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Mechanistic studies on the bioavailability of calcium fluoride for remineralization of dental enamel

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Summary

Bioavailability problems have been encountered for calcium fluoride as a fluoride source in the remineralization of dental enamel. Satisfactory performance of a prototype delivery system in vitro could only be achieved under conditions of small particle size (sub-micron) and high loading (5 mg) of calcium fluoride. The most likely causes of this particle size/load effect were investigated. Dynamic dialysis studies revealed that any increased intrinsic solubility for the sub-micron calcium fluoride would be too small to account for the greater efficacy of this material for remineralization. The technique also indicated that the surface area of calcium fluoride should not be a crucial factor influencing the fluoride release rate under normal solution conditions. Studies conducted under conditions simulating those used for in vitro remineralization, however, revealed a marked inhibition of calcium fluoride dissolution by other components of the remineralizing system. The particle size effect/load effect on bioavailability of calcium fluoride is interpreted as an indirect surface area dependence resulting from the formation of an inhibitory surface complex which involves both solution calcium and phosphate ions.

Introduction

A simple prototype fluoride delivery system has been developed in these laboratories for the remineralization of dental enamel, using calcium fluoride as a solid phase

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source of fluoride. Initial in vitro testing (Yonese et al., 1981), however, failed to demonstrate the full remineralizing capacity expected from the normal solution behavior of calcium fluoride. Additional tests conducted in the oral environment (Abrahams et al., 1980) also achieved limited success. Later attempts to optimize the delivery system (Spooner et al., 1982) revealed that the performance of the system could be greatly improved by applying a high load (5 mg) of small particle (sub-micron) calcium fluoride. Satisfactory fluoride uptake has also been achieved recently (Bello et al., 1982) with these formulation conditions in the oral environment. Because, however, the load of calcium fluoride used is far in excess of what was calculated for remineralization of the enamel site, examination of the problem was sought with a view to improving the effectiveness of calcium fluoride without adopting extreme formulation conditions.

Studies were undertaken to reveal the source of the particle size/load influence on remineralizing effectiveness of calcium fluoride. The approach describes a systematic evaluation of the most likely causes of this effect which are as follows: (1) an enhanced intrinsic solubility for the sub-micron calcium fluoride due to the extreme smallness of the particles (Gibbs-Kelvin effect) or polymorphic variations in this sample; (2) a simple surface area effect on the dissolution or solution reaction rate of the calcium fluoride; and (3) an indirect surface area effect on dissolution rate arising from effects from other components of the system or remineralizing environment.

Materials and Methods

The sub-micron and large particle size (10–30 μm) calcium fluoride samples were the same as those used in the in vitro testing of the delivery system (Spooner et al., 1982).

The delivery system, which had been used by Yonese et al. (1981) and Spooner et al. (1982), consisted of either: (a) calcium fluoride crystals spread evenly on the surface of bovine dental enamel (0.25 cm^2 window) and covered by a membrane; or (b) calcium fluoride crystals sandwiched between two membranes and attached to the bovine dental enamel by using dental inlay wax.

Studies were concentrated first on the fundamental properties of the calcium fluoride samples, that is, the intrinsic solubilities and simple surface area effects. Detecting any increased solubility for the sub-micron size calcium fluoride was not readily achievable by conventional equilibrium determinations due to problems experienced in ensuing removal of all undissolved particles from solutions sampled for analysis. The solubility behavior of the calcium fluoride samples was therefore interpreted using a dynamic dialysis system, as illustrated in Fig. 1. In most cases, experiments were conducted in doubly distilled water. The dialysis bag [1] had an effective surface area of 24.4 cm^2 and a solution volume of 10 ml. The bag solution was stirred at a constant speed of either 150 or 600 rpm. The external dialyzing solution had a 70 ml volume with a solution level equal to that in the bag, and was

[1] Spectropore Dialysis Membrane (6000–8000 molecular weight cutoff).

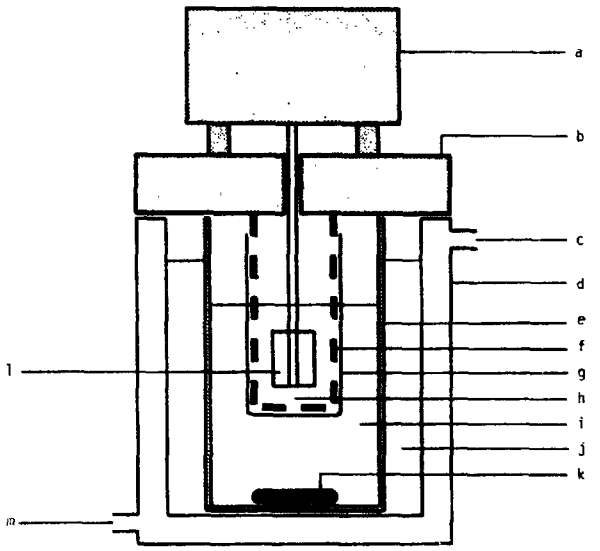

Fig. 1. Dynamic dialysis system. Key: (a) synchronous motor; (b) polyethylene cover; (c) water outlet; (d) jacketed beaker; (e) inner polyethylene container; (f) dialysis bag holding framework; (g) dialysis bag; (h) calcium fluoride suspension; (i) dialyzing solution; (j) water bath (30°); (k) Teflon stirring bar; (l) Teflon stirrer; (m) water inlet.

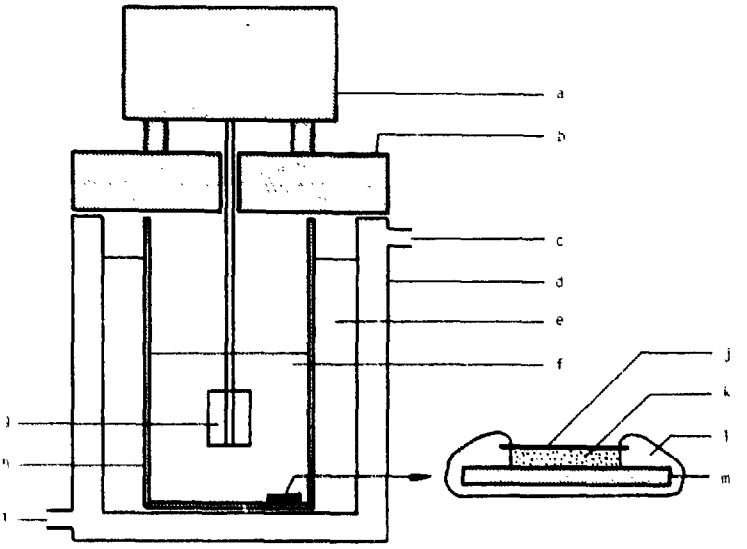

Fig. 2. Assembly for measuring release from the delivery system. Key: (a) synchronous motor; (b) polyethylene cover; (c) water outlet (d) jacketed beaker; (e) water bath (30°C); (f) dissolution medium; (g) Teflon stirrer; (h) inner polyethylene container; (i) water inlet; (j) ion-permeable membrane; (k) calcium fluoride slurry; (l) inlay wax; (m) glass slide.

stirred at a constant rate of 150 rpm. Calcium fluoride was added to the bag solution to produce a slurry density of between 0.025 and 2.0 mg · ml^{-1} of the sub-micron sample or 0.5–5.0 mg · ml^{-1} of the large particle size calcium fluoride. The temperature of the system was maintained at 30°C. The release of fluoride from the dialysis bag was monitored by removing samples of the dialyzing solution at appropriate time intervals and mixing these with a suitable volume of Total Ionic Strength Adjusting Buffer for analysis using a fluoride-sensitive combination electrode [2].

The influence of other components of the system or remineralizing environment on calcium fluoride dissolution was investigated under conditions which simulated those used in the in vitro remineralization experiments. Delivery systems were mounted onto glass slides as shown in Fig. 2. A slurry of calcium fluoride, prepared with 10 μl of doubly distilled water, was spread directly onto the surface of the slide, covered with an ion-permeable membrane [1], and enclosed with dental inlay wax so that 0.25 cm^2 of the membrane upper surface was left exposed. The lower membrane introduced recently into the delivery system (Spooner et al., 1982) was superfluous in these experiments and thus omitted. The rate of fluoride release under controlled hydrodynamic conditions was measured into 50 ml of standard remineralizing solution, consisting of 0.1 M acetate buffer at pH 4.5, containing 12 mM calcium and phosphate, with an ionic strength adjusted to 0.5 with NaCl, or into this solution without either the calcium or phosphate supporting ion.

Additional experiments were performed on the dissolution rate of large particle size calcium fluoride, suspended in some of the above solutions. These suspensions were stirred at 600 rpm in polyethylene containers, and samples removed for fluoride analysis were first passed through a membrane filter [3] to remove undissolved particles.

Results and Discussion

Dialysis experiments

The calcium fluoride suspensions released fluoride from the dialysis bag at a constant rate for a reasonable length of time as illustrated in Fig. 3. Thus steady-state release rates could be determined from the solution F concentrations in the suspensions. These rates are shown as a function of slurry density of both calcium fluoride samples in Fig. 4. The uppermost plot shows release rates from the sub-micron sample which increase rapidly to attain a limiting value at slurry densities as low as 1 mg · ml^{-1}. These rates were independent of stirring speeds used in the bag of between 150 and 600 rpm. The limiting or zero-order release rate is characteristic of the equilibrium solubility being attained in the bag, which occurred in this case at slurry densities of around 1 mg · ml^{-1} and greater.

Solution calcium depressed the dissolution rates of the sub-micron sample as shown by the lower curve in Fig. 4, although the limiting rate was again attained at

[2] Orion Research.

[3] Millipore filter, 0.22 μm pore size.

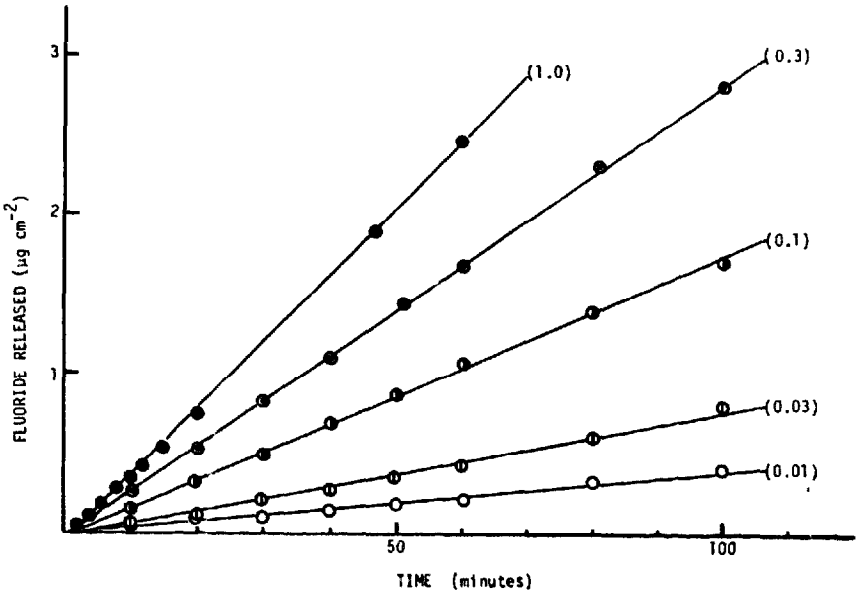

Fig. 3. Fluoride released from the dialysis bag with time. Values in parenthesis are the slurry densities $mg \cdot ml^{-1}$ of the sub-micron size calcium fluoride particles suspended in water.

around 1 $mg \cdot ml^{-1}$. The reduction in this rate was predictable from the reduced equilibrium solubility of calcium fluoride in the 12 mM calcium solution used. Solution calcium, therefore, merely exerted a simple common-ion effect on the

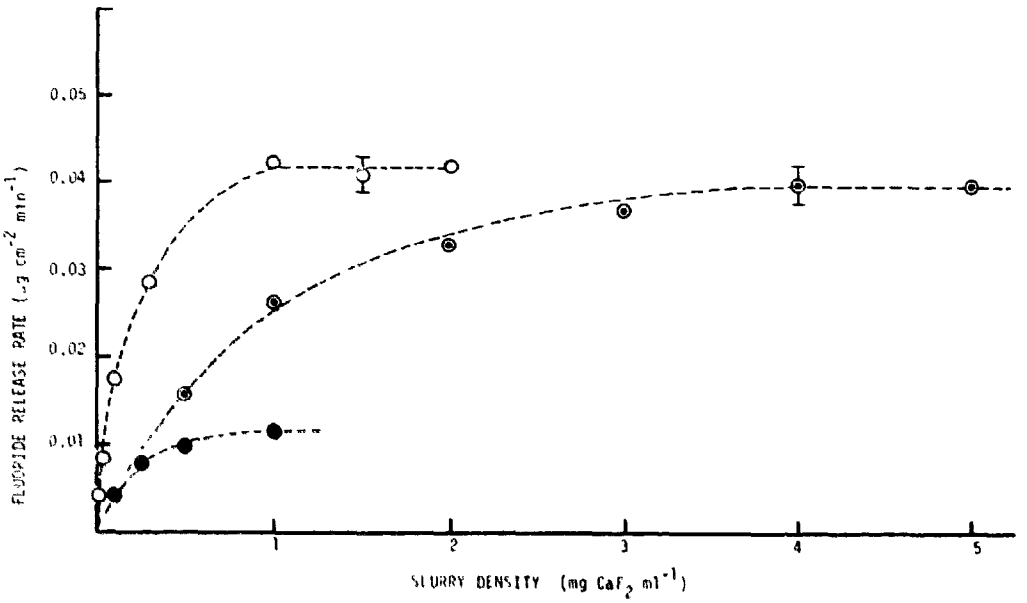

Fig. 4. Fluoride release rates from the dialysis bag as a function of slurry density for the sub-micron size calcium fluoride particles in water (○——○) and 12 mM calcium solution (●- - -●), and for the large particle size calcium fluoride in water (⊙——⊙). The bars represent the standard deviation for the points which were replicated.

equilibrium solubility without appearing to affect the rate at which this was attained.

As shown in Fig. 4 the large particle size sample did not achieve limiting release rates until their slurring densities were around 4 $mg \cdot ml^{-1}$ and greater. This slower attainment of equilibrium solubility in the bag is expected from the smaller specific surface area of this sample. Stirring in the bag for these cases was maintained at 600 rpm to ensure complete suspension of the particles.

The mean zero-order release rates for the sub-micron and large particle size samples were determined to be 0.0416 ± 0.0018 and 0.040 ± 0.0030, respectively. Equilibrium solubility differences could therefore be assumed to contribute little to the contrasting bioavailability observed for the two calcium fluoride samples in the in vitro studies.

Solid surface area would not be expected to have a direct bearing on fluoride availability from the delivery system, judging by the ease with which equilibrium levels of fluoride were attained in the dialysis bag with relatively low slurry densities. The slurry densities used in the delivery system were greater than those producing equilibrium solubility in the dialysis experiments by at least 50-fold for the sub-micron sample and at least 10-fold for the large particle size sample. So, even without considering that the stagnant nature of the slurry in the system should further diminish the importance of solution reaction rate and thus solid surface area, the conditions employed for the delivery system are fully expected to provide optimum fluoride levels based on the fundamental dissolution behavior. This inconsistency

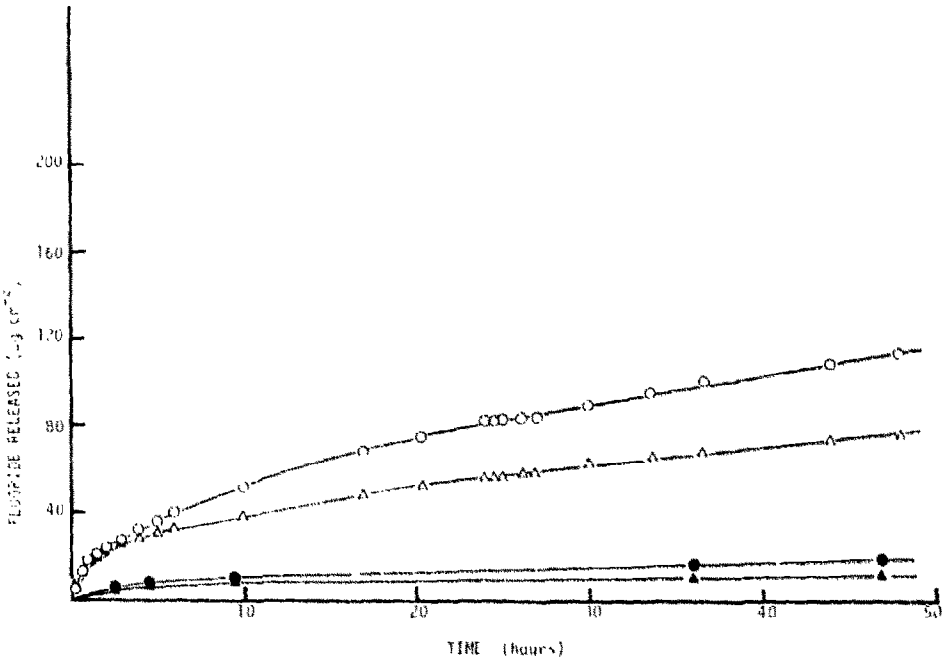

Fig. 5. Fluoride released as a function of time from the delivery system into the standard remineralizing solution. Delivery system containing sub-micron calcium fluoride at 5 mg (○——○) and 0.5 mg (●——●) load, and large particle size calcium fluoride at 5 mg (△——△) and 0.5 mg (▲——▲) load.

between the normal dissolution behavior of calcium fluoride and its performance in testing of the delivery system implies the influence of other factors, as described in the following.

Dissolution under remineralizing conditions

The rate of fluoride release from the delivery system into the standard remineralizing solution is shown in Fig. 5, for the same combinations of particle size and load of calcium fluoride as used in the remineralizing experiments (Spooner et al., 1982). These release rates are non-linear and become quite small after only a few hours, especially for the low loadings of calcium fluoride used. The ability of the various particle size/load combinations to release fluoride under these conditions is in the same order as their remineralizing effectiveness observed in vitro.

The fluoride release rates from the delivery systems into remineralizing solution without phosphate are shown in Fig. 6. These are constant over the same time period and much greater after the first few hours than in the previous case with phosphate ion present.

The release rates into the remineralizing solution without calcium were also linear and 1.2–1.9 times greater than in the absence of phosphate. Higher rates were expected from eliminating the common-ion calcium effect from solution.

The differences between the rates of fluoride release for the various combinations shown in Fig. 6 are probably associated with the ability of the solid to remain well dispersed in the delivery systems. That is, low loadings and large particles will tend to settle in the systems, thereby increasing the mean diffusional distance for dissolving ions. Even so, unlike those produced in the complete remineralizing

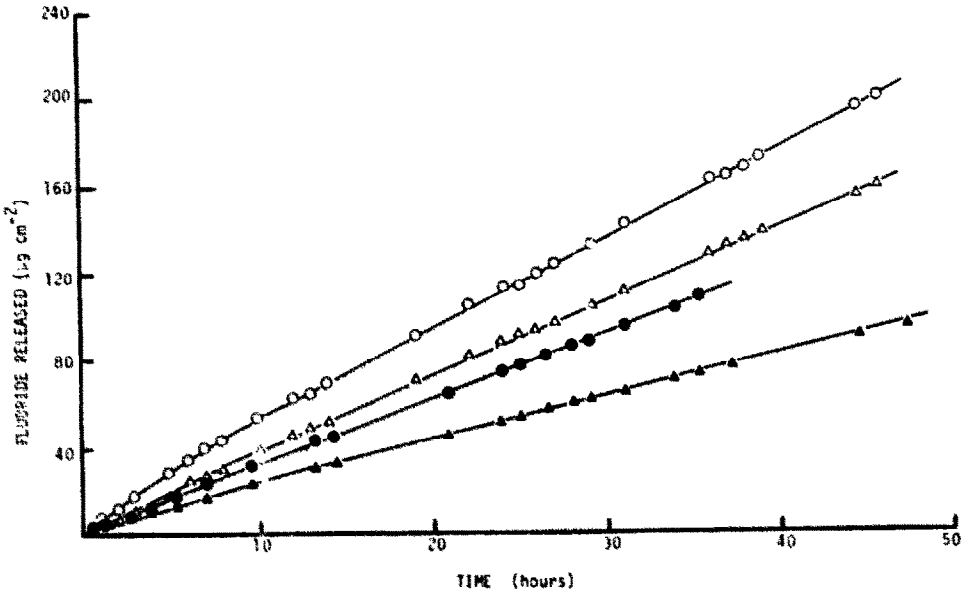

Fig. 6. Fluoride released as a function of time from the delivery system into the remineralizing solution without phosphate ions. Key as in legend to Fig. 5.

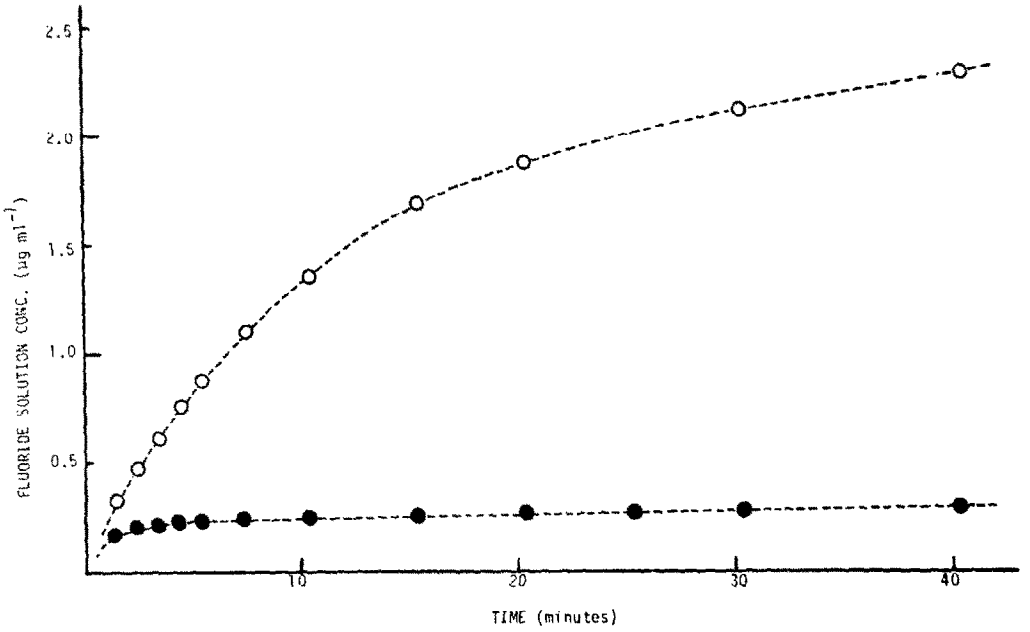

Fig. 7. Concentration of solution fluoride as a function of time found in suspensions of 1 mg·ml^{-1} of large particle size calcium fluoride in the standard remineralizing solution (●———●) and the remineralizing solution without phosphate (○———○).

solution, these settling differences are not considered large enough to account for the formulation effects observed in vitro.

Suspension studies

Supplementary studies were conducted on calcium fluoride suspensions to confirm that the supporting remineralizing components directly inhibit calcium fluoride dissolution. As shown in Fig. 7 the presence of phosphate in the dissolution medium of remineralizing components dramatically inhibits the dissolution of suspended calcium fluoride particles. This demonstrated that the inhibitory effect was not associated in any way with the ion-permeable membrane of the delivery system, but to the contrary, it appeared to be accentuated by a more direct contact with supporting remineralizing ions. Some fluoride could be recovered from the polyethylene vessel used for the dissolution study in phosphate-containing solution, by rinsing with 0.5 N HCl. However, this was only a small fraction of the reduction in fluoride resulting from having phosphate in solution, which was in excess of 100 μg. Also, when the phosphate was incorporated only after the suspension was allowed to become saturated, no reduction occurred in the fluoride level. These results show that losses of fluoride to surfaces within the apparatus were not responsible for the reduced appearance of solution fluoride when using the full complement of remineralizing ions.

Conclusions

The results reveal a marked inhibitory effect of the supporting remineralizing ions on the dissolution rate of calcium fluoride particles. This effect was found to be critically dependent on the presence of both calcium and phosphate ions in the remineralizing medium.

It would seem reasonable to predict that an insoluble surface complex is responsible for impeding the solution process, although the nature of any such reaction remains speculative. It is possible that an inhibitory complex forms which is apatitic in nature, since normally dissolving calcium fluoride will readily create a supersaturation with respect to fluorapatite ($C_{10}[PO_4]_6F_2$), the mineral intended for the remineralization process. Calcium fluoride particles were not found to provide effective nuclei for bulk deposition of fluorapatite from a solution highly supersaturated with this mineral. However, this finding does not preclude the occurrence of an inhibitory surface reaction, involving relatively small numbers of ions. This would also account for the failure of conventional IR examination to detect any compositional changes in calcium fluoride samples during treatment with the solution of supporting remineralizing ions. This aspect of the work will proceed using techniques more specifically adapted for surface analysis of solids and a more complete characterization of the process is anticipated.

The work has provided useful insight into factors affecting the bioavailability of calcium fluoride for remineralization of dental enamel, drawing attention to the influence of other components of the remineralizing environment. Means of alleviating this problem must await a more complete characterization of the process, which is currently being undertaken. The result, however, should allow a more enlightened approach toward the further development of this and other fluoride delivery systems utilizing solid phase sources of fluoride.

Acknowledgement

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