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Light scattering study on the size and structure of calcium phosphate/hydroxyapatite flocs formed in sugar solution

by

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ABSTRACT

The formation, flocculation and sedimentation of calcium phosphate particles are among the main physico-chemical reactions that occur during sugar manufacture. The mechanisms through which processes occur in sugarcane juice are still poorly understood. This study (being part of a comprehensive investigation to unravel these mechanisms) reports on the size and structure of calcium phosphate particles and aggregates in water and sugar solutions at 20 °C using the small angle laser light scattering technique. The average size of the primary calcium phosphate particles was in the range 10.4 μm to 17.5 μm and the scattering exponents, which describe the structure of the calcium phosphate flocs, varied from 1.97 to 2.76. The flocs formed without flocculant are more compact in water than those formed in sugar solution. The compactness of the flocs was also affected by pH of the solutions. This effect has been explained by considering the electrical double layer phenomenon.

INTRODUCTION

There are numerous areas in the processing of cane sugar manufacture that are not clearly understood. As outlined by Doherty and Edye1, one of those areas is the mechanism of cane sugar clarification. Poor understanding of juice clarification is due to the complex physico chemical reactions occurring during the process. However, the main chemical reaction during the process is that between soluble phosphate in the juice and added free calcium ions (in the form of lime/lime saccharate) to raise the juice pH and prevent sucrose degradation. This reaction results in the formation of calcium phosphate flocs. An anionic polymer, a co-polymer of acrylamide and acrylic acid, is added to bridge the flocs and enhance their sedimentation.

Deben2 determined that the precipitation reaction is controlled by the initial reaction:

\[ Ca^{2+} + HPO_4^{2-} \rightleftharpoons CaHPO_4 \]

The calcium phosphate formed is insoluble in water and is unstable, undergoing a secondary reaction to form a more stable compound (hydroxyapatite).

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Honig, Deben and Jourani et al. found that the dominant calcium phosphate phases formed were as follows:

\[ \text{Ca}^{2+} + 2H_2PO_4^- \rightarrow \text{Ca}(H_2PO_4)_2 \]  

\[ \text{Ca}^{2+} + HPO_4^{2-} \rightarrow \text{CaHPO}_4 \]  

\[ 2\text{CaHPO}_4 + 2\text{Ca}_3(PO_4)_2 \rightarrow \text{Ca}_4H_2(PO_4)_6 \]  

\[ \text{Ca}_3(PO_4)_2 + 2\text{Ca}^{2+} + HPO_4^{2-} + H_2O \rightarrow \text{Ca}_6(PO_4)_3OH + 2H^+ \]

Tetra calcium phosphate (Ca₄(OH)₂(PO₄)₅) may also form.

There are a number of issues associated with calcium phosphate precipitation. Firstly, the nature of the phase of the calcium phosphate precipitate is highly dependant on the calcium and phosphate concentrations, pH, and the surface that precipitation is occurring on. Secondly, in the presence of organic acids (such as citric acid and isocitric acid), the precipitation of calcium phosphates is inhibited. Also, carbonates and magnesium ions, even at low concentrations, will affect the rate of precipitation of phosphates.

The structure of flocs is important in solid/liquid separation systems. A floc consisting of a more open structure will firstly help to trap insoluble components in the fluid and secondly it may allow for an increased settling rate as fluid can flow through the floc as well as around it. Sutherland and Tan tested the hypothesis that an open floc structure allowed for the flow of fluid through the pores. They found that under most conditions the assumption that the floc contains immobilised fluid holds true, except in the case where hindered settling occurs. As the solution becomes thicker and settling velocities fall, the importance of permeation velocity increases. This is relevant in the case of settling of calcium phosphate flocs in sugarcane juice, as settling occurs by the hindered settling mechanism. Alternatively, it may be that the higher the porosity the greater the drag force. That is, the greater the surface area for interaction between the solution and the floc, the higher the expected drag force. Experimentally, it has been shown that porous spheres experience higher drag than impermeable spheres. Surface area effects rather than internal fluid flow have explained this. This was detected at higher Reynolds numbers where the surface roughness plays an increased role.
There are various techniques that allow the measurement of the fractal dimension of particles. They range from small angle X-ray scattering, small angle neutron scattering to SALLS. All of the mentioned techniques, except SALLS, can be used to study colloids. Due to limitations of the techniques, they can not be used successfully to measure the aggregates formed due to flocculation. The reason for this is that the aggregates formed during flocculation are larger than colloids and can range in size from a few microns to a few millimetres.

The theory underlying the interpretation of the scattering data is the Rayleigh-Gans-Debye (RGD) theory. The theory involves the assumptions that the primary particle size is comparable to the wavelength of the incident beam, that the particles have low refractive index and that multiple scattering is negligible. The interpretation of SALLS raw data involves finding the scattered light energy momentum transfer vector, Q, from the scattering intensity of the flocs with respect to their scattering angle, as shown in Equation 6.

\[ I(Q) \propto M(R_g)^2 P(Q)S(Q) \]  

Where \( I(Q) \) is the scattering intensity, \( P(Q) \) is the form factor of the primary particle and \( S(Q) \) is the interparticle structural factor. \( Q \) is related to the refractive index of the particles, the wavelength of the light and the angle of the detector to the incident beam, as shown in Equation 7.

\[ Q = \left( \frac{4n\pi}{\lambda} \right) \sin\left( \frac{\theta}{2} \right) \]

Where  
- \( n \) - refractive index of the liquid  
- \( \lambda \) - wavelength of the laser  
- \( \theta \) - angle between the laser beam and detector

RGD theory allows the fractal dimension of the floc to be determined from the following simplified relation.

\[ I \propto Q^{d_f} \]

where \( I \) is the scattering intensity, \( Q \) is the momentum and \( d_f \) is the fractal dimension. The power law scattering behaviour of a fractal object can be determined from a log-log plot of the scattering intensity as a function of the momentum transfer, where the slope of the line gives the fractal dimension.

Recent work by has developed a model to describe the settling behaviour of fractal aggregates of calcium phosphate. Compact calcium aggregates, with fractal dimensions varying from 2.3 to 2.8 were used in this study. Though the study reported producing calcium phosphate flocs in sucrose systems, the effect the sucrose molecule on the size and structure of calcium phosphate particles was not presented nor discussed. This present study is an extension of the work of Tang and co-workers and examines the effect of pH and sucrose on the size and structure of calcium phosphate.

**EXPERIMENTAL**
Preparation of calcium phosphate flocs

Calcium hydroxide suspension was prepared by mixing 5.6 g of analytical grade calcium hydroxide with 25 mL of nano-pure water. The nano-pure water was prepared by using deionised water that was filtered through activated carbon, ion exchange resin and a membrane filter.

Calcium phosphate flocs in water were prepared by dropwise addition of freshly prepared calcium hydroxide suspension to 500 mL of 5.74 gL\(^{-1}\) phosphoric acid to reach the endpoint of either pH 7.6 or pH 7.8. The solution was stirred continuously during the addition of the calcium hydroxide suspension. pH values of 7.6 and 7.8 were chosen because in sugar milling, clarification is carried out around these pHs. The working temperature selected for this investigation was \(\sim\)20 °C.

Calcium phosphate flocs in sucrose solution were prepared by adding freshly prepared calcium hydroxide suspension to 500 mL of 5.74 gL\(^{-1}\) phosphoric acid containing 70 g of analytical grade sucrose.

A stock solution (5 gL\(^{-1}\)) anionic flocculant was prepared with nano-pure water. This was diluted to 1 gL\(^{-1}\) prior to use.

Analysis with small angle laser light scattering (SALLS)

The Malvern Mastersizer 2000 was used to obtain light scattering data from which size and fractal dimension could be calculated.

A preliminary evaluation on the optimum particle concentration for accurate calculation of the floc size and structure indicated that 3 mL of calcium phosphate solution (prepared as described in the previous section) in 500 mL of nano-pure water adjusted to pH of 7.6 or 7.8 was required.

To ensure accurate measurements of the primary particle size of the unflocculated calcium phosphate particles, a solution of the particles was sonicated for 7 minutes immediately before measurement in the Malvern Mastersizer. For the measurement of aggregate size and structure the technique above was modified because the aggregates were delicate in nature and easily broken up. The sample cell contained nano-pure water adjusted to the required pH. The aggregates were then extracted from the beaker with a pipette with a broad spout to prevent damaging them. Caution was used also when injecting them into the cell so that no great shear forces were exerted on them that could potentially damage them.

The calcium phosphate flocs were investigated by SALLS both in the presence and absence of a flocculant, and in water and sucrose solutions.
A Leica zoom 2000 dissecting microscope and an Olympus C800L digital camera was used to capture black and white photographs of calcium phosphate flocs. Due to the three-dimensional structure of the flocs not all of the flocs were able to be in focus simultaneously. As a result it was only possible to focus on different levels of the floc. The structures were investigated at three different magnifications (2.8, 6.3 and 13.2 times).

X-ray powder diffraction of calcium phosphate flocs

The crystallographic forms of the calcium phosphate flocs were determined by X-ray powder diffraction (XRD) using a PANalytical X’Pert MPD with CuK$_\alpha$ radiation of wavelength 1.5418 Å.

RESULTS

X-ray powder diffraction results

The XRD spectra of the samples are presented in figure 1. All the patterns show weakly diffracting microcrystalline samples. Although the sample prepared in water at pH 7.8 show no identifiable compound, the other samples contained hydroxyapatite as the major component. The results of figure 1 show that the presence of sucrose had no influence on the crystallographic form of calcium phosphate formed.

![X-ray powder diffraction pattern of calcium phosphate flocs](image)

**Figure 1. X-ray powder diffraction pattern of calcium phosphate flocs**

**Calcium phosphate without flocculant addition**

*Particle size*
To ensure measurement of primary particles only, and not aggregates, shear forces were applied to the particles by stirring the solution before measurement in the Mastersizer.

The 50th percentile range diameter \( (D_{(v,0.5)}) \) of the particles over 10 measurements was recorded over a period of 5 minutes in water and sugar solutions. The 50th percentile diameter allows for a comparison of average sizes under differing conditions. Table 1 gives the size of the particles in water and sugar solutions.

<table>
<thead>
<tr>
<th>System pH</th>
<th>( D_{(v,0.5)} ) (μm)</th>
<th>Scattering exponent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Sugar solution</td>
</tr>
<tr>
<td>7.6</td>
<td>10.4</td>
<td>13.6</td>
</tr>
<tr>
<td>7.8</td>
<td>13.6</td>
<td>17.5</td>
</tr>
</tbody>
</table>

The results indicate that larger sized particles were formed in sugar solutions and at higher solution pH. The differences observed between the particle sizes of calcium phosphate in water and sugar solution is probably due to increased solubility of lime in sucrose. This effect would increase calcium phosphate supersaturation, reduce the number of nucleating sites and thereby increase the crystal growth of hydroxyapatite. The increase in calcium phosphate particle size from a solution of pH 7.6 to a solution of pH 7.8 is due to increase in hydroxyl ion concentrations. The rate of formation of hydroxypatite from amorphous tricalcium phosphate is dependent on the hydroxyl ion concentration.

As the sizes of the primary particles are not comparable in size to the wavelength of the incident light, this assumption in RGD theory is no longer valid. Consequently, it was not possible to determine the fractal dimension of the particles. However, the scattered light still contains information about the structure of the floc and can be used for comparative purposes. To distinguish our measurement from a true fractal dimension, the measured quantity will be called the “scattering exponent”.

### Scattering exponent

The power law scattering behaviour of flocs can be used to investigate the structure of flocs, by a log-log plot of the scattering intensity \( (I) \) as a function of the momentum transfer \( (Q) \). Figures 1 and 2 illustrates the \( I \) vs \( Q \) trends. Figure 1 shows that for the calcium phosphate flocs in water (at both pH 7.6 and 7.8), there is a ‘kick’ in the \( I \) vs \( Q \) plot. The ‘kick’ observed here is not as large as that found by Wong et al.\(^{21}\) with polyacrylamide flocs. Wong and co-workers found that this behaviour was unique to flocs produced in neutral or basic pH with polymers of low charge per unit length and with a high molecular weight. They postulated that the ‘kick’ is caused by the
compression of the electrical double layer. This is therefore likely to have occurred with the calcium phosphate flocs in this study.

The results of the scattering exponents for the calcium phosphate flocs are given in table 1. The scattering exponent decreased as the pH increased for both the water and sugar systems. Jung and coworkers (1995) found that for similar sized primary particles, a higher scattering exponent implies a higher packing density. In this work as the primary particles do not vary significantly under the two different pH values, it can be assumed that the increased scattering exponent directly implies a higher packing density.

There are two factors that influence the scattering exponent of aggregates formed in the absence of a flocculant: speciation and compression of the zone of influence. Speciation is not an influencing factor for the aggregates formed in this project because the main component is hydroxyapatite. However, compression of the zone of influence could potentially account for the discrepancy between the sucrose and water solutions at the same pH. The reason is that there is the possibility of ionisation of the hydroxyl ions on the sucrose molecule. Hence, the sucrose solution would have a relatively greater number of hydroxyl ions that could lead to compression of the electrical double layer allowing particles to approach each other more closely and be dominated by Van der Waals forces. This will lead to an increase in the rate of flocculation, and hence impact on the type of floc structure.

![Figure 2. Scattering intensity vs momentum of calcium phosphate flocs in a water solution at pH 7.8](image)
Size of flocs

There was a great variety in the sizes of the aggregates formed without the use of a flocculant. Microscopic analysis of the flocs confirmed that the aggregates formed were loosely held. Figure 4 shows the structure of the aggregates formed in water at pH of 7.8. The aggregates varied between 300 μm to > 1000 μm (past the detection limit of the Mastersizer). With stirring it was noted that any large aggregates that had formed were broken up by shear forces. For instance, aggregates formed in water (pH 7.8) had a mean diameter of 390 μm without shear forces. With shear forces the average size of the aggregates was reduced to 54 μm within 30 s of the stirrer in the Mastersizer being switched on. Over longer time intervals the results indicate that aggregates increase in size again until a balance between shear forces and aggregate size is achieved.

The radius of gyration was calculated for the aggregates formed in the water solution at pH 7.6 and 7.8 and was 262.3 μm and 293.6 μm respectively. The radius of gyration of the aggregate formed in a pH of 7.8 was approximately 12% higher than that at 7.6. The larger size of the aggregates formed in a solution pH of 7.8 may be explained by double layer compression due to availability of hydroxyl ions. As the particles can then approach each other more closely, there is the enhanced probability of Van der Waals forces playing a role in keeping the particles together.
Calcium phosphate flocs formed in the absence of a flocculant

Size and structure of flocs

Calcium phosphate formed on addition of calcium hydroxide slurry to a solution of phosphoric acid was flocculated with a co-polymer causing aggregation of the primary flocs into secondary flocs. The sizes of many of the flocs were ~1 mm in diameter, and because of this, no accurate floc size could be obtained with the Mastersizer. The Mastersizer only has the capability for measuring particles in the range 1 μm to 900 μm. Figure 5 illustrates calcium phosphate flocs formed in sucrose solution in the presence of a flocculant. These flocs are denser than those of figure 4 formed in the absence of flocculants.
As in the case with calcium phosphate flocs formed without flocculant, the fractal dimension of the floc could not be measured because of the large primary particle sizes. However, the scattering exponents could be calculated. Figure 6 is a typical plot of the scattering intensity, I, vs Q for calcium phosphate particles with flocculant. The plot illustrates that at low Q values, deviation from the power law behaviour occurred. Wong and coworkers (1988) found that with flocs produced with polyacrylamide, interparticle interference dominated at low Q values while intraparticle behaviour occurred at high Q values. Hence the observed behaviour in this study could be accounted for by interparticle interference.

![Figure 6. A typical plot of scattering intensity vs. momentum for calcium phosphate flocs formed in the presence of flocculants](image)

**Figure 6.** A typical plot of scattering intensity vs. momentum for calcium phosphate flocs formed in the presence of flocculants

*Effect of pH and flocculant dosage*

Table 2 gives the results of the scattering exponents formed under different conditions. The results show that, as expected, the use of a flocculant results in less compact flocs. (cf. table 1). Also, there is an increase in the scattering exponent with increase in flocculant concentration. Contrary to the results obtained in the absence of flocculant, the calcium phosphate flocs formed in sugar solutions were in general less compact than those formed in water.

The results of table 2 also show that, in general, the scattering exponent was lower at the higher pH, similar to the studies were no flocculant was used.
Two of the major considerations in the flocculation of the particles are the amounts of flocculant needed for effective flocculation and the effect of restructuring. It is expected that the speed of formation of the floc would be inversely proportional to the density of the floc and hence speed of formation will impact on floc structure. A floc that forms rapidly would be expected to be less dense as the particles have not had time to arrange themselves to form the best packed structure.

The amount of flocculant (based on surface area) would also be a significant factor to consider because, as bridging flocculation occurs in these systems, the flocculant must contact the particles and attach to them. Hence, to achieve a high proportion of removal of the particles in solution it is necessary to ensure that there are enough sites for the flocculant to attach to in order for well-structured flocs to form.

In a typical sugar milling factory the solid load to the clarifier varies depending on a number of factors including the cane variety, the soil type and whether the cane was harvested after a rainfall. Therefore, dosing flocculant based on solid size and loading can probably achieve a more effective flocculation than dosing by solution volume as is presently practised by sugar factories. It is therefore necessary to develop an on-line device to measure the amount of solid loadings in secondary juice prior to clarification and relate it to flocculant dose.

**CONCLUSION**

The diameter of the primary particles of calcium phosphate in water and sugar solutions were in the range 10.4 and 17.5 μm, and so could not be used to calculate the fractal dimension of calcium phosphate aggregates. However, the SALLS technique was used to determine the scattering exponent and hence describe the structure of the calcium phosphate flocs.

Calcium phosphate aggregates formed without flocculant are more compact than those formed with flocculant. However, the flocs formed with flocculant are stronger and hence are able to resist shear forces. The results show that the compactness of the flocs is affected pH and the precipitating medium and have been explained using the electrical double layer phenomenon.
Further investigation is required on the effect of sugarcane juice impurities (soluble and insoluble) on calcium phosphate floc size and structure.

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