Chromatography Today Help Desk

Silica gone bad

The Chromatography Help Desk

The help desk in this issue will be looking at a common problem that is observed with older generation silica columns primarily, but is occasionally seen with some newer generation silica based columns. The issue is around the batch to batch reproducibility and trying to get an understanding of the issues that separation scientist face, and the challenges that the manufacturers face in trying to ensure consistent product quality. It is one of the most frustrating aspects of separation science, that on occasion a method is developed on a particular column, and subsequent columns with the same part number do not perform the separation in the same manner. There are a range of reasons why this occurs, some of which the manufacturers have thankfully addressed, but some of which are inherent issues with the technology that is used.

Labelling issues

In the early days of column production, there was very little automation and so consequently manual handling issues were a problem, which could result in columns occasionally being mislabelled. This has resulted in chromatography users developing methods on effectively unknown columns. The helpdesk has come across examples where 50 mm columns have been labelled as a 100 mm column, which is relatively easy to identify as a labelling error, however this is not so easy if the stationary phase has been mislabelled. There are examples in the helpdesk inbox, which would suggest that the column used to develop an assay is not the same as that used for the subsequent validation studies, despite having the same part number. Fortunately, the use of electronic logging systems, bar codes and greater use of automation means that this is no longer a significant issue.

Effect of silica variability

Of greater significance for the separation scientist is the variability that is inherent within the manufacturing process. The primary reason for the variability is the substrate material that is commonly used, namely silica. The types of silica manufactured and the effect that silica can have on a separation has been discussed on several occasions in the helpdesk articles. It is also important to be aware of the effect that the packing process can have on the performance of a column, since column packing is still more of an art than a fully understood science

Why two nominally equivalent stationary phases, say C18, have very different retention mechanisms has been addressed in many articles previously, however it is always useful to go through the underlying theory to explain the situation. Ultimately it relates to two phenomena;

- The different forms of silanol groups that can exist at the surface of a silica particle
- The inability to completely cover the substrate surface, which results in the substrate being involved in the separation mechanism.

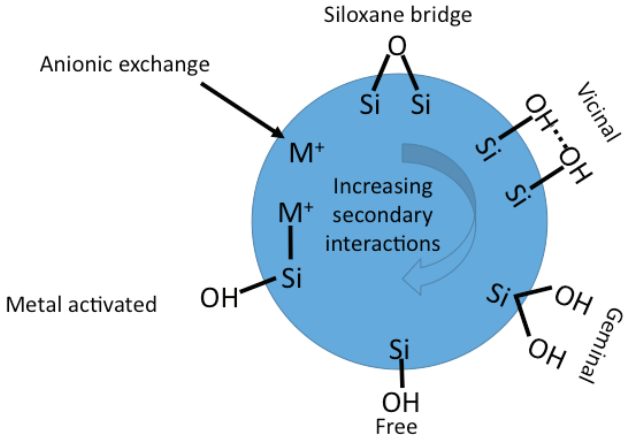

Figure 1. Schematic diagram of the different modes of interactions caused by the different surfaces of silica

Figure 1 is a schematic of the different forms of silica that can exist at the surface. Each of these forms of silica has different chemical properties, specifically different levels of acidity or degree of interaction with basic compounds. Manufacturers have realised that this causes issues and so with the newer phases, much effort is put into ensuring that the surface is relatively homogeneous. There are a variety of process that manufacturers can employ to reduce the variability of the silica surface including chemical treatment and heat treatment, however it could be suggested that despite the best efforts of the manufacturers a completely inert substrate material has still not been developed. The move to smaller particles presents some further challenges as some of the approaches that have been employed to overcome the physical stability issues of porous silica actually result in an increased activity of the substrate material.

The majority of the first type of spherical silica manufactured was derived from the polymerisation of a metal silicate, which resulted in an acidic surface due to a relatively high metal content. This process is still employed for many of the earlier generation of silica particles that are manufactured, and these silicas, often referred to as type 1 silica are often associated with increased tailing when they are used to separate basic compounds. Current

silicas are significantly less acidic which results in less activity associated with bases, i.e. less tailing.

The amount of tailing will give an indication generated by a base interacting with the acidic silanols, of the degree of acidity associated with the phase.

For some separations the increase in acidity is a beneficial factor as it will increase the amount of separation, and clever separation scientists will be aware of this and use it to their advantage. As a consequence of this it is can be stated that there are no bad stationary phases, just an inappropriate stationary phase selection. Thus a stationary phase which has a high degree of acidity may be ideal for separating certain compounds, where a silica which has reduced acidity is not an appropriate choice.

Peak Tailing factor

The peak shape that the chromatographer observes can have a substantial effect on the overall separation, this is particular the case when looking at impurity or degradation studies, where it is common to have one large solute peak in the presence of several much smaller impurity peaks. For compounds giving a similar response with a specific detector, the amount of separation that is required is less than when there is a substantial difference in the response of the detector for two peaks. Thus a separation that has a good resolution with two equally sized peaks may well not be good enough were one peak is much larger than the other. The peak shape will have a significant effect here, with tailing peaks being the cause of much concern amongst separation scientist looking at impurity studies. Reducing the degree of tailing by limiting the secondary interactions is therefore very beneficial in this case.

Effect of column packing method variability

The packing of the column with the stationary phase is often seen as more of an art form than a true science, surprising after nearly 40 years of commercial column packing, with manufacturers having a range of 'secret' recipes that will give them optimum packing performance. In truth there is substantially more research required in this area, in particular looking at a range of effects including;

Hardware, viscosity, temperature, flow, column packing pressure, packing solvents, rheology, particle morphology, particle strength, sonication, centrifugation, frit configuration, particle size distribution to name but a few.

To obtain a highly efficient column it is essential that at least these parameters are considered, however within the field of chromatography this is often seen as not being as exciting as developing new stationary phases, smaller particles, or a range of different morphologies, as this edition of Chromatography Today exemplifies. However, without at least a basic understanding of these fundamental parameters, which will vary dependent on the nature of the substrate and also the stationary phase, even the most exciting new stationary phase morphology development may have limited success. There is a lack of research in this area, which the help desk finds of some concern, in particular given the nature of particle development, which will necessitate the development of optimum packing methodologies to realise the incredibly high performance that the academics and then ultimately the end-user require. There are some notable exceptions to this and the interested reader is directed towards [1-11]. It should be noted though that the nature of the particle that is being packed will have a significant effect of the performance of the packing process, and this may be one of the biggest challenges that is facing separation scientists in ensuring optimal chromatographic performance as this is very reliant on a somewhat secretive manufacturing industry. The help desk would encourage manufacturers to work closely with academic groups in gaining a better understanding of the packing process, and not being reliant on the use of 'secret' packing recipes which will ultimately not perform with the development of novel stationary phases, due to the lack of fundamental knowledge.

Poor packing protocols can result in either peaks that front or tail resulting in poor column efficiency. Very poor protocols will result in fracture of the particles, which results in fines being produced and causes high column back pressures due to the blockage of outlet frits or interstitial spaces between the particles. Fines are very small particles will eventually move to the outlet frit and either result in blocking the frit, or potentially worse actually, if small enough, end up in the detector. Reversing the column and applying flow can remove these fines, however this is not an approach that the Help Desk would recommend. The help desk is aware of some columns that would only ever have an outlet frit (fortunately this is no longer the case), which caused a degree damage when the column was reversed to clear the exit frit, since the column was connected to an expensive mass spectrometer.

Stability of column packing material

It has already been mentioned that the packing material particle has a significant effect on the packing efficiency. One of the aspects that becomes more prevalent as smaller particles are employed in separation science is the compressibility of the particle. Porous particles will not necessarily behave as non-compressible particles under high pressure column packing and there may be a degree of elastic deformation that occurs, which on depressurisation of the column results in the creation of voids. This has been seen most significantly with organic polymers, however the compressibility of the particle can also be used advantageously under the right conditions.

Conclusion

The development of novel particles and stationary phases for separation science is exciting; however, it is essential that there is a consideration of the substrate material and the appropriate packing technology is employed to ensure that the best performance column is obtained. Manufacturers have started to address the purity of the substrate with high purity silicas, but there is still a lack of knowledge with regard to the column packing process. There are a variety of parameters that can be varied, some of which have been highlighted in this article, however it is evident that development of novel stationary phases has to be in conjunction with the development of column packing. Chromatography should be about the separation of Gaussian peaks and invariably this is not the case and better understanding of how to control the substrate and the packing process will go a substantial way to improving the current situation.

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