ANNEX 5

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From: Manuel Valiente <manuel.valiente@uab.es>
To: Anna Torrado <Anna.Torrado@uab.es>

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From: abc@Springer.de
To: manuel.valiente@uab.es

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Title : THE EFFECT OF RESINS PARTICLE SIZE ON THE RATE OF IONS RELEASE. INTERACTIONS IN MIXED BED SYSTEMS.
Corresponding author : Dr. Manuel Valiente

Dear Dr. Valiente,

I am writing to you on behalf of Editor Prof. Günter Gauglitz.

We are pleased to inform you that your manuscript ABC-00525-2003.R2, entitled "THE EFFECT OF RESINS PARTICLE SIZE ON THE RATE OF IONS RELEASE. INTERACTIONS IN MIXED BED SYSTEMS.", has been accepted for publication in Analytical and Bioanalytical Chemistry.

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THE EFFECT OF RESINS PARTICLE SIZE ON THE RATE OF IONS RELEASE. INTERACTIONS IN MIXED BED SYSTEMS.

A.Torrado and M. Valiente*

Departament de Química, Unitat Analítica, Centre GTS, Universitat Autònoma de Barcelona, Facultat de Ciències, Edifici Cn, E-08193 Bellaterra (Barcelona), Spain

Key words. Kinetics release, particle size, ion-exchange, Calcium, Phosphate, Fluoride.

Abstract. The aim of the present study is to evaluate the influence of the resins particle size towards the rate of ions release of a mixture of ion-exchange resins (named NMTD) which supplies calcium, fluoride, phosphate ions as the main mineral content, and to elucidate the different phenomena taking place through the related ion-exchange process. The final goal of the study, related to dental application (enamel restoration), is limiting the particle size range since the rate of ions release is a key parameter to a successful achievement of such objective. Weak type ion-exchange resins, loaded with the appropriate ions, were grinded and sieved into granulometric fractions of bead diameters between 0.1-0.075 mm, 0.075-0.063 mm and 0.063-0.05 mm. Particle size was controlled by a laser diffraction particle distribution analyzer. The experiments on the kinetics of ions release were carried out under batch conditions in artificial saliva desorption solution thermostatized at 37ºC. The release of Ca^{2+} and F^- was determined by corresponding ion selective electrodes automatically controlled, whereas $H_2PO_4^-$ was measured spectrophotometrically by the inductively coupled plasma-optical emission technique (ICP-OES). The results of this study show that the process of the ion-exchange for the

different particle size fractions of resins is critical for the study of the kinetics release of the ions immobilized in the corresponding mixed bed polymeric matrices. In fact, despite the apparent narrow range of particle sizes of the mixed bed systems studied, appreciable differences in the rate of ions release are obtained. Since the ion release rate is depending on the contact surface, an increase of factor of 2 in particle size represents an increase of an order of magnitude of the resin contact surface due to the resin porosity. In this concern, it has been observed that the rate of ions release increases when particle size decreases. The interactions occurring during the ion release from the mixed bed resins (containing calcium, fluoride and phosphate loaded resins) can be interpreted by the following phenomena: $H_2PO_4^-$ that hardly modifies its rate of release in the presence of Ca^{2+} and F^- in the mixture, promotes a considerable increase on the rate of Ca^{2+} release due to the formation of a calcium dihydrogen phosphate soluble complex. F^- also produces an acceleration in the rate of Ca^{2+} release due to the formation, on the surface of cationic resin particles, of solid CaF_2 that, on the contrary, it leads to a decrease in the rate of F^- release.

***Correspondence to:** Manuel Valiente, Departament de Química, Unitat Analítica, Centre GTS, Universitat Autònoma de Barcelona, Facultat de Ciències, Edifici Cn, E-08193 Bellaterra (Barcelona), Spain

Phone number. +34 93 581 2903

Fax number: +34 93 581 1985

E-mail: Manuel.Valiente@uab.es

1. Introduction

The wide applicability of ion-exchange resins in laboratory, industry [1,2], and reactive polymers [3], is very well known. It's a long time since they have been used in galenic applications like disintegrating additive for pills [4], drugs stabilization for treatment of several diseases [5], controlled release preparations [6], and so on. Several physico-chemical parameters can affect the adsorption/desorption kinetics of the active principle; with appropriate selection of resins, preparations for specific application can be designed. In this concern, particle size is one of the most studied parameter in the process characterization of materials [7,8], because several properties in the final application will depend on it. In these sense, zeolites with small particle size provide a relatively high external surface area to volume ratio and reduced mass transfer resistance what make them suitable for several industrial catalytic, sorption, and ion exchange processes [9]. Also, in the use of titanium dioxide as a photocatalyst in the removal of organic matter from wastewater streams, the control of the particle morphology and size distribution are key points [10]. A similar situation can be found in the biomedical materials field. Thus, for example, the powder properties of hydroxiapatite ($Ca_{10}(PO_4)_6(OH)_2$, biocompatible *in vivo*), such as crystallinity, surface area and particle size will determine its effectiveness for the specific application [11].

In this work, a new material (formulation) based on an ion-exchange resins mixture, also known as mixed bed, has been prepared. Different particle size fractions have been separed by the use of sieves. The formulation is a mixture of weak type ion-exchangers for dental application. Several studies use ion-exchange resins in calcium and fluoride forms for dental tissues treatment against tooth decay [12, 13], as well as calcium, fluoride and phosphate for the same objective [14]. The particle size characterization of

these samples was performed by the laser diffraction technique. The effect of the particle size towards the rate of ions release from the polymeric matrix and the interactions involved were evaluated. For this purpose, a 37 ºC thermostatized artificial saliva solution was used.

2. Experimental

2.1. Materials and equipment

Highly purified ion-exchange resins: carboxylic Lewatit S8528 (weak cationic) and tertiary amine Lewatit S3428 (weak anionic), were kindly supplied by Bayer Hispania Industrial, S.A. $CaCl_2$ 1 mol L^{-1} and 0.3 mol L^{-1}, KCl 0.01 mol L^{-1}, NaCl 0.008 mol L^{-1}, NaF 0.9 mol L^{-1}, $NaNO_3$ 0.5 mol L^{-1}, NaOH 2.2 mol L^{-1} and 0.5 mol L^{-1} solutions were prepared from the corresponding Panreac analysis quality solid salts. HCl 1 mol L^{-1}, H_3PO_4 0.8 mol L^{-1} and 0.32 mol L^{-1} solutions were prepared by dilution of the corresponding concentrated Panreac analysis quality acid. In all cases deionized water of Milli-Q quality (Millipore, USA) was used.

In this work, artificial saliva is considered as a solution which composition consists of 0.01 mol L^{-1} KCl and 0.008 mol L^{-1} NaCl.

2.2. Analytical determination

The release of $Ca^{2+}$and F^- was determined by an ion selective electrodes system (Orion, USA) automatically controlled by a PC, whereas $H_2PO_4^-$ was controlled spectrophotometrically by inductively coupled plasma atomic emission technique (ICP-OES) (ARL, model 3410 minitorch, USA), taking aliquots of the liquid phase

periodically. All samples were analyzed with an uncertainty less than 1.5% and all experiments were run in triplicate.

Particle size distribution was determined with a particle size distribution analyzer by laser diffraction with a detector window of 0.688-704.0 microns (Microtac, USA). For this purpose, the moist method was used by dispersing the resin sample in water. Mean values deviations between replicates were less than 2%.

Qualitative analysis of the elements present in the polymer surface were carried out by an X-Ray elemental analysis. This technique takes advantage of the X-Rays emitted by a sample covered with carbon, after being bombed by a primary electron beam from a SEM (Scanning Electron Microscopy) instrument (Jeol model JSM-6300, Japan) equipped by an X-Ray Energy Dispersive Spectrometer (EDS) (Link ISIS-200, England) incorporating a Super ATW Light Element Detector (138 eV resolution, elemental range from B to U), for the obtention of the X-Ray spectra.

An optic microscope (Nikon Labophot2-POL, Japan) with coupled digital camera was used for the morphologic ion-exchange resins study.

2.3. Resins loading

Resins were conditioned following a standard procedure [15]. The conversion of the resins to the desired ionic form was carried out in a reactor under batch conditions. It is interesting to remark that fluoride loading was carried out on an anionic resin previously loaded with phosphate (in the form of $H_2PO_4^-$ species). In this case, it was observed that the fluoride did not replace completely the loaded phosphate (a 25% of the resin capacity remained loaded with phosphate species).

After complete loading of the resins with respective loading solution, the resin phases were rinsed with water to remove the excess of electrolyte or acid solution, depending on the case, unloaded from the reactor quantitatively, centrifuged and dried in an horizontal drier. Resins, in different ionic forms, were stored in completely sealed containers.

2.4. Determination of loading capacity

Determination of the loading capacity for the different resins towards Ca^{2+}, F^- and $H_2PO_4^-$ was carried out under dynamic conditions in ion-exchange columns by following a standard procedure described thoroughly in the literature [16]. A weighed portion of each specific resin was introduced in a small column and the target counter-ion was eluted either with acid (in the case of the cationic resin) or with strong base (or $NaNO_3$) in the case of anionic exchangers. Eluates were collected in glass volumetric flasks for later analysis. Adsorption or desorption effects of Ca^{2+}, F^- and $H_2PO_4^-$ at or from the glass surface were negligible, taking into account the magnitude of the working concentrations. The results of the analysis were used to determine the resins specific capacity, q_s according to the equation:

$$q_s = \frac{VC_i}{W_i} \tag{2.1}$$

where V is the volume of eluted solution, C_i is the concentration of the solution containing the eluted ion and W_i is the weight of resin which contains the corresponding ion. Specific capacities determined experimentally are shown in Table 1.

2.5. Grinding and sieving

A portion of each loaded and dried resin was ground in a mechanical agate mortar Retsch RMO (Germany) and then sieved using a set of standard stainless steel sieves in a

mechanical siever, CISA (Spain). Thus, separate fractions of the resins were collected with particle sizes of diameter comprised between 0.1-0.075 mm, 0.075-0.063 mm and 0.063-0.05 mm. Formulations were prepared by mixing after sieving the corresponding weak resins loaded with Ca^{2+}, F^-, $H_2PO_4^-$ and Zn^{2+} (small amount) in the adequate proportions [14] (weak formulation).

Because of the relatively small content of zinc ions in the resins mixture and after the observation that its presence does not influence on the ion exchange process of the other ions, the present study does not include the related characterization on zinc ion-exchange.

2.6. Characterization of ion-exchange resins morphology

In order to verify the validity of the grinding and sieving processes, a characterization of the obtained resin particles has been carried out by means of optical image analysis.

Figure 1 shows the irregular shape of the weak formulation particles which is a consequence of the fracture caused by the mortar action. Practically there are no particles with a filiform shape, so, the perception of disagreement between the sieving process and the measurement of particle size distribution by laser diffraction methods is not caused by the particles shape.

As it is known, the measurement of particle size by the mentioned laser technique, depends on the position at which the particle disperses light which leads to a particle size that can be considerably over or under the size fraction selected. A thorough observation of images of the corresponding fractions under study reveals small differences between particles at first sight.

To verify the particle size distribution analysis, one individual sample was analyzed by two commercially different instruments. The agreement between results of this experiment indicates the validity of all particle size distribution curves.

On the other hand, the observed differences between the sieving process for particle size discrimination and the corresponding higher particle size measured, can be attributed to the resins swelling properties in water, since the measurements are carried out by a suspension of the insoluble powder in the mentioned solvent.

2.7. Determination of Ion-exchange kinetics

Experiments to evaluate the kinetics of ions release of the different finely powdered formulations were carried out under batch conditions by using the "limited volume" technique [17]. A small portion of resin was placed in a dialysis cellulose membrane tube closed hermetically by both sides and placed in a *Franz modified cell* [18] which was introduced in a 37 ºC thermostated cell containing 50 or 60 ml of artificial saliva vigorously agitated. The moment when the sample got into contact with artificial saliva was considered as the starting time (time zero) for the kinetics experiment.

3. Results and discussion

3.1. Determination of the degree of conversion

The experimental data obtained either by ion selective electrodes or ICP-OES were expressed in terms of degree of conversion, *F*, of the resins by applying the expression:

$$F = \frac{V_{as} c(t_i)}{q_\infty} \qquad (3.1)$$

where V_{as} is the volume of artificial saliva contained in the cell, $c(t_i)$ is the concentration of the target ion at the time of the experiment t_i and $q_\infty = mpq_s$ is the total capacity of the

resin (in mmols), being m the total weight of resin or resins mixture, p is the percentage of resin in the corresponding ionic form in the mixture and q_s (mmol/g) the specific capacity of that resin towards the corresponding ion determined as described in the section 2.4.

3.2. Effect of the precision of sieving on the ions release rate

As observed in Figure 2a the appropriate sieving is determinant to get a better particle size distribution, that leads to narrower and higher bands. Figure 2b shows the different patterns for the three fractions of particle size obtained under the optimized sieving. In the three cases the mean value of particle size of the corresponding fraction is considerably displaced to higher values (i.e., 0.0807, 0.096 and 0.114 mm instead of 0.0565, 0.069 and 0.0875 mm, respectively). This might be caused by the fact that laser diffraction measurements are performed in a water suspension of the resin samples, what takes account of the swelling of the resins in water. Furthermore and despite the optimization of the sieving process, the obtained fractions have a remarkable similarity, thus overlapping each other in a considerable extent.

On the other hand, a comparison of the results obtained on the calcium release before and after carrying out the optimization of the sieving procedure is shown in Figure 3. The decrease of the rate of release is due to the removal of most of the lower particle size fraction. As noticed, these small particles have a high contribution to the increase of the rate of ions release.

3.3. Evaluation of the effect of particle size towards the rate of ions release from monocomponent weak acid type ion-exchange resins samples

To carry out the study of the interactions of the ions when released from the ion-exchange resins mixture, a systematic characterization has been undertaken including the individual behavior of each resin component of the formulation, i.e., cationic resin loaded with Ca^{2+}, anionic resin loaded with F^- and the anionic resin loaded with $H_2PO_4^-$. In a second step of the study, binary mixtures were analyzed and related results are discussed below. Figures 4a-b show no significant differences on release rate for the various particle sizes, that is probably due to the small variation of size between the studied fractions. On the other hand, the fact that the degree of conversion of anions is considerably higher than the one of Ca^{2+}, is because the weak acid cationic resin has a higher affinity towards Ca^{2+} than to Na^+/K^+ ions present in artificial saliva [19], which are responsible for the corresponding ion-exchange. In the case of anions, the observed higher rate of release for F^- vs $H_2PO_4^-$ is due to the relatively higher affinity of the weak anion exchange resin towards $H_2PO_4^-$.

3.4. Binary mixtures of weak acid type ion-exchange resins. Evaluation of the effect of particle size towards the rate of ions release

Two binary formulations including the mixture of the calcium loaded resin and an anionic resin in either F^- or $H_2PO_4^-$ form were prepared. The molar ratio between the ionic components loaded in resins was the one used in the weak formulation. In Figures 5a and 5b kinetic release curves versus particle size are presented for the Ca^{2+}: F^- system, whereas Ca^{2+}: $H_2PO_4^-$ system is shown in Figure 6. The higher release of anions vs calcium is also observed in the binary mixtures. However, some differences are appreciated in the behavior of calcium. Thus, calcium release has a slight increase in

presence of fluoride ions while facing phosphate the release is 20 fold times higher. The increase of release can be attributed to the interaction between calcium and the respective ions F^- or $H_2PO_4^-$. In the case of fluoride, it has been observed the formation of solid CaF_2 at the surface of the calcium resin particles (see EDS analysis in Fig. 10b and respective comments below). This fact can explain the strong decrease on fluoride release with respect to the one observed in absence of Ca^{2+}. In fact, such a decrease is apparent since our data correspond to the fluoride in solution and do not account for the precipitated fluoride. Furthermore, the increase on Ca^{2+} release may be a consequence of the shift of the ion-exchange reaction by the mentioned formation of solid CaF_2. It is remarkable that the observed solid formation phenomenon is taking place at the resin phase since in solution the corresponding solubility product is not achieved to form the solid CaF_2 mostly because of the low calcium concentration. On the contrary, at the resin phase, the local concentration of Ca^{2+} is much higher, leading to a precipitation of solid CaF_2 on the surface of the lower kinetics ion exchanger, that is the cationic one.

In the case of phosphate, the mentioned ionic interactions are enhanced by the formation of weak Ca^{2+}-phosphate complex species [20]. These interactions are also reflected in the slight increase of phosphate release, which is expected to be relatively lower than calcium release because of the high difference in absolute values.

On the other hand, note that in both systems a correlation between the rate of the corresponding ions release and particle size is obtained for both calcium and anions release. This effect, which was not practically noticed in the corresponding monocomponent systems, is enhanced when a manifest chemical interaction occurs at the solid-liquid interface (in the monocomponent systems, the interactions of calcium with chloride are much weaker) [21].

3.5. Study of the interactions between ions released from mixed bed (weak formulation)

A comparison of the kinetic curves for loss of Ca^{2+}, F^- and $H_2PO_4^-$ in resins mixture with those corresponding to samples of monocomponent and binary mixtures, reveals the expected multiple interactions between these ions. Figures 7a-c show an increase in the rate of Ca^{2+} release when both anions are present in the resins mixture, whereas the cation presence promotes a decrease in the rate of F^- release and an slight increase in the corresponding phosphate liberation. These observations follow the previous findings for binary mixtures (i.e. increase on calcium and phosphate release rate and a decrease on the fluoride discharge with respect to the monocomponent resins). It is remarkable that fluoride has a higher decrease when phosphate is present. This particular effect can be attributed to the increase of calcium release in presence of phosphate, as the presence of calcium lowers the fluoride release.

From the concentrations found at the end of the experiment, and taking into account that the ICP technique allows us to quantify the total phosphorous content present in the solution whereas ion selective electrodes only determine charged free species in solution (in this case Ca^{2+} and F^-), the distribution diagram of the species was built with the help of the Equilibrium calculations [22] program. At pH=5.5 (which corresponds to the working pH conditions) the predominant species in solution result Ca^{2+}, F^-, $H_2PO_4^-$ and HPO_4^{2-} and positively charged calcium fluoride or calcium dihydrogen phosphate complexes, with concentrations of 10^{-4}, 10^{-4}, $6.3\ 10^{-4}$, 10^{-5}, $3.2\ 10^{-8}$ and 10^{-6} mol L^{-1} respectively. So, this theoretical predictions fit well with the observations above commented. In order to verify the potentiometric data of fluoride, it was checked that the

dihydrogen phosphate presence does not affect to fluoride signal through the evaluation of the response of the ion selective electrode towards F^- in the presence and absence of dihydrogen phosphate in solution.

At this point a more exhaustive analysis of the results obtained from the three studied systems was carried out with the help of a surface resin particle study by scanning electron microscopy.

3.5.1. Resins surface qualitative analysis by SEM-EDS.

A complementary study to support the findings on the interactions of mixed bed system was carried out.

Samples of different systems, i.e., binary mixtures in dried form, both before and after treatment with artificial saliva as well as dried monocomponents have been analyzed by SEM-EDS. Also, samples of individual unloaded resins were analyzed by SEM-EDS. Corresponding spectra results are collected in Figures 8-11.

Unloaded resins spectra show no signal corresponding to target ions. Monocomponent samples (Figures 8a-b) reveal the presence of each immobilized ion in the corresponding matrix. However, the fluoride anionic resin presents signals for F and P ($H_2PO_4^-$, originally present, remains from the fluoride loading process, see experimental section 2.3).

It must be noticed the relatively weak spectral signal corresponding to Na^+ (1.041 keV, practically non detected) and to F^- [23].

Spectra of binary mixtures including either Ca^{2+} and $H_2PO_4^-$ or Ca^{2+} and F^- loaded resins show a different behaviour. Thus, in Figure 9a (Ca^{2+}-$H_2PO_4^-$ system) it is observed that no contamination between cationic and anionic components occurs before the treatment

with artificial saliva as a consequence of the absence of reaction when no ionic solution contacts the loaded resins mixture. After desorption with artificial saliva, the observed signals correspond, as expected, besides the original ions (the ion-exchange is not complete), to Cl^- that appears from the anionic exchange. Na^+ is not observed because of its low intensity signal commented before.

For the Ca^{2+}: F^- binary system, the spectra results shown in Figure 10a indicate that before desorption, the signals are similar to the previously described monocomponents for the anionic component (in this case, fluoride is practically non detected). After desorption, the signal of Cl^- also appears on the anionic particles due to the ion-exchange process. The absence of F^- signal can be attributed to both the weakness of the corresponding signal as well as the non plain surface of the target resin particle. Spectra that results from the microanalysis of calcium particles treated with the desorption solution (Fig. 10b) appear with a fluoride signal. This indicates that a solid deposit of insoluble CaF_2 on the cationic resin particles has been formed; the higher rate of F^- release affects the ion-exchange equilibrium

$$R_2\text{-}Ca^{2+} + 2\ Na^+/K^+ \leftrightarrow 2\ R\text{-}Na^+/K^+ + Ca^{2+}$$

by inducing a displacement to the right and on the other hand, F^- are surrounding the cationic resin particles and the appearance of Ca^{2+} leads to the formation of the mentioned precipitate.

In addition, as observed in Figure 10a, the spectra resulting from the microanalysis of cationic particles also shows an unexpected fluoride signal, since this sample has not been treated with artificial saliva solution and it is simply a mixture of the two resins loaded with corresponding Ca^{2+} or F^-. In a magnification of the image of a cationic particle, when different zones are analyzed, two different situations can be clearly

observed. Thus, there are zones where there is no presence of fluoride, whereas the ones that give fluoride signal are accompanied by the corresponding phosphorous signal (See Figure 11a). This would indicate that, in this mixture, the cationic resin particles in Ca^{2+} form are surrounded with anionic resin in F^- form by electrostatic attraction. So, fluoride detected on cationic particles does not form solid CaF_2.

On the contrary, after desorption, the fluoride signal that appears in the related spectra, Figure 11b, is not accompanied by the corresponding phosphorous one, indicating the formation of solid CaF_2 on the cationic resin particles in Ca^{2+} form.

From the findings that results of both the resin particles characterization by electronic microscopy and the ion release curves shown in Figures 7a-c, the interactions occurring during the ion release from the sample of resins mixture (containing the three components) can be interpreted by the following phenomena: $H_2PO_4^-$ that hardly modifies its rate of release in the presence of Ca^{2+} and F^- in the mixture, promotes a considerable increase on the rate of Ca^{2+} release due to the formation of a calcium dihydrogen phosphate soluble complex. Furthermore, this increase of calcium release is affected by the presence of fluoride in the mixture. Thus, when resin loaded with F^- is added to the Ca^{2+}: $H_2PO_4^-$ binary system, the rate of Ca^{2+} release experiments a clear decrease; this is a consequence of the insoluble solid CaF_2 formation on the surface of the resin particles in Ca^{2+} form, that had already been detected by the scanning electron microscopy technique. On the other hand, the observed decrease of F^- release rate with Ca^{2+} presence and even more if $H_2PO_4^-$ is added to the system is due to the increase of the mentioned formation of solid CaF_2 as the Ca^{2+} released to the system increases considerably under these conditions.

The use of an alternative technique (scanning electron microscopy), to ascertain the formation of a solid CaF_2, verifies the lack of fluoride by potentiometric measurements.

4. Conclusions

The results obtained in the present study lead to the following conclusions:

The rate of ions release increases when particle size decreases (Figure 12).

The interactions occurring during the ion release from the mixed bed resins (containing calcium, fluoride and phosphate loaded resins) can be interpreted by the following phenomena: $H_2PO_4^-$ that hardly modifies its rate of release in the presence of Ca^{2+} and F^- in the mixture, promotes a considerable increase on the rate of Ca^{2+} release due to the formation of a calcium dihydrogen phosphate soluble complex. F^- also produces an acceleration in the rate of Ca^{2+} release due to the formation, on the surface of cationic resin particles, of solid CaF_2 that, on the contrary, it leads to a decrease in the rate of F^- release. Such opposite behavior can be interpreted as the buffering effect provided by the solid CaF_2 which enhances the low release of calcium from the carboxylic resin and diminishes the high release of fluoride from the anionic resin.

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Table 1. Specific capacity of the resins towards Ca^{2+}, F^- and $H_2PO_4^-$

Figure 1. Optic microscopy images of the weak formulation fractions at 200 magnification. (a) 0.0875 mm (b) 0.069 mm (c) 0.0565 mm

Figure 2a. Comparison of particle size distribution curves of the 0.069 mm weak formulation fraction before and after re-sieving

Figure 2b. Comparison of particle size distribution curves of the 0.0875, 0.069 and 0.0565 mm weak formulation fractions after re-sieving

Figure 3. Kinetics release of Ca^{2+} ion from weak formulation samples of different particle size at 37ºC. Evaluation of the re-sieving effect

Figure 4a. Kinetics release of Ca^{2+} ion from monocomponent weak type resin samples in Ca^{2+} -form of different particle size at 37ºC

Figure 4b. Kinetics release of F^- and $H_2PO_4^-$ ions from monocomponent weak type resin samples in F^- and $H_2PO_4^-$-forms respectively of different particle size at 37ºC

Figure 5a. Kinetics release of Ca^{2+} ion from samples binary mixture of weak type resins in Ca^{2+} and F^- -forms of different particle size at 37ºC

Figure 5b. Kinetics release of F^- ion from samples binary mixture of weak type resins in Ca^{2+}:and F^- -forms of different particle size at 37ºC

Figure 6. Kinetics release of Ca^{2+} and $H_2PO_4^-$ ions from samples binary mixture of weak type resins in Ca^{2+} and $H_2PO_4^-$ -forms of different particle size at 37ºC

Figure 7a. Comparison of the kinetics release of Ca^{2+} ion from weak formulation (NMTD) and Ca^{2+} -form monocomponent samples of different particle size at 37ºC

Figure 7b. Comparison of the kinetics release of F^- ion from weak formulation (NMTD) and F^- -form monocomponent samples of different particle size at 37ºC

Figure 7c. Comparison of the kinetics release of $H_2PO_4^-$ ion from weak formulation (NMTD) and $H_2PO_4^-$ -form monocomponent samples of different particle size at 37ºC

Figure 8a. X-Ray microanalysis of the cationic polymeric matrix with immobilized Ca^{2+} ions and the anionic one with immobilized $H_2PO_4^-$ ions

Figure 8b. X-Ray microanalysis of the anionic polymeric matrix with immobilized F^- ions

Figure 9a. X-Ray microanalysis of a sample binary mixture of weak type resins in Ca^{2+} and $H_2PO_4^-$ - forms not submitted to desorption

Figure 9b. X-Ray microanalysis of a sample binary mixture of weak type resins in Ca^{2+} and $H_2PO_4^-$ -forms submitted to desorption

Figure 10a. X-Ray microanalysis of a sample binary mixture of weak type resins in Ca^{2+} and F^- -forms not submitted to desorption

Figure 10b. X-Ray microanalysis of a sample binary mixture of weak type resins in Ca^{2+} and F^- -forms submitted to desorption

Figure 11a. X-Ray microanalysis of a magnified image of a calcium particle of a sample binary mixture of weak type resins in Ca^{2+} and F^- -forms not submitted to desorption

Figure 11b. X-Ray microanalysis of a magnified image of a calcium particle of a sample binary mixture of weak type resins in Ca^{2+} and F^- -forms submitted to desorption

Figure 12. Effect of the resins particle size on the rate of the corresponding F^- and $H_2PO_4^-$ ions release from binary mixtures of weak acid type ion-exchange resins

Table 1

	Capacity (mmol g^{-1})		
	Ca^{2+}	**F^-**	**$H_2PO_4^-$**
Lewatit S8528	3.47± 0.03	-	-
Lewatit S3428	-	2.73±0.04	3.38±0.01

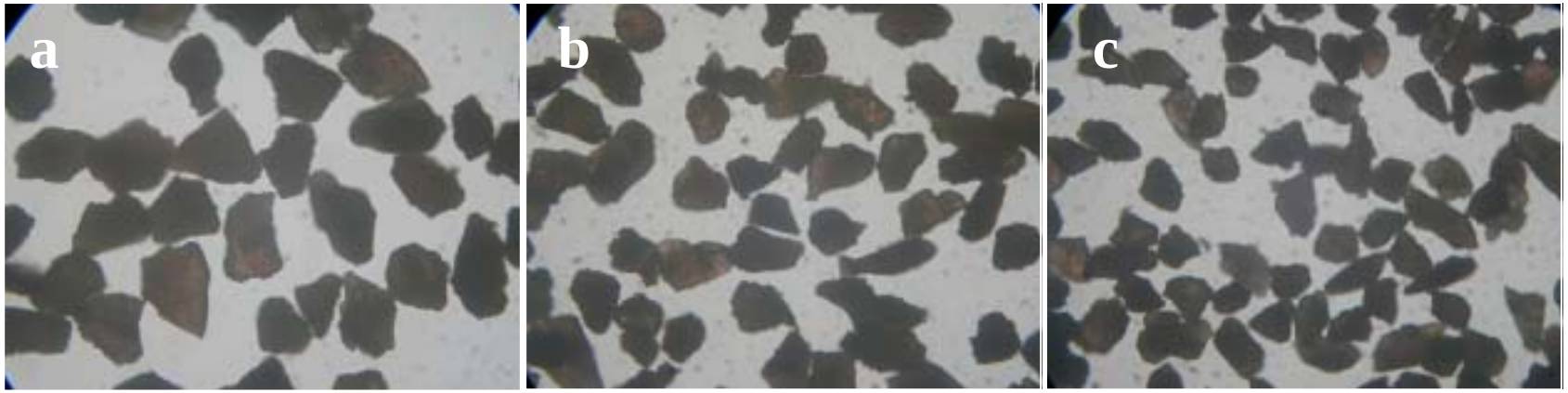

Figure 1

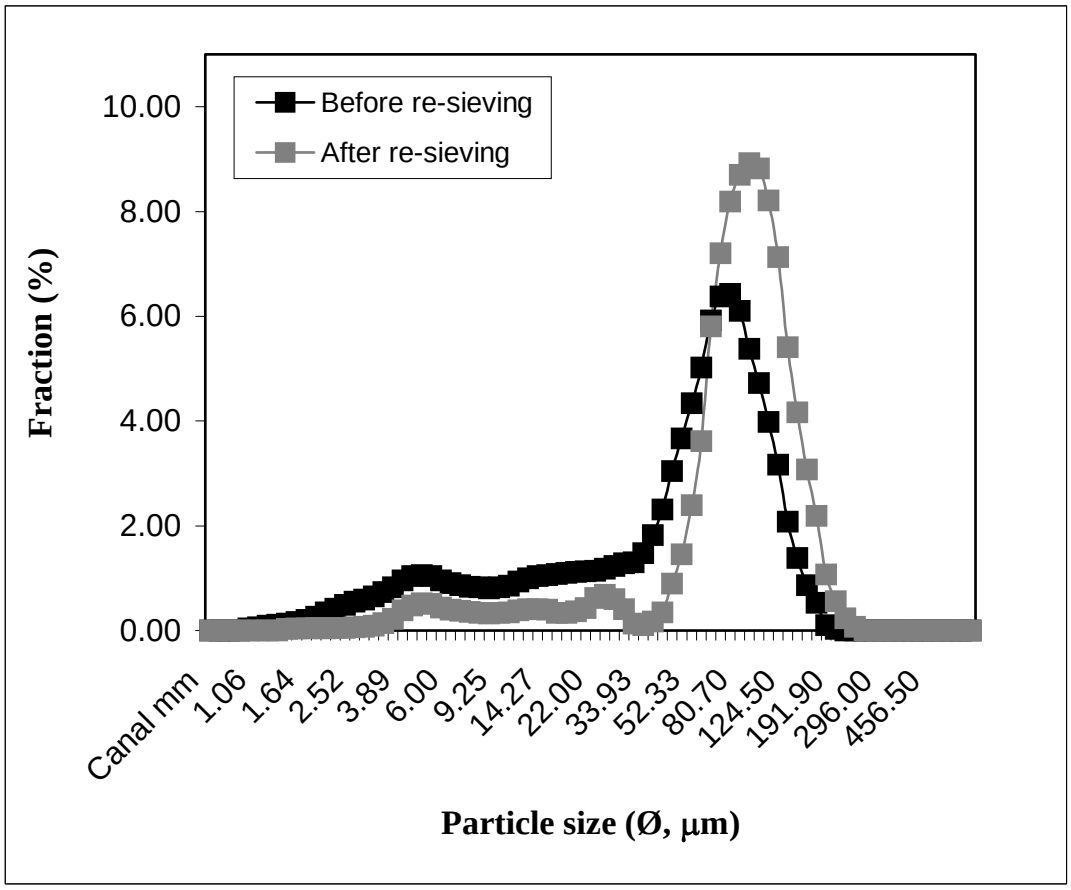
Before re-sieving
After re-sieving
10.00
8.00
6.00
4.00
2.00
0.00
Fraction (%)
Canal mm
1.06
1.64
2.52
3.89
6.00
9.25
14.27
22.00
33.93
52.33
80.70
124.50
191.90
296.00
456.50
Particle size (Ø, μm)

Figure 2a

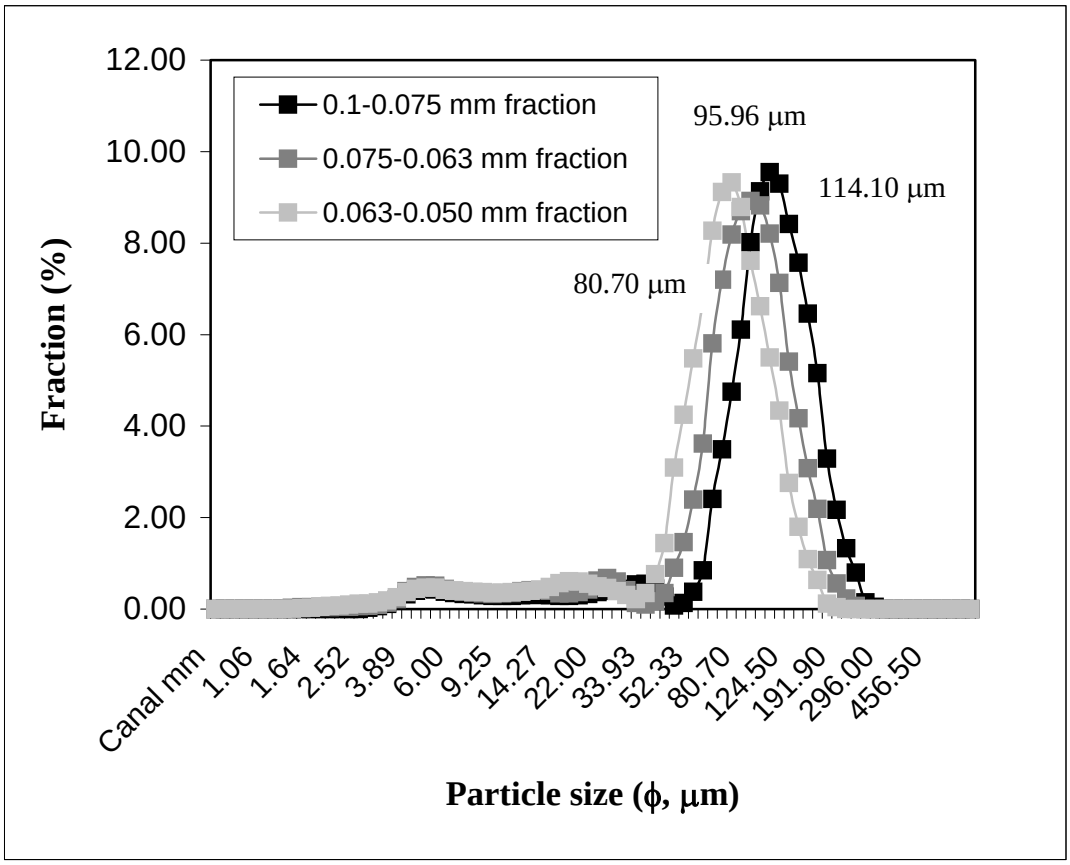
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Fraction (%)
0.1-0.075 mm fraction
0.075-0.063 mm fraction
0.063-0.050 mm fraction
95.96 μm
114.10 μm
80.70 μm
Canal mm
1.06
1.64
2.52
3.89
6.00
9.25
14.27
22.00
33.93
52.33
80.70
124.50
191.90
296.00
456.50
Particle size (ϕ, μm)

Figure 2b

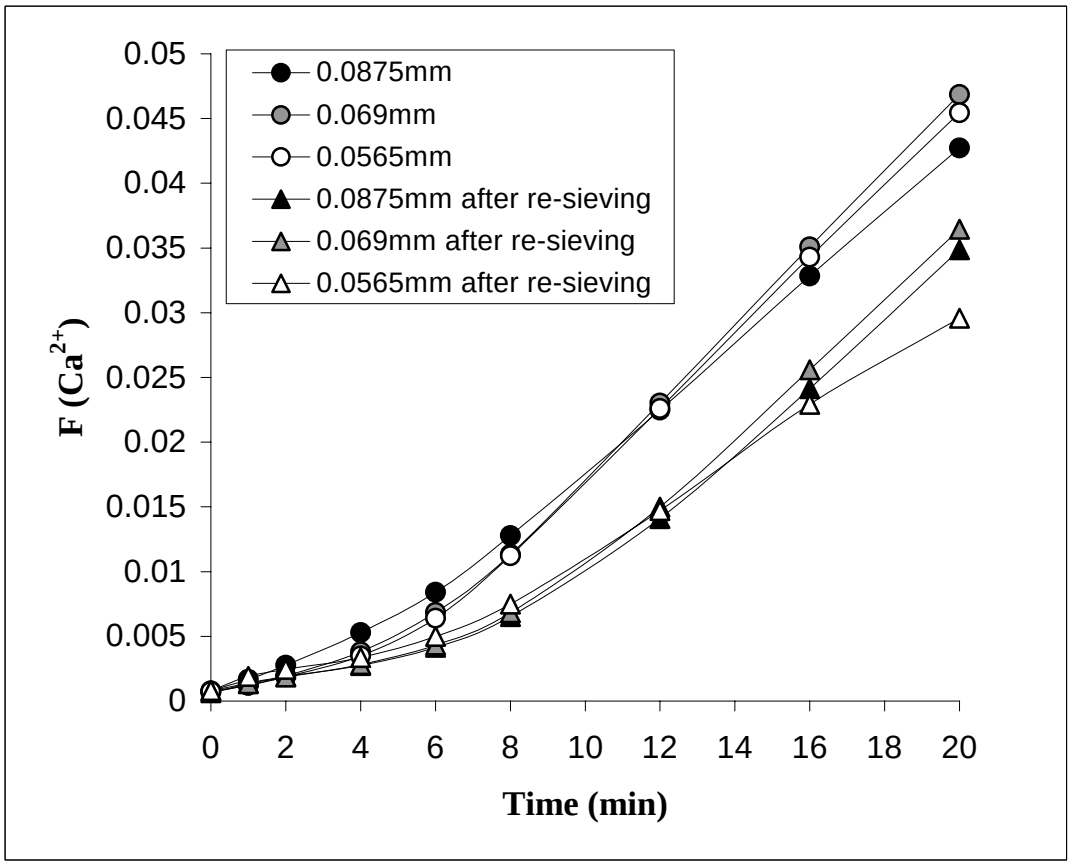
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
F (Ca2+)
0
2
4
6
8
10
12
14
16
18
20
Time (min)
0.0875mm
0.069mm
0.0565mm
0.0875mm after re-sieving
0.069mm after re-sieving
0.0565mm after re-sieving

Figure 3

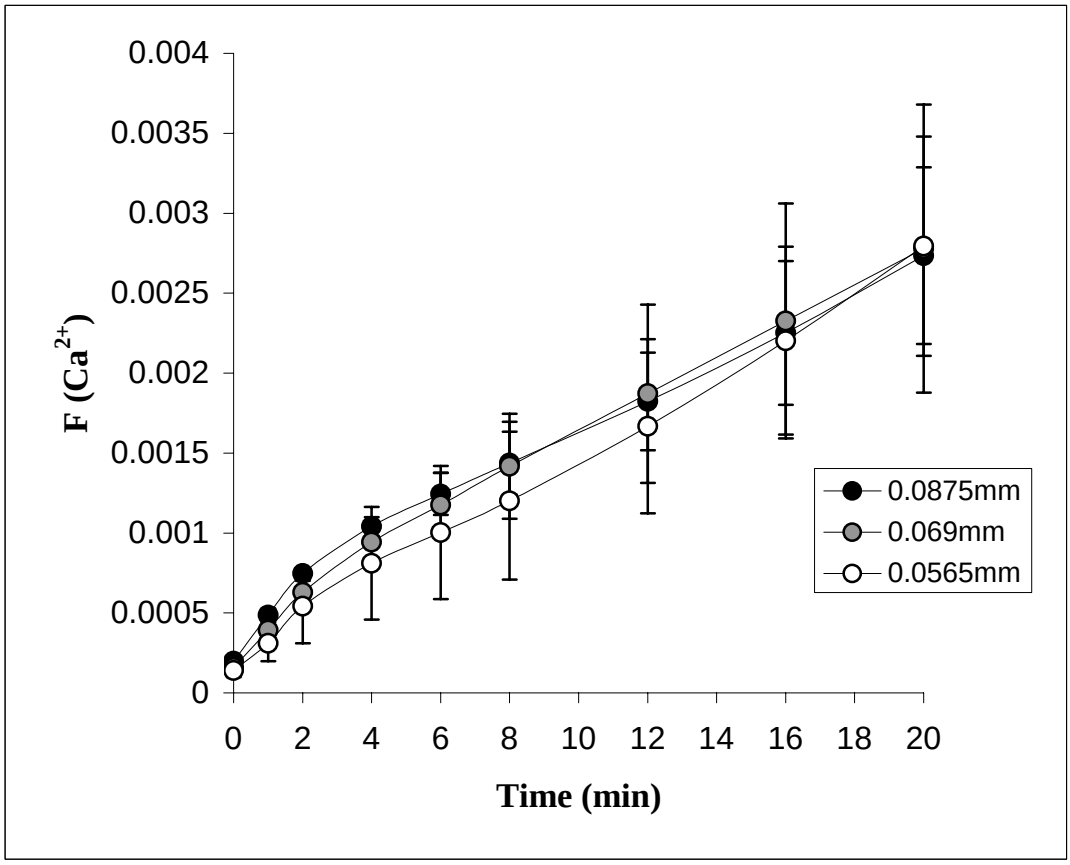
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
F (Ca2+)
0
2
4
6
8
10
12
14
16
18
20
Time (min)
0.0875mm
0.069mm
0.0565mm

Figure 4a

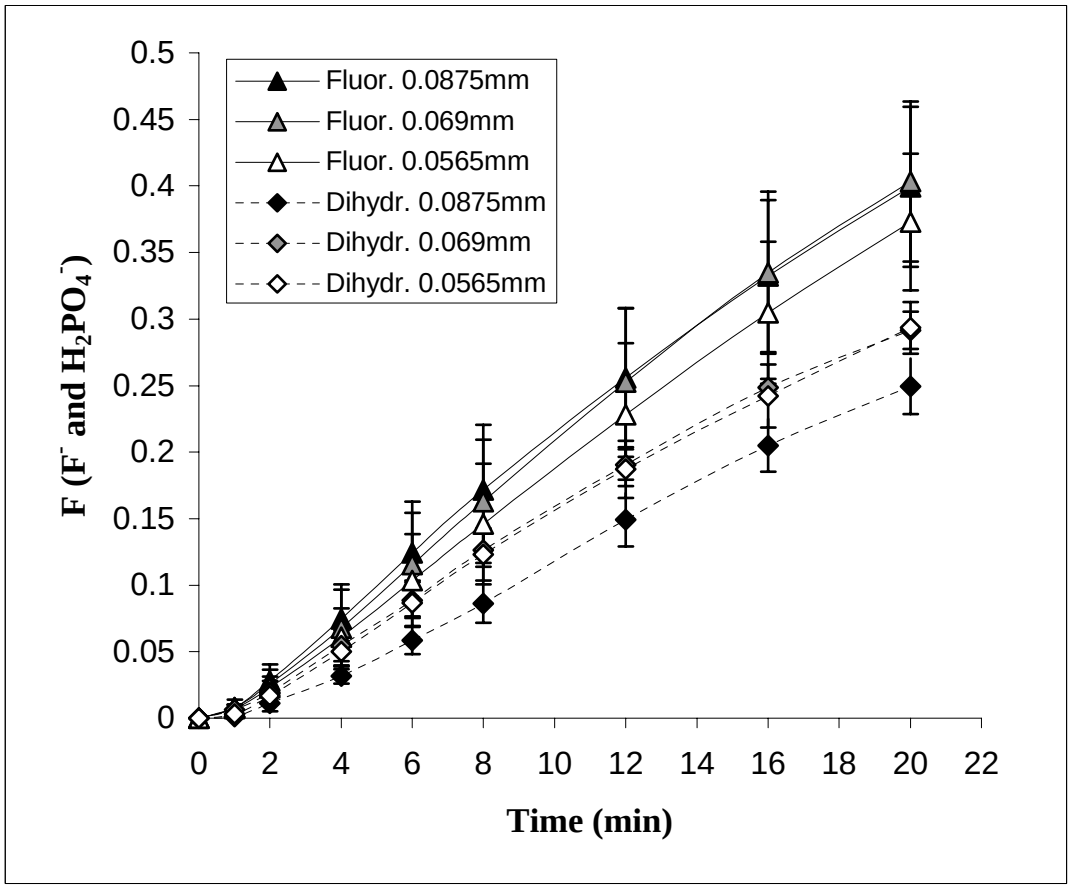
Fluor. 0.0875mm
Fluor. 0.069mm
Fluor. 0.0565mm
Dihydr. 0.0875mm
Dihydr. 0.069mm
Dihydr. 0.0565mm
F (F⁻ and H2PO4⁻)
Time (min)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0
2
4
6
8
10
12
14
16
18
20
22

Figure 4b

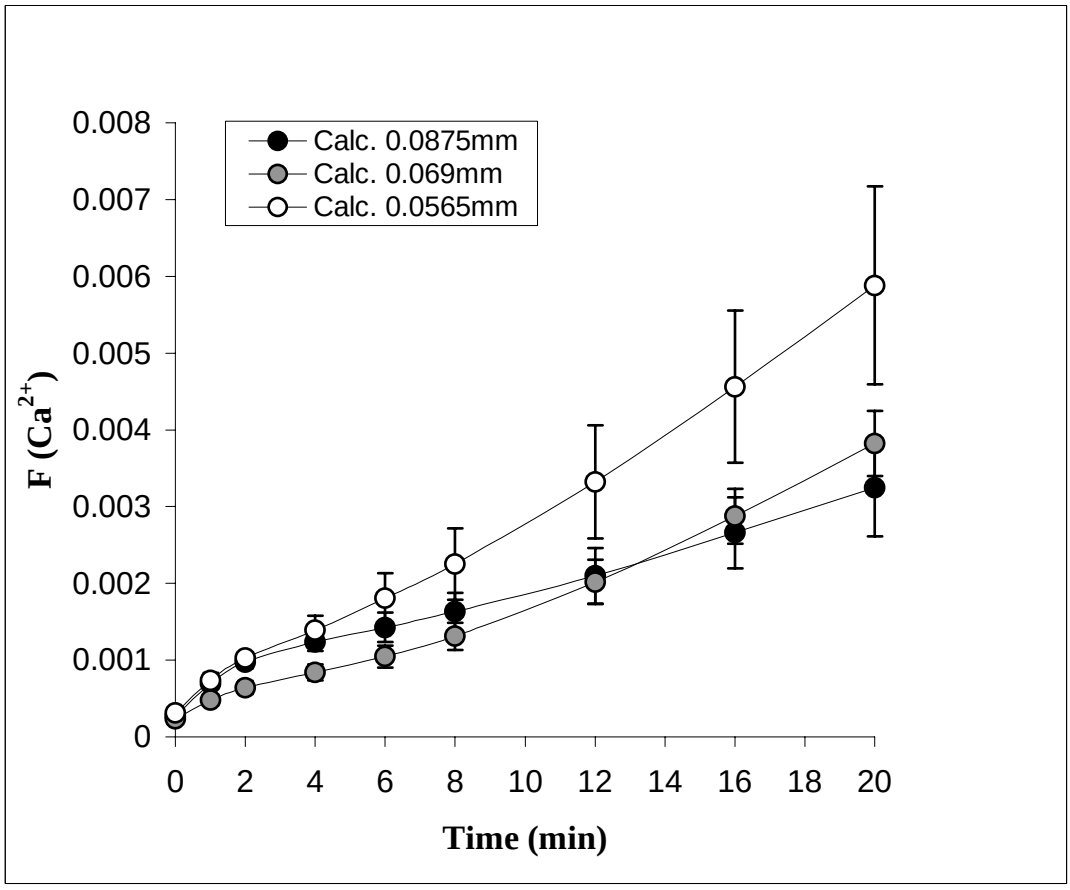
Calc. 0.0875mm
Calc. 0.069mm
Calc. 0.0565mm
F (Ca2+)
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
2
4
6
8
10
12
14
16
18
20
Time (min)

Figure 5a

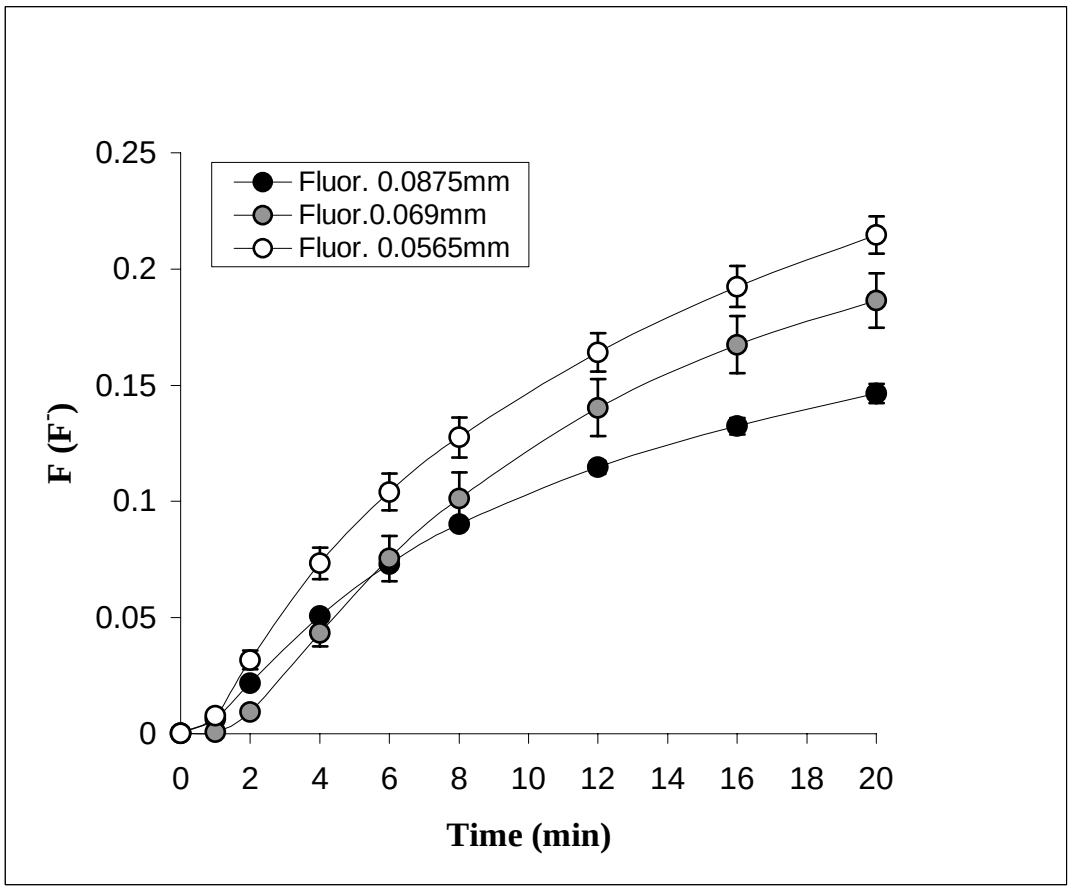
Fluor. 0.0875mm
Fluor.0.069mm
Fluor. 0.0565mm
F (F')
Time (min)
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
12
14
16
18
20

Figure 5b

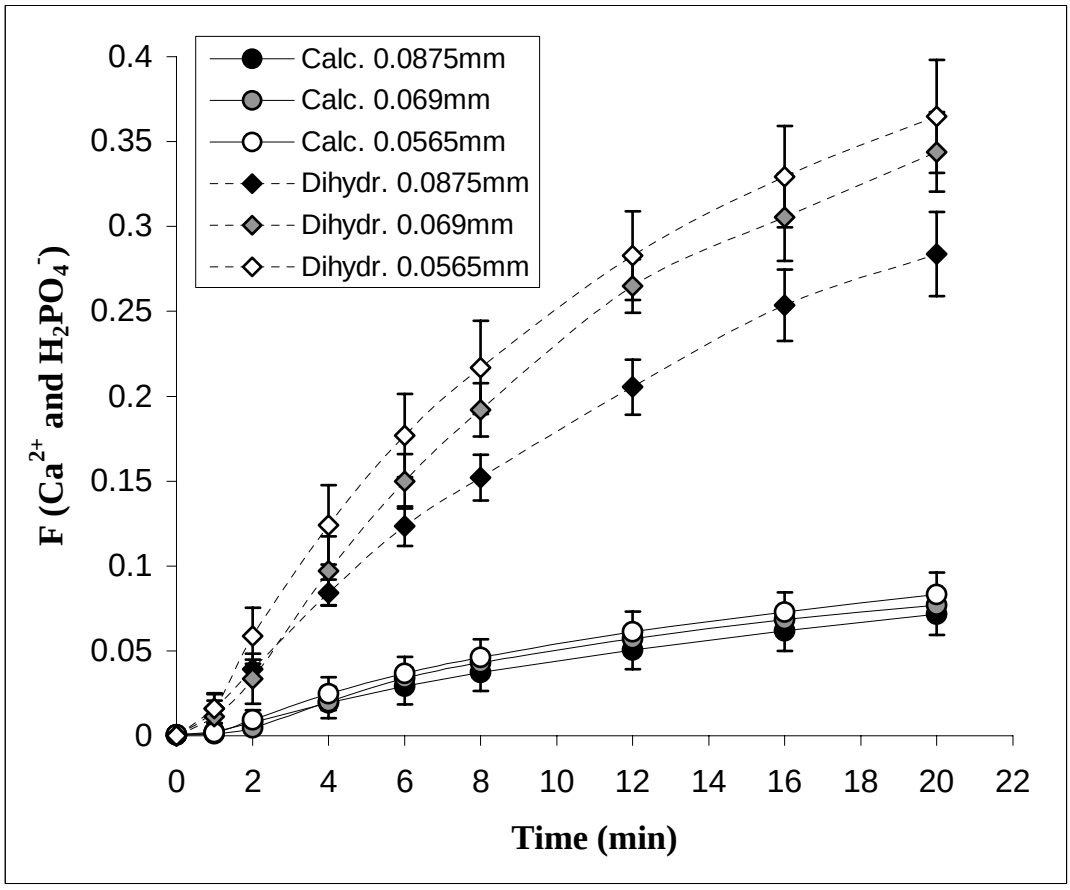
Calc. 0.0875mm
Calc. 0.069mm
Calc. 0.0565mm
Dihydr. 0.0875mm
Dihydr. 0.069mm
Dihydr. 0.0565mm
F (Ca2+ and H2PO4-)
Time (min)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0
2
4
6
8
10
12
14
16
18
20
22

Figure 6

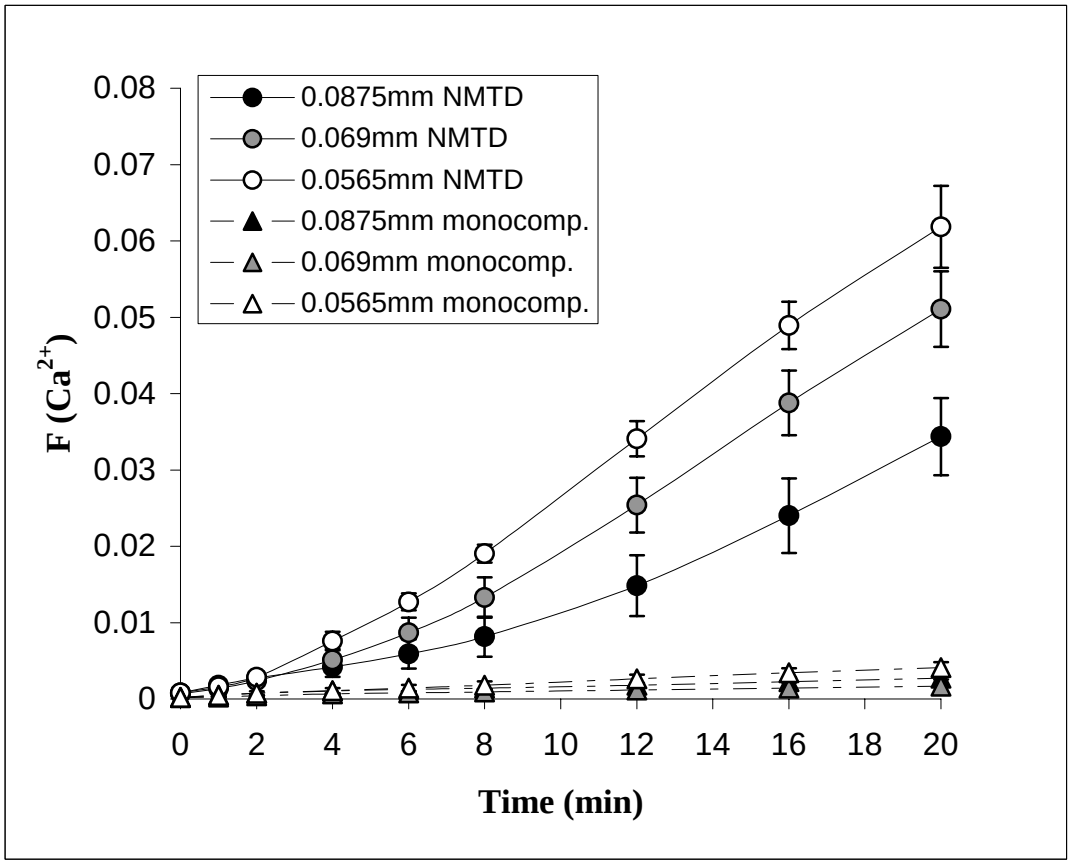
0.0875mm NMTD
0.069mm NMTD
0.0565mm NMTD
0.0875mm monocomp.
0.069mm monocomp.
0.0565mm monocomp.
F (Ca2+)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
12
14
16
18
20
Time (min)

Figure 7a

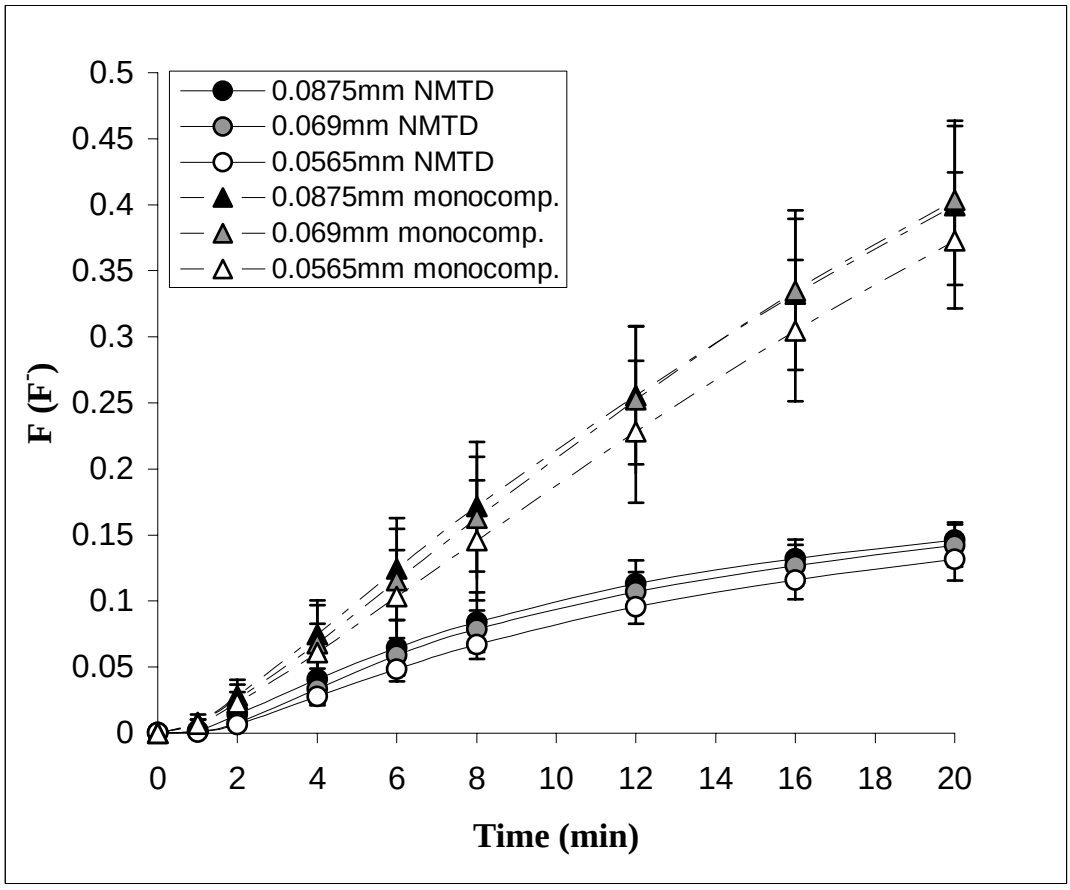
0.0875mm NMTD
0.069mm NMTD
0.0565mm NMTD
0.0875mm monocomp.
0.069mm monocomp.
0.0565mm monocomp.
F (F-)
Time (min)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0
2
4
6
8
10
12
14
16
18
20

Figure 7b

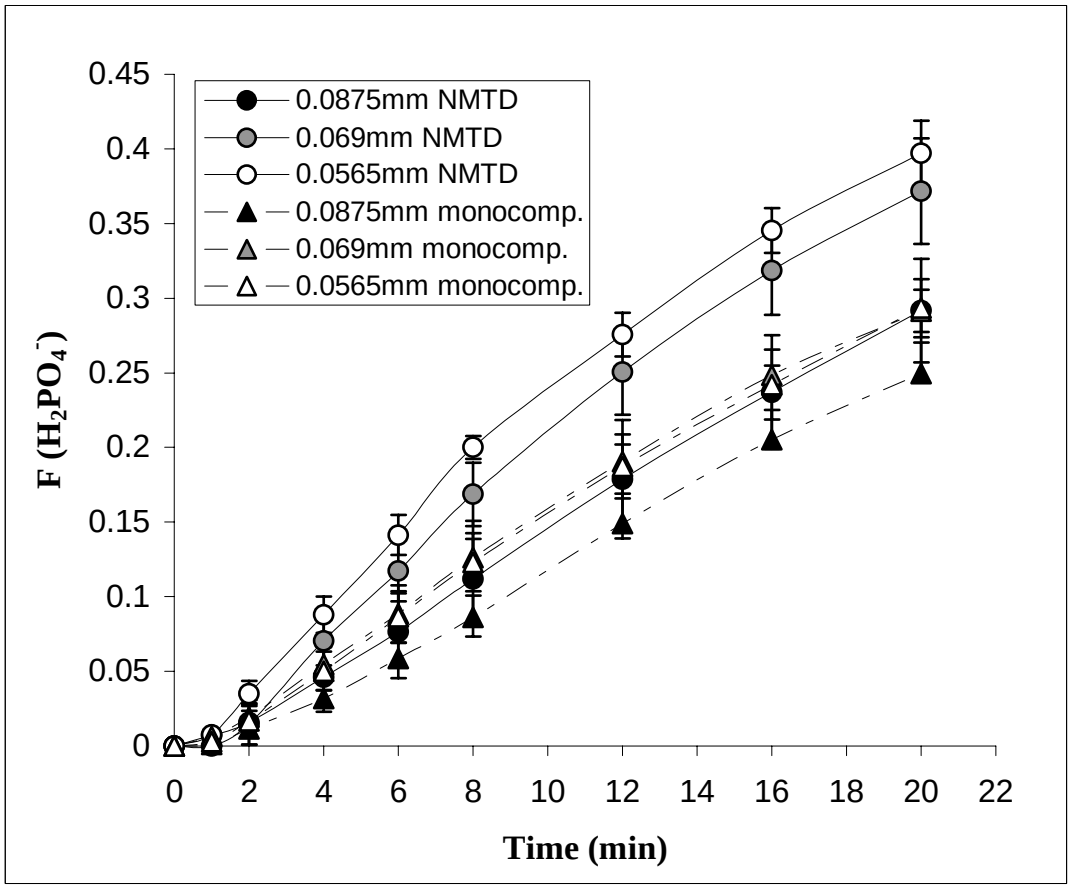
0.0875mm NMTD
0.069mm NMTD
0.0565mm NMTD
0.0875mm monocomp.
0.069mm monocomp.
0.0565mm monocomp.
F ($H_2PO_4^-$)
Time (min)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0
2
4
6
8
10
12
14
16
18
20
22

Figure 7c

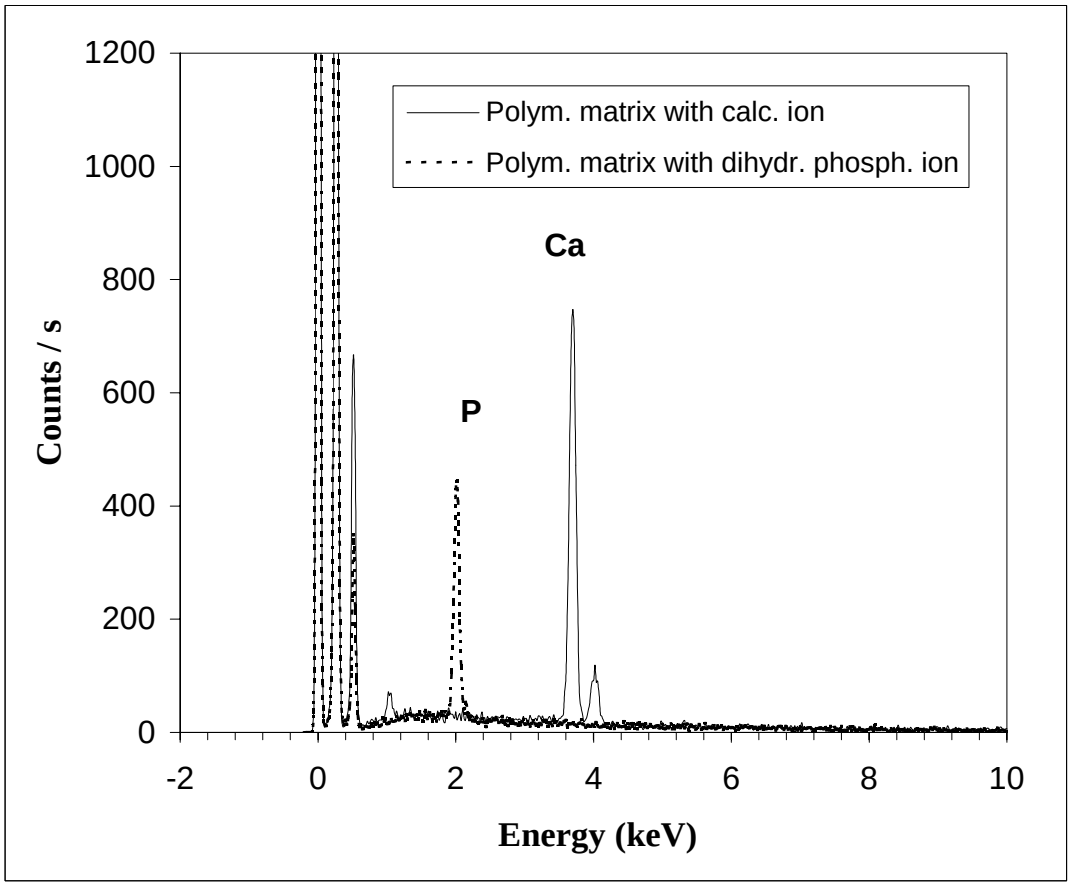
1200
1000
800
600
400
200
0
Counts / s
-2
0
2
4
6
8
10
Energy (keV)
Polym. matrix with calc. ion
Polym. matrix with dihydr. phosph. ion
Ca
P

Figure 8a

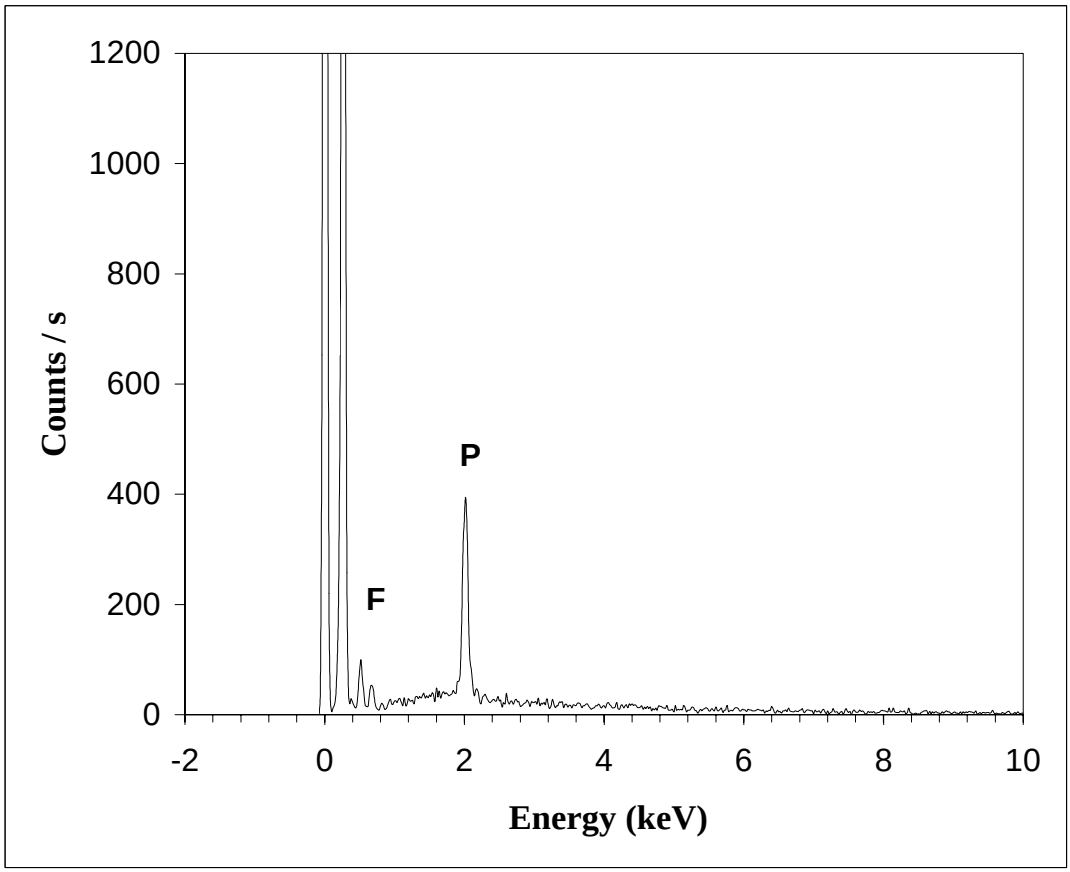

Figure 8b

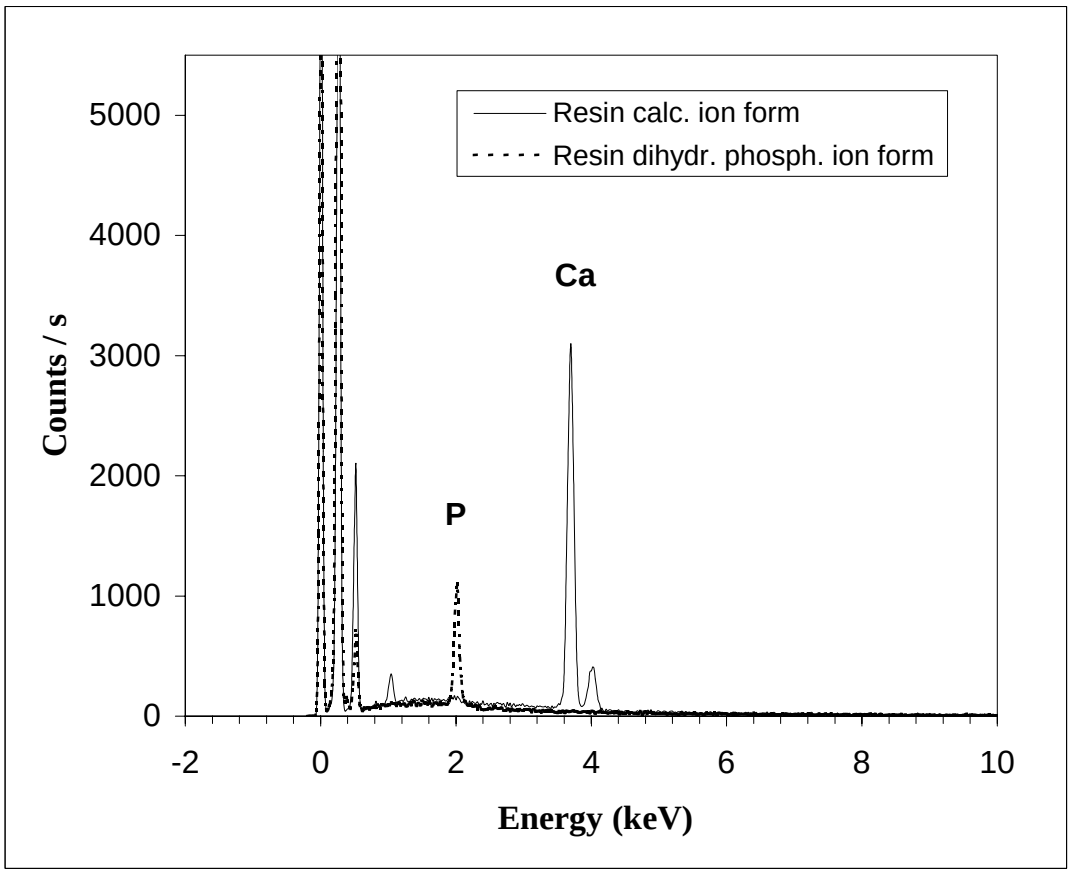
Resin calc. ion form
Resin dihydr. phosph. ion form
Ca
P
Counts / s
Energy (keV)
5000
4000
3000
2000
1000
0
-2
0
2
4
6
8
10

Figure 9a

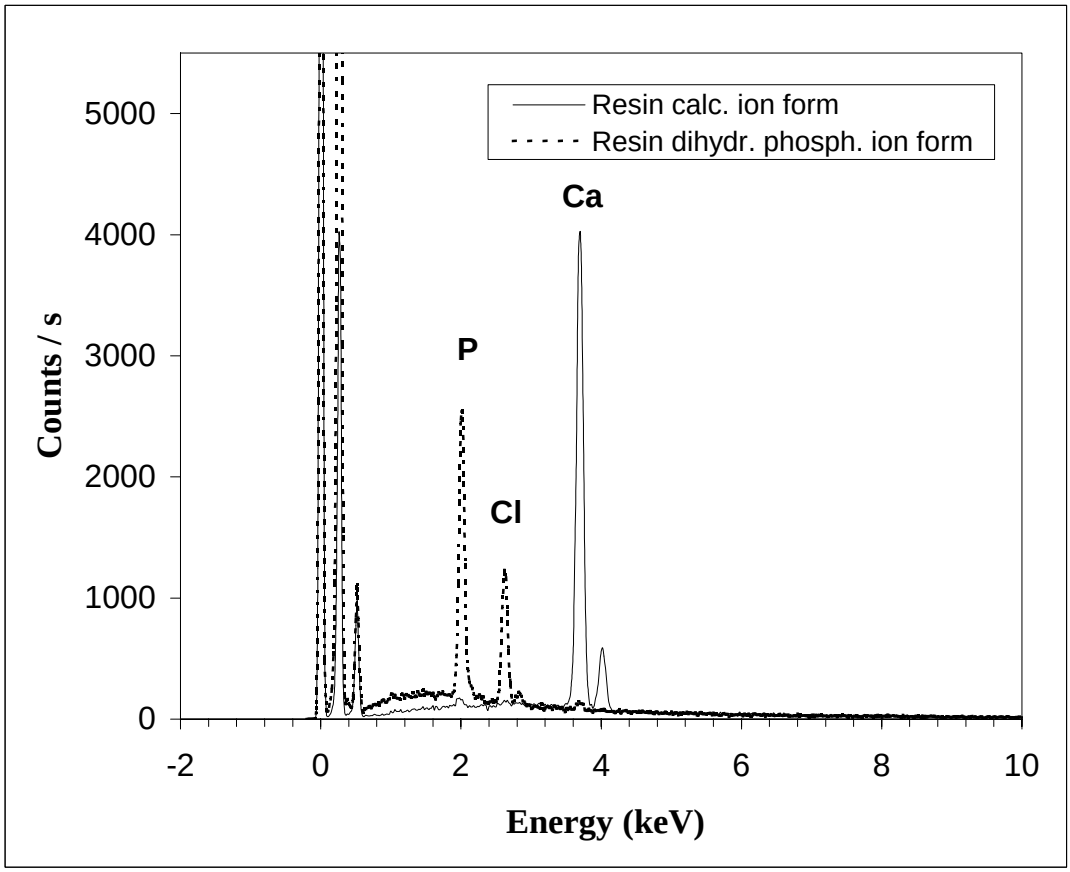
Resin calc. ion form
Resin dihydr. phosph. ion form
Ca
P
Cl
Counts / s
Energy (keV)
5000
4000
3000
2000
1000
0
-2
0
2
4
6
8
10

Figure 9b

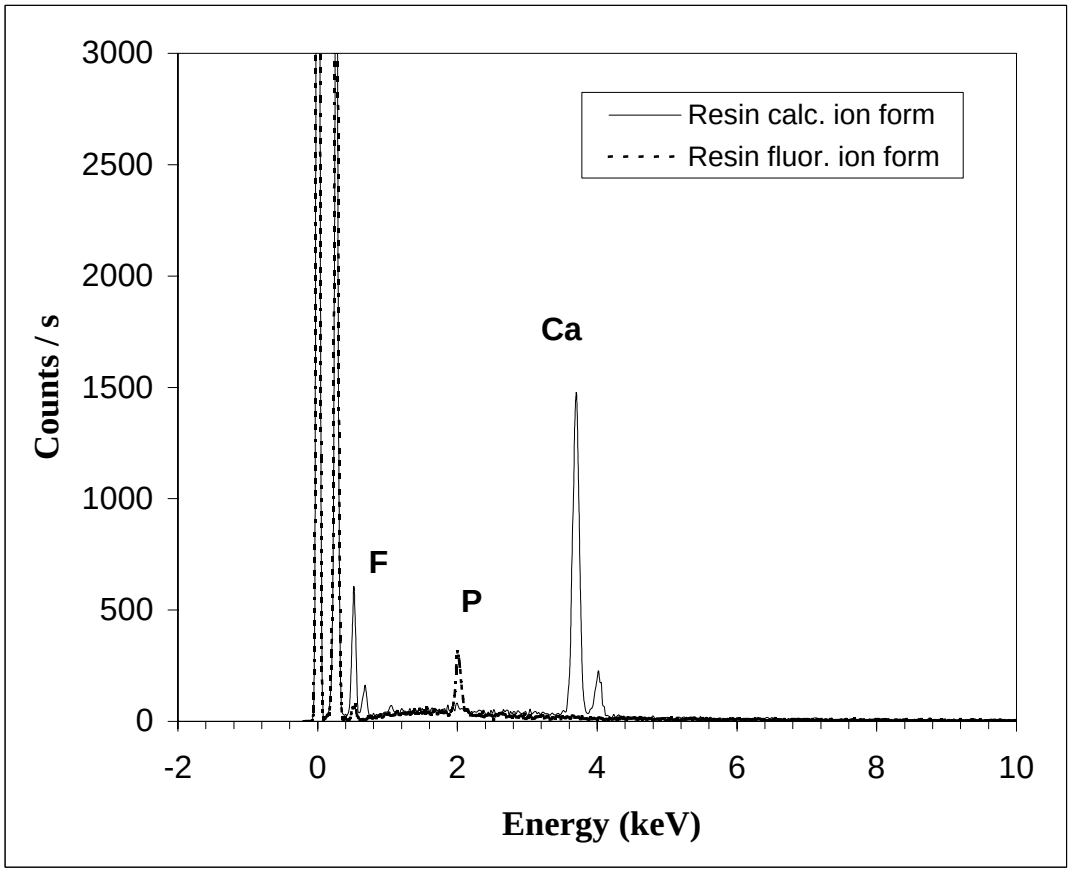
3000
2500
2000
1500
1000
500
0
Counts / s
-2
0
2
4
6
8
10
Energy (keV)
Resin calc. ion form
Resin fluor. ion form
Ca
F
P

Figure 10a

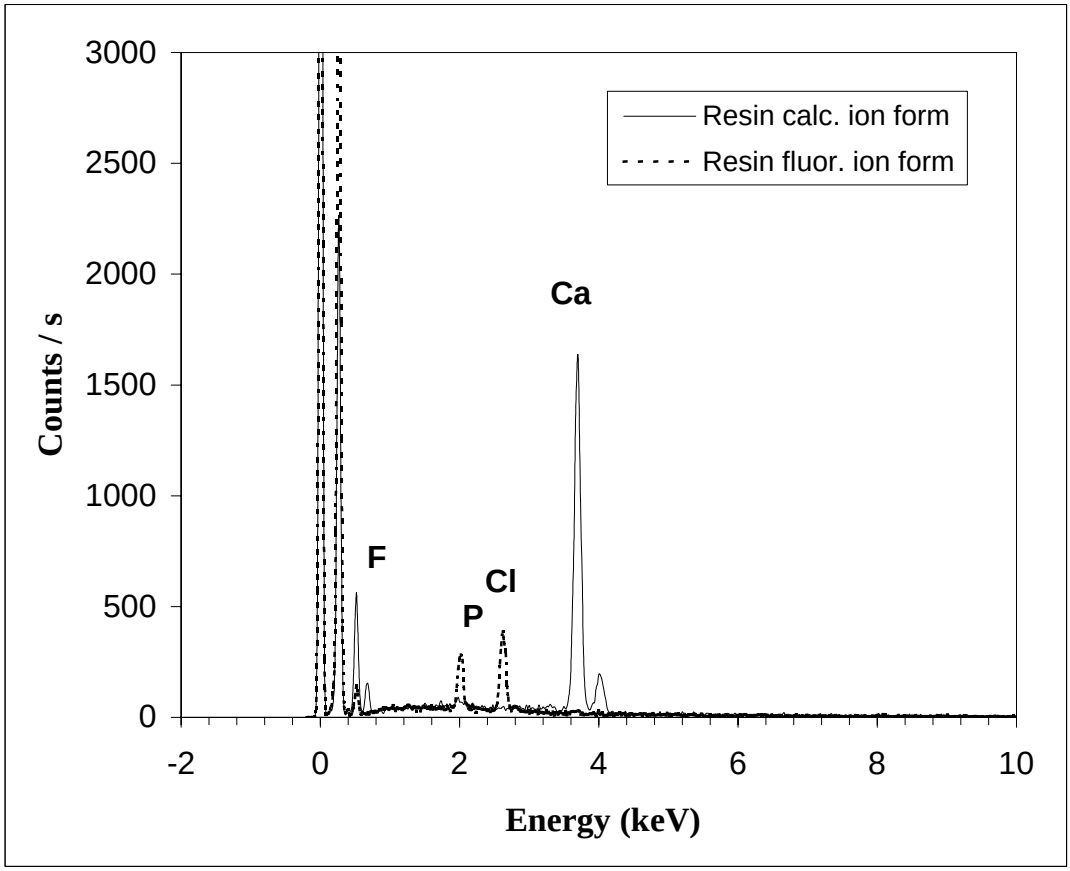
3000
2500
2000
1500
1000
500
0
Counts / s
-2
0
2
4
6
8
10
Energy (keV)
Resin calc. ion form
Resin fluor. ion form
F
P
Cl
Ca

Figure 10b

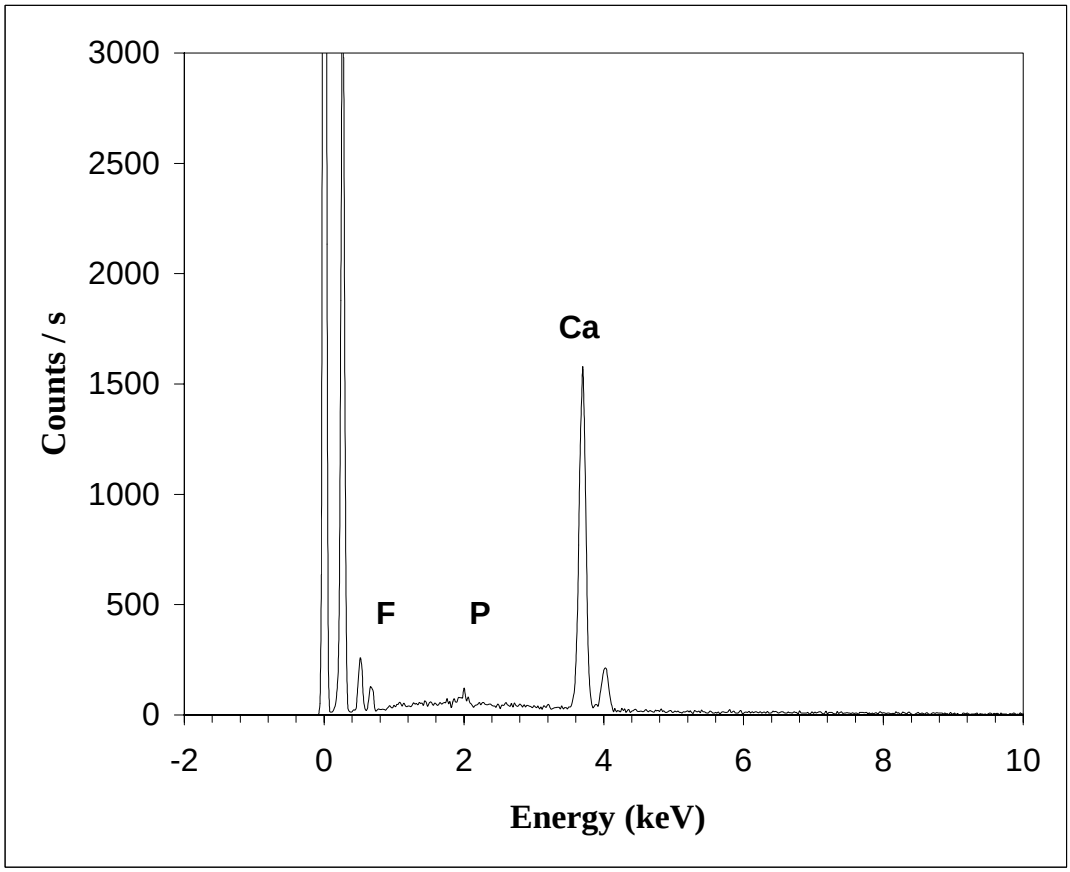
3000
2500
2000
1500
1000
500
0
Counts / s
Ca
F
P
-2
0
2
4
6
8
10
Energy (keV)

Figure 11a

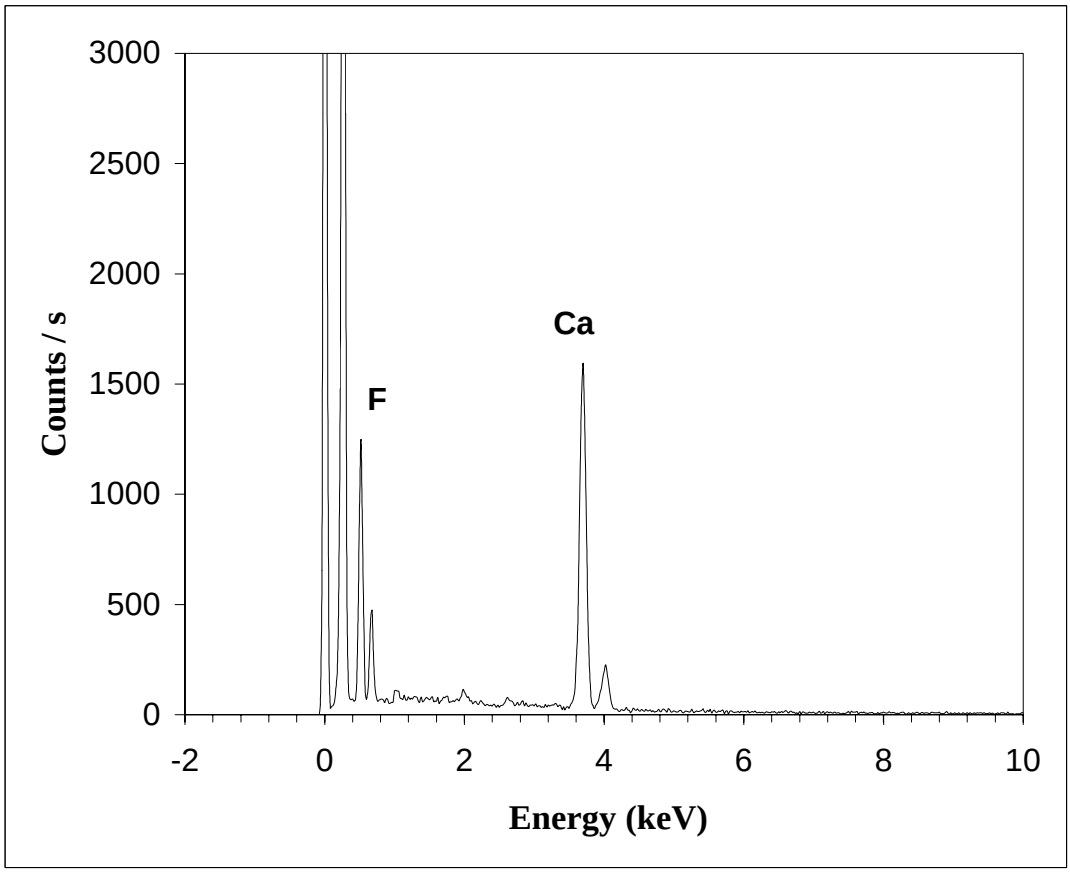
3000
2500
2000
1500
1000
500
0
Counts / s
F
Ca
-2
0
2
4
6
8
10
Energy (keV)

Figure 11b

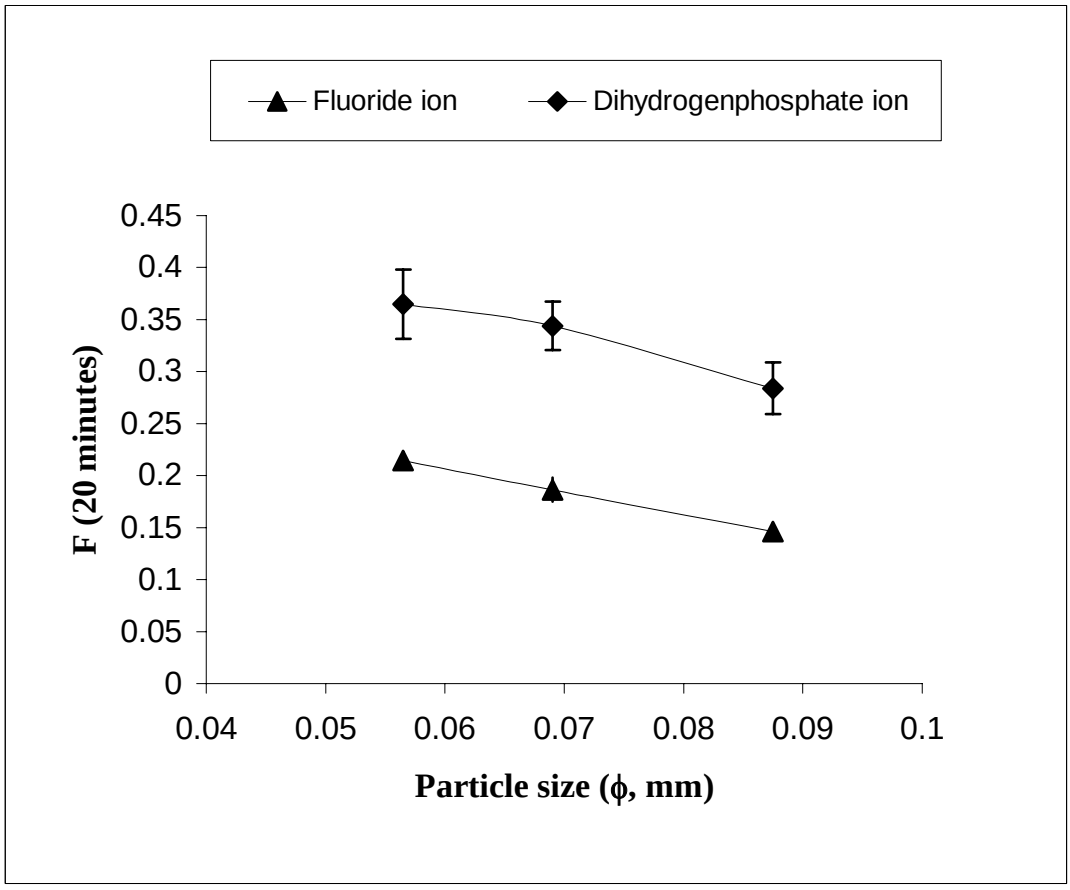
Fluoride ion
Dihydrogenphosphate ion
F (20 minutes)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.04
0.05
0.06
0.07
0.08
0.09
0.1
Particle size (ϕ, mm)

Figure 12

ANNEX 6

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau

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27 June 2002 (27.06.2002)

PCT

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(71) Applicant (for all designated States except US): SOCIEDAD LIMITADA PARA EL DESARROLLO CIENTIFICO APLICADO [ES/ES]; Passeig de Gracia, 77 Pral B, E-08008 Barcelona (ES).

(72) Inventors; and

(75) Inventors/Applicants (for US only): TORRADO BONALS, Ana [ES/ES]; Taneryas 34 3°, E-08240 Manresa (ES). VALIENTE MALMAGRO, Manuel [ES/ES]; Paseo Apeadero, 87, E-08195 Sant Cugat del Valles (ES).

(74) Agent: PONTI SALES, Adelaida; Comte d'Urgell, 322, E-08007 Barcelona (ES).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

as to the identity of the inventor (Rule 4.17(i)) for the following designations (AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG))

as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations (AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG))

of inventorship (Rule 4.17(iv)) for US only

Published:
— with international search report

(48) Date of publication of the international search report:
3 January 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DENTIFRICE PRODUCT

(57) Abstract: Comprises a surfactant, a cellulose derivative, additives and at least one resin charged with Ca^{+2}, F^-, PO_4^{-3} o Zn^{+2} cations or anions, with the calcium, fluoride and phosphate ions in a molar ratio of 2:1:1 and the zinc ions in dry-weight proportion with respect to the resin or resins of between 0.5 and 2 %. It is used as a dental-tissue remineralising agent or for making a medicine for the treatment of buccal diseases and, in particular, for the treatment of caries.

WO 02/049588 A3

OFICINA ESPAÑOLA DE PATENTES Y MARCAS

INSTANCIA DE SOLICITUD DE:

NUMERO DE SOLICITUD

FECHA Y HORA DE PRESENTACION EN LA O.E.P.M.

20 DIC 2000

FECHA Y HORA PRESENTACION EN LUGAR DISTINTO O.E.P.M.

CIDF

[X] PATENTE DE INVENCION [ ] MODELO DE UTILIDAD

(1)
- [ ] SOLICITUD DE ADICION
- [ ] SOLICITUD DIVISIONAL
- [ ] CAMBIO DE MODALIDAD
- [ ] TRANSFORMACION SOLICITUD EUROPEA

(2) EXPED. PRINCIPAL O DE ORIGEN

MODALIDAD
NUMERO SOLICITUD
FECHA SOLICITUD

MODALIDAD
NUMERO SOLICITUD
FECHA SOLICITUD

(3) LUGAR DE PRESENTACION: BARCELONA — CODIGO: 08

(4) SOLICITANTES	APELLIDOS O DENOMINACION JURIDICA	NOMBRE	DNI
	SOCIEDAD LIMITADA PARA EL DESARROLLO CIENTIFICO APLICADO		

(5) DATOS DEL PRIMER SOLICITANTE

DOMICILIO Pg. de Gràcia, 77, pral. B
LOCALIDAD BARCELONA — TELEFONO
PROVINCIA BARCELONA — COD. POSTAL 08008
PAIS RESIDENCIA ESPAÑA — COD. PAIS ES
NACIONALIDAD ESPAÑOLA — COD. NACION ES

(6) INVENTORES — (7) [ ] EL SOLICITANTE ES EL INVENTOR [X] EL SOLICITANTE NO ES EL INVENTOR O UNICO INVENTOR — (8) MODO DE OBTENCION DEL DERECHO [ ] INVENC. LABORAL [X] CONTRATO [ ] SUCESION

APELLIDOS	NOMBRE	NACIONALIDAD	COD. NACION
TORRADO BONALS	ANA	ESPAÑOLA	ES
VALIENTE MALMAGRO	MANUEL	ESPAÑOLA	ES

(9) TITULO DE LA INVENCION

PRODUCTO DENTIFRICO.

(10) INVENCION REFERENTE A PROCEDIMIENTO MICROBIOLOGICO SEGUN ART. 25.2 L.P. [ ] SI [ ] NO

(11) EXPOSICIONES OFICIALES

LUGAR — FECHA

(12) DECLARACIONES DE PRIORIDAD

PAIS DE ORIGEN	COD. PAIS	NUMERO	FECHA

(13) EL SOLICITANTE SE ACOGE A LA EXENCION DE PAGO DE TASAS PREVISTA EN EL ART. 162 L.P. [ ] SI [ ] NO

(14) REPRESENTANTE

APELLIDOS	NOMBRE	CODIGO
Ponti Sales	Adelaida	388/3

DOMICILIO	LOCALIDAD	PROVINCIA	COD. POSTAL
Consell de Cent, 322	Barcelona	Barcelona	08007

(15) RELACION DE DOCUMENTOS QUE SE ACOMPAÑAN

- [ ] DESCRIPCION. Nº DE PAGINAS ....
- [X] REIVINDICACIONES. Nº DE PAGINAS ... 2
- [ ] DIBUJOS. Nº DE PAGINAS ....
- [ ] RESUMEN
- [ ] DOCUMENTO DE PRIORIDAD
- [ ] TRADUCCION DEL DOCUMENTO DE PRIORIDAD
- [X] DOCUMENTO DE REPRESENTACION
- [ ] PRUEBAS
- [X] JUSTIFICANTE DEL PAGO DE TASAS
- [ ] HOJA DE INFORMACIONES COMPLEMENTARIAS
- [X] OTROS DECLARACION INVENTOR

FIRMA DEL FUNCIONARIO

FIRMA DEL SOLICITANTE O REPRESENTANTE

Adelaida Ponti Sales
Colegiado Nº 320

(16) NOTIFICACION DE PAGO DE LA TASA DE CONCESION

Se le notifica que esta solicitud se considerará retirada si no procede al pago de la tasa de concesión; para el pago de esta tasa dispone de tres meses a contar desde la publicación del anuncio de la concesión en el BOPI, más los diez días que establece el art. 81 del R.D. 10-10-86.

3. Recibo solicitante

ILMO. SR. DIRECTOR DE LA OFICINA ESPAÑOLA DE PATENTES Y MARCAS

UNE A-4 MOD. 3101

OFICINA ESPAÑOLA DE PATENTES Y MARCAS

DATOS DE PRIORIDAD (31) NUMERO	(32) FECHA	(33) PAIS	A1	(12) PATENTE DE INVENCION
				(21) NUMERO DE SOLICITUD 200003138
				(22) FECHA DE PRESENTACION 20/12/2000

(71) SOLICITANTE (S)
SOCIEDAD LIMITADA PARA EL DESARROLLO, CIENTIFICO APLICADO — NACIONALIDAD ESPAÑOLA

DOMICILIO Pg. de Gràcia, 77, pral. B
BARCELONA 08008 BARCELONA ESPAÑA

(72) INVENTOR (ES) ANA TORRADO BORALS, MANUEL VALIENTE MALMAGRO

(73) TITULAR (ES)

(11) N.º DE PUBLICACION	(45) FECHA DE PUBLICACION	(62) PATENTE DE LA QUE ES DIVISIONARIA	GRAFICO (SOLO PARA INTERPRETAR RESUMEN)

(51) Int. Cl.

(54) TITULO
PRODUCTO DENTIFRICO.

(57) RESUMEN

PRODUCTO DENTÍFRICO

Comprende un tensoáctivo, un derivado celulósico, aditivos y, por lo menos, una resina cargada con cationes o aniones Ca^{2+}, F^-, PO_4^{3-} o Zn^{2+}, encontrándose los iones calcio, fluoruro y fosfato en una relación molar 2:1:1 y los iones zinc en una proporción en peso respecto a la o las resinas en seco entre 0,5 y 2%.
Se utiliza como agente remineralizante de tejidos dentales o bien para la preparación de un medicamento destinado al tratamiento de enfermedades bucales y, en particular, para el tratamiento de la caries.

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

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NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

To:

PONTI SALES, Adelaida
Consell de Cent, 322
E-08007 Barcelona
ESPAGNE

Date of mailing (day/month/year) 28 December 2001 (28.12.01)	
Applicant's or agent's file reference A-152558	IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/IB01/02709	20 December 2001 (20.12.01)	20 December 2000 (20.12.00)

Applicant: SOCIEDAD LIMITADA PARA EL DESARROLLO CIENTIFICO APLICADO et al

Title of the invention: DENTIFRICE PRODUCT

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

- [x] was transmitted to the International Bureau on 28 December 2001 (28.12.01)
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DATOS DE PRIORIDAD

(31) NUMERO | (32) FECHA | (33) PAIS

A1

(12) PATENTE DE INVENCION

(21) NUMERO DE SOLICITUD
200003138

(22) FECHA DE PRESENTACION
20/12/2000

(71) SOLICITANTE (S)
SOCIEDAD LIMITADA PARA EL DESARROLLO, CIENTIFICO APLICADO

NACIONALIDAD
ESPAÑOLA

DOMICILIO Pg. de Gràcia, 77, pral. B
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Comprende un tensoactivo, un derivado celulósico, aditivos y, por lo menos, una resina cargada con cationes o aniones Ca^{2+}, F^-, PO_4^{3-} o Zn^{2+}, encontrándose los iones calcio, fluoruro y fosfato en una relación molar 2:1:1 y los iones zinc en una proporción en peso respecto a la o las resinas en seco entre 0,5 y 2%.
Se utiliza como agente remineralizante de tejidos dentales o bien para la preparación de un medicamento destinado al tratamiento de enfermedades bucales y, en particular, para el tratamiento de la caries.

CAMPO DE LA INVENCIÓN

La presente invención se refiere a un producto dentífrico que comprende una resina encapsulada dentro de una matriz a través de la cual se liberan iones.

La presente invención también se refiere a la utilización de dicho producto en la remineralización de los tejidos dentales.

ANTECENDENTES DE LA INVENCIÓN

La presente invención se refiere a un producto dentífrico con efecto remineralizante.

Se conocen productos dentífricos que comprenden un tensioactivo, un derivado celulósico y aditivos. En el estado la técnica no se conocen pastas de dientes que incorporen resinas de intercambio iónico encapsuladas dentro de una matriz. En algunos de los productos conocidos se utiliza el proceso de intercambio iónico con el fin de sintetizar componentes que actúen como agentes bactericidas (US patent 4621120,1986), inhibidores de la caries dental (DE patent 3605656,1987), componentes responsables de las propiedades de la pasta de dientes: su abrasividad (Hertzenberg, E. P. et al., Stud. Surf. Sci. Catal., 24:589-596 (1985)), su irritabilidad (US patent 4391798, 1983), etc. Otras incorporan en su formulación materiales inorgánicos tipo zeolitas (US patent 4349533, 1982, EP patent 297563, 1989).

Existen numerosos estudios en el estado de la técnica sobre la remineralización , siendo característico la aparición de iones calcio, fluoruro y fosfato en forma de sales inorgánicas varias. Se conocen algunos casos en que las pastas comprenden únicamente componentes aniónicos ya sea sólo fluoruro (Mellberg, J. R. et al., Caries Res., 25:65-69 (1991)) o mezcla de fluoruro y fosfato (US patent

4177258, 1979). Este tipo de composiciones dan lugar a la formación de compuestos insolubles al poner en contacto los diferentes iones, ya que, una fracción importante de la formulación de la pasta es agua, de manera que se produce una disminución de la cantidad efectiva de iones disponibles para llevar a cabo la remineralización. También se conoce el uso de dos compartimentos separados, uno para los componentes catiónicos y otro para los aniónicos (US patent 5858333, 1999), de este modo el precipitado insoluble se forma en el momento en que se ponen en contacto ambas partes, quedando depositado directamente sobre la lesión, de manera que aumenta el poder remineralizador de la pasta.

En el estado de la técnica se conoce un procedimiento que implica dos etapas (Magas, S. et al., Czas. Stomatol., 43(6):323-327 (1990)); una primera etapa basada en un tratamiento con una pasta que comprende componentes aniónicos (sales de fosfato, monofluorofosfato y fluoruro) y una segunda etapa basada en un tratamiento con una pasta que comprende sales de calcio y otros cationes.

Los estudios sobre la remineralizacón se pueden llevar a cabo mediante tres modalidades distintas:

-In vitro (Cheng, C. et al., Beijing Yike Daxue Xuebao, 23(4):305-307 (1991), Marinelli, C. B. et al., Caries Res., 31(6):418-422 (1997)) con dientes extraídos.

-In situ (Mellberg, J. R. et al., Caries Res., 25:65-69 (1991)) incorporan dientes extraídos, lesionados al medio biológico natural.

-In vivo (De Kloet, H. et al., J. Dent. Res., 65(12):1410-1414 (1986), Mellberg, J. R. et al., J. Dent. Res., 65(8):1078-1083 (1986)) diente no extraído.

Recientemente han aparecido estudios en los que se intenta desarrollar productos en los que aparte de conseguir una remineralización se mejoran características

como el emblanquecimiento y la eliminación de manchas (WO patent 2000047173, 2000).

DESCRIPCIÓN DE LA INVENCIÓN

En la presente invención se ha determinado el efecto del encapsulamiento de resinas en la velocidad de iones en matrices de pasta de dientes. De esta forma, se ha estudiado el efecto del tensioactivo y del derivado celulósico en dicha velocidad.

El producto dentífrico de la presente invención se caracteriza por el hecho que comprende por lo menos una resina de intercambio iónico cargada con cationes o aniones comprendiendo dicha o dichas resinas iones Ca^{+2}, F^{-}, PO_4^{-3} o Zn^{+2}. Preferentemente, comprende un derivado celulósico desprovisto de grupos secuestradores de iones calcio y, aún más preferentemente, un tensioactivo no iónico. Cuando los iones calcio, fluoruro y fosfato están presentes en la mezcla de resinas, estos se encuentran preferentemente en una relación molar 2:1:1. Los iones Zn^{+2} comprendidos en la o las resinas se encuentran en una proporción en peso seco entre 0,5-2%.

Dicha resina o mezcla de resinas comprende:

- Resina catiónica tipo ácido débil o ácido fuerte cargada con iones calcio (R-Ca).
- Resina catiónica tipo ácido débil o ácido fuerte cargada con iones cinc (R-Zn).
- Resina aniónica tipo base débil o base fuerte cargada con iones fosfato ($R-PO_4$)
- Resina aniónica tipo base débil o base fuerte cargada con iones fluoruro (R-F).

Aún más preferible que la composición de la pasta de dientes comprenda una proporción en peso de la resina o mezcla de resinas entre 1-15%.

En la Tabla 1, se muestran los componentes principales de las distintas bases de pasta de dientes utilizadas.

Tabla 1: Composición principal de las distintas bases de pasta de dientes.

	Base 1	Base 2	Base 3	Base 4	Base 5
Laurilsulfato sódico	✓	-	✓	-	-
Polioxil 40 de aceite de castor hidrogenado	-	-	-	-	✓
Carboximetilcelulosa sódica	✓	✓	-	-	-
Metilcelulosa MP424C	-	-	✓	✓	✓
Silice coloidal anhidra	✓	✓	✓	✓	✓
Glicerina	✓	✓	✓	✓	✓
Propilenglicol	✓	✓	✓	✓	✓
Agua	✓	✓	✓	✓	✓

A partir de estas bases se han preparado distintas muestras de pasta de dientes conteniendo las concentraciones de monocomponente y NMTD que se indican a continuación:

Tabla 2: Concentración de monocomponente y NMTD en las distintas muestras de pasta de dientes expresadas en porcentaje en peso.

	% Monocomponente				%NMTD
	$R\text{-}Ca^{2+}$	$R\text{-}Zn^{2+}$	$R\text{-}F^{-}$	$R\text{-}PO_4^{3-}$	$R\text{-}Ca^{2+}$ + $R\text{-}Zn^{2+}$ + $R\text{-}F^{-}$ + $R\text{-}PO_4^{3-}$
Base 1	10	10	10	10	-
Base 2	10	-	-	-	-
Base 3	10	-	-	-	-
Base 4	10	-	-	-	-
Base 5	10	-	10	-	10 / 15

Por último se ha evaluado el efecto que ejerce sobre la liberación de iones la incorporación de algunos

de los aditivos más utilizados en las formulaciones de pastas de dientes convencionales. Éste es el caso del óxido de titanio (TiO_2) típicamente utilizado para blanquear la pasta y poder añadir a continuación el colorante deseado. Se ha trabajado con muestras de base de pasta 5 conteniendo TiO_2 y un 10% de NMTD.

Preferiblemente, los derivados celulósicos, utilizados en la invención, se seleccionan entre metilcelulosa; hidroxietilcelulosa; hidroxipropilcelulosa o hidroxipropilmetilcelulosa.

Preferiblemente, el tensioactivo no-iónico se selecciona entre polioxietilensorbitan monolaureato; polioxietilensorbitan monopalmitato; monoestearato; polioxietilensorbitan monooleato; polioxietilensorbitan trioleato; polioxietilen estearato; polioxietilen laureato ;polioxil 40 de aceite de castor hidrogenado; polioxil 35 o 60 de aceite de castor y aceite de ricino polietoxietilenado.

Paralelamente se ha llevado a cabo también un estudio "in vitro" sobre la acción remineralizante del producto NMTD incorporado, en una proporción del 10 y 15% respectivamente, a la base de pasta dentífrica 5.

Los resultados obtenidos del estudio de las muestras formadas por base 1 +10% de monocomponente han mostrado que la liberación de los aniones en presencia de pasta de dientes se lleva a cabo más rápidamente. En cambio, para los cationes se da el efecto totalmente contrario. Al mismo tiempo se ha verificado la no presencia de los iones de interés en la matriz base de pasta de dientes (blanco). Ambas observaciones sobre las diferencias apreciadas en la liberación de cationes y aniones frente a la de los respectivos componentes puros se atribuyen a la presencia de tensioactivos iónicos (laurilsulfato sódico) y a compuestos con grupos iónicos residuales en la base de la pasta (carboximetilcelulosa).

Así pues con el propósito de optimizar la liberación de todos los iones de los respectivos monocomponentes-pasta dentífrica se ha procedido a la preparación de nuevas matrices de pasta de dientes modificadas para llevar a cabo un estudio de los componentes responsables de la baja liberación de los cationes. Se ha seguido preferentemente al calcio pues el zinc es componente minoritario en la mezcla que constituye el producto final.

Del estudio de la velocidad de liberación del ión Ca^{2+} de las muestras: base 2 +10% Ca-monocomponente, base 3 +10% Ca-monocomponente y base 4 +10% Ca-monocomponente se ha observado que la liberación del ión Ca^{2+} de las distintas matrices de pasta de dientes está claramente influenciada tanto por el tensioactivo como por la carboximetilcelulosa. Sin embargo, el efecto que ejerce esta última es superior a la del tensioactivo y la muestra sin ambos componentes es la que presenta una liberación del ión equivalente al comportamiento observado en el sistema resina en ausencia de matriz de pasta de dientes. (Ver la figura 1 adjunta)

Las curvas cinéticas de liberación de los iones Ca^{2+} y F^- de la muestra: base 5+ 10% del respectivo monocomponente muestran que la liberación, tanto de los cationes como de los aniones, se lleva a cabo prácticamente a la misma velocidad que en el caso en que tengamos los respectivos componentes sin pasta de dientes.

Así, el estudio de la liberación de los iones de la muestra base 5 +10% de NMTD, ha conducido a concluir que no hay diferencias significativas entre la liberación de los iones en la mezcla de resinas en presencia de esta matriz de pasta dentífrica de aquella que se da en ausencia de dicha pasta. (Ver la figura 2 adjunta)

El estudio de la evaluación del efecto que ejerce la concentración del principio activo (NMTD) en las

muestras de base de pasta de dientes del tipo 5 sobre la liberación de los iones, indica nuevamente que éstos se liberan a una velocidad totalmente comparable a la obtenida en el principio activo en ausencia de matriz de pasta dentífrica.

Los resultados de liberación de la muestra de bases de pasta 5 conteniendo TiO_2 y un 10% NMTD no han mostrado diferencias significativas entre la liberación de los iones en NMTD encapsulado y sin encapsular.

De acuerdo con los resultados obtenidos, la presencia de carboximetilcelulosa y laurilsulfato sódico en la base de la pasta de dientes provoca una aceleración en la velocidad de liberación de los aniones mientras que retarda la de los cationes. Este hecho se debe por un lado, al comportamiento desplazante de aniones que ejercen dichos componentes de la pasta de dientes y por otro lado, la interacción (complejación) entre estos componentes y los iones Ca^{2+} y Zn^{2+} contribuye al retardo de su liberación.

La optimización de la base de la pasta de dientes mediante la sustitución de la carboximetilcelulosa por metilcelulosa y del tensioactivo iónico por uno no-iónico permite conseguir una pasta de dientes compatible con la liberación de todos los iones integrantes del material NMTD responsable de la remineralización de los tejidos dentales.

La liberación de los iones calcio, fluoruro y fosfato no se ve afectada por la cantidad de principio activo introducido en la matriz de pasta de dientes así como tampoco por la presencia de aditivos tales como el agente blanqueante, óxido de titanio.

Los resultados obtenidos del estudio "in vitro" de la base de pasta 5 conteniendo un 10 o un 15% de producto NMTD han mostrado que la mayor obliteración de túbulos dentinarios, en comparación con todos los materiales que

se han usado para llevar a cabo dicho estudio, se ha conseguido con la pasta de dientes conteniendo un 15% de NMTD (Ver figura 3 adjunta). Además las pastas de dientes que contienen el producto NMTD tienen una mayor afinidad sobre la dentina que el resto de productos estudiados, poniendo de manifiesto el hecho de que el material NMTD puede mantener su capacidad de actuación a pesar de su encapsulamiento dentro de una matriz de pasta de dientes.

El producto dentífrico descrito en la presente invención, que comprende por lo menos una resina de intercambio iónico puede ser usada en la remineralización de tejidos dentales. Debido a este efecto, en la zona del diente en contacto con la pasta de dientes, este material es también beneficioso para el tratamiento preventivo o sintomático de infecciones bucales, como la caries y la gingivitis.

FIGURAS

- FIGURA 1: se evalúa el efecto del tensoactivo y derivados celulósicos sobre la velocidad de liberación del ión calcio en las distintas matrices de pasta de dientes.

- FIGURA 2: muestra el grado de conversión del ión calcio del componente base 5 + 10% NMTD (sistema PLS, en discontinuo y a 37°C).

- FIGURA 3: muestra el porcentaje de túbulos dentinarios oblierados.

REIVINDICACIONES

1. Producto dentífrico, que comprende un tensioactivo, un derivado celulósico y aditivos, caracterizado por el hecho que comprende por lo menos una resina cargada con cationes o aniones, comprendiendo dicha resina o resinas los iones Ca^{+2}, F^{-}, PO_4^{-3} o Zn^{+2} .

2. Producto dentífrico según la reivindicación 1 estando el derivado celulósico desprovisto de grupos secuestradores de iones calcio y siendo el tensioactivo no iónico.

3. Producto dentífrico, según la reivindicación 1, donde la resina o resinas comprenden iones calcio, fluoruro y fosfato en una relación molar 2:1:1.

4. Producto dentífrico según la reivindicación 1 donde la resina o mezcla de resinas comprende:

- Resina catiónica tipo ácido débil o ácido fuerte cargada con iones calcio (R-Ca).
- Resina catiónica de ácido débil o ácido fuerte cargada con iones cinc (R-Zn).
- Resina aniónica de base débil o base fuerte cargada con iones fluoruro (R-F).
- Resina aniónica de base débil o base fuerte cargada con iones fosfato ($R-PO_4$).

5. Producto dentífrico, según la reivindicación 1, donde la mezcla de resinas se encuentra en una proporción en peso entre 1-15%.

6. Producto dentífrico, según la reivindicación 1, donde el ión Zn^{+2} se encuentra en una proporción en peso respecto a la resina en seco entre 0,5-2%

7. Producto dentífrico, según reivindicación 1, donde dichos derivados celulósicos se seleccionan entre metilcelulosa; hidroxietilcelulosa; hidroxipropilcelulosa o hidroxipropilmetilcelulosa.

8. Producto dentífrico, según la reivindicación 1, donde dicho tensioactivo no-iónico se selecciona entre polioxietilensorbitan monolaureato; polioxietilensorbitan monopalmitato; monoestearato; polioxietilensorbitan monooleato; polioxietilensorbitan trioleato; polioxietilen estearato; polioxietilen laureato; polioxil 40 de aceite de castor hidrogenado; polioxil 35 o 60 de aceite de castor y aceite de ricino polietoxietilenado

9. Producto dentífrico, según cualquiera de las reivindicaciones anteriores, para su uso en la remineralización de tejidos dentales.

10. Utilización de un producto dentífrico, según cualquiera de las reivindicaciones anteriores, para la fabricación de un medicamento destinado al tratamiento de infecciones bucales.

11. Utilización de un producto dentífrico, según cualquiera de las reivindicaciones anteriores, para la fabricación de un medicamento destinado al tratamiento de la caries.

FIGURA 1

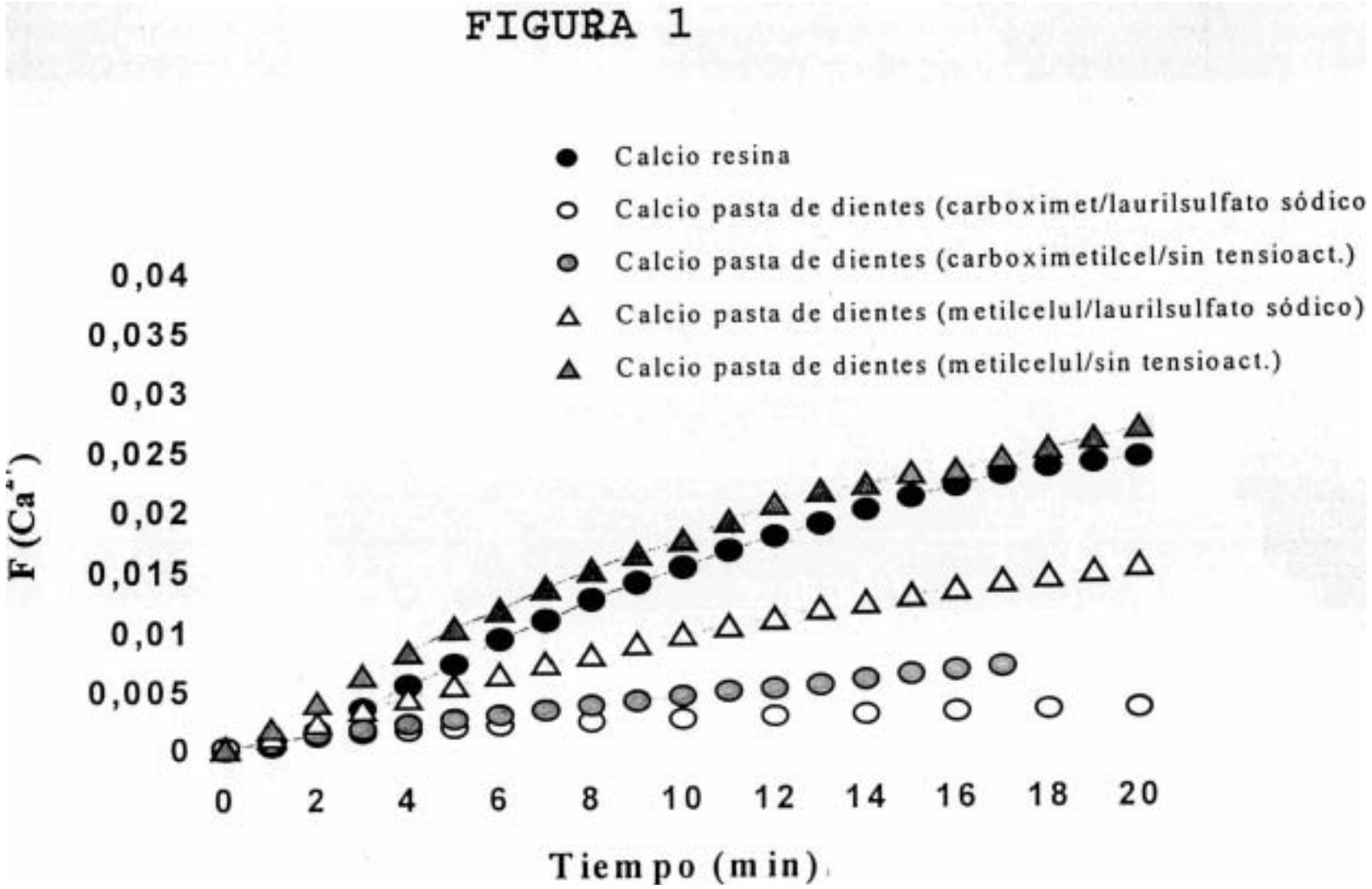

Calcio resina
Calcio pasta de dientes (carboximet/laurilsulfato sódico
Calcio pasta de dientes (carboximetilcel/sin tensioact.)
Calcio pasta de dientes (metilcelul/laurilsulfato sódico)
Calcio pasta de dientes (metilcelul/sin tensioact.)
0,04
0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
F (Ca++)
0
2
4
6
8
10
12
14
16
18
20
Tiempo (min)

FIGURA 2

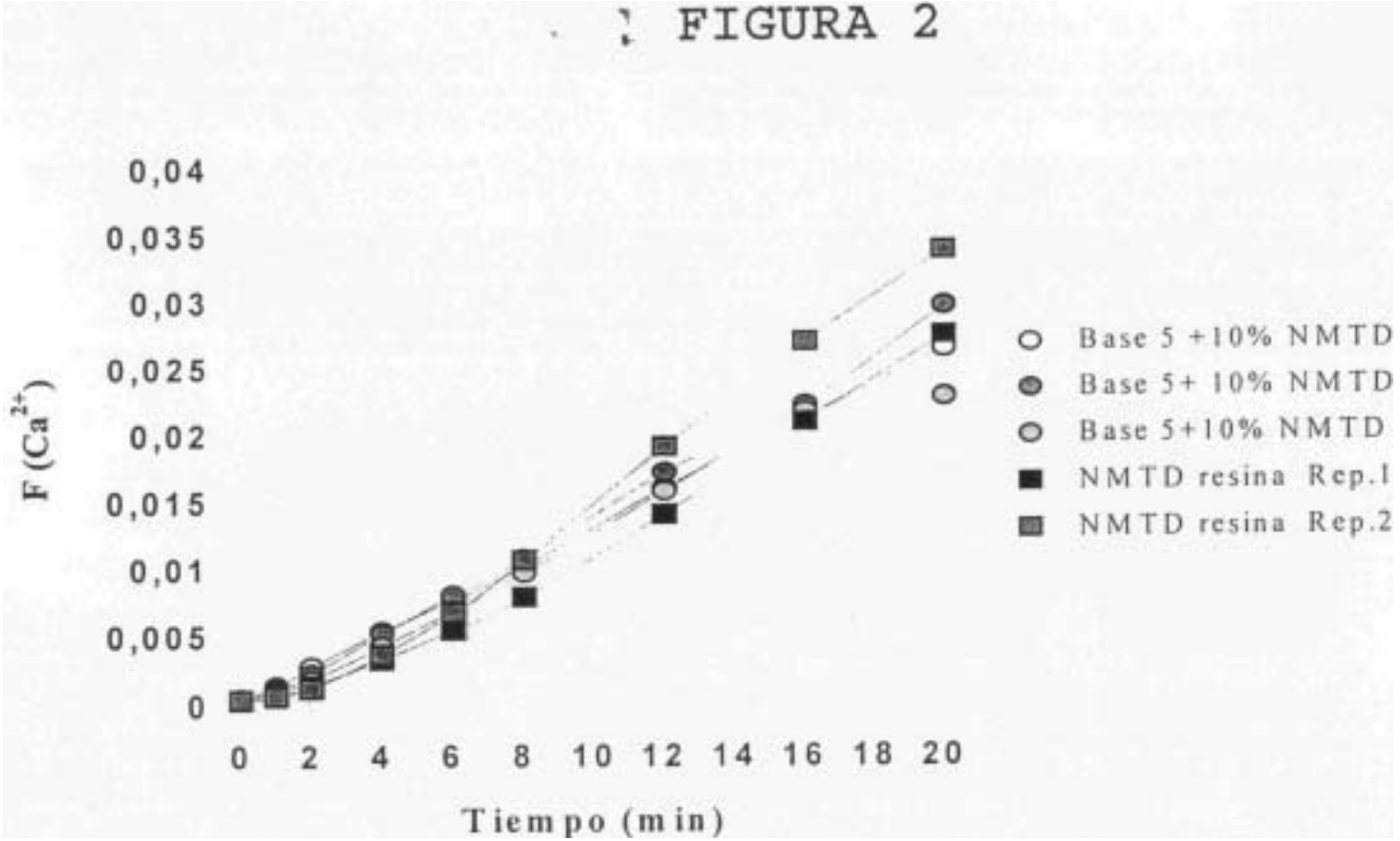
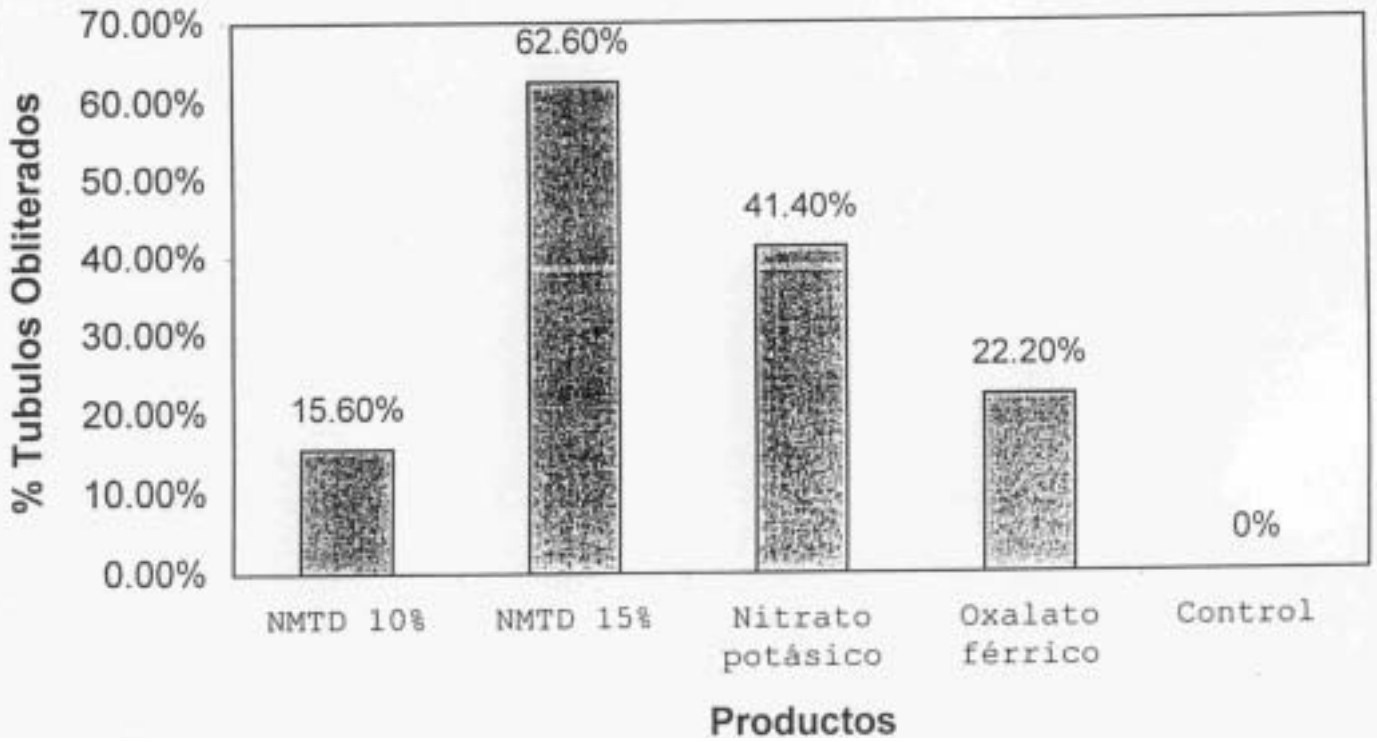

0,04
0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
F (Ca2+)
0 2 4 6 8 10 12 14 16 18 20
Tiempo (min)
Base 5 +10% NMTD
Base 5+ 10% NMTD
Base 5+10% NMTD
NMTD resina Rep.1
NMTD resina Rep.2

FIGURA 3


70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
% Tubulos Obliterados
62.60%
41.40%
22.20%
15.60%
0%
NMTD 10%
NMTD 15%
Nitrato potásico
Oxalato férrico
Control
Productos

ANNEX 7

IN VITRO CYTOTOXICITY OF A NEW TOOTHPASTE BASED ON AN ION-EXCHANGE RESINS MIXTURE RELEASING CALCIUM, FLUORIDE, PHOSPHATE AND ZINC IONS

Anna Torrado [2], Manuel Valiente[2], Wu Zhang[1], Yiming Li[1] and Carlos A. Muñoz[1]

[1]School of Dentistry, Loma Linda University, Loma Linda, CA 92350, USA
[2]Quimica Analitica, Universitat Autonoma de Barcelona, Bellaterra, E-08193, Spain

Corresponding author:

Carlos A. Muñoz, DDS, MSD

School of Dentistry

Loma Linda University

11092 Anderson St.

Loma Linda, CA 92350

Phone: 909-558-0656

Fax: 909-558-0270

E-mail address: **cmunoz@sd.llu.edu**

Keywords: cytotoxicity, ion-exchange, dentifrice, toothpaste, fluoride

Running Head: Cytotoxicicity of an Ion-exchange resins toothpaste

In-vitro Cytotoxicity of a New Toothpaste Based on an Ion-Exchange Resins Mixture Releasing Calcium, Fluoride, Phosphate and Zinc Ions.

Abstract.

Objective: This study evaluated the effects for cytotoxicity of two dentifrices: a toothpaste commercially available (Crest® Extra-whitening toothpaste) and a new experimental toothpaste based on a mixture of ion-exchange resins (named NMTD) that supplies calcium, fluoride, phosphate and zinc ions.

Methods: Cultures of mouse fibroblasts cells L929 were used in a MTT assay for *in vitro* cytotoxicity of the dentifrices. Cells were cultured in Eagle's minimal essential medium supplemented with 10 % fetal bovine serum. Cultures were incubated at 37 ºC in a humidified atmosphere of 5 % CO_2 and collected by tripsinization (0.05 % trypsin / 0.5 mM EDTA). A 96-well microplate method was employed for the MTT colorimetric assay. Positive control consisted of 10 µl of phenol in 5 ml of 6 % media, a dose that produces zero percent cell survival. Negative control was prepared by adding 0.5 ml of HBSS to 4.5 ml of 6 % media. The plates were incubated for 24 and 48 hours at 37 ºC in a 5 % CO_2 atmosphere.

Results: Means and standard deviations of absorbance values for each group and percentage inhibitory dosage (% ID) for each test material were calculated. None of the dentifrices resulted in a percentage of inhibition higher than 50 % and did not observe marked increases in cytotoxicity with time of incubation. The positive control gave almost zero percent cell survival, whereas the negative control gave a hundred percent cell survival. Analysis of the results indicated that test dentifrice dose had no significant effect towards the cell viability ($P<0.05$).

Clinical Significance: The new experimental toothpaste is not cytotoxic despite presenting a higher fluoride availability potential than a conventional commercial dentifrice.

Introduction

Fluoride is the thirteenth most abundant element on the earth. The major source of intake comes from drinking water and diet and from involuntary swallowing during and after the use of fluoride containing products like dentifrices, mouthrinses, tablets and gels, developed because of fluoride cariostatic properties. Everybody agrees in that fluoride taken in high amounts is toxic and can be lethal. Fluoride acute toxicity affects gastrointestinal, neurological and cardiovascular systems, and blood chemistry. Depending on the dosage, the treatment will be different: either milk ingestion, as a way of calcium administration, or quick hospitalization.[1] Therefore, care must be taken in order to prevent undesirable risks, that is why several recommendations, mainly concerning dentifrices, are made: [2,3] (1) dentifrice tubes should advice about the use of the product by young children, (2) all dental products should be equipped with secure caps (3) manufacturers should consider the production of dentifrices with low fluoride concentration for the children use and (4) parents should supervise their children's toothbrushing and teach them no to swallow the toothpaste.

The general metabolism of ionic fluoride is shown in Figure I. Commonly, fluoride is absorbed and enters the body fluids by way of the gastrointestinal system or the lungs. Fluoride uptake by calcified tissues or excretion in the urine are the major mechanisms by which fluoride is eliminated from the plasma and other body fluids.[2] Fluoride uptake by bone is inversely proportional to age basically because crystallites of younger bone are

smaller, more abundant, well organized and larger surface area due to hydration. Urine pH will be determining in the excretion of fluorides from the body. If the urine is relatively alkaline, fluoride proportionally exists in ionic form, which will remain in the tubule for later being excreted because of its charge and size. On the contrary, in acidic environment, fluoride proportionally exists in the un-dissociated form, hydrogen fluoride (HF), which will be re-absorbed and incorporated into the general circulation.

A new experimental toothpaste containing a mixture of ion-exchange resins that supply calcium, phosphate, fluoride and zinc ions (named NMTD)[4] was developed with the aim of preventing early caries formation and improving remineralization effects. Cytotoxicity testing is an important component of the biological evaluation of dental materials [5-7] and it is a required part of the standard screening procedures. The purpose of this *in vitro* study was to evaluate the effects of two dentifrices: a toothpaste commercially available (Crest® Extra-whitening toothpaste) and the new experimental toothpaste including a mixture of resins (NMTD) mentioned above, for cytotoxicity to mouse fibroblasts.

Methods and Materials

Test Dentifrices

A commercial, sodium fluoride dentifrice containing 0.16 % (w/v) fluoride ion[a], and an experimental formulation based on a mixture of ion-exchange resins (identified as NMTD)[b], which releases calcium, fluoride, phosphate and zinc ions, were evaluated for cytotoxicity. A specified quantity of toothpaste was weighed and a suspension of toothpaste and Hank's balanced solution (HBSS)[c] was prepared.

Cell Culture

Cultures of mouse fibroblasts cells L929 (ATCC CCL 1, NCTC clone 929 strain L)[d] were used in a MTT assay for *in vitro* cytotoxicity of the two dentifrices. Cells were cultured in Eagle's minimal essential medium (MEM)[c] supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, non-essential amino acids (1x), penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml). This media is referred as to complete media. Cultures were incubated at 37 ºC in a humidified atmosphere of 5% CO_2 and collected by tripsinization (0.05 % trypsin / 0.5 mM EDTA).

Cytotoxicity testing in the MTT assay

A 96-well microplate method was employed for the MTT colorimetric assay, based on the reduction of the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT)[e] to an insoluble formazan product by mitochondrial dehydrogenases of viable cells [8,9]. Cells grown near to confluence were washed with HBSS, treated with trypsin, and suspended in complete media. A 0.1 ml aliquot of a cell suspension of density 1 x 10^4 cells/ml was placed into each well. The plates were incubated for 24 hours at 37 ºC in a 5 % CO_2 atmosphere.

Concentrates of test agents were prepared for each group using HBSS. Five milliliters of test media were prepared by adding 0.5 ml of concentrate to 4.5 ml of 6 % media produced by dilution of complete media. A positive control consisted of 10 μl of phenol in 5 ml of 6 % media, a dose that produces zero percent cell survival. A negative control was prepared by adding 0.5 ml of HBSS to 4.5 ml of 6 % media. The media in wells was removed by aspiration and 0.1 ml of either test media, positive control or baseline, was added to each corresponding well. Media blank was prepared by adding 0.1 ml of test

media to wells without cell culture. The plates were incubated for 24 and 48 hours, respectively, at 37 ºC in a 5 % CO_2 atmosphere.

After incubation, 10 µl of MTT stock (5 mg/ml MTT in HBSS) were added to each well, including the cell-free media blank. The plates were incubated for four additional hours. The purple formazan product was dissolved in 0.1 ml of 10 % sodium laurylsulphate / 0.01 N HCl by gently shaking the plates. Then plates were incubated overnight at 37 ºC and 5 % CO_2 atmosphere to allow the solution to solubilize, and evaluated using a microplate reader (Bio-Rad, USA) at 570 nm.

Statistical Analysis

Means and standard deviations were calculated for absorbance values for each group and percentage inhibitory dosage (% ID) for each test material. The data were analyzed using a one-way analysis of variance (ANOVA) followed by a Student-Newman–Keuls method ($P<0.05$) to determine the significance among the means.

Results

Results of the variability of the percentage of inhibition with the test dentifrice dose are shown in Figures 1 and 2. The points on each line represent the mean of six wells per dose. The percentage of inhibition corresponds to the extent of cytotoxicity of the material. Neither of the dentifrices produced a percentage of inhibition greater than 50 %, the value at which the degree of cytotoxicity starts to be appreciable. Furthermore, there were no marked increases in cytotoxicity with either incubation time. The positive control had almost zero percent cell survival and a 97 ± 2 % inhibitory dosage. The negative control had a 100 % cell survival rate and a 0 % inhibitory dosage. Close examination of Figures 1and 2 indicates that the 48-hour incubation test resulted in a

higher percentage of inhibition than the 24-hour test. Statistical analysis of the data presented in Tables 1 and 2 indicated that the test dentifrice dose had no significant effect on cell viability.

Discussion

The MTT cytotoxicity assay is an *in vitro* screening test for dental materials. The results of this investigation indicated that the new dentifrice formulation based on a mixture of ion-exchange resins that release calcium, zinc, fluoride, and phosphate ions is safe for intra-oral use. The fact that the experimental formulation had a higher level of available fluoride (approximately 1400 ppm) than Crest® Extra Whitening toothpaste (control group) (1100 ppm) was reflected in the slightly higher percentage of inhibition of the experimental formulation in the 24-hour incubation test. However, this outcome did not result in cell cytotoxicity, because in the 48-hour test the differences between them were not appreciable. One conceivable rationale for adding the ion-exchange resin mixture to the toothpaste matrix was to provide a controlled release system with long-term action. In this way, calcium, phosphate, zinc and mainly fluoride ions can be slowly introduced into the media over time**.** In fact, the theoretical fluoride dose differed from the measured level as a result of the ion-exchange process. The new experimental toothpaste was not cytotoxic even after 48 hours of incubation. Apart from the MTT chemical evaluation of cell survival, viable cells were observed under the optic microscope during the incubation procedure. Direct cell observation, another way of verifying the toxic effect of fluoride on cell growth [10] showed no visible effect on cell health for both dentifrices and MTT assays, since cell density and characteristic shape were maintained. After the 48-hour incubation periods, higher dentifrice concentrations showed that a higher percentage of

inhibition could produce cytotoxicity. Because no signs of cytotoxicity were observed, it was concluded that fluoride dentifrice dosages above 500 ppm acted as a barrier for cell growth. It has already been reported[11] that low levels of cytotoxicity are associated with fluoride levels above 10 ppm. The highest dentifrice concentrations used in this study were approximately 7.4 ppm of fluoride for the new experimental toothpaste and 5.5 ppm of fluoride for the Crest® Extra Whitening dentifrice. Both of these products contain fluoride but at levels below that reported limit for fluoride cytotoxicity.

It was concluded that the experimental toothpaste based on a mixture of ion-exchange resins (named NMTD) containing calcium, fluoride, phosphate and zinc ions that was evaluated in this study was not cytotoxic despite having a potentially higher fluoride release than the existing commercial dentifrice, Crest® Extra Whitening toothpaste.

Acknowledgements

This work was supported by an F.I. Research Grant from the Autonomous University of Barcelona. The authors wish to thank Professor Josep Mª Suñé from the Industrial Pharmaceutical Technology Department of the University of Barcelona for his collaboration in the development of the new experimental toothpaste.

a.- Crest® Extra Whitening toothpaste, Procter & Gamble, OH, USA
b.- NMTD - Spanish Patent 9700016, Desarrollo Científico Aplicado, S.L., Barcelona (rights owner).
c.- Sigma Chemical Co., St Louis. MO, USA
d.- American Type Culture Collection, Rockville, MD, USA
e.- Acros. organics, New Jersey, USA

Tables and Figures

Table 1.

Statistical evaluation of the NMTD experimental toothpaste dose on percentage of inhibition in a 24-hour and a 48-hour MTT cytotoxicity assay

Table 2.

Statistical evaluation of Crest® Extra Whitening toothpaste dose on percentage of inhibition in a 24-hour and a 48-hour MTT cytotoxicity assay

Figure I.
The metabolism of fluoride

Figure 1.
Percentage of cell growth inhibition after 24 hours **(A)** and 48 hours **(B)** incubation with the NMTD experimental toothpaste

Figure 2.
Percentage of cell growth inhibition after 24 hours **(A)** and 48 hours **(B)** incubation with Crest® Extra Whitening toothpaste

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[4] Spanish Patent 9700016, Desarrollo Científico Aplicado, S.L., Barcelona (right owner).

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Table 1.

24-hour Incubation			48-hour Incubation		
Dentifrice dose (μg/ml)	**Absorbance**	**SD**	**Dentifrice dose (μg/ml)**	**Absorbance**	**SD**
Positive control*	0.018	0.017	**Positive control***	0.021	0.008
75.75	0.409	0.034	**5050**	0.608	0.050
252.5	0.423	0.034	**0.750**	0.737	0.080
7.575	0.427	0.020	**7.575**	0.753	0.100
126.5	0.43	0.034	**2525**	0.759	0.010
0.750	0.459	0.020	**75.75**	0.76	0.050
505.0	0.469	0.053	**1010**	0.771	0.070
5050	0.479	0.147	**126.5**	0.773	0.100
1010	0.488	0.064	**252.5**	0.781	0.060
Negative control*'	0.582	0.014	**Negative control*'**	0.924	0.045

Groups connected by brackets are not statistically different P >0.05

* Positive control: Phenol 10 μl/5ml in 6% MEM

*' Negative control: HBSS

Table 2.

24-hour Incubation			48-hour Incubation		
Dentifrice dose (μg/ml)	**Absorbance**	**SD**	**Dentifrice dose (μg/ml)**	**Absorbance**	**SD**
Positive control*	0.03	0.008	**Positive control***	0.016	0.007
129.6	0.423	0.057	**5183**	0.476	0.045
77.40	0.443	0.072	**2591**	0.695	0.056
1037	0.449	0.023	**1037**	0.73	0.074
259.1	0.451	0.040	**259.1**	0.738	0.032
7.770	0.454	0.036	**77.40**	0.821	0.070
2591	0.458	0.036	**7.770**	0.858	0.059
0.770	0.463	0.033	**129.6**	0.872	0.104
518.3	0.473	0.041	**0.770**	0.901	0.038
Negative control*'	0.508	0.031	**Negative control*'**	0.949	0.017

Groups connected by brackets are not statistically different P >0.05

* Positive control: Phenol 10 μl/5ml in 6% MEM

*' Negative control: HBSS

Figure I.

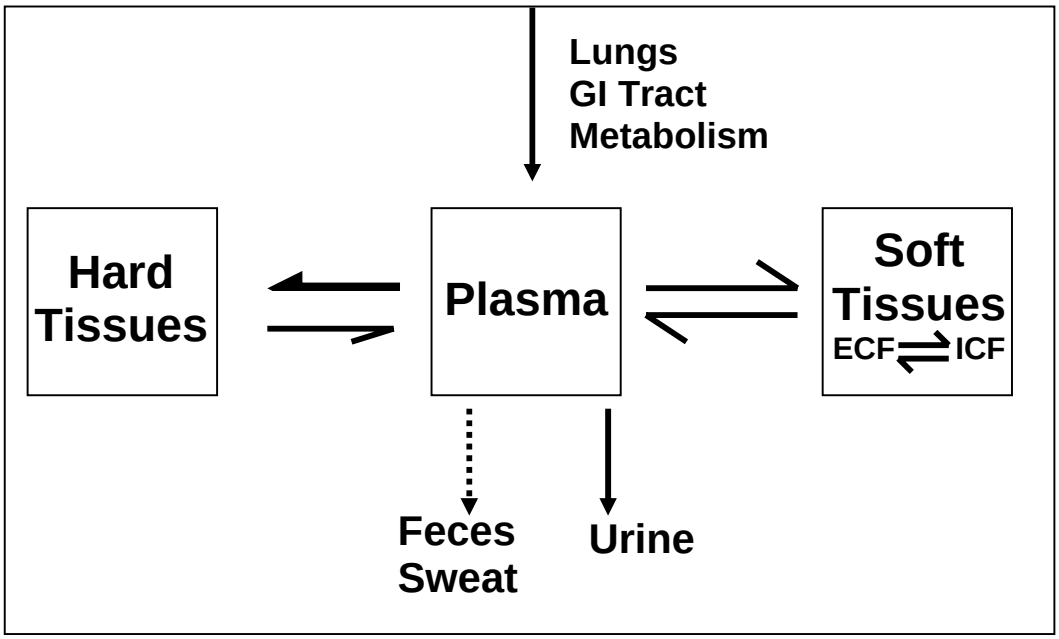
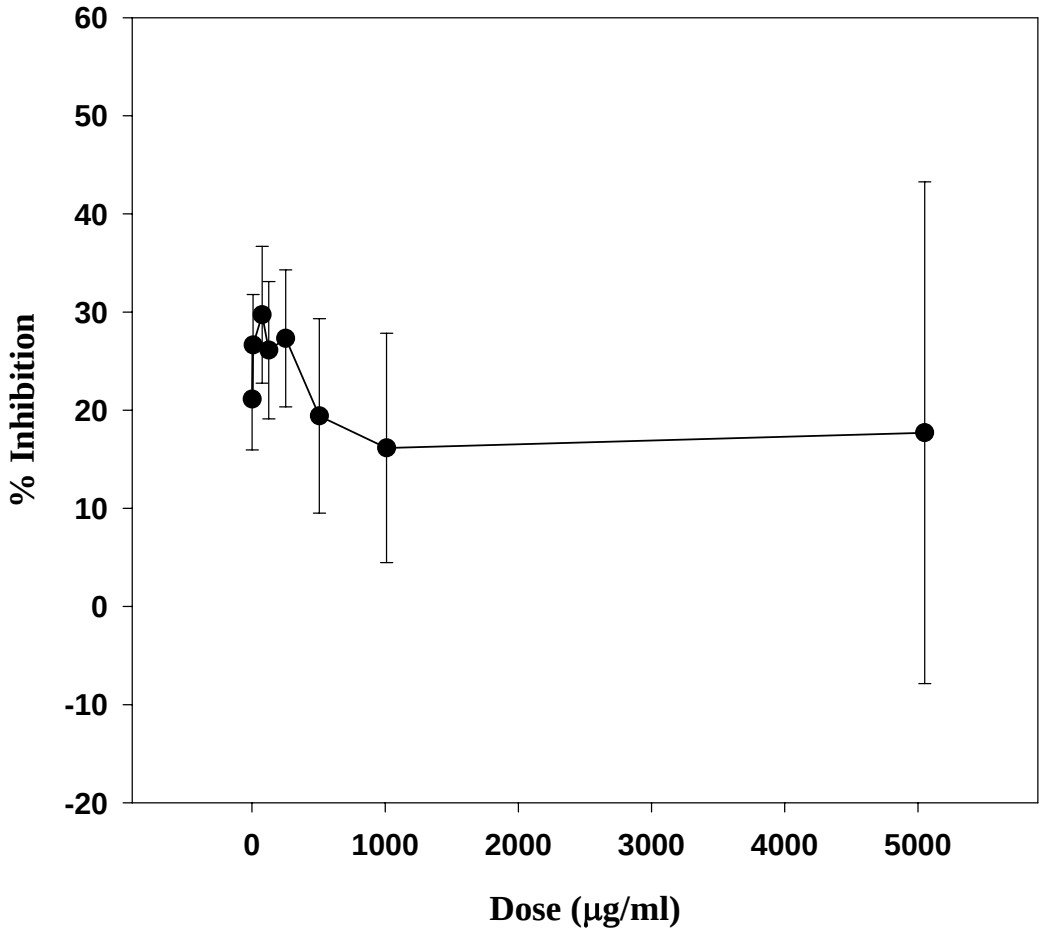

Lungs
GI Tract
Metabolism
Hard Tissues
Plasma
Soft Tissues
ECF ⇌ ICF
Feces
Sweat
Urine

Figure 1A.


60
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-10
-20
% Inhibition
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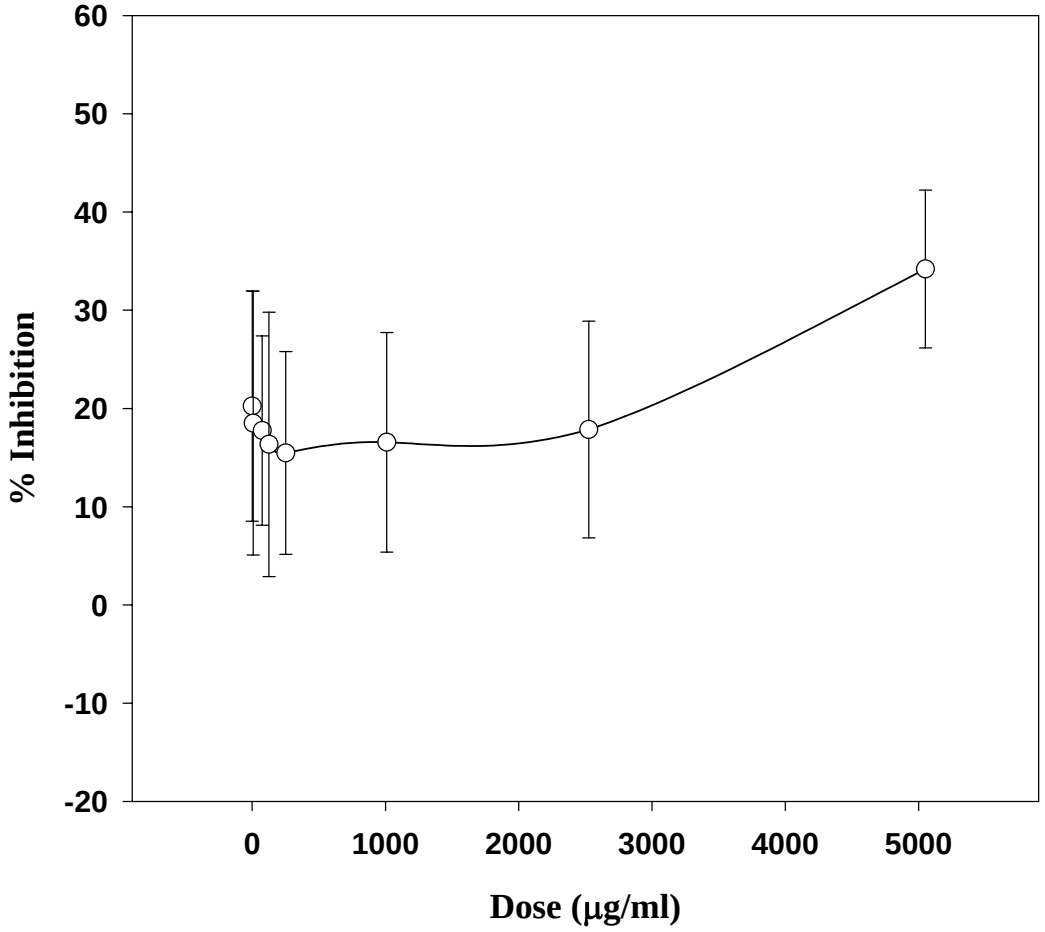
1000
2000
3000
4000
5000
Dose (μg/ml)

Figure 1B.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% Inhibition
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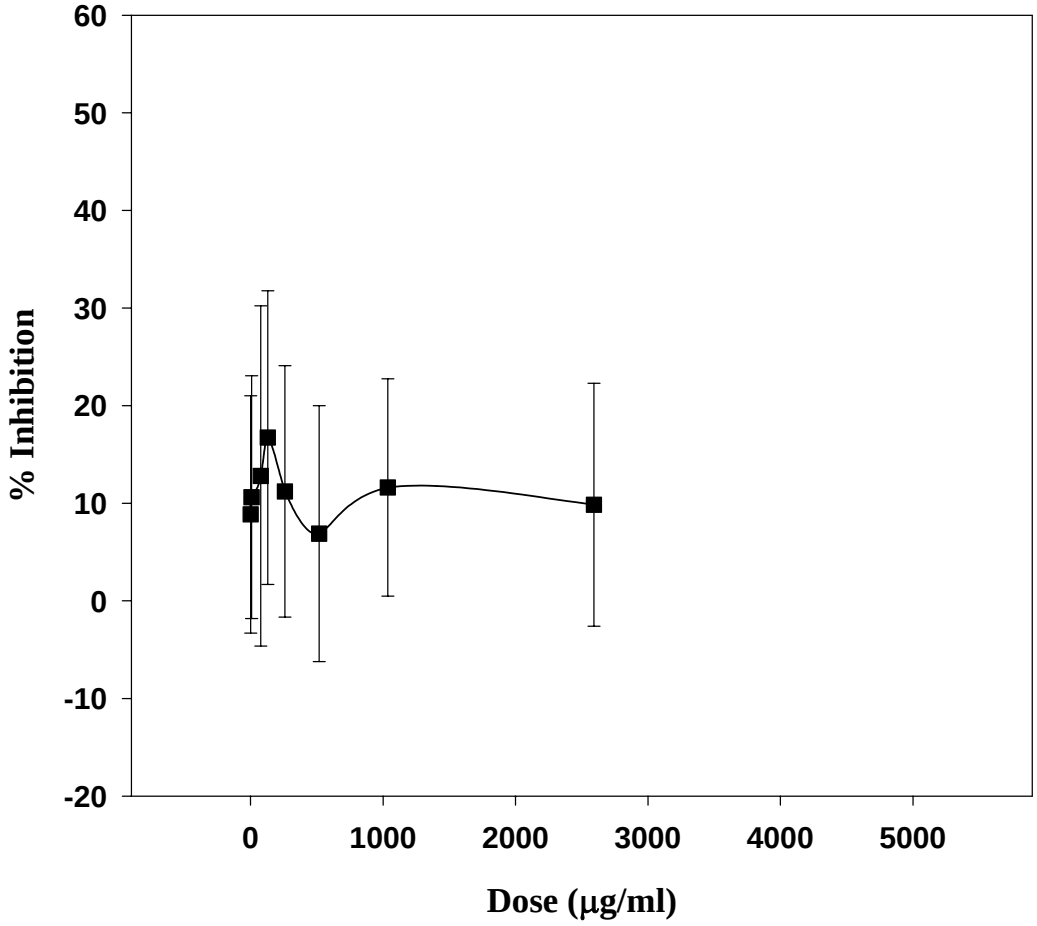
Dose (μg/ml)

Figure 2A.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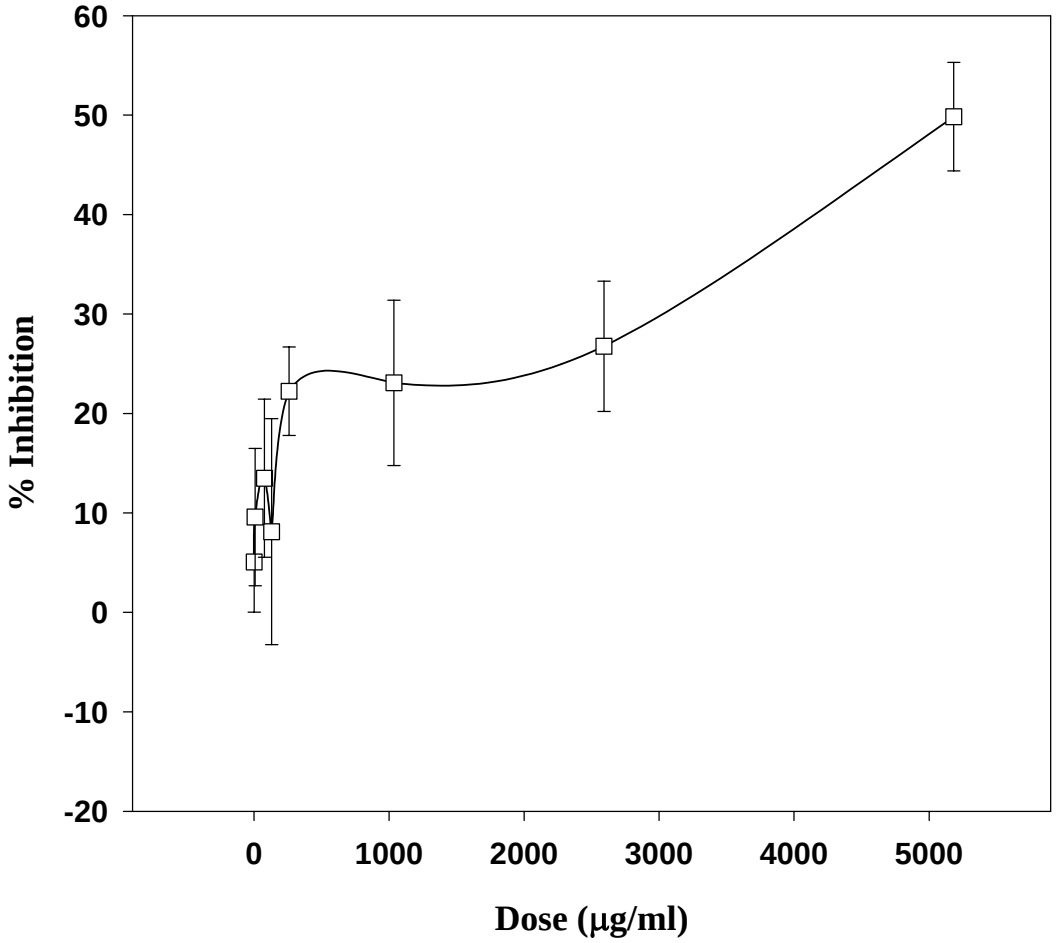
Dose (μg/ml)

Figure 2B.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% Inhibition
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Dose (μg/ml)

ANNEX 8

[Fwd: Acceptance of Article] Carlos Munoz

Subject: [Fwd: Acceptance of Article]
Date: Thu, 28 Aug 2003 15:39:43 -0700
From: Carlos Munoz <cmunoz@pd.lla.edu>
To: Anna Torrado <Anna.Torrado@uab.es>, Anna Torrado Bonals <Anna.Torrado@uab.es>

Subject: Acceptance of Article
Date: Wed, 27 Aug 2003 19:55:49 -0700
From: "Joseph Chartrem" <herschp@comcast.net>
To: <cmunoz@pd.lla.edu>

Dear Carlos,

After some disagreement among reviewers your manuscript entitled, "Remineralization Potential Of A New Toothpaste Based On An Ion-Exchange Resins Mixture Releasing Calcium, Fluoride And Phosphate Ions: An In-Vitro Study", has been accepted for publication in the Winter issue of the JCDP. The disagreement centered around the appropriateness of the topic for the JCDP and not the research itself.

I will need biographical sketches and photographs of your co-authors by October 1, 2003 to include in the final article.

Congratulations and thank you for your submission to the JCDP.

Sincerely,

Joe

REMINERALIZATION POTENCIAL OF A NEW TOOTHPASTE BASED ON AN ION-EXCHANGE RESINS MIXTURE RELEASING CALCIUM, FLUORIDE AND PHOSPHATE IONS: AN IN VITRO STUDY

A.Torrado [2], M. Valiente[2], W. Zhang[1], Y. Li[1] and C.A. Muñoz[1]

[1]School of Dentistry, Loma Linda University, Loma Linda, CA 92350, USA
[2]Quimica Analitica, Universitat Autonoma de Barcelona, Bellaterra, E-08193, Spain

Remineralization Potential of a new Toothpaste based on Ion-Exchange Resins

Corresponding author:
Carlos A. Muñoz, DDS, MSD
School of Dentistry
Loma Linda University
11092 Anderson St.
Loma Linda, CA 92350
Phone: 909-558-0656
Fax: 909-558-0270
E-mail address: **cmunoz@sd.llu.edu**

Key words

Remineralization, toothpaste, ion-exchange, fluoride

Abstract

The aim of the present study was to determine the ability of a dentifrice containing a mixture of ion-exchange resins (named NMTD), which supplies calcium, fluoride, phosphate and zinc ions, to promote remineralization and/or inhibit demineralization of dental human enamel in a pH cycling model in vitro. A fluoride toothpaste was used as the control. The enamel specimens were tested for microhardness before and after 10 days and 16 days of the demineralizing and remineralizing treatments. The results of this study showed that both dentifrices were effective in limiting in vitro enamel demineralization although the effects were not significantly different from each other. Inclusion of calcium and phosphate ion-exchange resins in the dentifrice containing a fluoride ion-exchange resin maintained a similar net outcome of the conventional dentifrice in demineralization-remineralization process under the experimental conditions employed.

Introduction

Despite the fact that caries affects a large percentage of the population, its rates have declined substantially due to fluoride use. The protective role of fluoride against dental caries was not recognized until the mid-1930s, when epidemiologic studies[1] demonstrated that children drinking naturally fluoridated water had fewer caries than those in populations with water supplies low in fluoride concentration.

The presence of fluoride in saliva has been correlated with increased rates of remineralization and decreased caries incidence. Even trace concentrations of fluoride ions are effective in promoting calcium hydroxyapatite formation from supersaturated solutions of calcium and phosphate. For this reason fluoride is added to toothpastes, mouthrinses[2] and drinking water as an anticaries agent. The use of fluoride-containing toothpastes has proven to reduce the incidence of caries in numerous clinical studies. Most toothpastes available in the United States contain about 1100 ppm fluoride.

However, fluoride's ability to promote remineralization in the oral environment is limited by the presence of calcium in saliva. Demineralization and remineralization can be considered a dynamic process, characterized by the flow of calcium and phosphate out of and back into tooth enamel, that should be balanced in order to prevent the progression of caries. This means that the formation of a cavity will be prevented if the average amount of demineralization that occurs is equal to or exceeded by the average amount of remineralization. The pH at which demineralization and remineralization occurs depends on the concentration of calcium and phosphate in saliva and plaque fluid.

The new mineral, that is formed if fluoride, calcium and phosphate are present in adequate proportions, contains hydroxyapatite and fluoroapatite ($Ca_5(PO_4)_3F$), both of which are less soluble than the original carbonated calcium hydroxyapatite. [3]

The relevant chemical reactions are schematized in Figure 1.

Probably the most effective caries-preventive treatment available today is fluoridation of municipal water supplies (optimal concentration of fluoride in water ranges from 0.7-1.0 ppm) and the use of fluoride-containing toothpastes. However with the introduction of fluoridated water in some countries and in addition dental products containing fluoride in most countries, there has been an increase in exposure to fluoride in children and also increased risk of toxicity and dental fluorosis.[4] In this sense, the current fluoridated toothpastes have an important shortcoming that is identified by a high concentration of fluoride released into biological fluids. For this reason, rational precautions when using fluoride in children less than 6 years old will reduce any risk considerably.

Future perspectives of fluoride utilization could be found in optimizing the control and/or slow release of fluoride in the oral environment. Furthermore, the essence of the remineralizing concept might be achieved by simultaneously supplying calcium, phosphate and fluoride ions to the teeth, in order to induce the formation of calcium fluoroapatite which remineralizes and strengthens the tooth. In this sense, a toothpaste with a controlled release of mineral ions based on an ion-exchange system instead of the dispensing system that keeps the calcium source separate from phosphate and fluoride salts,[5] is proposed. This new product allows mixing all the ions together and preventing them from entering in contact and precipitating before the application. Ion-exchange resins are insoluble high molecular weight compounds carrying ionic

functional groups that can react with ions in solution through the ion-exchange mechanism.[6] The majority of these resins are not toxic and some of them are used in the food industry,[7] pharmaceutical industry and in medical applications.[8] The application of ion-exchange materials has advantages in comparison with the conventional chemical reagents. These materials do not introduce undesirable ions into the solution, ions release is carried out only by the ion-exchange mechanism, they are characterized by practically neutral pH values and they can also adsorb bacteria on the surface. On the other hand, it provides a controlled release system for the anti-carious treatment of dental tissues. Although it is a long time since fluoride ion-exchange resins started to be used in the dental field,[9-11] no data about simultaneous release of calcium, phosphate and fluoride by ion-exchange resins mixture in tooth paste has been found in the literature.

The aim of the present study was to determine the ability of a dentifrice containing a mixture of ion-exchange resins (named NMTD)[12] which supplies calcium, fluoride, phosphate and zinc ions, to promote remineralization and/or inhibit demineralization of dental human enamel in a pH cycling model in vitro.

Materials and methods

Preparation of carious lesions

Enamel samples from third molar human teeth were sectioned in four parts by using a diamond disc. Each part was mounted in 0.6 cm diameter plastic rods with a dental adhesive (Optibond Solo Plus, Kerr, USA) and in order to prevent adhesive from hydrolyzing the interface enamel-plastic rod, it was covered with a second dental adhesive (Optibond FL, Kerr, USA). All specimens were ground and polished and

twenty specimens presenting a perfectly flat and intact surface were selected and divided in two groups. Acid resistant nail varnish was used to cover all sides of the specimen except the surface.

Carious lesions representing the preliminary stage of subsurface enamel demineralization were produced by suspending each rod, containing each enamel specimen, into 13 ml of 0.1 M lactic acid / 0.2 % polyacrylic acid (Carbopol C907) / 50 % saturated hydroxyapatite solution at pH 5.0 for 72 hours.[13] After creating the lesion an average surface hardness of 107 ± 21 Knoop hardness number (KHN) was obtained (sound enamel: 310≤ KHN ≤ 350).[14]

Test Dentifrices

Two test dentifrices were compared in this study: a sodium fluoride toothpaste containing 0.16 % (w/v) fluoride ion (Crest® Extra-whitening toothpaste, Procter&Gamble, OH, USA) and an experimental toothpaste based on a mixture of ion-exchange resins, named NMTD, which releases calcium, fluoride, phosphate and zinc ions. Free ionic fluoride was evaluated from the supernatant of a centrifuged 0.4 % (w/w) dentifrice:miliQ water suspension whereas total fluoride was evaluated directly from the suspension without centrifuging. Fluoride available in both toothpastes and a placebo, containing neither fluoride ion nor any ion-exchange resin, were evaluated following a modification of the hexamethyldisiloxane (HMDS) microdiffusion method developed by Taves.[15] In a polystyrene Petri dish, 1 ml of toothpaste suspension and 2 ml of miliQ water were placed. The trapping solution, 100 μl NaOH 0.075 N, was placed in droplet form on the lid which was next sealed with Vaseline. One ml of HMDS-saturated 3 N H_2SO_4 solution was introduced into the dish through a 1-mm hole in the lid and the hole was quickly covered with

Vaseline. All samples were gently hand agitated and left overnight. After this time, the trapping solution was neutralized with 50 μl $HClO_4$ 0.15 N, and analyzed in TISAB background with a fluoride ion-selective electrode (Orion, USA).

pH-cycling experiments

Specimens were randomized in groups of five specimens each. The pH cycling treatment regimen was a variation of the method originally developed by Ten Cate and Duijsters.[16] It consisted of one-minute soaking of each group in 10 ml of 33 % (w/w) dentifrice/natural human saliva slurry, four times per day (at 8:30 a.m., 9:30 a.m., 2:30 p.m. and 3:30 p.m.) to simulate tooth brushing exposure. Between treatments with dentifrice, the treatment groups were immersed in 15 ml 350 rpm agitated natural human saliva (pH= 7.8 ± 0.5) at 37 ºC for 1 hour periods, to effect remineralization through an acquired pellicle (saliva was collected by wax stimulation each day). In order to simulate the daily acid challenges from plaque bacteria, specimens were immersed daily at 10:30 a.m. during three hours in synthetic acid demineralization solution (same composition as lesion preparative solution). The continuous demineralization-remineralization cycles were carried out for 16 days. Over the weekend, specimens were refrigerated in a 100 % humidity atmosphere.

Surface Microhardness analysis

Three indenter penetration measurements were made initially, after demineralization and after 10 and 16 days of treatment, using a Leco M-400-H1 hardness testing machine with a 200 g load. The rods containing the enamel specimens were held perpendicular to the indenter path by using an specially designed specimen holder. Knoop harndess measurements were determined from the extent of the indenter lengths.

Data Analysis

Results obtained on microhardness determination were analyzed by a one-way ANOVA test. The differences among mean values were compared by Student-Newman-Keuls multiple comparison test ($P<0.05$).

Results

Fluoride availability

Fluoride availability results are shown in Table 1. We can see that the placebo has no fluoride in its formulation whereas the new toothpaste has a considerable higher content of total fluoride than the commercial one. Because it is known that the absence of fluoride content does not promote remineralization, the placebo formulation was not tested for the pH-cycling experiments and only the positive control was used as reference for the comparison of results. However, it can be seen that both toothpastes have a comparable level of free ionic fluoride. That is because the particles of fluoride ion-exchange resin constituting the active principle of the new toothpaste are surrounded by a layer of soluble NaF, which was used for the fluoride ion-exchange preparation. So, when the new toothpaste enters in contact with the natural human saliva, fluoride appearance into the environment will take place through two mechanisms: first, by direct solubilization of fluoride from the NaF salt and second, through an anion-exchange process between fluoride immobilized in the organic matrix and anions present in the natural human saliva, i.e. chloride.

The results shown in Figure 2, concerning the kinetics of total fluoride available, indicate an increasing difference between the concentrations of fluoride from NMTD paste and NaF paste present in solution. This behavior corresponds to the ion-

exchange mechanism present in the novel toothpaste. At the beginning of both experiments the fluoride release is practically the same due to the same source of fluoride, as indicated before, NaF was also present in the NMTD toothpaste. In the case of NMTD paste, the ion-exchange resin is encapsulated in the toothpaste base matrix, so the exchanging solution must first diffuse through the toothpaste base and then through the particles of the resin. Furthermore, is very well known that ion-exchange resins need a previous period of swelling in order to help ions diffuse through the inner paths of the ion-exchange resins,[6] apart from the time of the ion-exchange reaction itself. So, it must be noted that the mechanism that involves the fluoride releasing from the new toothpaste is complex and long-lasting.

Surface Microhardness results

The surface microhardness evaluations of the lesions are shown in Table 2, in which the hardness numbers are indicated for initial lesions and post pH-cycling lesions. The same data is presented in Figures 3 and 4, in terms of Knoop hardness number or percentage of remineralization versus time, respectively. Each point on each line represents the mean of the treatment of ten specimens per group. In general, fluoride dentifrices effect significant increases in surface hardness of the specimens during pH cycling remineralization conditions, what can be verified in Figures 3 and 4.

Both fluoride-based toothpastes limited very effectively lesion progression. Both types of dentifrices were not statistically different from each other ($p>0.05$) and in each case there were no significant differences after 10 and 16 days of treatment ($p>0.05$).

Discussion

The in vitro model reported here provides a test to measure the inhibition of demineralization and the enhancement of remineralization. It reveals whether the overall caries progression can be remarkably inhibited in a cycling demineralization/remineralization situation. The results of this study indicate that the new toothpaste based on NMTD, having a smaller release of fluoride into saliva, achieves similar results to the commercial control NaF toothpaste. In this sense, both toothpastes inhibit demineralization and enhance remineralization as in 16 days a 50% increase in enamel hardness is achieved. These results confirm the hypotheses originated by Koulourides and coworkers surrounding the importance of fluoride-enhanced early lesions remineralization towards conferring acid resistance to enamel.[17-19] Free ionic fluoride supplied by the dentifrices and the fluoride's ability to incorporate into the mineral part affected by the carious lesion are mainly responsible for the remineralization potential of both toothpastes. Note that the new toothpaste also supplies calcium, zinc and phosphate ions. The role of zinc ion is anti-bacteriological, that is, prevention from the formation of plaque acid. On the other hand, treatment of enamel with calcium and phosphate has been shown to promote fluoride uptake by enamel.[20-21] A higher fluoride content will reduce enamel solubility due to hydroxyapatite transformation to fluoroapatite by OH^-/F^- substitution and, the dissolution of calcium hydroxyapatite in an acidic media is a reversible reaction, the direction of which is dependent on the pH of the solution and the concentrations of calcium and phosphate ions present. This way, during remineralization the inclusion of higher concentrations of ionic calcium and phosphate in the media will result in an increase in the degree of supersaturation, and thereby an increase in the rate of deposition of mineral into the enamel.

The novelty of the product assayed then, is that is capable of supplying calcium, phosphate and fluoride ions through a controlled release mechanism depending on the tooth demand. In this sense, if a tooth has a mineral defect due to an acidic attack, undergoing a carious lesion, the product is able to regenerate the natural composition of the tooth as well as to regulate the pH conditions in order to prevent calcium hydroxyapatite dissolution.

Concerning the content of calcium and phosphate ions in the new toothpaste, it is lower than the ones present in the experimental toothpaste assayed by Schemehorn et al., which concluded that supplementation of salivary calcium and phosphate concentrations with a fluoride-containing dentifrice promotes the remineralization of enamel.[2] It must also be considered that the in vitro cyclic regimen of treatment may underestimate the protective effects of the toothpaste because the enamel specimens are thoroughly rinsed after each treatment, and any residual toothpaste is washed away. Under these conditions the concentration of fluoride, calcium and phosphate ions in the media will not be optimized. In addition, clinically, any tooth brushing will not last more than one minute, which was the time used for the present study. The presence of NaF in the corresponding resins mixture of NMTD conditions to some extent, the results obtained. A future work with absence of NaF in the resin, will contribute to clarify the mechanism of fluoride release in such complex systems. Taking into account these considerations and the parameters influencing the ions release from a resin (i.e. strong/weak character, particle size, available surface, etc.), further studies should be carried out to complete a methodical knowledge of this system. At this point, we suggest a revision of calcium and phosphate ions content in the dentifrice and further demineralization-remineralization in vitro and in vivo studies.

Conclusions

The new toothpaste containing NMTD which supplies calcium, fluoride, phosphate and zinc ions was effective in limiting in vitro enamel demineralization. Inclusion of calcium and phosphate ion-exchange resins in the dentifrice containing also a fluoride ion-exchange resin, did not affect the net outcome of the demineralization-remineralization process under the experimental conditions employed.

Acknowledgements

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Legends

Table 1. Fluoride availability

Table 2. Enamel surface microhardness measurements

Figure 1. Some chemical reactions relevant to the caries process involving fluoride

Figure 2. Kinetics of total fluoride availability

Figure 3. Enamel surface hardness evolution during the demineralization and remineralization process. Each line joins mean data points for each test group

Figure 4. Remineralization percentage exerted by toothpastes tested

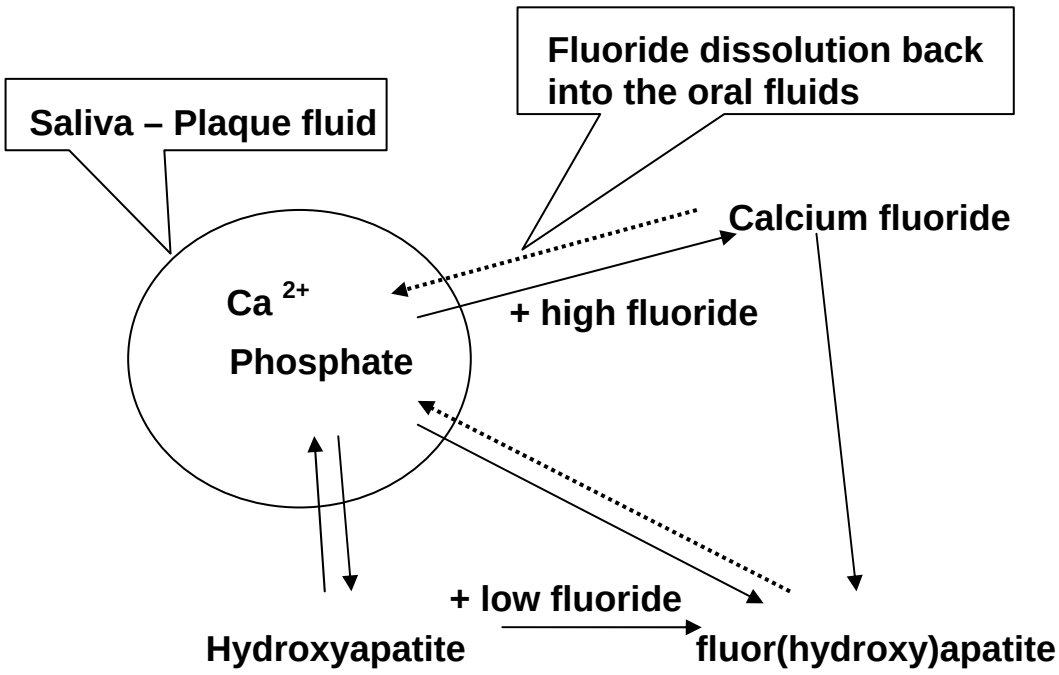
Fluoride dissolution back into the oral fluids
Saliva – Plaque fluid
Calcium fluoride
Ca 2+
+ high fluoride
Phosphate
+ low fluoride
Hydroxyapatite
fluor(hydroxy)apatite

Figure 1

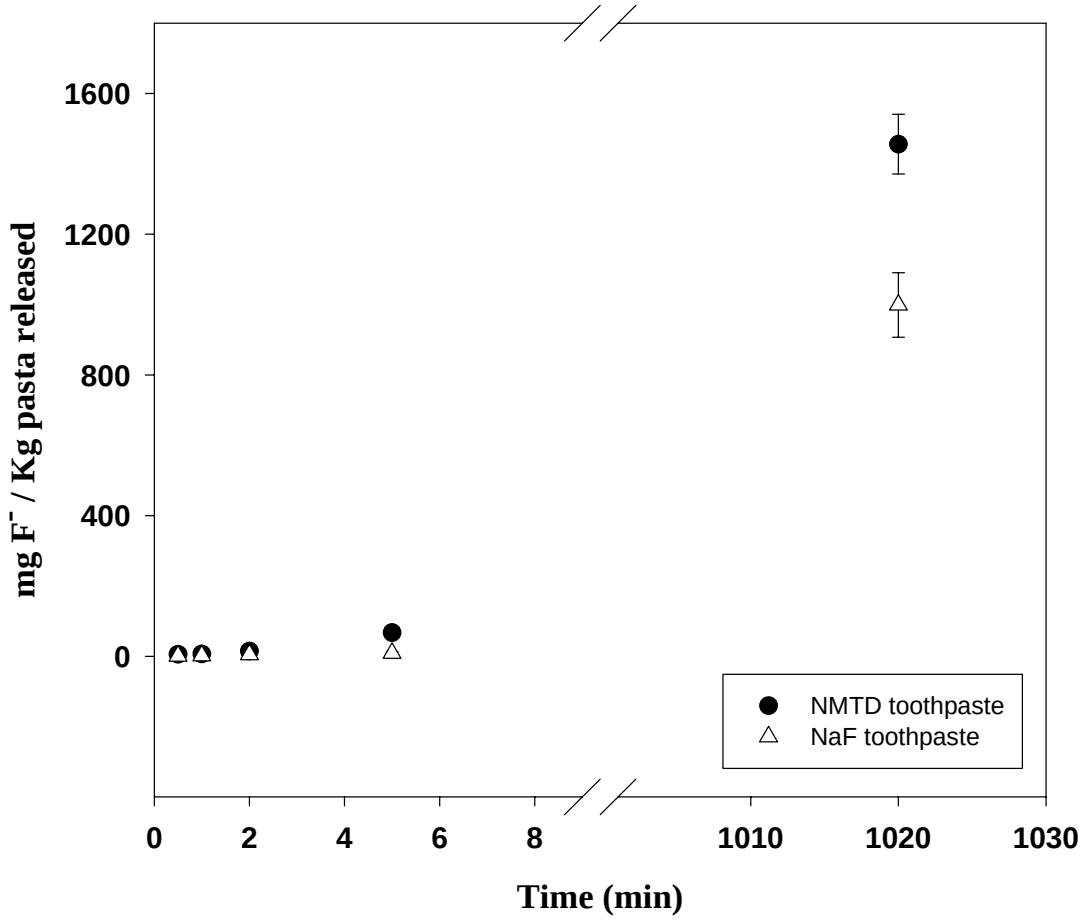
1600
1200
800
400
0
mg F- / Kg pasta released
0
2
4
6
8
1010
1020
1030
Time (min)
NMTD toothpaste
NaF toothpaste

Figure 2

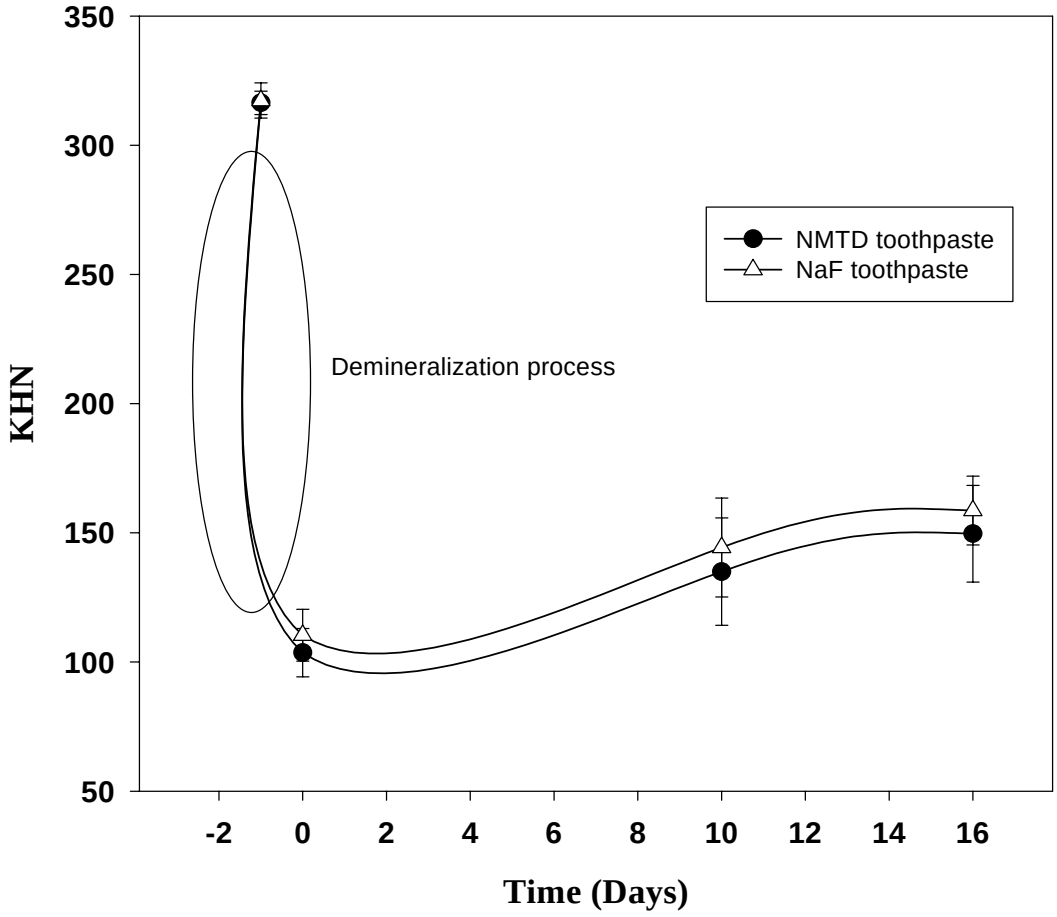
350
300
250
200
150
100
50
KHN
-2
0
2
4
6
8
10
12
14
16
Time (Days)
NMTD toothpaste
NaF toothpaste
Demineralization process

Figure 3

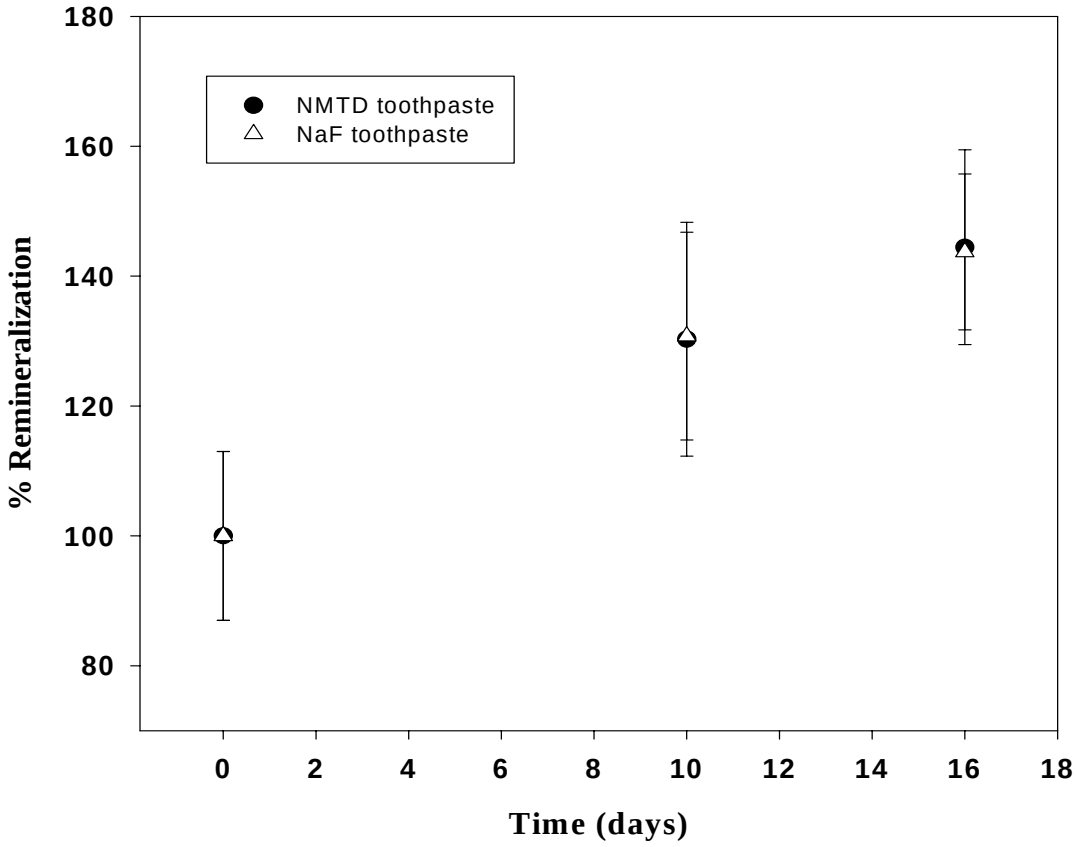
180
160
140
120
100
80
% Remineralization
NMTD toothpaste
NaF toothpaste
0
2
4
6
8
10
12
14
16
18
Time (days)

Figure 4

Table 1

Treatment group	Total fluoride (ppm)	Free ionic fluoride (ppm)
Placebo	5 ± 2	3 ± 1
NMTD toothpaste	1456 ± 126	965 ± 85
NaF toothpaste	999 ± 92	1032 ± 92

Table 2

Treatment group	KHN Initial	KHN After 10 days	KHN After 16 days
NMTD toothpaste	104 ± 9 (a)	135 ± 21 (c)	150 ± 19 (b) (c)
NaF toothpaste	110 ± 10 (a)	144 ± 19 (b) (c)	159 ± 13 (b)

(a), (b), (c) test groups not significantly different at $p < 0.05$ level

ANNEX 9

[Fwd: Revised manuscript] cmunoz

Subject: [Fwd: Revised manuscript]
Date: Mon, 28 Oct 2002 07:43:13 -0800
From: cmunoz <cmunoz@sdlhu.edu>
To: Anna Torrado <Anna.Torrado@uab.es>

Anna:

Hoy recivi confirmacion de aceptacion del primer articulo. Aqui te envio copia.

Carlos

Subject: Re: Revised manuscript
Date: Sun, 27 Oct 2002 22:57:08 -0500
From: godoy@nova.edu
To: cmunoz <cmunoz@sdlhu.edu>
References: 1

Dr. Munoz:

I received the revised paper.It has been accepted for publication.

Before publication you will receive galleys for your approval.

Best regards,

Franklin

In Vitro Study on Cleaning Power and Abrasivity of a new Toothpaste based on Ion-Exchange Resins

Anna Torrado, PhD Graduate Student, Quimica Analitica, Universitat Autonoma de Barcelona, Bellaterra, Spain.

Manuel Valiente, Professor, Quimica Analitica, Universitat Autonoma de Barcelona, Bellaterra, Spain.

Carlos A. Muñoz, DDS, MSD, Professor and Director, Center for Dental Research, Loma Linda University School of Dentistry, Loma Linda, CA.

Corresponding author:
Carlos A. Muñoz, DDS, MSD
School of Dentistry
Loma Linda University
11092 Anderson St.
Loma Linda, CA 92350
Phone: 909-558-0656
Fax: 909-558-0270

In Vitro Study on Cleaning Power and Abrasivity of a new Toothpaste based on Ion-Exchange Resins

Abstract

Purpose: This laboratory study compared the stain removal efficacy and enamel abrasivity of a new experimental dentifrice based on an ion-exchange resins mixture that releases calcium, fluoride, phosphate and zinc ions (named NMTD[1]), to five commercially available dentifrices: Crest® Extra-whitening toothpaste, Crest® Tartar Protection toothpaste, Crest® Cavity Protection toothpaste and Colgate® Fluoride Cavity Protection toothpaste. Calcium pyrophosphate was used as a control for the efficacy of the staining removal experiment. **Materials and Methods:** Cleaning power evaluation was made following the method developed by Stookey et al. at Indiana University Oral Research Institute. The abrasion of the toothpastes was determined by means of a brushing machine, using 2000, 4000 and 8000 strokes and a 250g toothbrush load. Bovine specimens were prepared and abrasion was measured by a surface profilometer system. **Results** showed that the new experimental dentifrice does not remove stains whereas Crest® Extra-whitening and Crest® Cavity Protection dentifrices produce statistically significantly improved stains removal when compared with calcium pyrophosphate control. Abrasion studies demonstrated that abrasion was linearly correlated to the number of strokes and the abrasion rates proved to be non significant for Colgate® and the new experimental dentifrices, but being significant for Crest® Extra-whitening, Crest® Tartar Protection and Crest® Cavity Protection.

Clinical Significance: This study demonstrated that the use of a dentifrice based on an ion-exchange mixture was not effective at removing stains when compared to other commercially available toothpastes.

Key words*:* stain removal, abrasion, toothpaste, ion-exchange

Introduction

Cleaning of the tooth surfaces is one of the most important functions of dentifrices. Human teeth have a natural color that is determined by the dentine and modified by the thickness and translucency of enamel. Discoloration of teeth can be classified in terms of intrinsic or extrinsic staining. Intrinsic stains are the result of the presence of chromogens within the enamel and dentin whereas extrinsic ones are those found on the surface of the teeth deposited on or in the acquired pellicle, which is a structureless glycoprotein film. Some substances like tea, coffee, tobacco and certain drugs may accelerate stain accumulation. The ideal cleaning system must show the capability of removal of unwanted deposits with minimal reactivity on enamel and dentin surfaces. The best oral hygiene practice to remove plaque and extrinsic stains is toothbrushing with the use of a toothpaste. One would tend to think that toothbrushing action is responsible for the damage that may be caused to the oral tissues. However, hard tissue abrasion is a function of the toothpaste since the toothbrush only contributes in the sense of transporting the toothpaste over the surface.[2,3] So, dental abrasives play an important role in the cleaning power of a dentifrice. The degree of abrasion shown by an agent is directly influenced by its own properties such as chemical composition, crystal structure, cleavage, friability, hardness, particle shape, surface features and particle size distribution, solubility, concentration and compatibility with other ingredients of the toothpaste. Nowadays the development of better cleaning formulations has been further complicated by the tendency towards the use of ingredients such as fluoride, antimicrobials, desensitizing agents and tartar control chelants. It was a long time ago since the protective role of fluoride against dental caries was recognized.[4] Probably the most effective caries preventive treatment available today is fluoridation of municipal water supplies and the use of fluoride-

containing toothpastes. However, this dental products supply a high concentration of ionic fluoride directly to the biological fluids increasing the risk of toxicity and dental fluorosis.[5] In this sense future perspectives of fluoride utilization are found in optimizing the control and/or slow release of fluoride in the oral environment. For this reason, a toothpaste with a controlled release of mineral ions based on an ion-exchange system, specifically an ion-exchange resins mixture that releases calcium, fluoride, phosphate and zinc ions (named NMTD), was developed. The application of ion-exchange materials has advantages in comparison with the conventional chemical reagents. These materials do not introduce undesirable ions into the solution, ion release is carried out only by the ion-exchange mechanism, they are characterized by practically neutral pH values and they can absorb bacteria on the surface. Once again, it is essential to formulate an adequate toothpaste matrix compatible with the function of anticarious treatment of dental tissues of the ion-exchange resins mixture, which is going to be encapsulated.

Substances of many kinds, either natural or synthetic, are being used as abrasive agents in dentifrices. The most common abrasive used in toothpastes today is silica.[6,7,8] The interest in silicas derives from different advantages, including: compatibility with other toothpaste ingredients, controlled chemical purity and particle size and the possibility to vary its concentration in order to achieve the desired degree of cleaning.

The methodologies most widely used in the evaluation of the changes in tooth color are spectrophotometry, based on the color space L*a*b* coordinates (established by the Commission Internationale de L'Eclaraige (CIE)[9]) or scanning electron microscopy.[10,11] Likewise, several methods have been described to estimate dental

abrasion: measuring weight changes of the test object,[12] surface profile measurements,[3] and radioactivity measurements.[13] This paper reports the effect of stain removal efficacy and enamel abrasivity of a new experimental dentifrice based on an ion-exchange resins mixture that releases calcium, fluoride, phosphate and zinc ions (named NMTD), compared with five commercially available dentifrices.

Materials and methods

Test dentifrices

Four commercial dentifrices and a new toothpaste based on a mixture of ion-exchange resins called NMTD which releases calcium, fluoride, phosphate and zinc ions (NMTD toothpaste) were evaluated. The dentifrices were evaluated for effectiveness of stain removal and abrasivity.

- The dentifrices evaluated for efficacy of stain removal were: Crest® Extra-whitening toothpaste and Crest® Cavity Protection toothpaste.[a]
- The dentifrices evaluated for abrasivity effect on enamel were: Crest® Extra-whitening toothpaste, Crest® Cavity Protection toothpaste, Crest® Tartar Protection toothpaste [a] and Colgate® Fluoride Cavity Protection toothpaste. [b]

Calcium pyrophosphate, the ADA reference material, was used as a tooth surface cleaning reference in the staining removal experiment. Abrasive composition within these toothpastes was obtained directly from the package labeling. Each dentifrice was tested on 8 tooth specimens.

Specimen preparation

Bovine central incisors were grinded in order to obtain specimens to fit a 12x17 mm mold. This size was required to position the specimens in the specimen holder of a V8 cross-brushing machine. The enamel specimens were then embedded in an

autopolymerizing poly-(methacrylate) resin, in such a way that only the enamel surface were exposed. The enamel was then smoothed and polished on wet 320 grit silicon carbide sand paper followed by a 600 grit, under a constant flow of water. Final polishing was carried out on a polishing wheel with 1.0 μm aluminum oxide until the surface was devoid of surfaces scratches. Care was taken not to expose the dentin surface.

Cleaning power evaluation

Cleaning power evaluation was made following the method developed at Indiana University Oral Research Institute by Stookey et al.[14] Bovine enamel specimens were lightly etched to facilitate stain accumulation and adherence. This etching procedure consisted of a sequential immersion in three different solutions: 60 seconds in 0.12 N HCl[c] solution, 30 seconds in a super-saturated Na_2CO_3 [c] solution and finally 60 seconds in 1 % phytic acid [c] solution.

The specimens were then rinsed with deionized water for one minute and attached to the staining apparatus shown in Figure 1. The apparatus consisted of a continuously rotating wheel at 2 r.p.m. For each rotation the specimens were exposed to 30 seconds of air followed by 30 seconds in the solution. The specimens were exposed to a pellicle forming solution for 96 hours, composed of 2.7 g of instant coffee, 2.7 g of instant tea, 2.0 g of gastric mucin [c] and 26 ml of 24 h Sarcina lutea turtox culture [c] in 800 ml of sterilized trypticase soy broth. [c] The staining solution was kept at 37 ºC and was changed every 12 hours. With each change the specimens were rinsed with deionized water to remove any debris. After the four days period the specimens were removed from the apparatus, rinsed well and stored in the refrigerator at 100 % humidity.

Each specimen was placed on the V8 mechanical cross-brushing machine, [d] equipped with soft, nylon-bristle toothbrushes [e] The bristle pressure was calibrated to 150 grams [f] by a weight sensor as shown in Figure 2. The sensor was placed at the bottom of the trough of the V8 mechanical cross-brushing machine, and by the toothbrush resting on it, the toothbrush pressure was adjusted to the desired load by tightening and untightening the holding screw. The dentifrices were tested as a slurry consisting of 25 g of dentifrice mixed with 40ml of deionized water, mixed thoroughly and the specimens brushed for 800 forward and backward strokes. The positive control group, calcium pyrophosphate slurry, with an arbitrary cleaning value of 100, was prepared by adding 10 g in 50 ml of a 0.5 % carboxymethyl cellulose [c] solution. All the slurries were mixed very vigorously before placing them on the V8 mechanical cross-brushing machine. Eight specimens were brushed at a time.

Stained specimens were evaluated, before and after brushing treatment, for tooth color using a Chromameter. [g] The intrinsic color of the teeth was recorded in triplicate using the tristimulus L*a*b* color space. The individual L*a*b* color factors represent the value (lightness or darkness) and the chromaticity, red-green, yellow-blue parameters of human color perception. A change in color was evaluated by the following expression:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{(i)}$$

Results of the equation indicate the color difference from baseline to test treatment. In dentistry a ΔE* change greater than three is considered as perceptible to the human eye. As this study attempted to indicate the change in value, the a* and b* coordinates were not evaluated. Thus, only the L* component was used to balance the specimens before the test and after.

Abrasion Evaluations

After polishing, the specimens were directly placed on the V8 mechanical cross-brushing machine equipped with the toothbrushes described in the previous section. The bristle pressure was, in this case was calibrated to 250 g and held constant on the tooth surface. The dentifrices were tested as a slurry prepared identically as explained in the previous cleaning power evaluation. The specimens were brushed for 2000, 4000 and 8000 forward and backward strokes, respectively. All the slurries were mixed very vigorously each time before placing them on the V8 mechanical cross-brushing machine. The specimens were rinsed with deionized water and ultrasonically cleaned between treatments and dried with an absorbent tissue before carrying out the abrasion measurements.

Abraded specimens were evaluated, before and after the brushing treatment, for tooth surface roughness using a profilometer[h] equipped with a diamond stylus with a tip radius of 10 μm. The stylus of the profilometer was moved across the abraded tooth enamel surface perpendicular on the brushing action direction. The applied tracing speed was 6 mm/s and the transverse range was set to 10 mm.

To assess the surface roughness, the Surftest III is designed to assess and indicate the center line average Ra of a roughness curve. In Figure 3, if the total area below and above the center line is M, the Ra is given as:

$$Ra = \frac{M}{l} \qquad \text{(ii)}$$

where l is the length along the center line.

The surface roughness was measured in three different areas and the average value was calculated.

Data analysis

A one-way analysis of variance (ANOVA) was used to evaluate the differences among test groups. If differences were found, a Student-Newman-Keuls test ($P<0.05$) to identify the specific differences.

For the cleaning power evaluation, the treatment effects were assessed according to relative efficacy, as compared against the reference calcium pyrophosphate abrasive as follows:

$$\mathrm{PCR(Pellicle \cdot Cleaning \cdot Ratio)} = \frac{L_{Tf} - L_{Ti}}{L_{Cf} - L_{Ci}} \mathrm{x100} \qquad \text{(iii)}$$

where L_{Ti} and L_{Tf} are L* chromameter values for test dentifrice treated specimens initially and post brushing, and L_{Ci} and L_{Cf} were the values for the calcium pyrophosphate abrasive control.

Results

Stain Removal Ability

An *in-vitro* test procedure was used to evaluate the cleaning potential of a new experimental NMTD toothpaste and the results obtained were compared with two commercially available toothpastes that are commercially available. Changes in lightness values (L*) of bovine enamel specimens after brushing with the three dentifrices can be observed in Table 1 and figure 4. Statistical tests demonstrated that there were significant differences among the cleaning power of the tested dentifrices. Compared to the baseline scores, the experimental dentifrice had no significant effect on the extrinsic stains. Table 2 presents the intergroup comparisons of the ΔL* extrinsic stain scores. The Crest® silica-based dentifrices tested in this study showed

significantly better results in reducing the brownish pellicle than the experimental toothpaste. The inclusion of a pyrophosphate salt in the formulation resulted in a better degree of cleaning power. Thus Crest® whitening toothpaste has a cleaning power comparable, or even greater, to that of the positive control. Another way to view the same results is shown in Figure 5, where the cleaning power ability is expressed in terms of the Pellicle Cleaning Ratio (PCR), that is the percentage of stain removed with each toothpaste. Essentially the commercial toothpastes were more effective than the experimental one.

Abrasion

Table 3 shows the results of the surface roughness measurements for the toothpastes assayed. In this case, together with the commercial toothpastes assayed in the previous staining study, two toothpastes of known low and high abrasivity (Colgate® Fluoride Cavity Protection and Crest® Tartar Protection toothpastes) were compared to the experimental toothpaste as a negative and positive control respectively. Table 4 lists the abrasivity of the commercial dentifrices[7] used in the present study. Note that the dentifrice abrasivity toward enamel is measured by REA (radioactive enamel abrasion) technique, method based on the enamel specimens neutron bombardment resulting in the controlled formation of radioactive phosphorous ^{32}P within the specimens. During brushing, particles are abraded from the specimen producing radioactive ^{32}P source in the brushing slurry. The radioactivity is determined in a scintillation counter. Although the assessment of dentifrices abrasion is different, results obtained in the present study are similar to other already reported studies, as the dentifrice abrasivity sequence obtained in both cases was the following: Crest® Tartar Protection > Crest® Extra-whitening > Crest® Cavity Protection > Colgate® ≡ Experimental. In Figure 6, surface roughness dependence along with the number of

strokes is represented. Notice that, a first approximation to linearity can be appreciated between both parameters[15] except for the new experimental toothpaste and the Colgate one. Statistical analysis showed that there were significant differences among tested groups. In Table 5, further evaluation proved that the abrasion rates were non significant for Colgate® and the NMTD experimental dentifrices, but appreciable for Crest® Extra-whitening, Crest® Tartar Protection and Crest® Cavity Protection.

Discussion

The ability of a dentifrice to remove extrinsic stain from the tooth surface has been considered to be related to its abrasivity. In fact in this sense the results obtained from both studies correlate well within each other, as the experimental toothpaste which is of low abrasivity hardly removes any stain pellicle whereas Crest® Extra-whitening and Crest® Cavity Protection provide a higher abrasion rate and accordingly remove a higher percentage of extrinsic stains. So mechanical brushing with the new experimental dentifrice based on a silica abrasive cleaning system does not afford the expected results if compared with the conventional silica abrasive agent contained in Crest® formulations, that has a considerable effect.

Dentifrice abrasivity depends on the particle size and shape, hardness of the abrasive, pH and others factors unrelated to the dentifrice, such as frequency of brushing and hardness of the toothbrush bristles. Due to that fact that the latter factors remained constant among all dentifrices tested in each study, only the physical properties of the silica employed in each formulation would explain the disparity of the results obtained. Indeed, the average particle diameter of the abrasive determines the abrasion rate.[3] On the other hand, chemically identical abrasives can also have different cleaning/abrasion rates depending on the total dentifrice composition.[16] This fact may

explain why dentifrices using the same type of abrasive differ in accordance with their cleaning power potential. The abrasivity of a toothpaste must be determined on the basis of its complete composition and not only limited to its abrasive agent.

It should be noted that the formulation of the toothpaste matrix compatible with the ion-exchange resins mixture which is encapsulated in this new experimental toothpaste was not evident. Despite the low abrasivity of some toothpastes and judged to be not adequate to remove extrinsic stains, it has recently been reported that when using these toothpastes, stained pellicle can be controlled by increased brushing time.[17,18] On the contrary Lamb et al.[19] concluded that stained pellicle tends to accumulate on the teeth when using this kind of toothpastes and the quality of oral hygiene does not affect the degree of staining. Another consideration is that, dentin is more vulnerable towards abrasivity due to its lower hardness, that's why changes in enamel abrasivity are not the primary concern when conducting tooth abrasion tests. So globally, further studies on this new experimental toothpaste are recommended, such as dentin abrasivity tests in order to decide whether a formulation revision is necessary or not. The development of better cleaning dentifrices requires consideration of formulation compatibility, cleaning power and hard tissue safety.

The results of this laboratory study demonstrated that toothbrushing with a new experimental dentifrice based on an ion-exchange resins mixture that releases calcium, fluoride, phosphate and zinc ions (named NMTD), didn't reduce significantly extrinsic stains from bovine enamel teeth, while the commercially available dentifrices tested had significant effect on the stain. For the surface roughness tests, the results obtained were similar to the ones from the low abrasive Colgate® toothpaste.

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a. Procter&Gamble, OH, USA.
b. Colgate-Palmorive, NY, USA.
c. ICN Biomedicals Inc., OH, USA.
d. Sabri, Enterprises, Downers Grove, IL, USA.
e. Oral-B® Soft 40, Oral-B Laboratories, Belmont, CA, USA.
f. Copper Instruments & Systems, model DFI Infinity, Warenton, VA, USA.
g. Minolta CR221 Chromameter, Minolta Corp., Tokyo, Japan.
h. Mitutoyo, Surftest III, Tokyo, Japan.

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Legends:

Table 1

Dentifrice group	L*before brushing	SD	L* after brushing	SD
NMTD toothpaste	40.4	2.6	42.5	3.4
Crest® Cavity Protection toothpaste	40.3	1.3	57.0	2.5
Crest® Extra-whitening toothpaste	40.3	1.7	61.9	1.7
Calcium pyrophosphate	41	2.4	60.2	1.9

Table 2

Dentifrice group	L*values	SD
Crest® Extra-whitening toothpaste before brushing	40.3	1.7
Crest® Cavity Protection toothpaste before brushing	40.3	1.3
NMTD toothpaste before brushing	40.4	2.6
Calcium pyrophosphate before brushing	41	2.4
NMTD toothpaste after brushing	42.5	3.4
Crest® Cavity Protection toothpaste after brushing	57.0	2.5
Calcium pyrophosphate after brushing	60.2	1.9
Crest® Extra-whitening toothpaste after brushing	61.9	1.7

Table 3

	Ra values (μm)			
DENTIFRICES	**Initial**	**2000 strokes**	**4000 strokes**	**8000 strokes**
NMTD toothpaste	0.50 ± 0.03	0.46 ± 0.03	0.51 ± 0.03	0.54 ± 0.07
Colgate®	0.58 ± 0.04	0.58 ±0.05	0.58 ± 0.04	0.58 ± 0.06
Crest® Cavity Protection	0.6 ± 0.13	0.69 ± 0.16	0.79 ± 0.12	0.84 ± 0.13
Crest® Extra-whitening	0.62 ± 0.08	0.83 ± 0.11	0.95 ± 0.15	1.15 ± 0.11
Crest® Tartar Protection	0.53 ± 0.04	0.87 ± 0.11	1.00 ± 0.11	1.18 ± 0.19

Table 4

Dentifrice	Abrasive cleaning system	REA
Crest® Extra-whitening	Soft silica	4.1
Crest® Cavity Protection	Conventional Silica	2.8
Crest® Tartar Protection	Conventional Silica	5.7
Colgate®	Dicalcium Phosphate Dihydrate (DCPD)	1.4

Table 5

Dentifrice group	Strokes	Ra values (μm)	SD
NMTD toothpaste	0	0.50	0.03
Crest® Tartar Protection	0	0.53	0.04
NMTD toothpaste	8000	0.54	0.07
Colgate®	0	0.58	0.04
Colgate®	8000	0.58	0.06
Crest® Cavity Protection	0	0.60	0.13
Crest® Extra-whitening	0	0.62	0.08
Crest® Cavity Protection	8000	0.84	0.13
Crest® Extra-whitening	8000	1.15	0.11
Crest® Tartar Protection	8000	1.18	0.19

Figure 1

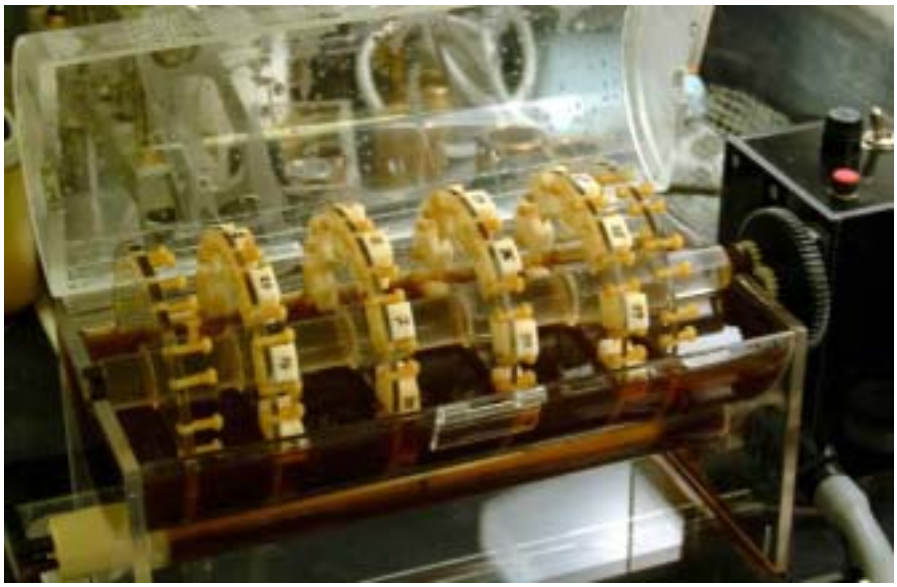

Figure 2

Figure 3

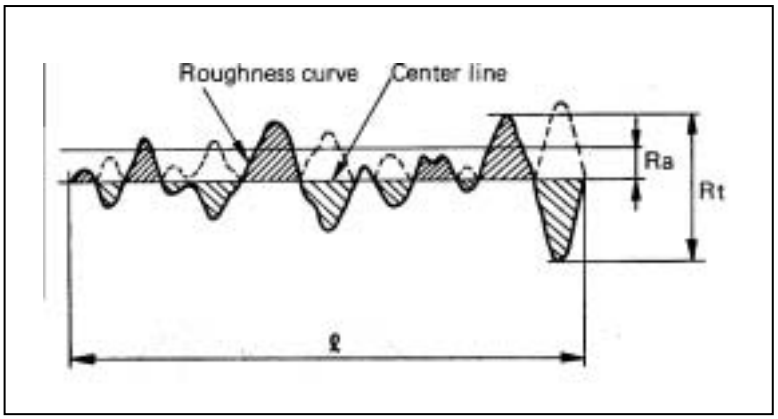

Figure 4

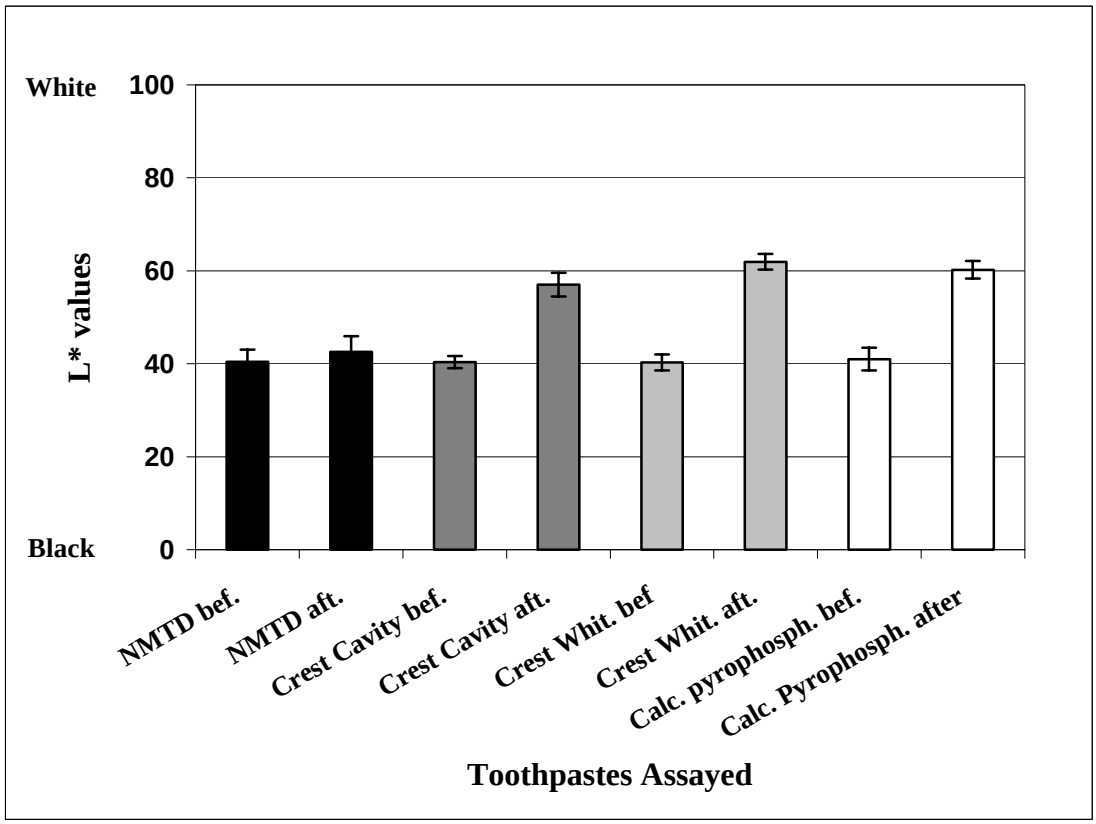

Figure 5

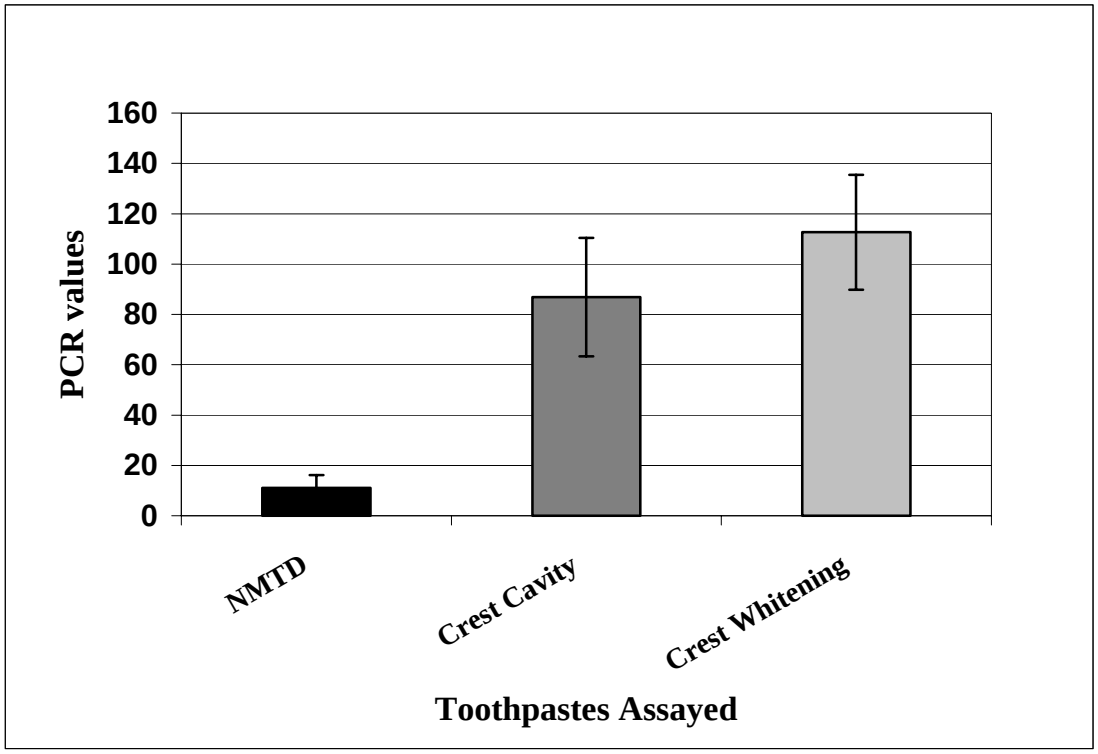

Figure 6