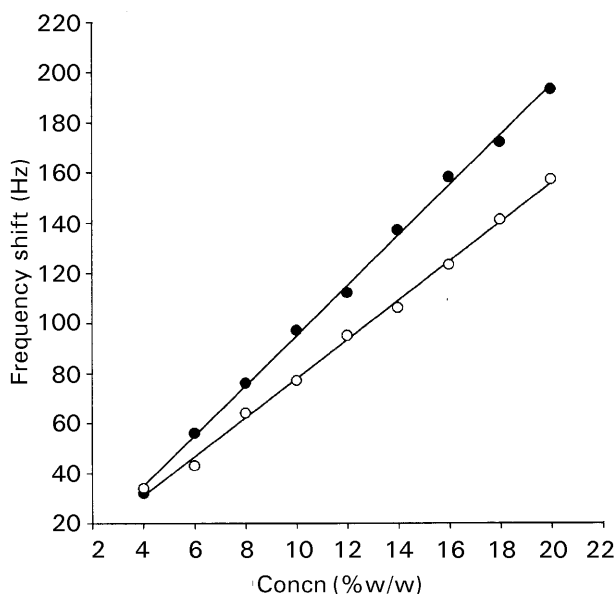


Figure 4. Frequency shifts observed with aqueous solutions of glycerol (●) and diethylene glycol (○).

the crystal which correlated linearly ($r^2 = 0.9979$) with glycerol concentration (C , %w/w), according to the equation:

$$\Delta F = -4.8222 + 9.9667C \quad (1)$$

Experiments with diethylene glycol resulted in smaller frequency shifts; these which also correlated linearly ($r^2 = 0.9971$) with diethylene glycol concentration (C , %w/w), according to the equation:

$$\Delta F = 0.0333 + 7.775C \quad (2)$$

These results show that glycerol and diethylene glycol induce different shifts in the resonance frequency of the crystal, and, as the slopes of the calibrations lines are different, greater absolute differences for solutions of equal concentration of each of the compounds can be observed for the most concentrated solution (20%). Similarly, for a contaminated glycerol sample, the difference between the frequencies obtained from contaminated and pure glycerol solutions will be higher for the most concentrated prepared sample, as long as crystal vibration is not impaired.

Figure 5 shows the frequency shifts observed with 20% (w/w) aqueous solutions prepared from mixtures containing different amounts of glycerol and diethylene glycol. Starting from a solution of pure diethylene glycol, the observed frequency shift decreases as diethylene glycol is replaced by glycerol. The minimum value corresponds to 49.67% glycerol, after which the frequency shift

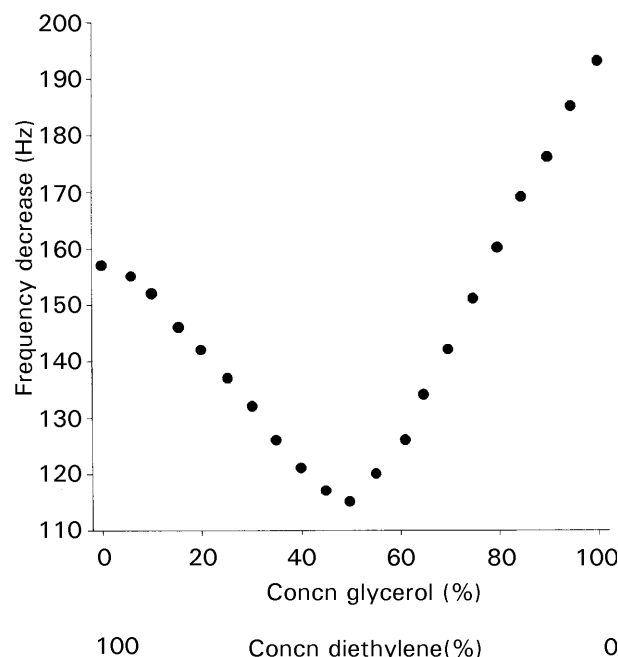


Figure 5. Frequency shifts observed with 20% (w/w) aqueous solutions of mixtures of glycerol and diethylene glycol.

increases, until the frequency of pure glycerol is attained. The linear regression after the 49.67% of glycerol mixture ($r^2 = 0.9976$) can be described by the equation:

$$\Delta F = 29.868 + 1.6298C \quad (3)$$

with a residual standard deviation $S_{y/x}$ of 1.9593. From this value, and the slope, calculation of the lowest amount of diethylene glycol that can be detected in contaminated glycerol yields the value 3.61%.

The graph presented in Figure 5 can be used to quantify diethylene glycol contamination of glycerol. If the frequency shift observed is above that of pure diethylene glycol (157 Hz), the result is straightforward and can be obtained simply from the graph. Otherwise, the graph gives two solutions, and a new reading must be taken after addition of glycerol, or diethylene glycol, to the sample to check, by observing the increase or reduction of the frequency shift, if the left or right linear branch of the graph should be used. Quantification of diethylene glycol can be achieved with an uncertainty of at most 3.98% ($\alpha = 0.05\%$), for concentrations above 50.33%, and with an uncertainty of at most 3.13% ($\alpha = 0.05\%$), for concentrations below 50.33%.

General procedure for detection and quantification of a contaminant

Determine the water content of the glycerol sample. Perform a QCM experiment, and record the frequency shift observed for a 20% (w/w) aqueous solution of the glycerol sample. Using Figure 3, obtain the expected frequency shift for a 20% (w/w) solution of pure glycerol with the same water content as the sample to be tested. Compare the two frequency shift values. If they are not statistically different, no contamination can be detected with the proposed method. Otherwise, the nature of the contaminant compound must be found by a suitable qualitative method of analysis, after which quantification can be performed by the QCM method. If the contaminant is diethylene glycol, Figure 5 can be used, otherwise, a similar graph must be obtained by analysing aqueous solutions containing a constant total mass of solutions with different mass proportions of glycerol and the contaminant.

Checking the purity of real samples

As an example, a sample of glycerol of unknown purity, bought in bulk, was tested for purity. A sample (4.0462 g) of the glycerol sample was dissolved in water (16.1917 g). This solution (0.5 mL)

was injected into the FIA/QCM system and the observed frequency shift for five replicates was recorded (172 ± 1 Hz). The density of the solution was 1.2552, and the water content of the sample, determined by Karl–Fischer titration, was 13.4 ± 0.3 g L⁻¹. From the glycerol linear calibration of Figure 3, a frequency shift of 192 ± 7 Hz would be expected for the sample after water-content correction. From comparison of the frequency obtained and the expected value ($\alpha = 0.05$) it can be concluded that the glycerol is contaminated.

Conclusion

The proposed method is simple, fast and inexpensive, and can be used to detect contamination of glycerol by any substance with density or viscosity different from glycerol. However, as the work is a direct consequence of the very serious incident reported in Haiti with diethylene glycol contamination, a complete study was performed with the aim of detecting and quantifying this particular compound in a glycerol sample. The method can detect diethylene glycol in glycerol at a concentration as low as 3.61%; this is not possible by carefully controlled FTIR spectroscopy with mathematical data manipulation.

The advantage of the proposed method—the possibility of detecting, with a single experiment, a large number of contaminants—is also its major drawback. Its lack of specificity implies that once it is established that the sample is contaminated, to quantify the contaminant by use of the proposed QCM method it is necessary to investigate the nature of the contaminant by other chemical tests or methods. However, it is worth emphasizing that an analysis that takes at most 4 min, without any previous sample preparation, could have prevented the deaths of 59 children.

Acknowledgements

We thank Carlos Ramos for the determination of the water content of the sample.

References

- Allanson, E., Charvill, A. (1987) Contamination of Glycerol, MAIL-Medicines Control Agency Journal May/June: 6–7
- Blecher, L., Mercill, A. (1998) Inactive \neq inert, progress in pharmaceutical excipients. Pharm. Technol. 22: 50–54
- Davies, J., Mortimor, D., Valovin, S. (1998) Quantification of glycerol contamination by diethylene glycol. Int. Lab. 28: 9A–9J
- Nomura, T., Okuhara, M. (1982) Frequency shifts of piezoelectric quartz crystals immersed in organic liquids. Anal. Chim. Acta 142: 281–284