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Notes

Influence of enzymes on the stability of polybutylcyanoacrylate nanoparticles

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Summary

The influence of three different enzymes (amylase, pepsin and esterase) on the stability of polybutylcyanoacrylate (PBCA) nanoparticles was studied. The study was performed as an in vitro investigation using side-by-side diffusion cells. A suspension of ^{14}C-labelled PBCA nanoparticles was placed into the donor chamber of the diffusion cell and incubated with the above-mentioned enzymes. Esterase caused a highly significant degradation of the nanoparticles that was proportional to the amount of the enzyme put into the donor chamber. Amylase and pepsin had no significant effect on the stability of the PBCA nanoparticles.

Peroral application is the most convenient way of drug administration especially in long-term treatment. However, many drugs are unstable in the gastrointestinal (GI) tract or are insufficiently absorbed, especially highly potent protein or peptide drugs. Investigations are under way to overcome these problems employing drug delivery systems such as PBCA nanoparticles. In order to be successful, oral drug carriers, such as PBCA nanoparticles, must fulfill two requirements: first, they should be stable enough in the GI tract to protect the incorporated drug; second, they must improve the absorption of the drug from the GI tract.

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Earlier studies have shown that cyanoacrylate nanoparticles (Maincent et al., 1986; Kreuter, 1991) and nanocapsules (Damgé et al., 1987, 1988, 1990) improve the absorption of several drugs like vincamin or insulin from the GI tract. Maincent et al. (1986) demonstrated the efficiency of PBCA nanoparticles as a drug delivery system for the GI tract by improving the absorption of vincamin by more than 60% compared to an equimolar solution.

Investigation of the parameters that influence the stability of nanoparticles in the GI tract is much more difficult because of the complexity of the GI tract. The pH changes from pH 2 in the stomach to pH 7–8 in the small intestine. Moreover, there are different enzymes at different parts of the GI tract and these enzymes are found in varying concentrations. Degradation studies with cyanoacrylate nanoparticles were performed

as early as 1972 (Wade et al.). Lenaerts et al. (1984) used rat liver microsomes to incubate polyisobutylcyanoacrylate (PBCA) nanoparticles. These microsomes contain a mixture of different enzymes, and thus it was difficult to determine which enzyme takes part in the degradation process. To help delineate the role of enzymes and related conditions in stability of PBCA nanoparticles, an in vitro study was undertaken using three enzymes with variation of the pH. Pepsin was chosen because it is the major enzyme in the stomach. Amylase was selected since dextran is used as a stabilizer during the polymerisation of PBCA nanoparticles and is partially incorporated into the nanoparticles (Douglas et al., 1985). Thus, the degradation of dextran could influence the stability of the nanoparticles. Esterase was employed because hydrolysis of the ester side chain is the main route of degradation of polyalkylcyanoacrylate nanoparticles (Couvreur et al., 1984).

Polybutylcyanoacrylate (PBCA) nanoparticles were prepared by emulsion polymerisation, according to a method described by Kreuter (1983). 500 μl of butylcyanoacrylate monomer (Sichel-Werke Hannover, Germany) was added dropwise to a solution of 50 ml 0.01 N HCl containing 500 mg dextran 70000 (Sigma, St. Louis, U.S.A.). The solution was stirred with a magnetic stirrer for 4 h. The resulting suspension was buffered with 0.1 N NaOH to pH 6. The radiolabelled PBCA nanoparticles were obtained from Amersham (U.K.). The preparation was performed in the same way as described above. However, in the latter case, butyl 2-[3-C^{14}]cyanoacrylate was used as the monomer. The radiolabelled particles were diluted with unlabelled particles to prepare a stock solution.

Butyl 2-[3-^{14}C]cyanoacrylate was chosen as monomer in order to ensure that the detected radioactivity was the result of a massive disintegration process of the nanoparticles. The labelling of the 2-butanol side chain leads to much less conclusive results because splitting of the 2-butanol at the surface of the PBCA nanoparticles leads to detection of a significant amount of radioactivity even if the nanoparticles remain intact.

0.5 ml of a PBCA nanoparticle suspension containing 0.1 MBq was added to 4.5 ml buffer solution in the donor side of Higuchi-type side-by-side diffusion cells (Precision Instruments, U.S.A.). The temperature was kept at 37°C and the cells were stirred vigorously. Each run was repeated six times ($n = 6$). Buffer solutions (USP) with pH values of 2, 5, 6, 7 and 8 were used and all buffer substances were of analytical grade (pH 2, hydrochloric acid buffer; pH 5, phthalate buffer; pH 6, 7 and 8, phosphate buffers). Amylase and pepsin (Sigma, U.S.A.) were added at concentrations of 100 IU to the nanoparticle suspension while esterase (Sigma, U.S.A.) was added at concentrations of 25, 50 and 100 IU, respectively. Drug-free PBCA nanoparticles consist of a number of oligomeric subunits with molecular weights of 600–3000 (Vansnick et al., 1985; Couvreur et al., 1986). Therefore, a dialysis membrane (Dianorm, Germany) with a molecular weight cutoff of 10000 was used to separate the donor and receiver side of the diffusion cell in order to allow passage of only the low molecular weight degradation products while intact particles and the enzymes were held back. After 60, 120, 180 and 240 min samples were taken out of the receiver side and replaced by fresh buffer. The samples were mixed with Biosafe II scintillation cocktail (Beckman, U.S.A.) and the amount of radioactivity was determined by liquid scintillation counting using a Beckman Scintillation Counter (Beckman, U.S.A.). The degree of degradation of the PBCA nanoparticles into low molecular weight products was calculated from these results.

The study showed significant degradation of PBCA nanoparticles at 37°C dependent upon the pH of the buffer solution. At pH 2 and 5 about 2.4% of the radioactivity appeared in the acceptor side of the diffusion cell after 4 h. This amount rose to 4.1% at pH 6 and about 8.5% at pH 8 (Fig. 1).

The addition of pepsin (Fig. 2) as well as of amylase (Fig. 3) to the buffer solution had no significant influence on the stability of the PBCA nanoparticles.

As mentioned above, dextran is partially incorporated into the cyanoacrylate polymer. For this

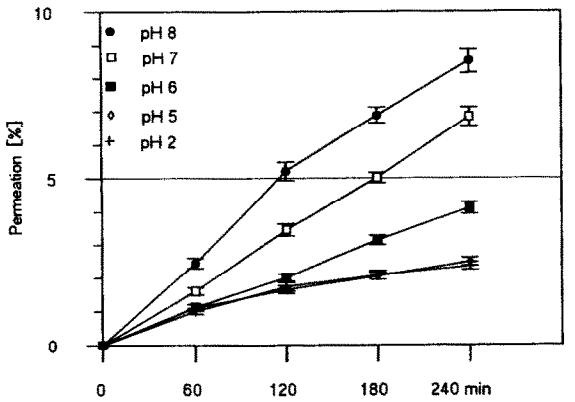

Fig. 1. Influence of pH on the stability of PBCA nanoparticles at 37°C (at pH 2, 5, 6, 7 and 8) ($n = 6$). The amount of radioactivity (cumulative in percent) that permeated into the receiver chamber is shown.

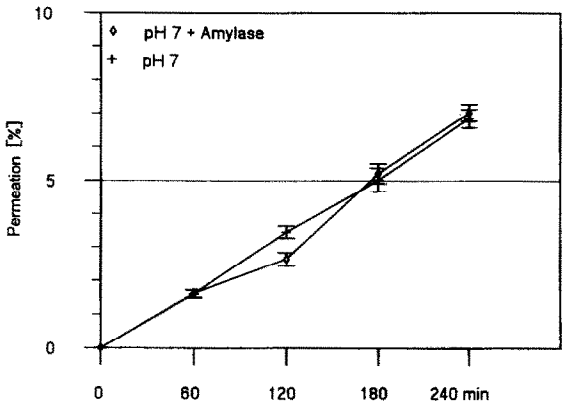

Fig. 3. Influence of the enzyme α-amylase (100 IU) on the stability of PBCA nanoparticles at 37°C and pH 7. Buffer solution with and without α-amylase was compared ($n = 6$). The amount of radioactivity (cumulative in percent) that permeated into the receiver chamber is shown.

reason, the influence of amylase on particle degradation was examined, since this enzyme could degrade incorporated dextran. However, the results show that dextran either was not incorporated to a significant degree or that its incorporation did not alter the regular non-enzymatic or esterase-dependent degradation pathway and velocity of this polymer in the form of nanoparticles because amylase cannot reach the incorporated dextran.

In contrast, esterase had a highly significant influence on the stability of the PBCA nanoparticles. The activity of the esterase depended on the pH of the buffer. At pH 6 about 6% of the radioactivity appeared on the receiver side, while after 4 h this value rose to 33.4% at pH 7 and 30.2% at pH 8 (Fig. 4).

These results show that the esterase demonstrated the highest activity of nanoparticle degradation at neutral pH values. In the slightly acidic environment of pH 6, esterase is only slightly effective. In contrast, considerable degradation occurs at pH 7 and 8. Experiments using different concentrations of esterase (25, 50 or 100 IU,

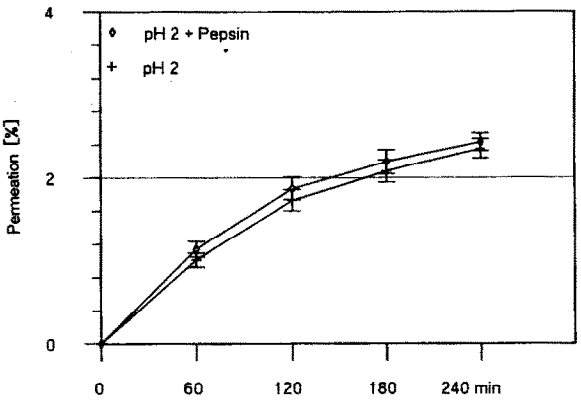

Fig. 2. Influence of the enzyme pepsin (100 IU) on the stability of PBCA nanoparticles at 37°C and pH 2. Buffer solution with and without pepsin was compared ($n = 6$). The amount of radioactivity (cumulative in percent) that permeated into the receiver chamber is shown.

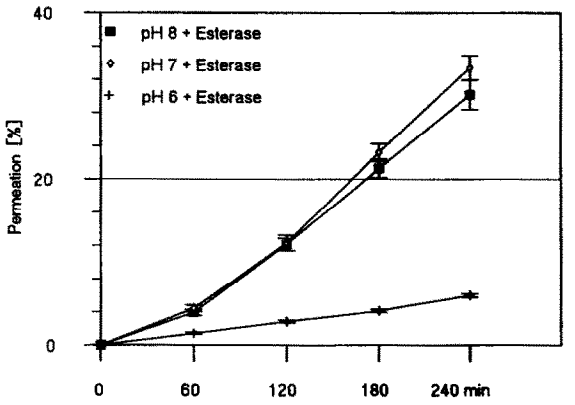

Fig. 4. Influence of the enzyme esterase (100 IU) on the stability of PBCA nanoparticles at 37°C and pH 6, 7 and 8 ($n = 6$). The amount of radioactivity (cumulative in percent) that permeated into the receiver chamber is shown.

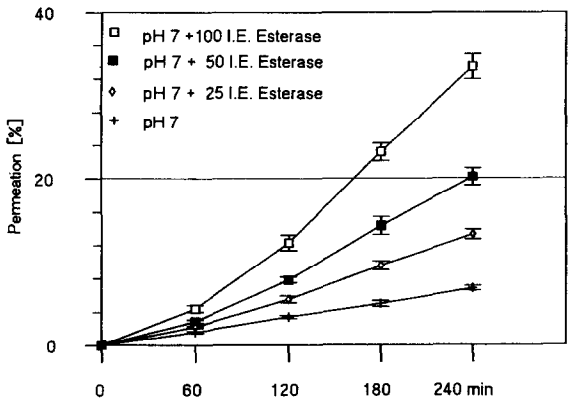

Fig. 5. Influence of different concentrations of the enzyme esterase (25, 50 and 100 IU) on the stability of PBCA nanoparticles at 37°C ($n = 6$). The amount of radioactivity (cumulative in percent) that permeated into the receiver chamber is shown.

respectively) showed that the degradation of the PBCA nanoparticles was proportional to the amount of esterase present (Fig. 5).

The experiments showed that there is a lag time of about 1 h before massive degradation due to esterase occurs. The results of this study suggest that the concentration of the esterase is the crucial factor for the stability of PBCA nanoparticles in the GI tract. Esterase is released by the pancreas into the lumen of the small intestine, however, a major part is located in the apical membrane of the gut wall. This suggests that degradation of PBCA nanoparticles does not occur before they reach the small intestine – the major site of absorption of drugs.

Even at the very high concentrations of esterase used in this study, the PBCA nanoparticles remained about 90% intact for 2 h. Therefore, PBCA nanoparticles are stable enough to protect drugs against degradation until they reach the major site of drug absorption.

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References

Couvreur, P., Lenaerts, V., Leyh, D., Guiot, P. and Roland, M., Design of biodegradable polyalkylcyanoacrylate nanoparticles as a drug carrier. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds), *Microspheres and Drug Therapy,* Elsevier, Amsterdam, 1984, pp. 103–115.

Couvreur, P., Lenaerts, V., Ibrahim, A., Grislain, L., Vansnick, L., Guiot, P., Verdun, C. and Brasseur, F., Targetable nanoparticles. In Gregoriadis, G., Senior, J. and Poste, G. (Eds), *Targeting of Drugs with Synthetic Systems,* Plenum, New York, 1986, pp. 147–164.

Damgé, C., Michel, C., Aprahamian, M. and Couvreur, P., Nanocapsules as carriers for oral peptide delivery. *J. Controlled Release,* 13 (1990) 233–239.

Damgé, C., Aprahamian, M., Balboni, G., Hoeltzel, A., Andrieu, V. and Devissaguet, J.P., Polyalkylcyanoacrylate nanocapsules increase the intestinal absorption of a lipophilic drug. *Int. J. Pharm.,* 36 (1987) 121–125.

Damgé, C., Michel, C., Aprahamian, M. and Couvreur, P., New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carrier. *Diabetes,* 37 (1988) 246–251.

Douglas, S.J., Illum, L. and Davis, S.S., Particle size and size distribution of poly(butyl-2-cyanoacrylate) nanoparticles. II. Influence of stabilizers. *J Colloid. Interface. Sci.,* 103 (1985) 154–163.

Kreuter, J., Evaluation of nanoparticles as drug-delivery systems: I. Preparation methods. *Pharm. Acta Helv.,* 58 (1983) 196–209.

Kreuter, J., Peroral administration of nanoparticles. *Adv. Drug Del. Rev.,* 7 (1991) 71–86.

Lenaerts, V., Couvreur, P., Christiaens-Leyh, D., Joiris, E., Roland, M., Rollmann, B. and Speiser, P., Degradation of poly(isobutylcyanoacrylate) nanoparticles. *Biomaterials,* 5 (1984) 65–68.

Maincent, P., Le Verge, R., Sado, P., Couvreur, P. and Devissaguet, J.P., Disposition kinetics and oral bioavailability of vincamine-loaded polyalkyl cyanoacrylate nanoparticles. *J. Pharm. Sci.,* 75 (1986) 955–958.

Michel, C., Aprahamian, L., Defontaine, P., Couvreur, P. and Damge, C., The effect of site of administration in the gastrointestinal-tract on the absorption of insulin from nanocapsules in diabetic rats. *J. Pharm. Pharmacol.,* 43 (1991) 1–5.

Vansnick, L., Couvreur, P., Christiaens-Leyh, D. and Roland, M., Molecular weights of free and drug-loaded nanoparticles. *Pharm. Res.,* 2 (1985) 36–41.

Wade, C. and Leonard, F., Degradation of poly-(methyl-2-cyanoacrylates). *J. Biomed. Mater. Res.,* 6 (1972) 215–220.