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Surface alkaline hydrolysis of 2-hydroxyethyl methacrylate gels

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The surface modification of glycol methacrylate intraocular lenses by selective alkaline hydrolysis was studied in order to find the conditions for improvement in biocompatibility and simultaneous preservation of good optical properties of these gels. It was found that the thickness of the modified layer can be influenced by the reaction temperature, NaOH concentration and reaction time. Using at least 30% NaOH, short reaction times and temperatures of at least 90 °C, lenses with good optical properties and with carboxylic groups in the surface layer were prepared. The method may be useful for obtaining hydrophilic medical materials with improved properties and increased biocompatibility.

1. Introduction

In a series of papers [1], it was shown that biocompatibility of polymers may be improved under some circumstances significantly by introducing carboxylic groups into the materials. The copolymers of 2-hydroxyethyl methacrylate with ethylene dimethacrylate (HEMA-EDMA) and methacrylic acid (MMA) in animals have been tested [2–7] and implantation of a similar copolymer containing 1% of MMA into the human eye has been successful [8]. On the other hand, an increased swelling of the modified materials in water or physiological milieu and the resulting refractive index decrease led in some applications, e.g. for intraocular lenses, to less satisfactory results. The conciliation of contradictory requirements, i.e. increased biocompatibility and simultaneous preservation of good optical properties might be achieved with materials in which the bulk would maintain the refractive index high enough while the surface, would, for example, consist only of carboxylic groups, ensuring high biocompatibility. The following methods for the preparation of such materials were considered:

(1) copolymerization of a mixture of MMA-EDMA into the surface layers of the optical member (formation of a "snake-cage" structure);
(2) chemical or radioactive grafting of MMA into the surface layers;
(3) surface hydrolysis of ester groups under the formation of carboxylate groups.

The last mentioned method of introducing carboxylate groups into the surface layers of the intraocular lenses is reported in this paper.

2. Experimental procedures

2.1. The preparation of G (glycerol)-type intraocular lenses

A mixture of 2-hydroxyethyl methacrylate (HEMA) (99.7 g), ethylene dimethacrylate (EDMA) (0.3 g), glycerol (20 g), and diisopropyl peroxycarbonate (0.4 g) was injected under CO_2 atmosphere into polypropylene moulds (radius 4.5 mm), in which the mixture was polymerized for 2 h at 60 °C and for 2 h at 70 °C. The lenses were extracted in boiling water under stirring for 60 h to remove soluble substances and then their optical power (22.5–22.7 dptr.) and swelling in water (38.5%) were determined. The lenses were dried on a Teflon plate in overheated steam at 120 °C to avoid deformations which occur using the usual drying procedures. Dried lenses were preserved in a desiccator over solid KOH.

2.2. Preparation of A-type intraocular lenses

The preparation was similar to the previous procedure except for the absence (A) of glycerol in the reaction mixture and the lenses were not extracted. Their swelling in water reached 37%.

2.3. Surface hydrolysis of intraocular lenses

Lenses of both types were hydrolysed in the same way: well dried lenses were placed in a perforated Teflon flask. The flask was heated for 20 min at temperatures of 90, 105 or 120 °C in a thermostat and then transferred to a stainless steel reactor containing 16 ml of 30 or 50% aqueous NaOH. The reactor was intensively stirred to ensure good exchange of liquid in the flask

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and removal of air bubbles. The hydrolysis was quenched by immersing the flask in ice-cold distilled water and the contents washed successively with 0.001 M HCl and distilled water.

2.4. Determination of the extent of hydrolysis

A swollen lens was placed into a holder made of a thin polypropylene membrane fixed in a special lid of a microtitration flask provided with a magnetic stirrer. The lid housed the glass (Radiometer G2222) and the calomel (Radiometer K4112) microelectrodes, three delivery tubes (for 0.001 M NaOH, 0.001 M HCl and CO_2-free water), a degassing tube provided with a magnetic valve, and two nitrogen inlet tubes, the first reaching to the bottom and provided with a magnetically operated vacuum branch and the second reaching above the level of the titrated sample. After removing air and filling the apparatus with nitrogen, the titration vessel was charged with 3.0 ml of 0.001 M NaOH and the contents were magnetically stirred for 10 min. Then a titration curve with 0.001 N HCl was scanned using the TitraLab 42 titrator until consumption of 3.5 ml of the acid, thus ensuring complete transformation of carboxylate ions. The solution was then sucked off and the contents of the titration vessel were washed three times with 8.00 ml of degassed water followed by a perfect removal of liquid. The procedure was repeated at least twice.

2.5. Microscopy

The G-type lenses were conditioned for at least 24 h in a phosphate buffer saline (PBS), cut to 0.5 mm thick slices and stained for 20 min with Ruthenium Red (0.1% in bidistilled water). The slices were washed with PBS at 4 °C for 30 days. The stained slices were cut into small pieces and treated with 1% osmium tetroxide. The samples were then routinely prepared for transmission electron microscopy (TEM).

2.6. Optical properties

The mean optical power of the starting and modified G-type lenses was measured in water using a contact lens analyser (Optimec, UK). A documator (Carl Zeiss, Jena, FRG) equipped with a measuring grid was used for determination of the maximal thickness of the modified layer of G-type lenses.

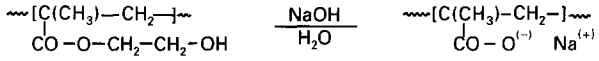
2.7. Biological testing

The lenses made from HEMA-EDMA copolymer (untreated or modified with NaOH) were subcutaneously implanted into the rat as previously described [2]. The implants were removed 9 days after surgery and the cells colonizing the implant surface were studied after staining with hematoxylin-eosin.

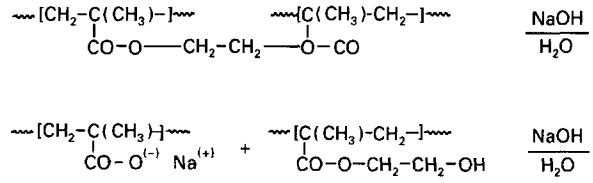
3. Results and discussion

The intraocular mould cast lenses prepared either in the absence (A-type lenses) or in the presence of glycerol (G-type lenses) were used for surface modification by alkaline hydrolysis. Before hydrolysis, the G-type lenses were extracted with boiling water to remove the solvent as well as the other soluble substances and then thoroughly dried in overheated steam at 140 °C to preserve perfect lense shape and optical properties.

For introducing carboxyl groups into the lense surface the preparation of a snake-cage structure, the surface grafting of methacrylic acid on to the HEMA–EDMA skeleton or the surface hydrolysis of ester groups, can be used. The third method, related to our previous studies of alkaline hydrolyses [9] and chemical transformations [10] of glycol methacrylate gels, seemed the most promising and therefore alkaline hydrolysis was chosen. Previous results showed that in all chemical transformations of glycol methacrylate gels the ester bonds, through which the pendent 2-hydroxyethyl or 2-(2-hydroxyethoxy)ethyl groups are bound to the main skeleton, are transformed, unlike crosslink ester bonds [9, 10]. In the fully swollen state, the crosslink ester groups were not hydrolysed by alkali before the pendent ester [9] (scheme 1a, b).



Scheme 1a Hydrolysis of HEMA structure unit in HEMA–EDMA copolymer



Scheme 1b Hydrolysis of EDMA structure unit in HEMA–EDMA copolymer

The same reaction selectivity was observed with other transformations [10] of the ester groups.

The reaction conditions of alkaline hydrolysis were chosen so as to result in the formation of a high concentration gradient between the modified and unreacted material: enhanced temperature, high concentration of NaOH, and compact state of the polymer (not previously swollen). The experimental results corroborated the assumption that, under these conditions, the reaction rate of hydrolysis would surpass the diffusion rate of NaOH into the polymer, which would result in the formation of a high concentration gradient of carboxylate groups.

The extent of hydrolysis was estimated from the results of indirect determinations of carboxyl groups (back titration with 0.001 M HCl of an excess of 0.001 M NaOH). The modified layer of the lens behaves as a weak acid cation exchanger and can be brought repeatedly into its H^+ form with an excess of 0.001 M HCl. The accuracy of the determination of the carboxyl content in lenses was not worse than 10%. The microtitration method can be used successfully for the determination of carboxylic groups if their amount exceeds 100 nM.

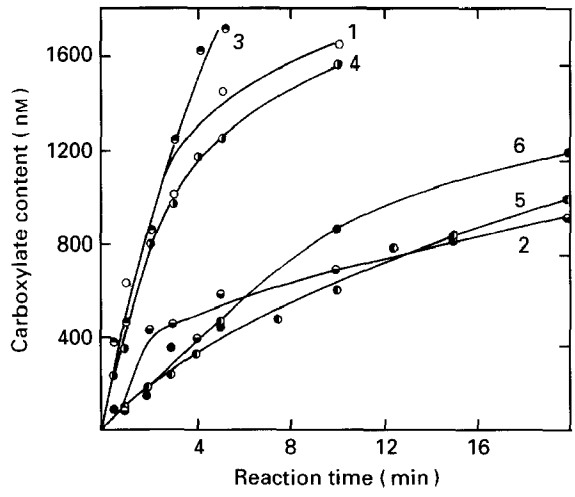


Figure 1 The content of carboxylate groups (nM) in the surface layer of the modified intraocular lens as a function of the type of lens (A, G), temperature (°C), reaction time (min) and concentration of NaOH (%): 1, 2 – type A; 3–6 – type G; 1, 3 – 120°C; 2, 6 – 90 °C, 4 – 105°C. 1–5 – 30% NaOH, 6 – 50% NaOH.

The courses of the hydrolysis of both G-type lenses and A-type lenses are presented in Fig. 1 as the dependence of the amount of carboxylic groups on the time of contact of the lens with the NaOH solution (30 or 50%) at 90, 105 and 120 °C. For A-lenses, the temperature dependence of hydrolysis with 30% NaOH can be seen from the comparison of curves 1 and 2 for 90 and 120 °C (Fig. 1). For G-type lenses, analogously, the temperature dependence can be followed from the comparison of curves 3, 4 and 5 at 90, 105 and 120 °C (Fig. 1). The increase in temperature enhances the degree of hydrolysis very substantially. Fig. 1 shows that G-type lenses, which were synthesized in the presence of glycerol, are more sensitive to NaOH at 120 °C than A-lenses. When the amount of carboxylic groups reached about 1700 nM, the modified surface layers are peeled off due to the swelling pressures exerted by carboxylate ions, and thus artefacts are formed which deteriorate the optical properties of the lenses. Contrary to expectations, the A-lenses undergo a faster transformation than the G-type lenses at the very beginning of the reaction carried out at 90 °C (compare curves 2 and 5 in Fig. 1). The carboxylic groups content in both types of lenses reach the same value (800 nM) after 13 min, due to the fact that the rate decrease with increasing conversion is more pronounced with the A-lenses. At contents above 800 nM the G-type lenses react more quickly.

The influence of the concentration of NaOH at 90 °C is demonstrated by curves 5 (30% NaOH) and 6 (50% NaOH): hydrolysis with 50% NaOH is faster at reaction times greater than 2 min.

The main parameters of G-type lenses (swelling in water, dioptric power after swelling in physiological solution and in 0.5% $NaHCO_3$ solution) were determined after extraction of soluble substances with water. The values for each lens were used as reference values for the properties of the modified lens. The modified layer is well observed at the edge of the lense in the direction of the optical axis and when the carboxylic groups are fully ionized, i.e. immediately after hydrolysis or after the reaction of $NaHCO_3$ solution with lenses in the H^+ form. With regard to the much higher degree of swelling of the modified phase, its refractive index is lower, so that the modified layer can be observed at the lens edge as a light ring and measured using a documator with a measuring grid. At higher conversions, corrugated edges (the result of osmotic pressure and differences between the swelling capacities of unreacted and modified materials) can be observed with the modified layer in the Na^+ form. The light edges disappear if the carboxylate ions are converted into carboxyl groups. This is because of a small difference between the swelling capacities (and refractive indices) of the material with carboxylic groups in the surface layer and the bulk material.

The results of the edge thickness measurements of the modified layer in 0.5% $NaHCO_3$ solution are presented in Figs 2–5. They confirm its increase with reaction time: the layer formed after 30 s modification of the lens with 30% NaOH at 90 °C shows a thickness of 0.05 mm. It grows almost linearly with increasing conversion and reaches 0.7 mm after 20 min (see Fig. 2). A similar, almost linear dependence was found with 30% NaOH at 105 °C; also here the edge thickness after 20 min rose to 0.7 mm. A different behaviour was observed at 120 °C: on the one hand, the edge thickness increased in the expected way (see Fig. 4), i.e. after 5 min modification, it was already 0.4 mm (cf. Figs 2 and 3); on the other hand, after 7.5 min, the modified layer began to peel off due to strong swelling forces.

The optical boundary lines observed in the edge thickness measurements arose as a consequence of the sharp concentration gradients of carboxyl groups. Direct evidence of gradients was given by microscopic observations of the sections in which the carboxyl groups areas were selectively stained by means of Ruthenium Red. The electronoptical analysis demonstrated that a 5-min modification with 30% NaOH at 90 °C yields a product with a very steep carboxyl group gradient (Fig. 6). The Ruthenium Red electrondense surface layer is about 2.5 μm thick (in the dry state).

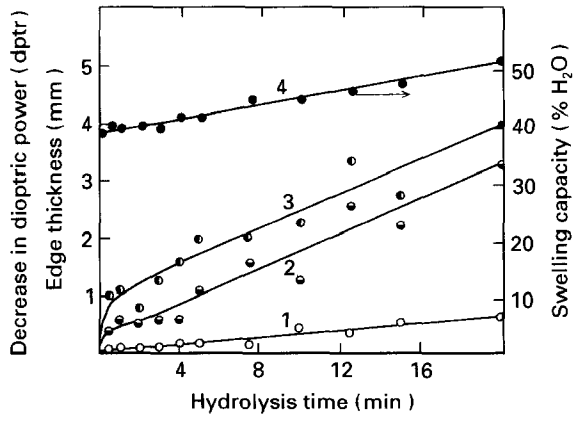


Figure 2 The properties of intraocular G-type lenses as a function of the hydrolysis time (min) (30% NaOH, 90 °C): 1 – edge thickness (mm) of the modified layer; 2 – decrease in the dioptric power (dptr) of the modified lens swollen in 0.9% NaCl solution; 3 – decrease in the dioptric power (dptr) of the modified lens swollen in 0.5% $NaHCO_3$ solution; 4 – swelling capacity in water (% of water).

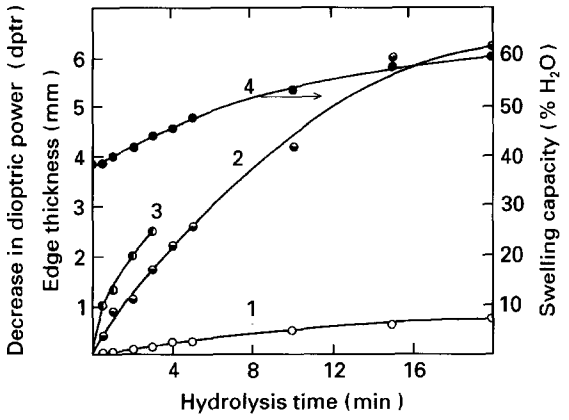


Figure 3 The properties of intraocular G-type lenses as a function of the hydrolysis time (min) (30% NaOH, 105 °C): 1–4, see Fig. 2.

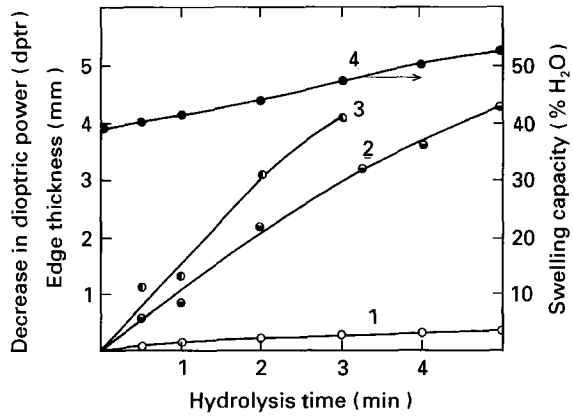


Figure 4 The properties of intraocular G-type lenses as a function of the hydrolysis time (min) (30% NaOH, 120 °C): 1–4, see Fig. 2.

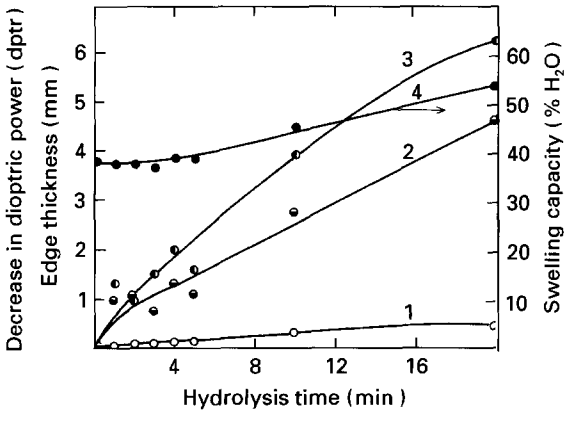


Figure 5 The properties of intraocular G-type lenses as a function of the hydrolysis time (min) (50% NaOH, 90 °C): 1–4, see Fig. 2.

The courses, similar to the time dependence of the edge thickness (Fig. 2), were measured at various temperatures (Figs 3, 4) and also for enhanced concentration of NaOH (Fig. 5). Similarly monotonous courses were measured for time and temperature dependences of the swelling capacity increase and dioptrical power loss (more pronounced in 0.5% $NaHCO_3$ than in physiological solution) (Figs 2–5).

The results show that the modified layer thickness can be controlled by the reaction time, temperature and concentration of hydrolytic agent. The practically useful range of conditions is (as judged according to the decrease in the dioptical power of the modified lens and the sharpness of the optical cross in the focometer) short to very short reaction times, high concentration of alkali (30% NaOH at least) and increased reaction temperatures (90 °C at least).

The important feature of the successful alkaline modification of the glycol or diglycol methacrylate gels is the selectivity of hydrolysis, which, under mild conditions, takes place only at pendent 2-hydroxyethyl- or 2-(2-hydroxyethoxy)ethyl groups. We have already mentioned that the hydrolysis of ester groups in crosslinks takes place after the pendent groups have been spent. The lower the reaction temperature, the higher the selectivity of hydrolysis.

This is a contradictory requirement to that of a high reaction rate in comparison with the diffusion of the hydrolytic agent inside the solid material in order to keep the highest reaction gradient. The selectivity of the hydrolytic reaction enables the formation of an insoluble modified gel on the surface of the lens. The modified gel is anchored to the unmodified material by the original crosslinks, which, due to the selectivity of the reaction, survived the severe conditions of the modification reaction. Due to these crosslinks, the modified gel does not dissolve in water or a physiological milieu, but it swells, maximum swelling occurring with ionized carboxyl groups and with weak ionic strength of the solvent. With highly modified gels, osmotic forces cause peeling of the surface layers, as was observed at 120 or 105 °C.

The gels modified by alkali differ from those prepared by "radiation-induced grafting by methacrylic acid" (RIGMA) as a result of perfect stability due to the chemical bonds of the modified gel to the main skeleton of the starting material. The physicochemical stability of carboxyl groups in the alkali-modified lenses are demonstrated by excellent stability of the staining, which could not be washed out by PBS for prolonged periods, in contrast to the behaviour of RIGMA lenses. RIGMA lenses immediately after "grafting" show, to some extent, the tendency to be stained diffusively, but this staining is weak and vanishes in a short time as soon as the stained PMAA is

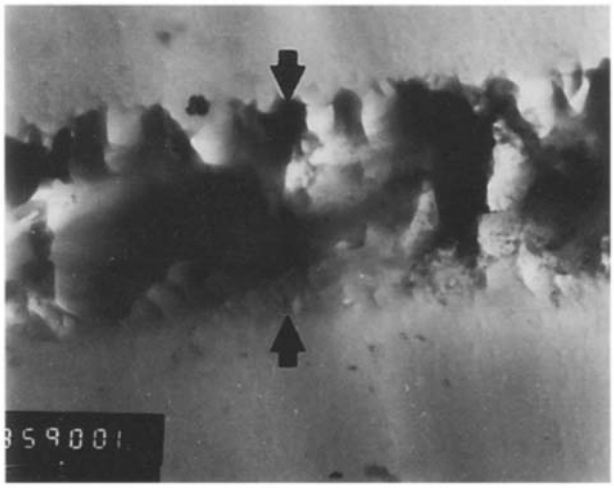


Figure 6 Ruthenium Red positive layer (between arrows) in NaOH hydrolysed lens. Magnification × 10000.

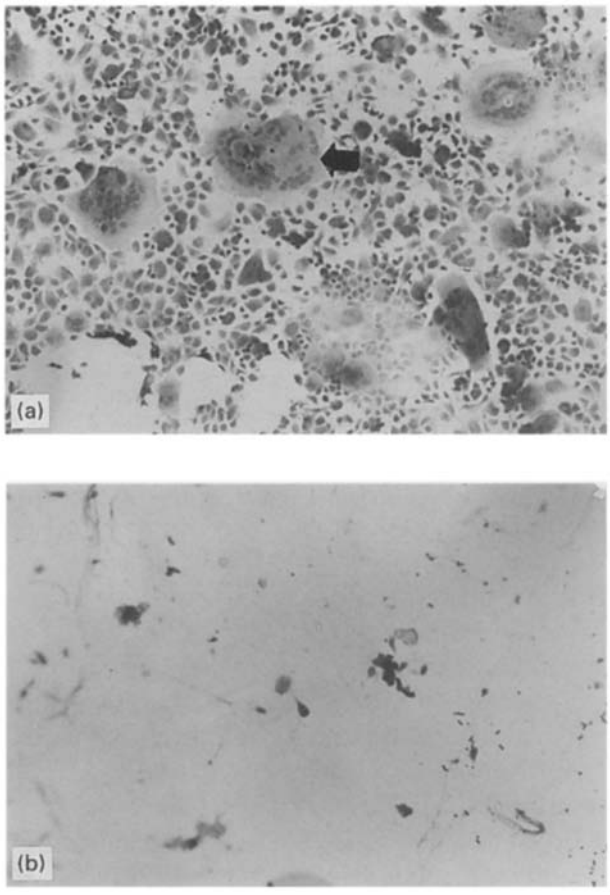


Figure 7 Surface of unmodified HEMA–EDMA lens (a) and NaOH-hydrolysed lens (b) after implantation (foreign body giant multinucleate cells (arrow)). Specimen stained with hematoxylin-eosin. Magnification × 120.

washed out from the lenses into the PBS. The solubility of the "grafted" poly(methacrylic acid) suggests that MMA has been only homopolymerized in the surface layers of the lenses and has not been grafted on to the skeleton. The washed RIGMA lenses could no longer be stained, which showed again that no MMA units remained grafted on to the washed lens (not shown). On staining, the alkali-modified lenses showed an intensive coloration with Ruthenium Red and a sharp boundary line between the modified and starting materials (Fig. 6), thus proving that the reaction rate of hydrolysis was high enough to follow the penetration of the hydrolytic agent.

The surface of NaOH-treated G-type lenses was colonized with poorly spread mononuclear macrophages without the occurrence of foreign body giant multinucleate cells (Fig. 7). This result contrasted with a high incidence of these multinucleate cells on the surface of the untreated HEMA–EDMA implants (Fig. 7). Our previous results [6, 7] showed a similar cytological appearance of the foreign body reaction, i.e. the absence of foreign body multinucleate cells on the surface of copolymer HEMA–EDMA–MMA (3% of MMA). However, the swelling of this copolymer is so extensive that its use for intraocular lens construction is impossible. The results obtained indicate favourable biocompatibility of NaOH-treated HEMA–EDMA copolymer for these applications because it is generally accepted that poorly tolerated implants induce the fusion of macrophages into multinucleate cells [10].

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