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SCANNING ELECTRON MICROSCOPY, X-RAY MICROANALYSIS AND IMMUNOHISTOCHEMISTRY ON WORN SOFT CONTACT LENSES

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Abstract

The deposits accumulated on the surfaces of soft contact lenses are a cause of problems for the wearer of these lenses, as the deposits are never completely removed by the available washing solutions. Therefore it appears of interest to investigate the composition of these deposits.

In this paper we review the major findings in the literature and, in addition, present our personal experience.

We have studied new, continuously and daily worn soft contact lenses by scanning electron microscopy (SEM), X-ray microanalysis and immunohistochemistry. We have carefully evaluated preparative methods, and we can conclude that SEM and X-ray microanalysis are best carried out on unfixed, air-dried lenses.

The deposits present consist mainly of mucus, especially on the tarsal side of the lenses. Chloride and potassium, coming from the tear fluid, as well as sulfur, derived from proteins, were found. Calcium was very rarely detected. IgG, IgA, IgE and C_{3c} complement fractions were found only on the outer surfaces and not witnin the lens.

We believe that the best characterization of the deposits is achieved by means of correlative techniques on the same lens. In fact, this approach integrates morphology and composition.

KEY WORDS: Scanning Electron Microscopy, X-ray microanalysis, immunohistochemistry, deposits, soft contact lenses.

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Introduction

The use of soft contact lenses (SCLs) has become increasingly common, as they provide an optimal correction of refraction and represent a "bandage" possibility for various corneal diseases.

With daily wear, deposits can accumulate on the lens surface, as they are not completely removed by the cleaning solutions. This can cause intolerance, lowering of the lens life-span, conjunctival and/or corneal hyperemia and decrease of visual acuity (Mondino et al., 1982). One of the most serious complications is the so called Giant Papillary Conjunctivitis, observed in both hard and soft contact lens wearers and in ocular prostheses wearers (Allansmith et al., 1977; Srinivasan et al., 1979). This disease is supposed to be due to the deposits which probably undergo a process of denaturation as the wear goes on.

Thus, it appears important to characterize the exact nature of these deposits. The easiest method is the observation of the lens with the aid of a slit-lamp, which provides a 7-10 x tridimensional image. This procedure is quite practical, but, of course, inaccurate as it identifies the deposits only on the basis of their morphology. In addition, the low magnification does not visualize the smallest deposits and crystals.

In our laboratory we have studied the deposits on SCLs by means of different and correlative techniques.

Scanning Electron Microscopy (SEM) is the best method to analyze the contact lens surfaces, both to detect possible irregularities or scratches and to investigate the various organic materials and microorganisms deposited on them. SEM is the approach subsequent to slit-lamp examination as it brings the investigation from the level of the naked eye to the ultrastructural level.

X-ray microanalysis detects elements within the deposits. Thus, it correlates morphology with composition of the deposited material.

Cytochemistry and immunocytochemistry provide evidence on the presence of organic substances and immunoglobulins, coming from the environment, on the surface of the lens.

In contrast with the major part of the published studies, which were based upon spoiled lenses, this study describes the deposits present while the lenses were still usable.

Materials

We have studied hydrophilic contact lenses (PT70 GALILEO) under various conditions of wear. Only one type of lens was used to avoid that the difference in the materials of which the lenses are composed would affect the wear and therefore the results. PT70 GALILEO are poli-Hema lenses, with a 70% hydrophilic rate.

We can divide the lenses studied into 3 groups.

GROUP A

5 lenses were used as controls. They were never worn and were directly removed from their vial of factory-sealed, sterile preserved saline. Care was taken to avoid contamination with finger prints.

GROUP B

30 lenses were removed by the finger-pinch method from 18 asymptomatic patients (10 males, 8 females, medium age 27) after a continuous wear of 5 months on average. A group of 14 patients wore lenses professionally cleaned by the optician, the other patients had been instructed to clean their lenses weekly with the available kit solutions. At the time of removal, the lenses had undergone the last cleaning procedure at least one week previously.

GROUP C

10 lenses were removed as described above from 10 asymptomatic patients (7 males, 3 females, medium age 24) after a daily wear of 11-12 months on average. All patients had been trained to clean their lenses every night with the proper solutions. At the time of removal 5 lenses were immediately processed as will be described in the next paragraph, the other 5 were first immersed in a storage solution and sent to us within two weeks.

All the worn lenses appeared unbroken immediately after their removal. Some were not transparent, but showed a pale brown staining. Large deposits were not visible to the naked eye.

Methods

All the lenses were cut into small fragments and then processed following these procedures:

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At least two pieces from all lenses of the 3 groups were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 for 1 h at 4°C, washed overnight in 0.15 M phosphate buffer at 4°C, washed for 2 h in distilled water and air-dried at room temperature for a minimum of 72 h. The pieces were then mounted (from the same lens: one piece with tarsal side up, another with the corneal side up) on aluminium stubs with a silver conducting painting, sputtered with a thin layer (20 nm) of gold and observed in a Philips 505 SEM, operated in the range of 10-20 kV.

An alternative procedure for SEM avoided the fixation step; the pieces were only air-dried and subsequently treated and observed as described above. In this case, the fragments from group A (non worn lenses) were briefly washed in distilled water before air-drying.

X-ray microanalysis

The analysis was performed only on some selected daily wear lenses (both fixed and unfixed) belonging to group C. The corresponding fragments of these lenses had previously been observed in the SEM and had shown a relatively large amount of deposits over the surfaces.

Pieces of lenses were air-dried at room temperature for a minimum of 72 h, mounted with double-sided adhesive tape to a carbon planchet fitting the specimen holder. The adhesive tape did not contain elements detectable by conventional energy-dispersive X-ray microanalysis and did therefore not contribute any characteristic peaks to the spectrum. A small amount of silver paint was used to reduce specimen charging and the specimens were in addition covered with a conductive carbon layer.

Analysis was carried out with a Tracor Northern 5500 X-ray microanalysis system in a JEOL 1200 EX electron microscope. The specimens were observed in the secondary electron mode and analyzed at an accelerating voltage of 20 kV. Quantitative analysis was carried out by comparing the ratio of characteristic intensity to the background intensity in the same energy region for specimen and standard (a gelatin matrix containing inorganic salts in known concentrations) (Roomans, 1981). The results are expressed in mmol/kg, but it should be taken into account that the analyzed volume may have exceeded the thickness of the deposits, and that also

lens material can have been excited. An increase in the thickness of the deposits would thus lead to an apparent increase in the elemental concentrations.

Immunohistochemistry

A fragment from each lens from all 3 groups was fixed in PLP (periodate-lysine-paraformaldehyde) freshly prepared according to McLean and Nakane (1974) for 2 h at 4°C, washed overnight in 0.1 M cacodylate buffer containing 2.7% saccarose and directly embedded in a hydrophilic resin (JB4, glycol-methacrylate, Polysciences Inc., Warrington, PA, USA). Sections 4 µm thick were cut with a Leitz ultratome and collected on very clean glass slides. Immunohistochemical reactions were performed utilizing anti-human IgG, IgA, IgE and C_{3c} complement fraction antisera conjugated with fluorescein isothiocyanate (FITC). The antisera were purchased from Biometics Inc., Kensington, MD (IgG, IgA) and Behringwerke AG, Marburg (IgE, C_{3c}).

The sections were first incubated in 0.1% pronase (non specific protease from Bacillus Amyloliquefaciens, Type VII, Sigma Chemical Co., St. Louis, MO) in 0.5 M Tris-HCl buffer, pH 7.5 as suggested by Casanova et al. (1983). After an overnight washing in 0.01 M PBS at 4°C, the sections were incubated in the antisera diluted 1:20 / 1:40 in 0.01 M phosphate buffer saline pH 7.2 for 30 minutes at room temperature in a moist chamber. The sections were then washed several times for 10 minutes in cold PBS in the dark. Glass coverslips were mounted with a drop of glycerol and finally the sections were observed with the Zeiss III RS photomicroscope equipped with an epifluorescent condenser. Photographs were taken at a magnification of 16 and 40 x and observed in a double blind test by two of us (PV, MCM).

As control reaction, serum specificity against immunoglobulins was evaluated by adsorption of the labeled antisera with an excess of the corresponding antigen (IgG, IgA, IgE, C_{3c}, Behringwerke AG, Marburg). A layer of the adsorbed serum was then placed on the control sections.

For 4 lenses of group B and 2 of group C we also performed an immunocytochemical analysis by using a pre-embedding technique in transmission electron microscopy (TEM). Anti-human IgG antiserum was purchased from Behringwerke AG, Marburg. Colloidal gold particles 17 nm in diameter prepared according to Frens (1973), were conjugated with Protein A (Pharmacia Fine Chemicals, Uppsala) following the method of Roth et al. (1978). The immunocytochemical reactions were performed as follows. One fragment of each lens was cut immediately after its removal from the eye and briefly (10 min) fixed in 2% paraformaldehyde, washed (20 min) in 0.01 M PBS, incubated in anti-human IgG antiserum diluted 1:50 in PBS, for 30 min. After a wash of 5 min in 0.01 M PBS, the fragment was incubated in the protein A-gold solution diluted 1:10 in PBS for 30 min at 4°C. The fragment was then dehydrated in ethanol and embedded in Araldite. In the control reactions, the first antiserum was omitted.

Ultrathin sections were obtained with a Reichert UM3 ultratome, counterstained with uranyl acetate and lead citrate and observed with a Zeiss EM9 TEM.

Results and Discussion

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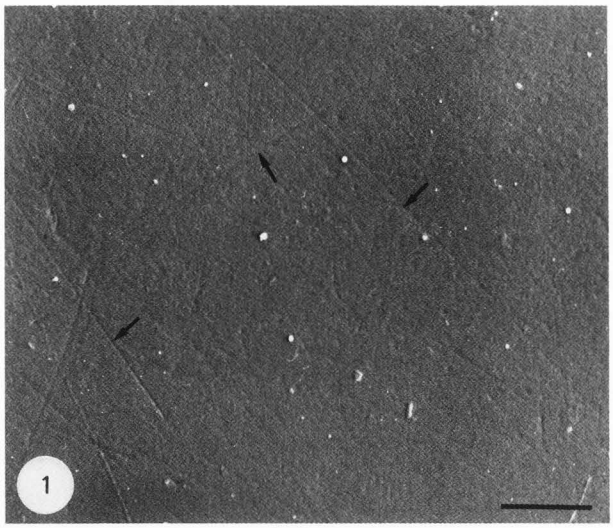
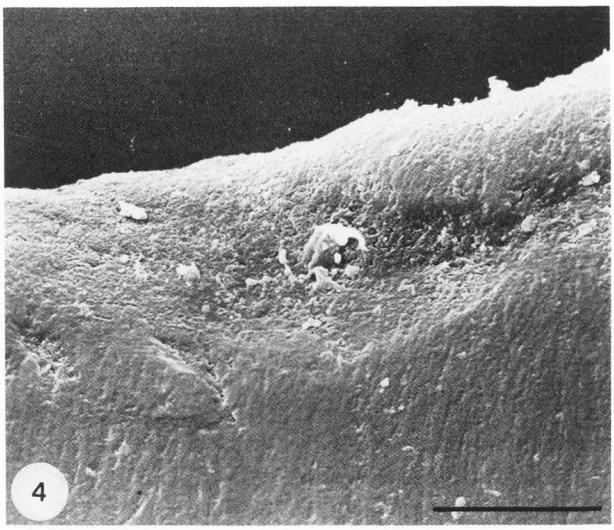
The first point to be assessed is how to process SCLs for SEM examination so that artefacts are avoided. Matas et al. (1972) claimed that freeze-dried and critical point dried specimens showed better preserved morphology than air-dried specimens. They observed on never worn hydrophilic lenses several polishing marks and irregularities, which could make the attachment of foreign materials easier. In a very recent paper Deg and Binder (1986) reach the opposite conclusion on the processing of SCLs for SEM. In a series of experiments on never worn lenses they found that critical point drying as well as ethanol dehydration have to be avoided. They suggest that the fixation step is necessary to preserve possible biological deposits and to stabilize the polymer. Air-drying, after a thorough wash in double distilled water, was found to be the optimum method of dehydration.

In the past years much work has been done on the surface morphology of worn lenses by SEM, both with and without correlative analytical techniques (Tripathi and Ruben, 1973; Holden et al., 1974; Conrads 1975; Kanai et al., 1977; Colin et al., 1979; Dreyer et al., 1979; Fowler et al., 1979a, b; Fowler and Allansmith 1980a,b, 1981; Miller 1980; Winterhoff et al., 1980; Hesse et al., 1982; Hovding and Seland 1984; Versura et al., 1985; Hart et al., 1986).

In our study, the never worn lenses of group A exhibited both smooth corneal and tarsal surfaces, with few polish marks (Fig. 1). Exfoliated cells were also observed, probably due to handling during the non-mechanized production steps (Fig. 2). Scattered debris of uncertain origin, possibly derived from the storage solution, was seen on both sides (Fig. 3). The edges of the lenses were quite regular, only in some areas did they not appear smooth





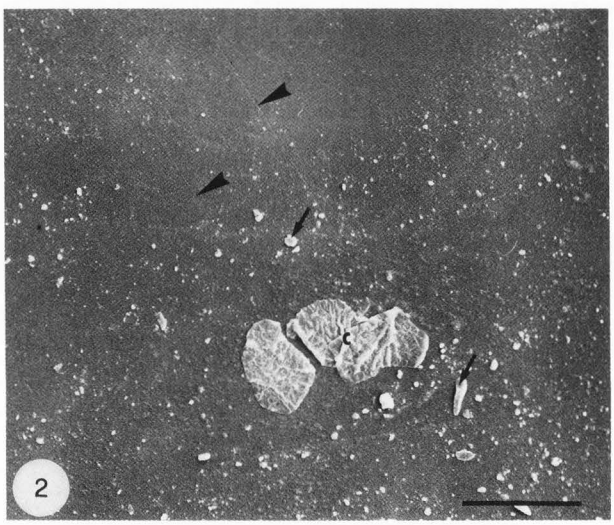
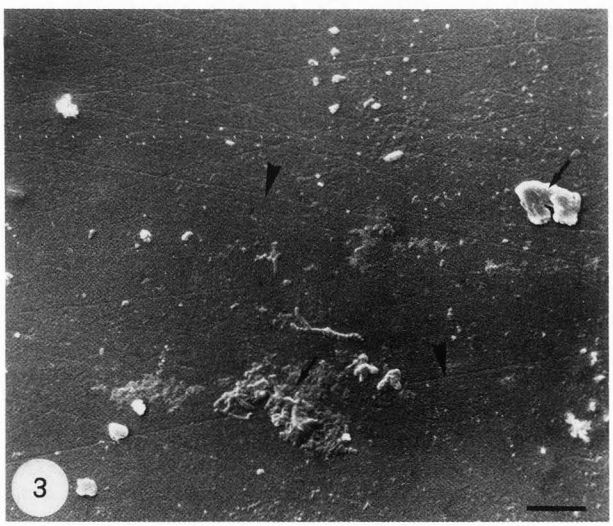


Fig. 1. Never worn SCL. The surface shows a homogeneous grey background with linear marks (arrows), probably due to polish manufacturing steps. SEM. Bar = 10 μm

Fig. 2. Never worn SCL. Exfoliated cells (c), scattered debris (arrows), polish marks (arrowheads) on the surface. SEM. Bar = 50 μm

Fig. 3. Never worn SCL. Scattered debris (arrows) and polish marks (arrowheads) on the surface. SEM. Bar = 10 μm

Fig. 4. Never worn SCL. Irregularity of the lens edge. SEM. Bar = 10 μm



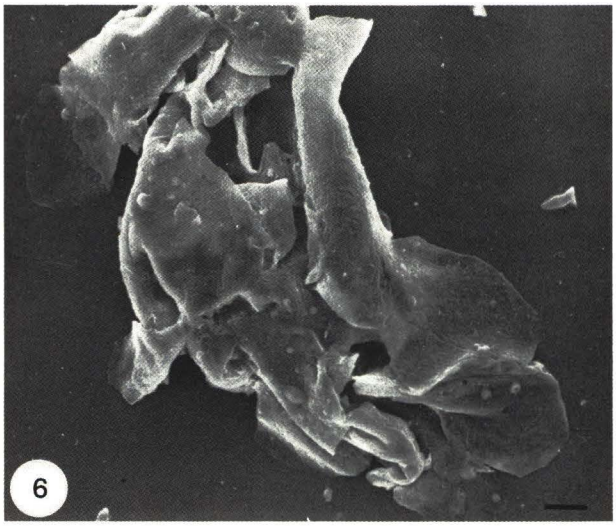
(Fig. 4). These results are in accordance with what has been described in literature. The surface morphology of unfixed and fixed lenses was exactly the same. No abnormal surface structure (resembling possible deposits) and surface wrinkling were observed; neither were cracks and scratches. The homogeneity of the surface of the new lenses is very important. In fact, it is believed that the deposits begin to adhere to the irregularities and even that these predispose to further deposition (Tripathi et al., 1980). Therefore, SEM observation is extremely useful to assess the perfect smoothness of new contact lenses and to suggest to the manufacturers which improvements are needed to achieve the most regular surface.

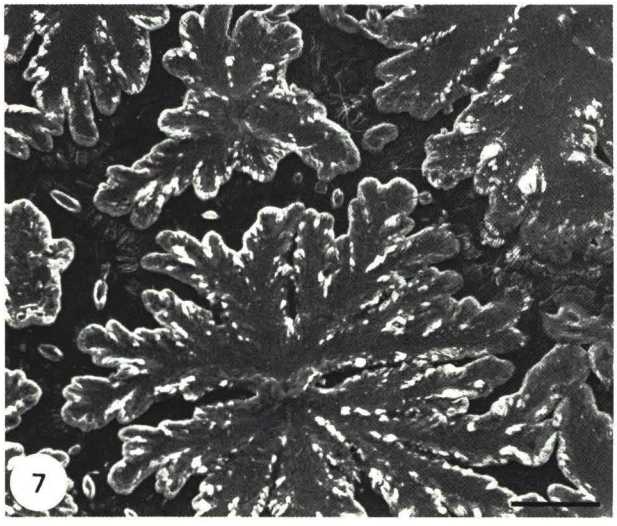
The continuously worn lenses of group B showed a surface heavily covered with a smooth matted coating (Fig. 5). This characteristic

Fig. 5. Continuously worn SCL, unfixed specimen. The surface heavily covered with a matted coating is shown. SEM. Bar = 10 µm

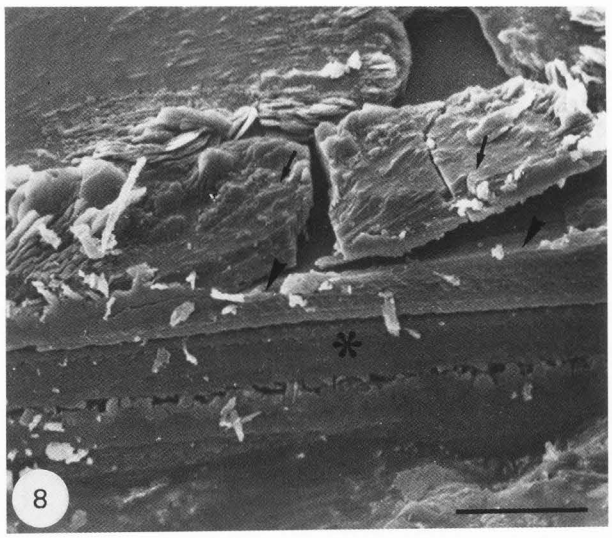
Fig. 6. Continuously worn SCL, unfixed specimen. Exfoliated cells on the surface. SEM. Bar = 10 µm

Fig. 7. Continuously worn SCL, fixed specimen. A deposit with an unusual ferning-like appearance is evidenced. SEM. Bar = 100 µm





feature was also reported by Fowler and Allansmith (1980 b; 1981) and Hovding and Seland (1984). The lens surfaces were dirtier in the optical zone than at the edges and also dirtier on the tarsal side than on the corneal side. Only a few exfoliated corneal or conjunctival cells were observed (Fig. 6) even if one cannot exclude that the cells were inside the coating and thus not visible. The cells were found more frequently on the tarsal surface, probably deposited due to rubbing during blinking. In none of the lenses observed, were any fungi or bacteria detected. Fowler et al. (1979 a) and Winterhoff et al. (1980) reported the presence of microorganisms in the surface-coating deposits. On this substrate bacteria can proliferate and represent a source of dangerous infections. Only in the fixed fragments did we find deposits of unusual morphology. These deposits appeared to have an organic core surrounded by an inorganic rim (Fig. 7). The deposits were not "explosions" of the surface, as in certain areas they were observed on top of the surface lens and not penetrating and disrupting the lens matrix (Fig. 8). As the deposits had an unusual ferning-like appearance, we concluded that they consisted of mucus, because of the particular ferning-like morphology of the ocular mucus when put on a glass slide and air-dried (Tabbara and Okumoto, 1982). We also observed, even if very rarely, lipid deposits (Fig. 9). Lipid deposits are not removed by the cleaning solutions and the dynamics of their deposition is yet unknown. Their presence has been related to the presence of calcium, protein, mucin, lipid or bacteria (Hart, 1984; Tripathi et al., 1980; Gasset et al., 1977; Liotet et al., 1983). Hart et al. (1986) in a histochemical and SEM study claimed that these deposits consisted of long and intermediate chain length cholesterol esters, triglycerides and waxy esters. This composition would match that of the tear film lipids secreted by the Meibomian gland. Small crystalline structures were also detected, showing different morphology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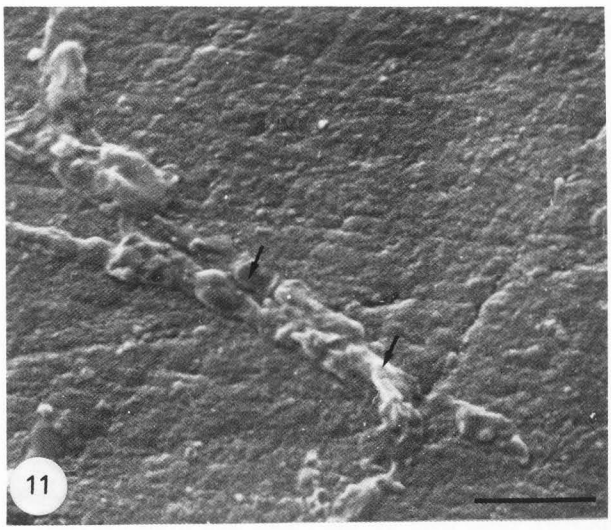
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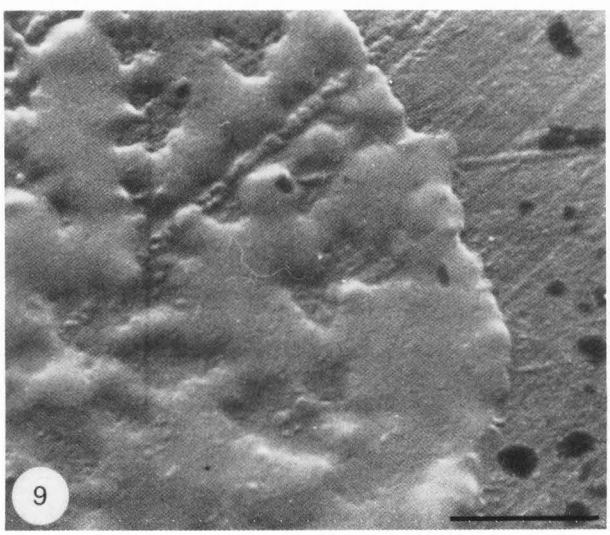
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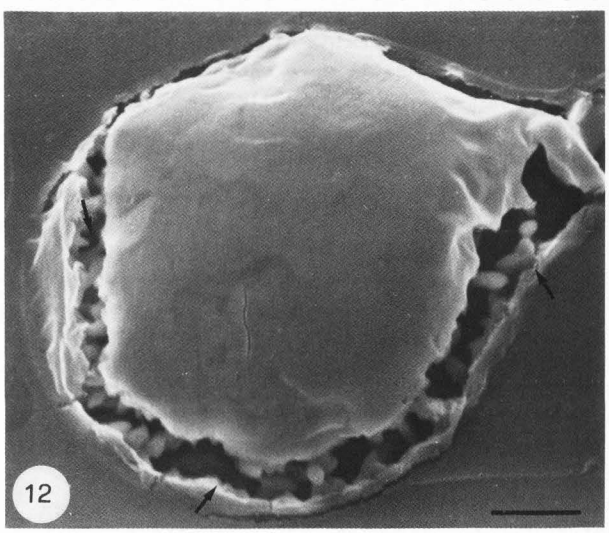
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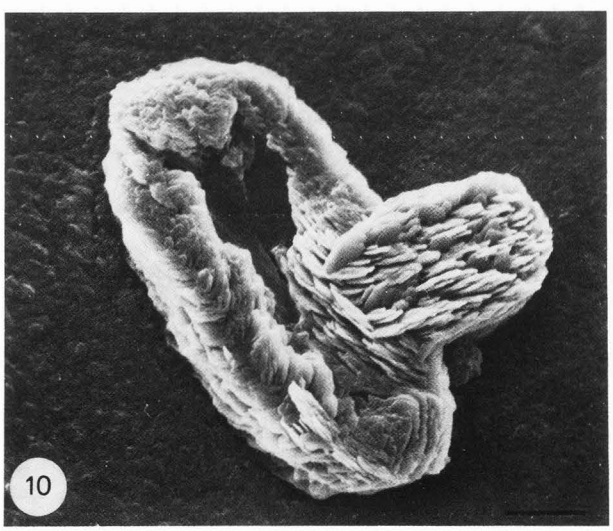
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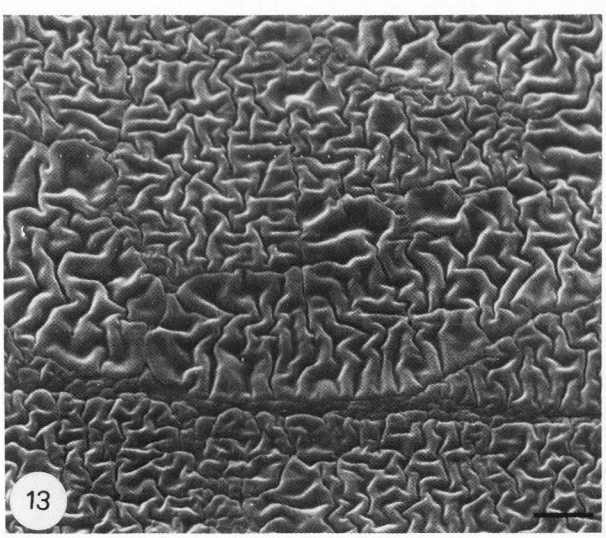
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Fig. 8. Continuously worn SCL, fixed specimen. The deposits of Fig. 7 (arrows) are located on the top of the lens surface (arrowheads) and do not penetrate the lens matrix. * = cut lens SEM. Bar = 50 μm

Fig. 9. Continuously worn SCL, unfixed specimen. A lipid deposit on the surface. SEM. Bar = 10 μm.

Fig. 10. Continuously worn SCL, unfixed specimen. A crystalline structure on the lens surface covered by a smooth matted coating. SEM. Bar = 10 μm

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Fig. 11. Daily worn SCL, unfixed specimen. Mucus-like material on the tarsal surface (arrows). SEM. Bar = 50 μm

Fig. 12. Daily worn SCL, unfixed specimen. An erupted bubble exposes the lens matrix (arrows). SEM. Bar = 10 μm

Fig. 13. Daily worn SCL, unfixed specimen. Wrinkling of the surface. SEM. Bar = 10 μm

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(Fig. 10). SEM cannot provide any information on the nature of these deposits and we can only speculate as to their identity. The definitive answer might be given by X-ray microanalysis.

The importance of the complete characterization of the organic and inorganic deposits is mainly related to the problem of the appropriate cleaning procedure of the lens surfaces. Fowler and Allansmith (1981) and Hesse et al. (1982) investigated by SEM the effectiveness of various cleaning solutions. They concluded that the coating of the lenses was not completely removed by any method they tested. As the wear goes on, the increasing thickness of the coating and the virtually complete absence of bare lens surface indicate that oxygen permeability through the lens to the cornea can be seriously affected, thus provoking corneal problems. In the patients who cleaned their lenses themselves, we found more deposits, especially of mucus, than in professionally cleaned lenses.

The surfaces of the daily worn lenses of group C were cleaner than those of the continuously worn lenses, as seen by SEM. However, the same types of deposits were found. Exfoliated cells, especially on the tarsal side, and crystalline structures were observed. Mucus-like material (Fig. 11) was mainly detected on the tarsal side, as was also found by Doughman et al. (1975). The heavier coating of the anterior surface was probably mainly due to the frequent drying when the eyes were open. In the fixed fragments we found the same deposits observed in the fixed fragments from continuously worn lenses, i.e., those with the ferning-like appearance supposedly consisting of mucus material. As this kind of deposit is completely absent in the fragments from never worn lenses, we conclude that these deposits are due to the interaction between mucus and one or more components present in the solutions used for processing the lenses. In the internal curve of the lenses we observed polymer alterations, namely gas bubbles "extruding" from the matrix of the lens (Fig. 12). This phenomenon was also described by Miller (1980), and it probably leads to intolerance as the lens surface becomes similar to emery paper. We also observed roughness and wrinkling of the surface (Fig. 13). Some authors state that these alterations are due to aging and incorrect cleaning of the lens (Tripathi and Ruben, 1973).

X-ray microanalysis

In the recent literature the value of X-ray microanalysis in characterizing the nature of the deposits has been stressed. Several authors have studied series of different contact lens types. Ruben et al. (1975) studied SCLs embedded in plastic for previous TEM analysis and found within the deposits calcium and phosphorus in a ratio matching the composition of calcium triphosphate $Ca_3(PO_4)_3$. They correlated the quantity of calcium present with the degree of opacification of the lens and concluded that calcium deposition is a cause of spoilage of hydrophilic contact lenses. The same results were obtained by Klintworth et al. (1977) in dry-eye patients with a high tear calcium content. Dreyer et al. (1979) found calcium in over two-thirds of the cases studied, the other elements were more variable and infrequent. Miller (1980) found variable amounts of several ions on SCLs: sodium, chloride, silicon, magnesium, aluminium, phosphorus, sulfur and potassium. Adenis et al. (1981) studied the nature of the deposits in continuously worn SCLs in aphakic eyes. They detected sodium and chloride, which most probably come from the tear fluid, and calcium in 8 out of 13 lenses. Hovding and Seland (1984) detected on SCLs, continuously worn for therapeutic purposes, elements belonging to both the normal tear fluid and lens storage solution, and sulfur, while calcium was very rarely found.

In all these studies there is a great

variability with regard to the type of lenses studied and the procedure applied to the specimens for the analysis.

In our correlative investigations on SCL deposits, we have applied X-ray microanalysis only to fragments of selected lenses, whose surfaces were previously judged by SEM as showing "characteristic features". This allowed a better correlation between morphology and composition of the deposits, which was the aim of the work.

In the group of lenses fixed and subsequently washed in buffer and water before air-drying, we found (Fig. 14) sodium and phosphorus, coming from the buffer, and sulfur. These elements were detected in the deposits with the morphology shown in Fig. 7. Therefore we conclude that these structures are a mixture of organic (sulfur deriving from mucoproteins) and inorganic (sodium and phosphorus deriving from the buffer) substances. These deposits are thus an artefact due to the interaction between mucus and buffer, as they are not present on the new, never worn lenses. Not only the buffer washing, but also fixation (as the fixative is dissolved in the buffer) should be avoided. Only negligible chloride and potassium were found, but some calcium could be detected, probably because it was bound to proteins or mucus.

The results of the X-ray microanalysis on unfixed, only air-dried lenses are summarized in Table 1. The level of sulfur is quite variable which indicates that the amount of proteins is also variable, as was also observed by SEM. Phosphorus was found in two lenses but always in correlation with sodium. This probably means that the components of the washing fluid were left on the lens (probably as a precipitate) and were not washed away by the tear film. There are variable amounts of sodium and chloride and low potassium; this reflects the tear fluid composition. Calcium levels were low. We noted that the calcium levels increased with increasing levels of sulfur; this probably means that proteins (mucins) can bind and sequester calcium. In Fig. 15 a typical spectrum of an unfixed lens is shown.

In Table 2 the results of the X-ray microanalysis on unfixed, that had previously been stored in a storage solution, air-dried lenses are shown. Sulfur is present in variable amounts. Sodium and chloride are found in relatively high amounts, and are due to the storage solution, which is basically a saline buffer. Low potassium, little or no calcium, little or no phosphorus were found. The composition of the deposits in the stored lenses is very close to that of the lenses in Table 1, except that on the average

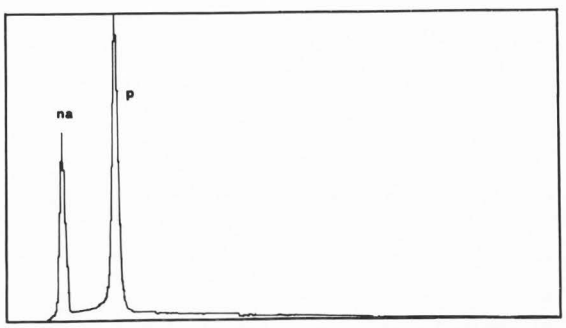


Fig. 14. Daily worn SCL, fixed, washed in buffer and water specimen. X-ray microanalytical spectrum.

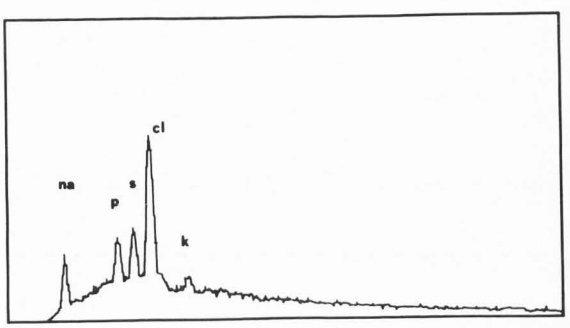


Fig. 15. Daily worn SCL, Unfixed spectrum. X-ray microanalytical spectrum.

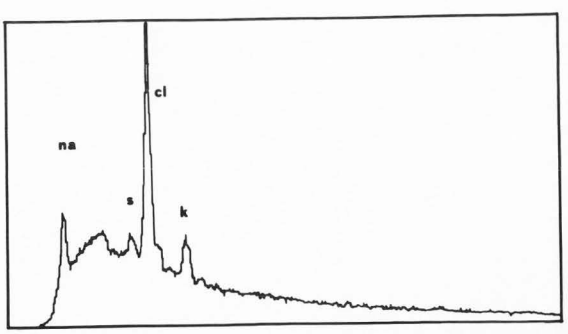


Fig. 16. Daily worn SCL, unfixed and stored in a storage solution specimen. X-ray microanalytical spectrum.

Table 1

Elemental concentrations (mmol/kg dry weight) in deposits on contact lenses of group C (unfixed) (mean of three measurements)

lenses	side	Na	Mg	P	S	Cl	K	Ca
n. 2	cor	231	0	3	85	275	28	6
	tar	442	6	674	328	789	85	26
n. 5	cor	369	0	186	132	192	30	13
	tar	205	0	7	86	249	33	3
n. 6	cor	166	0	4	62	257	19	7
	tar	269	3	5	312	477	113	12
n. 8	cor	188	0	3	85	167	13	2
	tar	282	1	12	186	204	25	6

cor = corneal surface; tar = tarsal surface

Table 2

Elemental concentrations in deposits on contact lenses of group C (unfixed, stored) (mean of three measurements)

lenses	side	Na	Mg	P	S	Cl	K	Ca
n. 2	cor	120	1	3	161	120	15	1
	tar	176	0	8	118	121	13	5
n. 6	cor	202	0	0	43	424	87	0
	tar	170	0	0	74	367	62	7
n. 8	cor	147	0	0	18	261	38	2
	tar	102	0	0	25	186	32	0

cor = corneal surface; tar = tarsal surface

they contain less sulfur. In Fig. 16 a spectrum of this group of lenses is shown.

On the basis of these data we cannot assess if a difference in the extent of the deposits exists between the corneal and the tarsal side. In 5 out of 7 cases the sulfur levels (indicative of mucus) were higher on the tarsal side. This would suggest an increased amount of mucus on the tarsal side, which was also observed in SEM.

Immunohistochemistry.

This type of approach has been attempted in the past by only a few authors. Dreyer et al. (1979) applied conventional histochemical stainings to detect mucopolysaccharides, proteins, lipids, fungi and bacteria to paraffin-embedded or frozen SCL sections. Mucopolysaccharides were found to be the main components of the deposits, as was already suggested by previous biochemical studies (Wedler, 1977; Hilbert et al., 1976; Hathaway and Lowther, 1976; Allen et al., 1978). Tuffery (1978) found that the coating over SCLs is composed of organic material and observed a frequent lack of calcium. Allen et al. (1978) found neutral mucopolysaccharides in the deposits together with calcium, protein and lipids. Doughman et al. (1975) detected almost exclusively lipids and lipoproteins in a series of SCLs, but no calcium. Gudmundsson et al. (1985) examined 5 SCLs from asymptomatic patients. They used an indirect immunofluorescence technique on paraffin sections to detect lysozyme, lactoferrin, IgG and IgA. These authors believe that these proteins are not denaturated, at least in part, as they were detected by antibodies against normal proteins. This was in contrast to previous findings by Refojo and Holly (1977) and Refojo and Leong (1979). In addition, it is still questionable if these proteins can or cannot penetrate inside the porosity of the hydrogel lenses (Refojo, 1985). This point is of importance in establishing the etiology of ocular allergy. In other words, the question is whether an immunoresponse to contact lenses is due to denaturated autologous proteins, or, more likely, to foreign substances from the environment which adhere to the lens surface.

In our work we have critically examined the method of processing the lenses for immunohistochemistry. The sectioned paraffin-embedded material appears to be unsuitable for immunofluorescent microscopy of the deposits which are located at the edge of the sections. As is well known, artefactual fluorescence can be detected due to the dye "under" the edges that do not adhere well to the glass slides, especially if the section is more than 5 µm thick. Gudmundsson et al. (1985) even prepared sections 8 µm thick. Therefore we used a plastic embedding medium for light microscopy of the lenses, JB4, a glycol-methacrylate previously used by Casanova et al. (1983) for the detection of immunoglobulins in human kidney biopsies. In this way, the resin does not need to be removed, the edges of the lens section are not at the border and therefore the artefactual fluorescence does not occur as in paraffin sections.

In Fig. 17 the clusters of fluorescence indicative for the presence of IgG are shown. They appear specifically located and are deposited as patches and not spread out over the entire lens surface. The results of our study on both continuously and daily worn SCL surfaces are shown in Table 3.

Table 3. Immunohistochemical detection of IgG, IgA, IgE and C_{3c} on both continuously (Group B) and daily (Group C) worn SCL surfaces.

	lenses	IgG	IgA	IgE	C_{3c}
Group B/n	1	-	+	+	-
	2	-	+	+	-
	3	-	+	-	-
	4	-	-	-	-
	5	+	-	+	+
	6	-	+	+	-
	7	+	+	+	-
	8	-	+	-	-
	9	-	+	-	-
	10	+	+	-	-
	11	-	-	-	-
	12	+	+	+	+
	13	-	+	+	-
	14	+	+	-	-
	15	+	+	+	+
	16	-	-	+	-
	17	-	-	+	-
	18	+	+	+	-
	19	+	+	-	-
	20	+	+	-	-
	21	-	+	+	-
	22	+	+	+	-
	23	+	-	+	-
	24	+	-	+	-
	25	-	+	-	-
	26	-	+	-	-
	27	+	+	-	-
	28	-	+	+	-
	29	+	-	+	-
	30	-	+	+	-
Group C/n	1	+	+	+	-
	2	-	-	-	-
	3	-	+	-	-
	4	-	+	+	-
	5	+	+	+	+
	6	+	+	-	-
	7	+	+	+	-
	8	-	+	-	-
	9	-	+	+	-
	10	+	-	-	-

+ = presence; - = absence

Fig. 17. Continuously worn SCL. The presence of IgG is indicated by small clusters of fluorescence (arrows). * = section of the lens. LM Bar = 100 μm

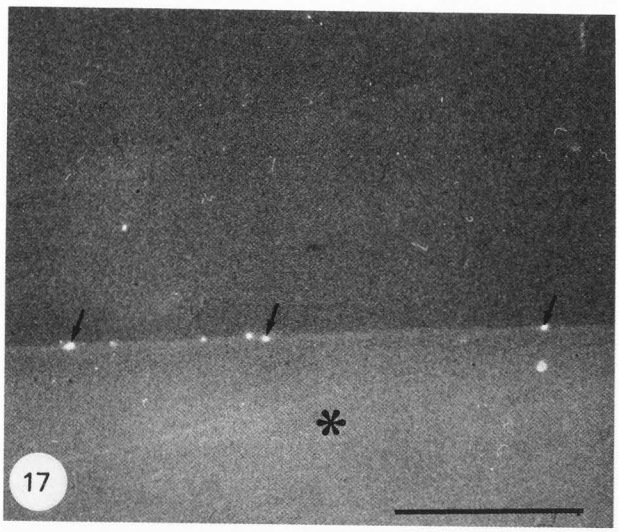


Fig. 18. Daily worn SCL. The gold particles indicate the presence of IgG in the surface deposits (arrows). * = section of the lens. TEM Bar = 1 μm

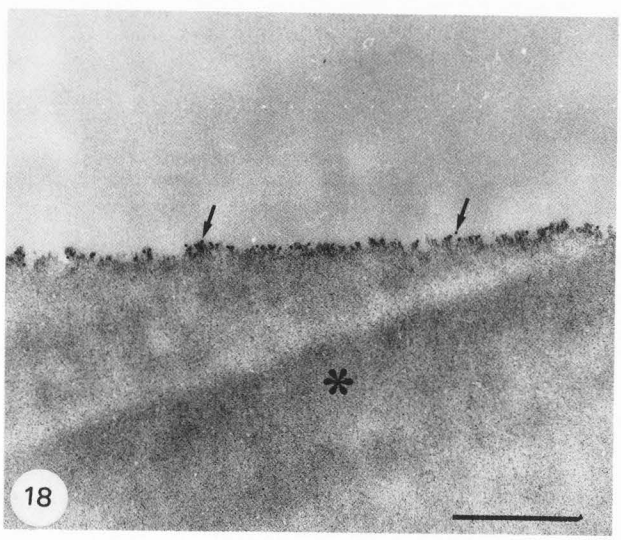
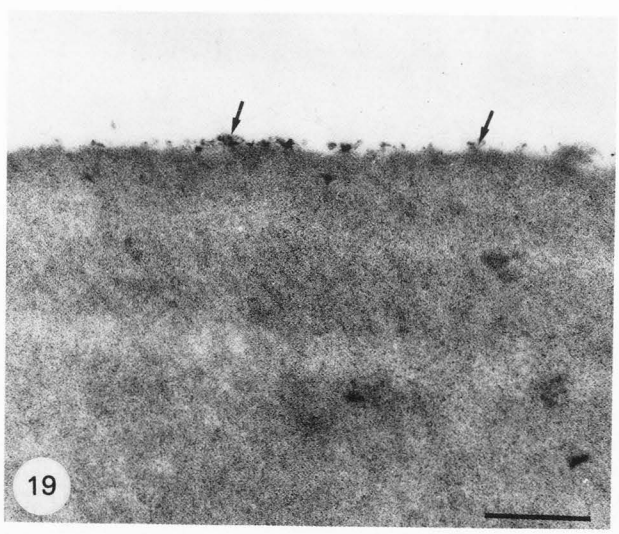


Fig. 19. Daily worn SCL. Control reaction. The surface deposits (arrows) are unlabeled by the protein A-gold complex. TEM Bar = 1 μm



We did not find any trace of fluorescence inside the material. As the reaction is performed on sections, the antisera can label even the cut surface and not only the outer surfaces. Therefore, it should be possible to detect undernaturated proteins inside the lens material. On the other hand, it is impossible to assess if any denaturated material is present because this may not show any fluorescence, as was also noted by Allansmith (1985). The never worn lenses did not show any fluorescence, except for a light autofluorescence of the polymer itself.

The low resolution of LM can of course be overcome by applying immunocytochemistry in TEM. For this reason we applied to 6 lenses, at the time of removal, a pre-embedding technique to detect even small amounts of immunoglobulins. In Fig. 18 the colloidal gold particles show the presence of IgG over the lens surface. In Fig. 19 the result of the control reaction is shown, performed by omitting the first antiserum. The few cases we have studied with this technique in TEM do not allow us to draw conclusions about a possible difference between the continuously and the daily worn lenses with regard to the presence of IgG in the surface deposits.

We cannot say at the moment if the immunoglobulins and the C_{3c} complement fraction were present on the lens as a specific sign of the ocular tissue reaction against the prostheses. It is well known (Chodirker and Tomasi, 1963; Covey et al. 1971) that in normal tears secretory IgA are the predominant immunoglobulins, that IgG are also present and IgE are often detected. The only specific sign of ocular reaction against the lens could be the presence of C_{3c}, which is not found in normal conditions (Selinger et al., 1979). Moreover, it cannot be excluded that C_{3c} is present for causes independent of the lens wear. In addition, we detected C_{3c} only in 4 out of 40 lenses; this fact does not allow us to conclude that the ocular reaction against SCL

specifically activates the complement pathway.

Conclusion

On the basis of our results we can state that the best procedure to obtain correlative information on SCLs is immediate air-drying after removal, with no previous fixation or washing, as the morphology of the organic deposits is still well preserved. This very easy method allows one to send the prostheses to specialized laboratories for SEM and X-ray microanalysis. These two techniques appear to be particularly useful and should be considered not only as sophisticated tools for research, but also, and mainly, always available to ophthalmologists interested in this important subject.

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Discussion with Reviewers

Reviewer III: What was the cutting procedure used to reduce the lenses into small fragments and what precautions were taken to prevent contamination? How do the authors control for ambient deposits during the 72 hour air-drying period?
Authors: The lenses were put on a piece of clean parafilm and cut with a new razor-blade with cleaned edges. The fragments obtained were collected in an extremely clean Petri dish, the cover of which was never removed during the entire 72 hour air-drying period.

Reviewer III: What do you mean by "Care was taken to avoid contamination with fingerprints"?
Authors: During the preparation the lenses were only touched at the edges with a forceps.

Reviewer III: What exactly was involved in the cleaning regimens used by opticians and the patients "using available kit solutions"? How did the regimens differ? How does this affect the results?
Authors: The cleaning regimens carried out on the continuously worn lenses of group B by both the opticians and the patients were exactly the same and consisted of the weekly use of proteolytic enzymes dissolved in saline. As the lenses cleaned by the opticians were found at SEM to be less heavily covered by deposits we conclude that the patients failed to carry out a correct cleaning procedure with the available liquids, even if they had been instructed in this respect.

As to the daily worn lenses of group C, the patients employed a liquid detergent containing three tensioactive components (two anionic and one amphoteric, with a combined action) and EDTA (ethylene diamine tetraacetic acid disodium salt dihydrate, a calcium and magnesium ion sequestrant). The lenses were cleaned each night for 1 minute with this solution and stored overnight in buffered sterile saline.

Reviewer III: The fact that the authors cannot determine whether the concentrations obtained by x-ray microanalysis are not greatly affected by the depth of individual deposits on the surface is a fundamental problem.
Authors: The problem is less serious than it might appear. If the deposits are small, the beam will completely penetrate the deposits and excite the lens material, which produces background radiation but no characteristic peaks. The "concentration" of the elements in the deposits will appear relatively low. As their size increases, less lens material is excited and the apparent concentrations will increase. A plateau is reached when the thickness of the deposits exceeds the maximal penetration of the electrons at the accelerating voltage. Except for very thick deposits, therefore, there is a positive correlation between deposit size and apparent concentration.