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Rapid Communication

Degradation of polybutyl 2-cyanoacrylate microparticles

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Summary

Polybutyl 2-cyanoacrylate microparticles were incubated in buffer solution at pH 3.0, 6.0 and 10.0 at room temperature and at 50°C, and in dog serum at 37°C. Complete degradation took place at pH 10.0 within 9 days (room temperature) or 24 h (50°C) and at pH 6.0 within 35 days (50°C). At pH 3.0 and 6.0 (room temperature) only slight degradation was observed. Almost complete hydrolysis occurred in dog serum within 3.5 h.

Polyalkyl 2-cyanoacrylates are often used as biodegradable tissue adhesives (Vinters et al., 1986) or lysosomotropic drug carriers in the form of nano- or microparticles (Couvreur et al., 1979; Couvreur, 1988). The monomer, which polymerizes by an anionic mechanism in aqueous medium, is prepared by Knoevenagel reaction of formaldehyde with an ester of cyanoacetic acid. The question quite often discussed in the literature is which pathway is followed during the degradation of the water-insoluble polymer (Leonard et al., 1966; Panl et al., 1968; Wade and Leonard, 1972; Vezin and Florence, 1980; Lenaerts et al., 1984). One possibility is an inverse Knoevenagel reaction which would lead to the water-soluble educts, the other is side chain hydrolysis which would yield alcohol and water-soluble poly-2-cyanoacrylic acid. The purpose of this study was to investigate the degradation pathway of polybutyl 2-cyanoacrylate microparticles in aqueous solutions with different pH values and in dog serum.

Butyl 2-cyanoacrylate (BCA) was supplied by Schering, Germany 0.01 N HCl was prepared from a stock solution (Titrisol, Merck, Germany) by dilution with deionized water. Buffer solutions of pH 3.0 and 10.0 (Riedel de Haen, Germany) and chloroform A.R. (Merck, Germany) were used as recieved from the manufacturer. Dog serum was prepared from the whole blood of a beagle and used within a few hours of preparation.

The preparation of the microparticles was performed by dispersion of 1 ml BCA in 100 ml of 0.01 N HCl, containing 0.1% Poloxamer 407, using ultrasound. The particles were separated by centrifugation and washed several times with water. Eventually they were suspended in buffer solution pH 3.0 or 10.0, in deionized water pH 6.0, each containing 0.1% Poloxamer 407, or in dog serum. The volume distribution of the particle sizes was determined by laser light scattering using a Master Sizer (Malvern, U.K.).

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For the quantitative determination of the BCA concentration of the suspensions, 1.0 ml of microparticle suspension was filtered through Millex FG 13 filter units (pore width: 0.2 μm, Millipore, Germany), washed three times with deionized water and dried. The residue was eluted from the filter with 1.0 ml chloroform. This solution was assayed by FT-IR spectroscopy (FT-IR spectrometer 710, Nicolet, U.S.A.) using the area of the ester bond between 1710 and 1825 cm^{-1} as a measure of the cyanoacrylate concentration (equation of the calibration line: area = 1.355 · concentration + 0.002, $r = 0.999$).

For the degradation experiments in aqueous solution, the microparticle suspensions and the corresponding particle-free media were stored at 50°C and at room temperature for 35 days. Immediately after preparation of the suspensions, after 15, 24 and 43 h and after 9 and 35 days, samples were withdrawn and filtered through 0.2 μm PTFE filter (Sartorius, Germany). The concentration of butanol was determined by GC (GC 5890, Hewlett Packard, U.S.A.) and expressed as % of $butanol_{max}$, the highest amount of butanol that could be found theoretically at a given mass of BCA.

The microparticle suspension in dog serum was incubated at 37°C for 3.5 h and assayed for butanol as described.

The suspensions consisted of cyanoacrylate particles with sizes mostly below 6 μm. The volume distribution showed a $d_{50\%}$ and $d_{90\%}$ of 2.4 and 5.9 μm, respectively.

At pH 3 and room temperature, a small amount of butanol was released after 35 days, while at 50°C 3.5% of the theoretically possible butanol was formed. At pH 6 and room temperature, as in the case of pH 3, 0.2% of butanol had been formed. Complete degradation occurred at 50°C. At pH 10 complete hydrolysis had taken place at room temperature after 9 days and at 50°C after 24 h. Butanol was not detectable in the particle-free media (limit of detection: 0.5 ppm). These data are summarized in Table 1, 82% of the highest theoretical amount of butanol was found after incubation of the microparticles in dog serum for 3.5 h. Pure dog serum and microparticle suspension contained no butanol (limit of detection: < 2 ppm). The degradation of the microparticles could be followed also by changes in the appearance of the suspension. An undegraded suspension appeared milky white (pH 3 and pH 6/room temperature) whereas degraded suspension was nearly transparent (pH 6/50°C and pH 10).

It is obvious from this study that a relationship between pH value, storage temperature and degradation of cyanoacrylate exists.

The higher the pH value, the more rapidly does degradation take place. The degradation rate is further enhanced by elevated temperature. These findings support the opinion of some authors that the degradation of cyanoacrylate follows hydrolysis of the butyl ester side chain rather than a breakdown of the backbone (inverse Knoevenagel reaction). Whether the latter took place was not examined in this study. Considerable degradation via inverse Knoevenagel reaction must, however, have led to destruction of microparticles, which would have changed the

TABLE 1

Formation of butanol from polybutyl 2-cyanoacrylate microparticles stored under different conditions

Storage conditions	Concentration of cyanoacrylate (mg/ml)	Concentration of butanol expressed as % of $butanol_{max}$					
		T_0	15 h	24 h	43 h	9 days	35 days
pH 3//r.t.	1.1	–	n.d.	–	–	0.1	0.2
pH 3/50°C	1.1	n.d.	–	0.1	0.2	1.3	3.5
pH 6/r.t.	1.2	0.8	n.d.	0.8	0.2	0.3	0.2
pH 6/50°C	1.2	n.d.	1.4	1.4	1.4	39.7	103.0
pH 10/r.t.	0.9	9.5	n.d.	55.9	66.1	98.0	99.1
pH 10/50°C	0.9	n.d.	92.5	102.5	105.2	107.0	106.1

r.t., room temperature; –, below limit of detection; n.d., not determined.

appearance of the suspension from milky white to transparent, as was the case at high pH. From the fact that this did not occur at pH 3 and pH 6/room temperature and that butanol was formed under the other three storage conditions, it can be concluded that backbone degradation does not play an important role in the degradation mechanism of cyanoacrylate in aqueous medium.

This is also true for degradation in serum. Almost complete hydrolysis was observed within 3.5 h. If the polymer had been degraded in serum only by pH-dependent hydrolysis, a much slower rate would have been expected on the basis of the results observed for degradation in the buffer solutions. Thus, it is likely that an enzymatic mechanism, such as that for an unspecific hydrolase, is involved in the degradation.

It can be concluded that BCA microparticles are degraded in aqueous medium by side chain hydrolysis and that this is also the main degradation pathway in dog serum. The temperature and pH value have a strong influence on the degradation rate. This rate is further enhanced in dog serum, probably by an enzymatic mechanism.

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