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Supercritical Fluid Extraction

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Introduction

Plastics are a mixture of the polymer itself and many small molecules. Some, such as antioxidants and plasticizers, are added to the polymer to alter the properties. Others, such as residual monomers, processing aids and feedstock contamination are present inadvertently. The levels of these compounds must be accurately known by manufacturers and regulators in order to assess whether the plastic is fit for its intended purpose. There are usually many compounds present in the plastic, which makes analysis of their levels whilst still in the plastic very difficult. Usually, therefore, the compounds must be separated from the bulk polymer before analysis. Conventional methods include liquid/solid extraction and dissolution followed by reprecipitation of the polymer. Conventional solvent extraction methods tend to be very slow, e.g. Soxhlet extraction may require 24 hours to complete, and the dissolution/reprecipitation methods may result in extracts contaminated by oligomeric ‘waxes’, requiring further clean up before analysis. Methods producing clean, fast extracts are therefore very useful. The techniques of supercritical fluid extraction (SFE), pressurized fluid extraction (PFE) and microwave assisted extraction (MAE) have been shown to decrease extraction times, with lower use of solvents than conventional methods.

The Extraction Process

In SFE and PFE, the matrix is held in a cell, and the solvent is pumped into the cell under pressure. Commonly in SFE, the solvent is pumped continuously past the sample (dynamic extraction), dissolving the analyte molecule and carrying it out of the cell to be collected. In PFE it is more common for the solvent to be pumped until the cell is full, and then left for a period of static extraction. The analyte dissolves in the solvent, which is then flushed by more solvent from the cell to the collecting vial. MAE is carried out in one container, in which sample and solvent are placed. The solvent is heated by microwaves, and the analytes dissolve in the solvent. The vessel must then be allowed to cool before opening, and the extraction liquid can be separated from the extracted polymer by simple filtering. In simple terms, extractions with all methods can be thought of as proceeding in two steps, movement of the extracting compound from the bulk polymer to the surface by diffusion, and then dissolution in the solvent. The rates of these two steps is influenced by several factors.

Factors Affecting Extraction Rate

Particle size The distance the molecule has to move affects the time it takes to reach the surface. Small particles and thin films are therefore preferred. For spherical particles, the rate of extraction is proportional to $1/(\text{radius})^2$. Thus grinding 3 mm beads to 0.5 mm particles should increase extraction rate 36 times. For complete extraction, it is the size of the largest particles which dictates the extraction time, not the average particle size. Sieving to remove the largest particles can increase extraction rates dramatically. Grinding polymers generates considerable heat, and this can cause loss of analytes by degradation or volatilization. Polymers are usually freeze-ground under liquid nitrogen to prevent loss of analyte. A problem with polymers is that the particles can easily stick together, increasing the effective particle size.

Rate of diffusion Diffusion is driven by the tendency of a material to move from an area of high concentration to low concentration. The rate of diffusion depends on the size of the concentration gradient and the diffusion coefficient, a measure of how easily the diffusing material can move through the matrix. The diffusion coefficient follows the Arrenius equation:

$$D = D_0 \exp(-E/RT)$$

where D is the diffusion coefficient, E is the activation energy, T is the absolute temperature and R the gas constant. The diffusion coefficient will be greatest when E is small and T is large. Several factors affect the rate of diffusion, as outlined below.

Size of diffusing molecule Smaller molecules can more easily move through the matrix, hence activation energy is lower for smaller molecules and diffusion is faster.

Polymer matrix The more open the matrix, the lower the activation energy, and the faster the diffusion. The 'openness' of the matrix is affected by several factors. For polymers of the same type, the density gives a good indication. Thus diffusion is faster through low density polyethylene than high density polyethylene. The crystallinity of the polymer also has a large influence. The molecules in crystalline parts of a polymer are more highly ordered and densely packed than the amorphous parts, and hence diffusion is much slower through crystalline than amorphous polymer. An important factor in extraction from polymers is therefore the glass transition temperature, Tg, at which the polymer moves from a glassy to an amorphous state. The diffusion is much faster at temperatures higher than Tg.

Temperature The temperature affects the diffusion directly, by the T term in the Arrenius equation, and through effects on the polymer. From the Arrenius equation, the temperature should be as high as possible to maximize D. However, at very high temperatures, the polymer will soften and melt. This will cause the particles to agglomerate, and hence slow down the extraction. High temperatures could also cause the extracting molecule to decompose or react. The extraction temperature should always be above Tg for the polymer, but below the softening point.

Solubility If the concentration of the material at the surface is higher that the solubility, the amount extracted will be limited to that which can dissolve in the solvent. This will in turn slow down the rate at which the material diffuses to the surface, as the concentration gradient will be smaller. In solubility limited extractions, the extraction rate can be increased by increasing the solvating power of the solvent. In SFE, this can be either by increasing the pressure or adding a modifier. Increasing the flow rate will dissolve more material in unit time, and, therefore, has a similar effect to increasing the solubility.

Models of Extraction

Mathematical models have been developed to describe the extraction process, using equations for diffusion and solubility. Two terms are defined, a diffusion term, D/a^2, and a term proportional to solubility, ha, where a is the radius of the particles. The proportion of the total extracted (m/m_0, where m is the amount remaining and m_0 the amount at time = 0) can then be defined in terms of these variables. Plotting $\ln(m/m_0)$ against time produces a characteristic shaped line, shown in **Figure 1**(A). The amount remaining in the polymer falls rapidly at first, as the surface analyte is extracted. The plot then becomes linear, as the surface concentration falls and the extraction is controlled by the diffusion rate. The stages occurring during extraction can be illustrated by the concentration of analyte across a particle. **Figure 2**(A) shows a completely diffusion limited case, where ha is infinite. The concentration at the surface rapidly falls to zero, and the extraction is then

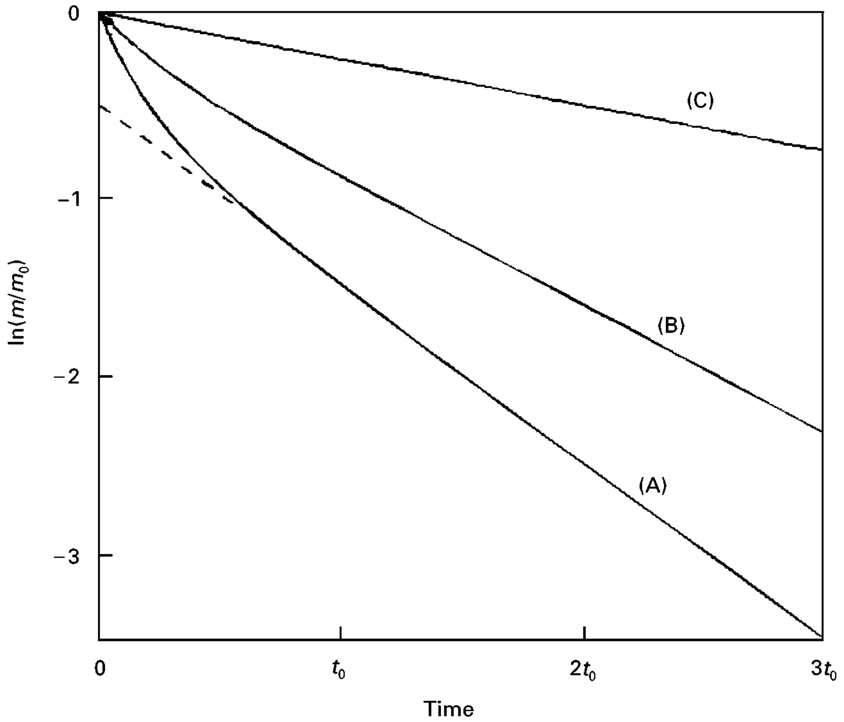

Figure 1 Plots of ln (m/m_0) for extractions with different solubilities. (A) = diffusion-limited extraction ('hot ball' model). (B) = intermediate solubility. (C) = solubility-limited extraction.

limited only by the rate at which analyte diffuses to the surface. This diffusion limited extreme is the 'hot ball' model, so called because it used modified equations describing the cooling of a hot sphere. The opposite extreme is shown in **Figure 2**(C), where, the diffusion is much faster than the ability of the solvent to remove analyte from the surface. The concentration gradient remains flat as the material is dissolved. The intermediate case shows the position where both solubility and diffusion play a significant part in the extraction. In each case, the initial step is the formation of the smooth concentration profile across the particle. This occurs during the sharply falling part of the ln(m/m_0) plot. After this, the profile retains the same shape, but reduces in size during the linear (exponential) part of the ln(m/m_0) plot.

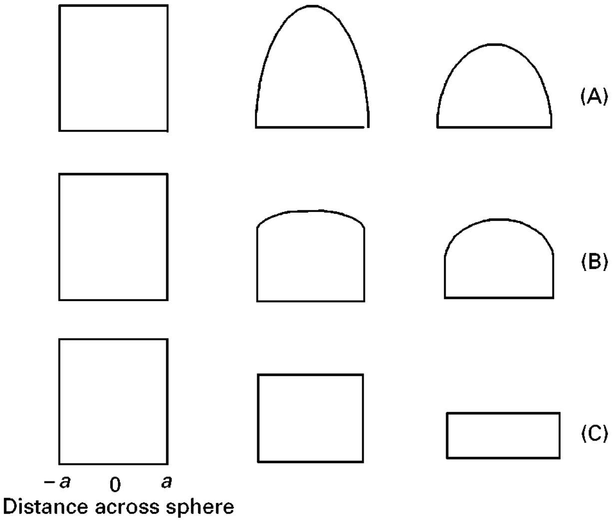

Figure 2 Concentration profiles during extraction for different values of solubility. (A) ha = infinite. Corresponds to Figure 1(A), the 'hot ball' model, extraction completely diffusion-limited. (B) ha intermediate. Corresponds to Figure 1(B), solubility and diffusion both contribute to extraction rate. (C) $ha = 1$. Corresponds to Figure 1(C). Solubility-limited extraction.

Diffusion- or Solubility-limited?

The strategies required to optimize an extraction often depend on the limiting factor, solubility or diffusion. Often, both will play a significant part in determining the extraction rate, but the dominance of one factor over the other can lead to short cuts in the optimization process. If diffusion is limiting (hot ball model), then greater swelling and higher temperatures will accelerate extraction. Increasing the flow rate or solubility will not help. In the case of solubility-limited extraction, greater swelling of the polymer will not help, and raising the temperature is likely to slow down SFE as the density of the solvent diminishes.

There are different methods for identifying the limiting factor in an extraction. A plot of $\ln(m/m_0)$ may show the classic 'hot ball' shape, indicating significant diffusion control (**Figure 1**(A)). If the line is straight, intersecting the origin, then this indicates solubility-limited behaviour (**Figure 1**(C)). A problem with these methods is that nonspherical particles, nonuniform initial distribution of analyte in the particle or a mixture of particle sizes can easily distort the shape of the curve. An alternative method is to measure extraction rates at different flow rates. If the extraction is diffusion-limited, then increasing the flow rate will have little effect. However, increasing the flow rate of a solubility-limited extraction will increase the extraction rate. **Figure 2** shows concentration profiles during extraction for different values of solubility.

Effect of CO_2 on Polymers

CO_2 dissolves in polymers, swelling, softening and plasticizing them. Material will diffuse much faster through the swollen polymer, and diffusion rates can be increased by several orders of magnitude. The *Tg* and softening points are also lowered. The extent of this lowering depends on the pressure and the nature of the polymer. CO_2 is mainly soluble in the amorphous part of a polymer, so the greater the amorphous content, the larger the effect of the CO_2. Increasing the pressure also causes more CO_2 to enter the polymer. The result is a complex interaction, with CO_2 lowering *Tg*, which increases the amorphous content, allowing more CO_2 to enter. This means that the softening point is not easy to predict from the usual softening point. In most cases, higher pressure of CO_2 will increase extraction rates in SFE from polymers, as this will enhance both solubility and diffusion, by increased swelling of the polymer. For highly amorphous or rubbery polymers, a maximum can be reached in the extraction rate with increasing pressure. This is because the softening point is lowered by the extra CO_2 dissolving in the polymer as the pressure increases. If it is lowered below the extraction temperature, the particles will agglomerate and slow the extraction. In these cases careful optimization of pressure and temperature is required for maximum extraction rates. Addition of a solvent as a modifier to the CO_2 can also swell the polymer, as well as increase the solubility of the analytes. A solvent known to swell the polymer at room temperature will have the greatest swelling effect.

SFE from Polymers

As indicated above, the optimization of SFE from polymers is not straightforward. Generally, the optimum conditions are likely to be at as high a temperature as possible, just below that at which the polymer melts, with as high a pressure of CO_2 as possible. The addition of a small amount (10%) of a cosolvent or modifier which is known to swell the solvent will accelerate extraction. There are many examples of polymer extractions with SFE, and extraction times are usually much shorter than conventional extraction times.

Pressurized Fluid Extraction

PFE is similar to SFE. The sample is held in a cell and the solvent is passed over it at elevated temperature and pressure. The commercial Accelerated Solvent Extraction (ASE^{TM}) is a trademark of Dionex Corp., and uses static extraction. Modified SFE equipment has also been used, and can use static or dynamic extraction. The solubility of polymer additives in liquid solvents at the high temperatures used in PFE is likely to be high enough that extractions are largely diffusion-limited. This is illustrated in **Figure 3** for the extraction of Irganox 1010 from polypropylene. The curve has been fitted assuming *ha* is infinite, i.e. completely diffusion limited (hot ball model). The fit is reasonably good, indicating that the assumption is valid. Pressure has much less effect on liquids than supercritical fluids. Liquids are almost incompressible, and therefore the pressure applied only has the effect of keeping the solvent liquid above its atmospheric boiling point.

The most difficult part of method development for PFE of polymers is selecting the solvent. Solvents used for atmospheric pressure extractions have usually been selected to swell the polymer, hence speeding up the extraction. However, when these solvents are used at high temperatures, the polymer dissolves. As the solvent cools in the transfer lines of the equipment, the polymer drops out of solution, causing blockages. Partially dissolved or melted polymer also agglomerates, increasing particle size and slowing down extraction rates. Solvents conventionally used for polymer extractions therefore cannot be used for PFE. The interaction between the solvent and polymer can be considered as a continuum, from no interaction (no swelling) through increasing swelling until eventually dissolution occurs which can be thought of as infinite swelling. The degree of interaction generally increases with temperature for a given solvent, and therefore, a solvent will just dissolve a polymer at a particular temperature, called the 'theta temperature'. We can assume that the extent of swelling is similar for each solvent at just below the theta temperature. The criteria for the best PFE extractions from polymers are

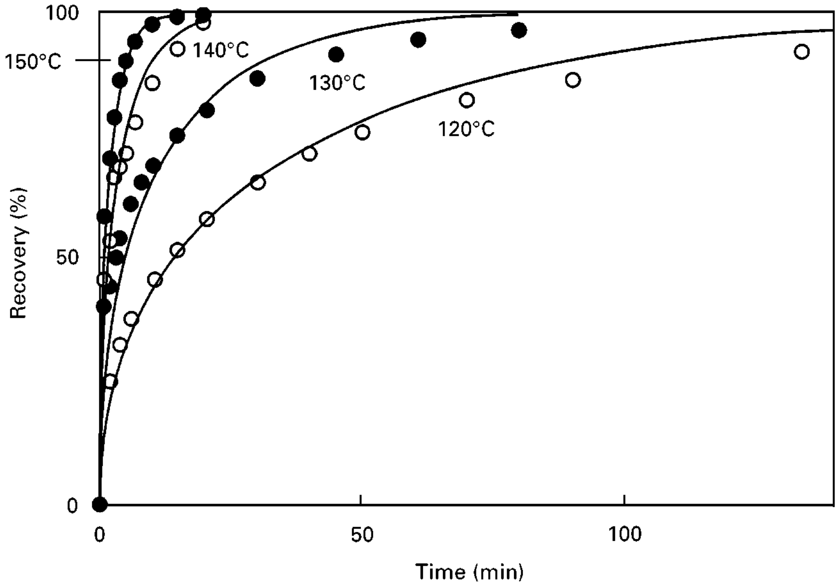

Figure 3 Extraction of Irganox 1010 from polypropylene by PFE. Symbols = experimental results, solid lines = fitted curve using the 'hot ball' model. (Reproduced with permission from Vandenburg, Clifford, Bartle *et al.* (1998) *Analytical Chemistry*, 70: 1943–1948).

as follows:

1. The temperature should be as high as possible to make D as large as possible. For a given 'openness' of the polymer structure, the higher the temperature, the faster the extraction. There will be an upper limit to the temperature, set either by the melting point of the polymer or the stability of the extracted analyte, which we can call $T_{\max}$.
2. The polymer structure should be as 'open' as possible. This effectively means that the polymer should be swelled as much as possible without dissolution or agglomeration of particles.

By combining the requirements of 1 and 2 above, what is required from the best extraction solvent is that the theta temperature is just above $T_{\max}$. The diffusion will then be maximized through both the highest temperature possible and the most open polymer structure. No other combination will give faster extraction.

Solvent Selection

Hildebrand Solubility Parameter

How can we select the best solvent? The use of solubility parameters is a good starting point. The Hildebrand solubility parameter is defined as the internal energy of vaporization divided by the molar volume. It is also called the cohesive energy density, and gives an indication of how strongly the material is held together. The principle of 'like dissolves like' applies, and solvents will dissolve polymers with a similar solubility parameter. The theta temperature tends to be higher the greater the difference in solubility parameter between solvent and solute. There are several more sophisticated measures of solubility in polymers, which take into account polar and hydrogen bonding interactions, but are not as simple as the Hildebrand parameter, which sums up the solubility behaviour as a single number. Hildebrand parameters are also widely available for polymers and solvents. Examples of common solvents and polymers is given in **Table 1**. However, the simplicity of the parameter also means that exact numeric accuracy is sometimes lost, and specific numerical predictions are difficult to make from this parameter alone.

Table 1 Solubility parameters of selected solvents and polymers

Material	*Solubility parameter ($MPa^{1/2}$)*
Hexane	14.9
Cyclohexane	16.8
Ethyl acetate	18.6
Chloroform	19.0
Dichloromethane	19.8
Acetone	20.3
2-Propanol	23.8
Ethanol	26.0
Methanol	29.7
Polypropylene	16.6
PVC	19.5
PET	20.5
Nylon 66	28
PMMA	19.0

Use of Binary Mixtures

A suggested scheme for PFE solvent selection is to use the solubility parameter as a guide for initial solvent selection of a binary mixture. The exact proportions of the solvents can then be determined with a few simple experiments. The two solvents should be a 'good' solvent, which has a solubility parameter close to that of the polymer, and a 'poor' solvent, with a solubility parameter distant from the polymer. Initially, T_{max} is determined by extracting with pure poor solvent for a set time at increasing temperatures. Initially, the amount extracted will increase with temperature as swelling and diffusion rate are increased. Eventually a maximum will be reached, representing either the melting of the polymer or the decomposition of the analyte. The maximum swelling at this temperature is then determined by a series of extractions with increasing amounts of the good solvent, again until a maximum is reached when the polymer begins to dissolve and agglomerate. If required, the proportions and temperature can be fine tuned to further maximize extraction rates. Often the initial values will be sufficient, and further marginal improvements not worth the extra effort. Once these conditions are identified, the time for the extraction can be determined by a series of extractions on the same sample until no further analyte is detected.

Examples of the best solvent mixtures suggested to date for some polymers are indicated in **Table 2**.

An advantage of this system of selection is that the solvents can be selected from any with a close and distant solubility parameter. The criteria may be cost, environmental considerations or simply those easily available. Equally good extractions could be obtained from a variety of different solvent mixtures. The extraction time for ground polymers is likely to be minutes rather than hours. Using the conditions in Table 2, the extraction times for polypropylene, nylon and PVC were all under 20 min. This is much faster than extractions using conventional extraction techniques.

Microwave Assisted Extraction

MAE can be either 'open focused', where the vessel is at atmospheric pressure, or 'closed vessel', where the solvent is kept liquid above the usual boiling point in a sealed, pressure resistant vessel. The effects of microwaves on extraction can be either purely thermal or through some other interaction of the microwaves with the matrix or analyte. For extraction from polymers, nonthermal effects have not yet been satisfactorily demonstrated, and the benefits for extraction may be primarily thermal. There are two components to the enhanced extraction rates for MAE:

1. Microwaves heat the solvent directly, resulting in much faster heating than conventional oven heating.
2. The closed pressure vessels allow heating above the atmospheric pressure boiling point of the solvent.

In MAE the extraction is always static. The solvent and polymer are placed in a vessel and heated by microwaves. The analyte dissolves in the solvent. Typical vessel sizes are 100 mL (e.g. MES 1000 by CEM Corp., USA).

In order to be heated by microwaves, the solvent must have a significant dielectric constant (relative permittivity). Some examples for common solvents and their dielectric constant are given in **Table 3**.

Optimization of extraction conditions with MAE is similar to that in PFE. Solvents and temperatures which are effective for PFE are also likely to be good for MAE, and extraction conditions can be worked out in a similar way. An extra requirement is that a major component of the solvent must have a significant dielectric constant, which does restrict the choice of solvents.

An alternative possible with MAE is the dissolution/reprecipitation approach. MAE does not have the problems of blocked transfer lines associated with PFE, and therefore it is not a problem if the polymer dissolves. The issue is whether the dissolution occurs faster than the extraction without dissolution. If the particles of polymer can be prevented from softening

Table 2 Suggested extraction conditions for PFE from some polymers

Polymer	*Poor solvent* (%)	*Strong solvent* (%)	*Extraction temperature* (°*C*)
Polypropylene	Propan-2-ol (97.5)	Cyclohexane (2.5)	140
PVC (plasticized with di-octylphthalate)	Hexane (60)	Ethyl acetate (40)	170
PET	Ethyl acetate (100)		190
Nylon 66	Hexane (60)	Ethanol (40)	170
PMMA	Hexane (70)	Ethyl acetate (30)	150

Table 3 Dielectric constant of solvents at 25°C

Solvent	*Dielectric constant*
Water	78
Nitrobenzene	35
Methanol	33
Ethanol	24
Ammonia	17
Hydrogen sulfide	9 (at − 85°C)
Benzene	2
Carbon tetrachloride	2
Cyclohexane	2

and agglomerating before dissolving, then it is likely that the dissolution will be fast. This is an area which requires further investigation.

MAE extractions of polymers have not been reported as much as SFE and PFE extractions, but those that have indicate very rapid extractions. In some cases, the most time-consuming part is the cooling of the vessels before opening. The advantages over PFE are more rapid heating and no possibility of blocking the transfer lines. The disadvantages are a more limited choice of solvents and longer cooling down times. SFE, PFE and MAE offer distinct advantages over conventional extraction methods, but at higher initial cost. The particular method chosen will depend on the exact requirements for extractions in the individual laboratory.

See also: **II / Extraction:** Microwave-Assisted Extraction; Supercritical Fluid Extraction. **III / Microwave-Assisted Extraction: Environmental Applications. Pressurised Fluid Extraction: Non-Environmental Applications.**

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POLYSACCHARIDES

Centrifugation

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Polysaccharides are, as their name implies, polymers of saccharide residues. The general formula of saccharide or carbohydrate residues is often quoted as $(CH_2O)_n$, although this is an oversimplification which needs to be modified in many cases to take into account, e.g. amino, sulfate and phosphate groups. Most saccharide residues are five or six membered ring structures with one member of the ring being oxygen.

Polysaccharides are becoming increasingly important in biomedical, pharmaceutical food and health products. The role of preparative centrifugation in polysaccharide development has not been significant compared to other classes of compounds such as nucleic acid and proteins. In part, this may be due to the considerable heterogeneity of polysaccharides, and techniques such as chromatography and precipitation-based methods have been more commonly applied. However, *analytical* centrifugation techniques are becoming increasingly used as a means of characterizing the size and shape of polysacchrides in solution as well as for investigating their interaction with each other and with other biopolymers such as pro-