Microencapsulation of Silver Nanoparticles for the Detection of Analytes

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Introduction

Microencapsulation, defined as "the process of enclosing", is a widely-used technique that has been applied to a broad range of materials including food flavorants, drugs, proteins and enzymes. (Deasy, 1984)
The purpose of this study is to combine microencapsulation with Surface Enhanced Raman Spectroscopy (SERS). SERS is commonly used to detect low concentrations of analytes and has been applied to areas as diverse as medicine, environmental analysis and forensic science. The technique is based on bringing the analyte to be studied into contact with a microscopically-roughened metal surface such as the surface of silver or gold nanoparticles. Under these conditions large increases in Raman scattering are observed, i.e. the analyte signals are surface-enhanced (Bell, 2001)

Here the main aim has been to find appropriate methods to preserve enhancing silver nanoparticles by encapsulating them before storage. Since the particles are prepared in water, encapsulation methods for aqueous media are required and here results for microspheres prepared by both the water in oil in water (W/O/W) double emulsion solvent evaporation technique and the alginate extrusion technique are reported. The prepared beads were characterised by various techniques to examine bead topography, porosity and particle diameter. Finally, Raman spectroscopy was employed to determine if silver nanoparticles had been successfully encapsulated within the alginate beads.

Materials and Methods

Preparing Microspheres Using the W/O/W Double Solvent Evaporation Technique
The microspheres were prepared by a modified solvent evaporation method based on the formation of a W/O/W emulsion. (Hermann, 1995)

Preparing Calcium Alginate Beads
A 3% w/v sodium alginate solution was prepared and then dropped from a pipette into a beaker containing 25 ml of a 0.1 molar calcium chloride solution and calcium alginate beads formed instantly. The beads were left in the hardening solution for approximately 15 minutes.

Scanning Electron Microscopy
Scanning Electron Microscopy was used to examine the particle diameter, surface roughness and topography of the beads. Various samples were analyzed using SEM.

Particle Diameter of Microspheres Prepared by W/O/W Double Emulsion Solvent Evaporation
Numerous microspheres prepared at various agitation speeds between 50 rpm and 500 rpm were placed on metal stubs and examined using SEM. From each of these batches, a microsphere was chosen at random and its diameter was measured.

Water Adsorption Study
A water adsorption study was carried out to investigate the porous nature of the alginate beads. 3 g of alginate beads were immersed in water and reweighed every 20 minutes.

Encapsulation of Silver Nanoparticles

• 2ml of silver colloid was added to a sample jar along with 0.5ml of crystal violet (1 x10^{-5} M). 1ml of 0.1M sodium chloride was then added to the solution. NaCl was used to aggregate the silver nanoparticles within the solution since this increase the enhancement factor and thus gives stronger Raman signals. A Raman spectrum of this solution was then taken.

• The solution above was then incorporated into a 3% w/v solution of sodium alginate in a ratio of 3:1. (3 parts sodium alginate). A Raman spectrum was then taken of this solution.

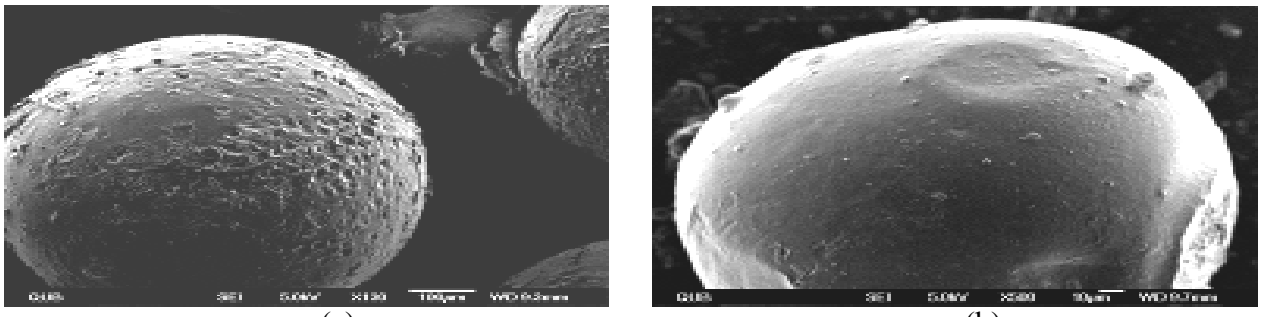
• The above solution was then dropped into a calcium chloride bath to make calcium alginate beads and a Raman spectrum was then taken of these beads.

Results and Discussion

Bead Topography

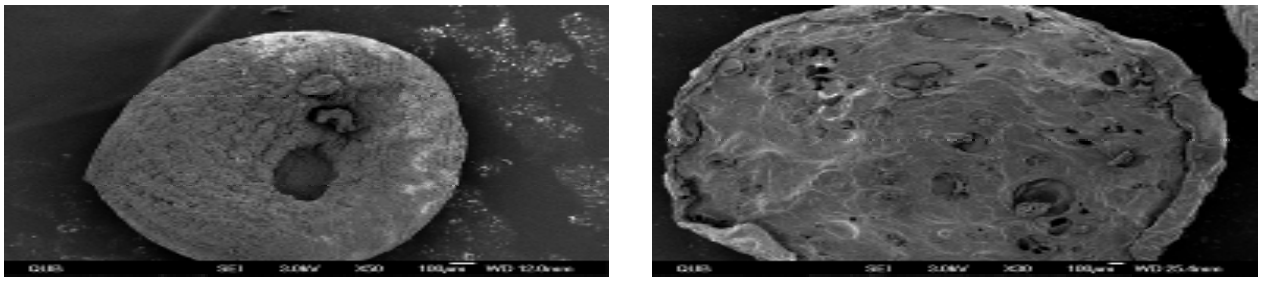
Figure 1(a) shows a bead prepared by the W/O/W technique at 50 rpm and it can be seen that the bead has an almost perfect spherical structure which is desirable. It is evident that the microsphere has a wrinkly appearance in certain areas and contains numerous pores. This type of porous nature would be unsuitable for the application of encapsulation of silver nanoparticles with the reason being that the nanoparticles need to be encapsulated until they are required at which point the microsphere can be burst open, releasing the nanoparticles from within.

Fig 1(b) shows a microsphere that was prepared at an agitation speed of 200 rpm. In comparison to the bead prepared at 50 rpm, this bead has a smooth non-porous appearance which is much more desirable for the application of nanoparticle encapsulation. The non-porous appearance of the microparticle would suggest that a slower polymer precipitation rate has occurred during the formation of the bead. Likewise the microspheres prepared at 300 rpm had a similar non-porous structure. However, the microsphere prepared at 500rpm was porous although the pore diameter was much smaller than the pores of the bead prepared at 50rpm. It appears that stirring speeds of 200-300rpm are within an intermediate stirring range which yield microspheres with a non porous appearance.



(a) (b)

Fig 1: (a) W/O/W bead prepared at 50 rpm (x130); (b) W/O/W bead prepared at 200 rpm (x500);



(a) (b)

Fig 2: (a) Alginate bead, uncut, air dried (x50); (b) Alginate bead, cut, freeze dried (x30);

Scanning electron micrographs of air dried and freeze dried calcium alginate beads are shown in Fig 2. Fig 2 (a) shows an alginate bead that was air dried for 24 hours. Detailed examination of the surface structure reveals that several wrinkles are present which is likely to be caused by collapsing of the polymer network during dehydration. In addition, it can be seen that the alginate bead retained a spherical structure.

In contrast, freeze drying of the alginate beads caused significant improvement on the maintenance of the surface of the beads and left them with a smoother surface. Fig 2(b) illustrates a cross section of an alginate bead. In particular, it is evident that the contents of the beads were preserved. It can be seen that they are composed of a matrix structure, composed of porous channels which run throughout the bead. Thus, in essence the beads do not exhibit properties of true 'shell and core' encapsulation but could be classed under the heading of 'matrix encapsulation'.

Particle Diameter of Microspheres Prepared by W/O/W Double Emulsion Solvent Evaporation
It is clearly evident from figure 3 that as agitation speed increased, the diameter of the microspheres produced decreased. This is due to the shear caused by the agitator. The greater the agitation speed, the greater the shear which caused the primary emulsion to be broken up more vigorously within the external phase which resulted in the production of smaller microspheres.

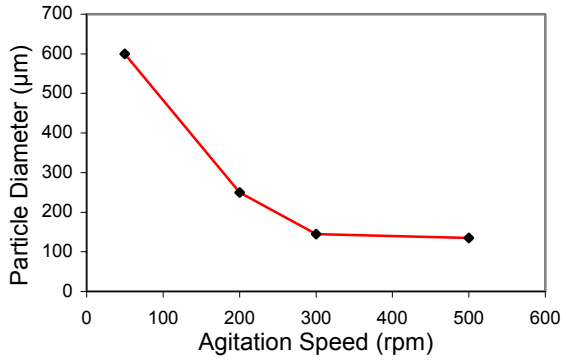


Figure 3: Graph of particle diameter of microspheres prepared at different agitation speeds.

Water Absorption Study
It can be seen that from figure 4 that from 0 minutes up to 100 minutes, the weight of the beads increases steadily until the beads have increased in weight by approximately 62%.

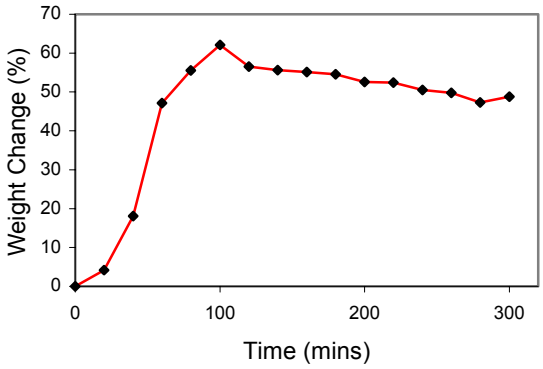


Figure 4: Water adsorption study of alginate Beads

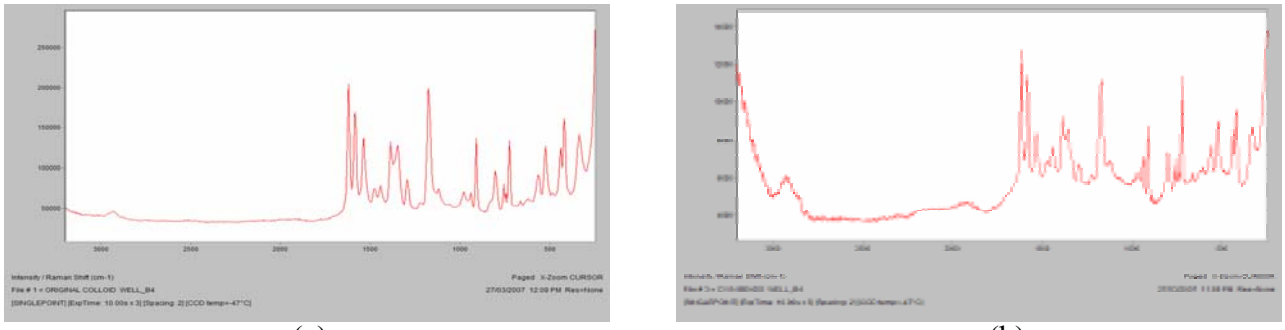
The weight gain is attributed to the beads absorbing water in order to fill the void regions within the beads that remain dehydrated until they reach the equilibrium state. Swelling of the calcium alginate beads in water lasted for about 100 minutes until the osmotic pressure equaled the force of the crosslinking bonds that maintain the structure of the polymer network stable. When these two forces were equal, no further water gain from the beads was observed. This study has shown that the

calcium alginate beads have a porous network through which water can be adsorbed. Therefore, it would be necessary to apply a further outer coating to the beads if they were to be stored for extended periods.

Encapsulation of Silver Nanoparticles

It was the objective in this experiment to utilise Raman spectroscopy to identify a test molecule, crystal violet, within a number of different media to prove that encapsulation of silver nanoparticles had occurred and that the particles still enhanced the Raman signals while encapsulated. Firstly, 0.5ml of crystal violet was added to 2 ml of silver colloid and a Raman spectrum of this solution was recorded (Fig 6(a)). This spectrum shows the unique spectral fingerprint of crystal violet.

The solution above was then incorporated into a 3% w/v solution of sodium alginate and a Raman spectrum was taken of this mixture. It was evident that the spectrum collected was almost identical to that of the first spectrum which showed that crystal violet along with silver nanoparticles were incorporated successfully within the sodium alginate solution and continued to enhance the crystal violet signal. Finally, the alginate mixture above was then dropped into a calcium chloride bath to produce beads and a Raman spectrum was taken of these beads (Fig 6(b)). Once again, the spectrum was almost identical to that of the first spectrum which illustrated that the silver nanoparticles along with the test molecule had been encapsulated successfully within the calcium alginate beads.



(a) (b)

Fig 6: (a) Raman spectrum of original silver colloid; (b) Raman spectrum of alginate bead containing silver colloid

Conclusions

● From the SEM images, it is evident that at stirring speed between 200 and 300 rpm yields W/O/W microspheres with a non-porous appearance

● SEM images of calcium alginate beads illustrated that freeze drying is a superior method to air drying in the preservation of the beads as shown by the smoother bead surface.

● As agitation speed increased, the particle diameter of the W/O/W microspheres decreased.

● A water adsorption study showed that the prepared alginate beads were quite porous. After 100 minutes in water, the beads increased in weight by 62%.

● By using Raman spectroscopy as an analytical tool, it was proved that silver nanoparticles were successfully encapsulated within the alginate beads.

References

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