

# Chemical Changes of Flattened Enamel Surfaces with In Vitro Weak Acid Demineralization

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*Infrared internal reflection spectroscopy (IRS) was used to study changes in surface chemistry of enamel after in vitro weak acid demineralization. Hypotheses that relate previous histomorphologic observations to current IRS findings are discussed.*

Carious degradation of enamel surfaces is generally thought of as a process of acid demineralization.<sup>1-5</sup> When the pH at the enamel surface decreases to the level at which it has a significant effect on adjacent equilibria, mineral components dissolve and are lost rapidly, either to solution or to surrounding molecular complexes.<sup>6</sup> Enamel caries results from a local environment imbalance in which the prolonged effects of acid demineralization exceed those of remineralization.<sup>6-9</sup>

Recent study of unaltered surface enamel with the scanning electron microscope (SEM) has shown a characteristic morphologic pattern of weak acid degradation.<sup>10</sup> Fractured surfaces of natural incipient carious lesions (extracted human teeth) also have been observed.<sup>11</sup> Comparison of SEM photomicrographs from the two studies shows the similar morphology of the carious and in vitro acid change. Structural degradation of enamel has been observed and related to submicroscopic morphologic units; however, the chemical changes of weak acid enamel breakdown have not been determined. This lack of knowledge is the result of the inability of current analytic methods to accomplish nondestructive molecular characterization.

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Infrared internal reflection spectroscopy (IRS) recently has been demonstrated as a useful, nondestructive, analytic method for the study of enamel surfaces.<sup>12</sup> IRS is reproducible under varying conditions, sensitive to subtle differences of sample composition, and comparable to transmission infrared methods.<sup>12</sup> The IRS variation of infrared spectroscopy permits repeated analyses of a sample surface at sequential stages of chemical transition.<sup>13</sup> Thus, IRS seems an ideal method for determination of time-lapsed chemical changes in enamel surfaces exposed to weak acid demineralization.

This study is an initial attempt to map the chemical transitions of surface enamel challenged in vitro by weak acid immersion. Accomplishment of such surface characterization would allow correlation of morphologic and chemical data for more meaningful interpretation of previous information about the pathology of dental caries.

## Materials and Methods

Samples for this study were flattened enamel slabs prepared from fresh, sound teeth.\* Specimens were prepared for multiple reflection by slicing off enamel slabs roughly parallel to the labial surface.<sup>12</sup> Slabs then were ground by serial polishing with garnet strips until the surface was flat and smooth. Sixteen of forty prepared slabs, approximately 20 to 40 mm<sup>2</sup> each, were selected for study on the basis of flatness and lack of dentin contamination. Specimens were washed in water and dried in air before IRS analysis. Base-line (control) spectra were run periodically to check for prism adequacy and possible contaminants. Base lines and infrared IRS analyses were done

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with the use of multiple reflection 2 mm KRS-5 prisms in an IRS accessory.\* Specimens were scanned from 2 to 25 micrometers ( $\mu\text{m}$ ) in an IR spectrophotometer.†

Initial (untreated) IRS spectra were obtained for each specimen. After the initial IRS analysis, slabs were immersed in 17% lactic acid-gelatin mixtures<sup>14</sup> (pH 3.0) at 37 C for specified periods of time. Specimens 1 through 5 were demineralized in two stages (one minute plus four minutes) for a total of five minutes. Specimens 6 through 16 were further demineralized in three additional stages (accrued time of 8 minutes, 12 minutes, and 20 minutes) for a total of 20 minutes demineralization. After each stage of acid immersion, the samples were removed and agitated in three successive 500 ml rinses of distilled water for three minutes each (a total of nine minutes). Specimens were removed, dried in air, and analyzed by multiple reflection IRS. Flattened slabs were thus subjected to either 5 or 20 minutes total demineralization in incremental stages. Infrared IRS analysis was performed with instrumentation realigned for maximal spectral resolution after each stage.

### Results

IRS spectra of untreated flattened enamel slabs showed a broad, unresolved absorption in the 900 to 990  $\text{cm}^{-1}$  range. The usual sharp apatitic  $\nu_3$  phosphorous-oxygen absorption (asymmetric stretch) was not observed. Carbonate absorption was noted as a doublet at 1,405 and 1,450  $\text{cm}^{-1}$ , and a definitive band was noted in the 860 to 880  $\text{cm}^{-1}$  range.<sup>12,15</sup> The  $\nu_4$  phosphorous-oxygen absorption band (asymmetric bend) was observed as a double degeneracy. The high energy  $\nu_4$  segment occurred as a sharp, well-defined band at approximately 600  $\text{cm}^{-1}$ , and the remaining segment (low energy level) was broadened and poorly resolved in the 540 to 570  $\text{cm}^{-1}$  range (Fig 1).

Definitive changes in IRS surface absorption were noted after weak acid etch. After one minute demineralization, the broad  $\nu_3$  band resolved into two separate peaks. Bands, although indistinct, were observed at 930 to 950  $\text{cm}^{-1}$  and 1,000 to 1,010

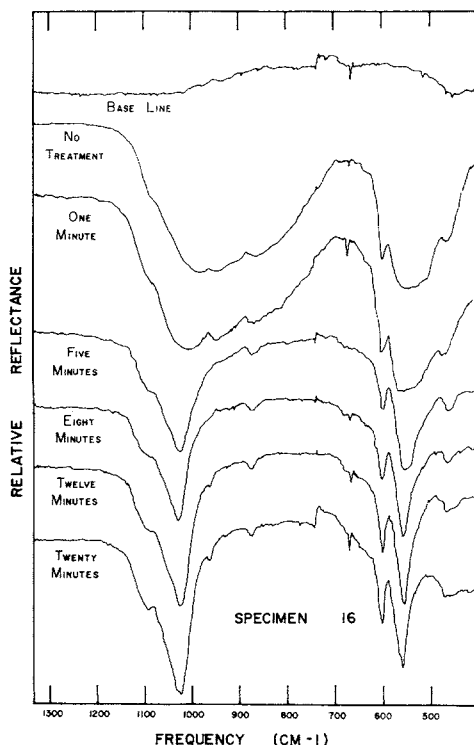


FIG 1.—IRS spectra of representative flattened enamel slab (specimen 16) through serial stages of weak acid demineralization. Note shifts of peak maxima to high energy levels with improved peak resolution as demineralization progresses. Carbonate absorption at approximately 870  $\text{cm}^{-1}$  persists after 20 minutes demineralization.

$\text{cm}^{-1}$ . Carbonate absorption at 860 to 880  $\text{cm}^{-1}$  was not affected significantly by the initial acid etch. Absorption above 1,300  $\text{cm}^{-1}$  diminished in intensity, but did not shift in frequency. The low energy  $\nu_4$  segment sharpened appreciably and was resolved at approximately 550 to 560  $\text{cm}^{-1}$ . Thus, poorly defined absorption bands of phosphate vibrations were defined more clearly after brief demineralization, with absorption maximums shifting to frequencies of higher energy (Fig 1).

After five minutes demineralization, further accentuation of previous changes were demonstrated by IRS. The low energy  $\nu_3$  segment continued to shift to approximately 1,030  $\text{cm}^{-1}$  as a well-defined peak. The  $\nu_3$  shoulder at about 1,090  $\text{cm}^{-1}$  became resolved, along with diminished intensity of

\* Wilks Model 45 Micro-ATR, Wilks Scientific Corp., So. Norwalk, Conn.

† Perkin-Elmer Model 337, Downers Grove, Ill.

carbonate absorption at 870 to 880  $\text{cm}^{-1}$ . At this stage, all but a trace of the once strong 900 to 990  $\text{cm}^{-1}$  maximum had disappeared. The  $\nu_4$  absorption was similar to that of the one minute demineralization stage, except for a continued accentuation and increased resolution of the low energy segment at 560  $\text{cm}^{-1}$  (Fig 1).

Increase of the total time of demineralization to 8, 12, and 20 minutes, respectively, had minimal effect on the chemical nature of the enamel surface, as demonstrated by IRS (Figs 1, 2). Peak resolution of low energy  $\nu_3$  and  $\nu_4$  bands improved slightly and approached classic needle points at 1,030  $\text{cm}^{-1}$  and 562  $\text{cm}^{-1}$ , respectively. In addition, absorption at 1,095  $\text{cm}^{-1}$  was resolved as a weak band rather than as the previous shoulder. Carbonate bands, although weak, were perceptible after a total of 20 minutes surface demineralization.

### Discussion

Infrared IRS analysis demonstrated that a distinct chemical change had taken place as specimens of flattened enamel underwent successive stages of demineralization. Broad and indistinct absorption bands observed in the IRS spectra of untreated slabs became resolved into distinct peaks after slab demineralization. In addition, absorption maxima shifted to higher energy levels (Figs 1, 2). These changes indicate that surface components responsible for broadened, ill-defined absorption in untreated slabs were dissolved preferentially in early stages of

acid demineralization. The remaining surface mineral probably became more crystalline, as indicated by improved band definition.<sup>16,17</sup> Continued demineralization accentuated these effects and indicated a gradual increase of surface homogeneity. Generally, IRS spectra of flattened enamel slabs became almost identical to IRS spectra of powdered enamel<sup>12</sup> reported previously with increased accrued time of enamel slab demineralization.

IRS analysis reported here indicates that surface carbonates were present, and persisted through several stages of weak acid enamel demineralization. The relatively high solubility of unbound carbonate would predispose this group to preferential dissolution in weak acid demineralization procedures. Although surface carbonate absorption diminished significantly in degree, definitive carbonate bands were shown some time after spectral evidence of amorphous phosphate loss (Fig 2). This observation is consistent with previous reports that carbonate is present in enamel, not only in soluble form, but at least partially as a more stable apatitic configuration.<sup>15,18</sup>

The honeycomb surface morphologic pattern of weak acid enamel demineralization has been demonstrated recently with the SEM.<sup>10</sup> Because experimental samples in this study were flattened enamel slabs, and natural enamel surfaces were used by Hoffman et al, data reported here should not be related directly to the SEM observations. However, it seems reasonable that weak acid demineralization would produce similar lesions in flattened and natural enamel surfaces. If this is assumed correct, a correlation between the IRS-determined chemical changes and morphologic degradation may be attempted.

Several theories have been proposed to explain the submicroscopic pattern of enamel demineralization.<sup>11,19,20</sup> Although evidence has been offered in support of each, no single hypothesis has gained complete acceptance. IRS data suggest preferential dissolution of relatively soluble surface mineral in the early stages of acid demineralization. Histomorphologically, this IRS observation is explained best by the hypothesis that the initial change of weak acid enamel demineralization is the loss (dissolution) of amorphous phosphate mineral from the intercrystalline space. Thin-section electron micro-

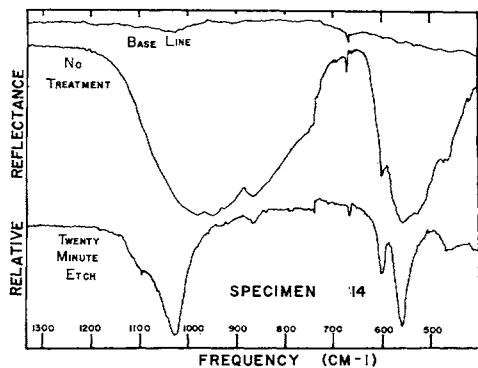


FIG 2.—IRS spectra of specimen 14, before and after 20 minutes of weak acid demineralization. Spectral changes (sharpening, and shifts to higher energy regions) were similar in all specimens tested.

scope studies of enamel have shown crystals in tightly packed and irregularly scattered distributions. In addition, the variation in crystallite angulation<sup>21</sup> is indicative that spaces between crystallites seen in EM photomicrographs are real,<sup>20</sup> rather than artifacts of preparation.<sup>22</sup> Although direct electron microscope evidence of an amorphous mineral phase is lacking at this time,<sup>21</sup> it is probable that noncrystalline material exists in the intercrystalline space. Loss during preparation or inadequate resolution could account for its apparent absence in electron photomicrographs. Interstices of crystallites must be rich with inorganic material to account for growth in crystallite size during maturation. Assuming that the space between crystallites is real, it is likely that the *in vivo* intercrystalline milieu contains high concentrations of inorganic mineral components in solution or amorphous form. When surface enamel is exposed to acid demineralization, superficial noncrystalline inorganic material is readily lost and creates a local sieve of apatite crystallites. Lack of acid-stable protein fibrils in the prism core<sup>23</sup> would result in chemical erosion of unsupported crystallites and yield a honeycomb surface pattern of enamel degradation.<sup>11</sup>

An alternative mechanism that may account partially for mineral loss during weak acid demineralization is differential solution of enamel crystallite constituents.<sup>20</sup> Initial mineral loss would then be governed by solubility properties of crystalline components rather than those of an amorphous phase. IRS data suggest preferential dissolution of relatively soluble surface mineral in the early stages of acid demineralization. Poorly resolved absorption characteristics of the specimens before treatment support the contention that a more amorphous component is present with the crystalline component. Also, crystalline minerals usually yield more definitive, highly resolved infrared spectra.<sup>17</sup> Theoretically, blunted absorption maxima could be attributed to the additive presence of a number of closely related crystalline species. However, the degree of broadening observed in the spectra of untreated slabs indicates that origin from crystalline mineral is highly improbable. IRS data, in addition to evidence reported previously,<sup>10,24</sup> suggest that preferential loss of crystallites on the basis of differential solubility, is a possible

but unlikely mechanism of mineral loss during initial carious degradation.

### Conclusions

Partial characterization of the chemical changes in the enamel surface at various stages of demineralization was achieved by the use of infrared IRS analysis. Sequential IRS analysis of flattened enamel slabs at different stages of demineralization produced evidence that characteristic chemical changes occur with exposure to weak acid. Spectral evidence suggests that relatively soluble amorphous phosphates are lost preferentially in early stages of weak acid enamel demineralization. Loss of amorphous phosphate mineral from the intercrystalline space is the most probable explanation. The persistence of surface carbonate absorption after successive stages of flattened enamel demineralization indicates that at least some carbonate is bound in a relatively stable apatitic form.

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