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INTERFACIAL POLYMERIZATION, A USEFUL METHOD FOR THE PREPARATION OF POLYMETHYLCYANOACRYLATE NANOPARTICLES

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ABSTRACT

An interfacial polymerization procedure was developed for the preparation of polymethylcyanoacrylate (PMCA) nanoparticles loaded with triamcinolone acetonide. The nanoparticles were characterized concerning their interior structure, size distribution, drug content, drug release and in vivo distribution. These results (except those for the in vivo distribution) were compared with those obtained with nanoparticles prepared by micell polymerization [5]. Both preparation procedures yielded particles with a mean diameter below 500 nm. The drug content of the nanoparticles prepared

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by interfacial polymerization ranged from 6,5 % w/w to 1,9 % w/w depending on the employed monomer concentration in contrast to 0,045 % w/w for nanoparticles prepared by micell polymerization [5]. In comparison to microcrystalline substance the drug release from the nanoparticles could be sustained in all cases, but there was no difference in drug release between the nanoparticles prepared by both methods.

After removal of surface adherent drug from nanoparticles prepared by both methods those prepared by interfacial polymerization had an about 12 times higher drug content and the remaining drug amount was released more slowly by these particles. Furthermore, using increasing monomer concentrations during interfacial polymerization (125 - 500 mg/100 ml emulsion) drug release was slowed down, but no further improvement could be achieved for monomer concentrations exceeding 250 mg/100 ml emulsion.

After intravenous injection of ^{99m}Tc labeled PMCA nanoparticles into rats they accumulated predominantly in liver, spleen and kidney, a distribution pattern usually found for colloidal particles.

INTRODUCTION

The idea of delivering drugs to specific target organs has prompted many attempts in modifying the

organ distribution of drugs in vivo [1-4]. One possibility to achieve this goal are polyalkylcyanoacrylate nanoparticles, a colloidal drug carrier system which was developed by Couvreur et al. [5,6]. These authors employed the spontaneous polymerization of monomeric alkylcyanoacrylates in the presence of water to adsorb drugs (mainly cytostatics) onto the surface of the particles. Hence the water solubility of the drug is important for achieving high drug contents, because its lipophilicity limits the payload.

The scope of the present investigation was to evaluate the feasibility of interfacial polymerization to produce nanoparticles loaded with a lipophilic drug and to compare their features with nanoparticles produced according to Couvreur [5]. Triamcinolone acetonide was used as a model compound because a further possible application - besides their systemic application - is local treatment of certain diseases as for instance asthma or arthritis.

MATERIALS AND METHODS

Preparation of PMCA Nanoparticles by Interfacial Polymerization A w/o emulsion consisting of 40 ml isooctan with 0,5 % w/w Span 85 (Atlas Chemie, Essen, W. Germany) (outer phase) and 2 ml methanol (inner phase)

was prepared by sonication with an ultra sonic device (Branson Sonifier Cell Disrupter, B 15, Branson Schallkraft GmbH, Heusenstamm, W. Germany). The inner phase was alkalized by shaking with solid sodium carbonate to obtain a pH of about 7 - 8. Furthermore, 20 mg of triamcinolone acetonide (TrA) (von Heyden GmbH, München, W. Germany) and a trace amount of ^{3}[H] TrA (New England Nuclear, Boston, Mass., USA) were dissolved in the methanol by gentle heating. This inner phase was added to the sonicated outer phase assuring a quantitative emulsification by placing the inner phase directly beneath the ultra sonic tip. During the emulsification step (30 min.) the mixture was maintained below a temperature of 15 $^{\circ}$C with an ice bath.

The formed emulsion was then diluted by an equal amount of outer phase, transferred into another vessel and stirred by a two blade stirrer at about 2000 rpm. To this emulsion 5 ml of chloroform containing 100, 200 or 400 mg of monomeric methylcyanoacrylate (Serva, Feinbiochemika, Heidelberg, W. Germany) was added in one aliquot. The spontaneous polymerization was allowed to proceed for 5 min, the resulting nanoparticles were separated by centrifugation at 15 000 rpm for 30 min. .

The pellets were redispersed in 0,5 % w/w Tween 20 solution (Atlas Chemie, Essen, W. Germany) with an ultra turrax (Janke und Kunkel GmbH, Staufen, W. Germany) and

again centrifuged. After repeating this procedure twice with water the nanoparticles were freeze-dried (freeze drier GT 2, Heraeus GmbH, Hanau, W. Germany) for about two days.

Preparation of PMCA Nanoparticles by Micell Polymerization The performed procedure was adopted from Couvreur et al. [5]. Briefly, to 5 ml 0,1 N hydrochloric acid 45 ml of a 0,5 % w/w Tween 20 solution which contained 25,4 µg TrA/ml and trace amounts of ^{3}[H] TrA were added. During one minute 640 mg of monomeric methylcyanoacrylate was dropped into the stirred solution and agitated for further 30 min. .

The suspension was filtered through a fritted glass filter (9-15 µm pore size) and centrifuged at 15 000 rpm for 30 min. . After washing three times with water the nanoparticles were freeze-dried.

Size Distribution The size distribution of the nanoparticle preparations was determined by scanning electron microscopy (SEM). About 2,5 mg of dry nanoparticles were dispersed in water, nebulized and the fine drops captured onto aluminium foil. The foil was dried overnight, coated with a 10 nm thick gold layer in a sputter device (Balzers Union Sputter Device, Balzers Union, Liechtenstein) and examined in a SEM (SEM S 450, Hitachi Deshi GmbH, Rodgau, W. Germany). Photographs of the samples were taken at different magni-

fications and about 500 - 1000 particles were measured from the negatives (usually 10) with the aid of an automatic picture analyzer (MOP AM 03, Kontron Meßgeräte GmbH, Eching, W. Germany).

Sample Preparation for Transmission Electron Microscopy (TEM) About 5 mg of dry nanoparticles were suspended in 2 ml of a 1 % w/w ethanolic uranyl acetate solution and kept in this solution for 1 h. After centrifugation and washing with ethanol to remove excess uranyl acetate, the particles were incubated with different epon - (epon 812 : dodecenylsuccinic-acidanhydride : methylnadicanhydride : 2,4,6, tris-(dimethylaminoethyl)-phenol 16,2 : 10 : 8,9 : 0,53) (all chemicals from Serva Feinbiochemika, Heidelberg, W. Germany) - ethanol mixtures in the following sequence: epon : ethanol 1 : 3 for 2h; epon : ethanol 2 : 2 for 24 h; epon : ethanol 3 : 1 for 24 h; pure epon for 24 h. After each incubation step the particles were sedimented by centrifugation, the supernatant was discarded, fresh embedding solution added and the particles were resuspended. After the last step the nanoparticles were suspended in pure epon and polymerized at 60 ^{o}C for 2 days.

The polymerized blocks were cut into about 100 nm thick samples with an ultra microtome (Ultrotome Typ 4802 A, LKB instruments, Great Britain) equipped with

a diamond knife. The cuts were contrasted with a saturated solution of uranyl acetate in 50 % w/v ethanol for 1 h followed by a lead citrate solution for 2 min. and then examined in a transmission electron microscope (EM 9, Zeiss, Oberkochen, W. Germany).

Drug Content and Drug Release Experiments The amount of encapsulated drug was determined by liquid scintillation counting (Packard Tricarb 300, Packard Instruments, Downers Grove, USA). Quench correction was performed by the external standard method and the counting efficiency was generally greater 30 %.

The drug release experiments were performed by two different approaches. To examine the drug release of nanoparticles with surface adherent drug, a membrane filtration method was employed. An amount of particles which was equal to 50 µg TrA was dispersed with the aid of an ultra sonic device in 10 ml of phosphate buffer (pH 7,35, 37 ^{o}C). The vessel was rotated at 25 rpm, and at different time intervals 1 ml of the suspension was filtered through a 0,1 µm membrane filter (Nucleopore Corp., Pleasanton, CA, USA). The amount of released drug was determined by liquid scintillation counting in a defined volume of the filtrate. During the whole release experiments sink conditions (drug concentration below 10 % w/v of saturation concentration) were maintained. To examine the drug release over extended periods of

time a dialysis procedure was employed. The particles were suspended in phosphate buffer, filled into dialysis bags and dialyzed against 7 ml of buffer (pH 7,35, 37 °C, 25 rpm). After each sampling time dialysis bags were put into fresh buffer, and the amount of released drug was calculated from the measured radioactivity.

Labeling of Nanoparticles with ^{99m}Tc To a suspension of 1,5 mg particles in 3 ml water 1 ml of pertechnetate solution (10 mCi) was added. The TcO_4^- was reduced by 0,1 mg of $SnCl_2$ dissolved in 0,1 ml of water. The efficiency of the procedure was checked by TLC on cellulose (methylethylketone : methanol 8:2), and it was confirmed that the labeling yield was greater than 90 % and that the label was stable over 3 h.

In Vivo Distribution Rats were given intravenously a suspension of ^{99m}Tc labeled nanoparticles. After 2h the animals were sacrificed, the whole organs (liver, spleen, kidney, lung, thyroid gland) and weighted amounts of bone marrow, muscle, bone and 1 ml of blood were removed and the radioactivity was measured in a γ-counter

RESULTS AND DISCUSSION

Preparation of the Nanoparticles After freeze-drying the described process yielded in all cases a light, white

powder which was dispersible in water forming a milky suspension which displayed the Tyndall effect. As can be seen in Tab. 1 the yield was independent of the amount of monomeric methylcyanoacrylate, which was used for the preparation of the particles. Furthermore, the yield of the interfacial polymerization process was significantly higher than the yield of the micell polymerization [5]. This was due to large agglomerates which were formed during dropping of the monomer into the drug solution. These agglomerates were removed by filtration and caused the reduced yield.

For the interfacial polymerization, the inner phase has to have an alkaline pH in order to allow a rapid and complete polymerization. Wood et al. [7] reported that they were able to produce stable microcapsules using 0,05 N sodium carbonate solution as the inner phase. The pH of the inner phase was found to be important because at pH 9 hydrolyzation of the freshly formed polymer occured. Therefore, a pH of 7 - 8 was found to be appropiate because polymerization was rapid and no degradation of the polymer could be observed.

Electron microscopic Investigations SEM examinations of suspended PMCA nanoparticles revealed small particles with a spherical shape (Fig. 1). To some extent the particles were aggregated because of the highly concentrated suspension used for sample preparation. There-

T A B L E 1

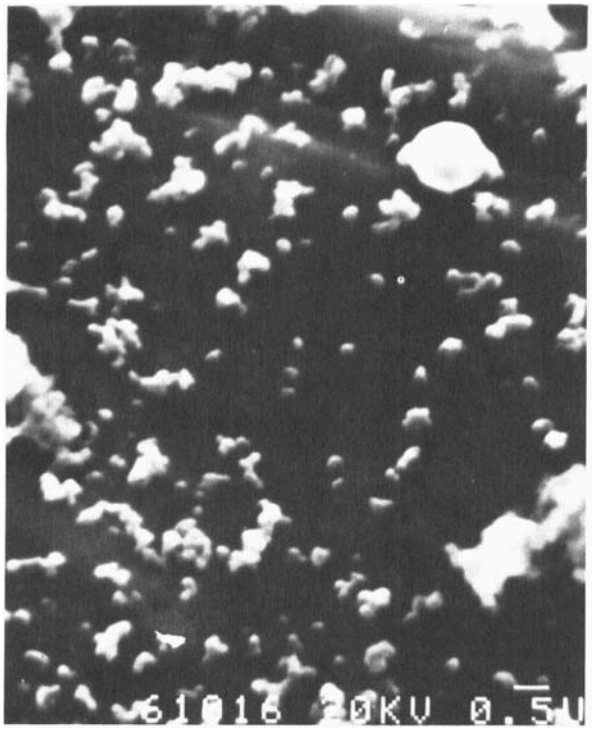
Features of PMCA Nanoparticles, n = 3

nanoparticle batch	yield[1] % w/w ± rel. S.D.	drug content % w/w ± rel. S.D.	encapsulation[2] efficiency % ± rel. S. D.	mean diameter[3] [nm] ± S.D.
PMCA 100	70,2 ± 21	6,5 ± 15	27,8 ± 26	90 ± 31
PMCA 200	79,4 ± 11	4,1 ± 21	36,3 ± 12	366 ± 93
PMCA 400	77,3 ± 11	1,9 ± 19	29,8 ± 15	298 ± 154
$PMCA_{Couv.}$ [5]	43,6 ± 8,5	0,045 ± 18	38,8 ± 31	184 ± 52,7

(1) $\text{yield} = \frac{\text{amount of employed polymer and drug}}{\text{amount of recovered nanoparticles}} \times 100$

(2) $\text{encapsulation efficiency} = \frac{\text{mg of drug bound by total amount of nanoparticles}}{\text{total amount of applied drug [mg]}} \times 100$

(3) n = 500 - 1000 measured particles

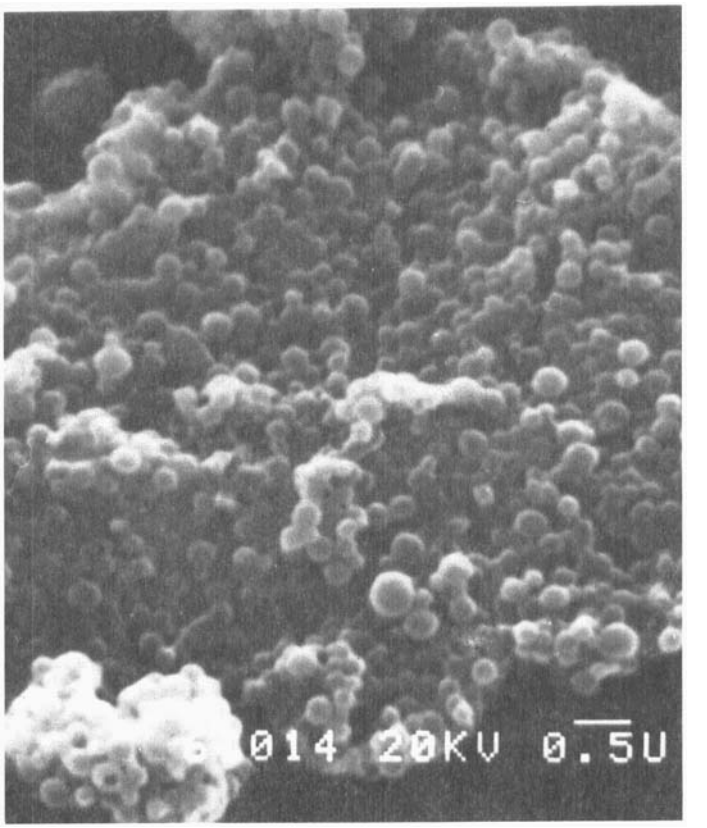


F I G U R E 1

SEM picture of nanoparticles prepared by interfacial polymerization, bar represents 0,5 µm magnification: 10 000 x

fore, during drying the particles stuck together, but filtration experiments showed that the nanoparticles passed through 0,8 µm membranes, if they were suspended in water.

The nanoparticles prepared according to Couvreur [5] were also spherical and had a smooth surface (Fig. 2). As discussed above these particles aggregated on the sample holder too but passed through 0,8 µm membranes. Couvreur et al. [5] showed that nanoparticles which were



F I G U R E 2

SEM photograph of nanoparticles prepared by micell polymerization [5], bar represents 0,5 µm magnification: 17 000 x

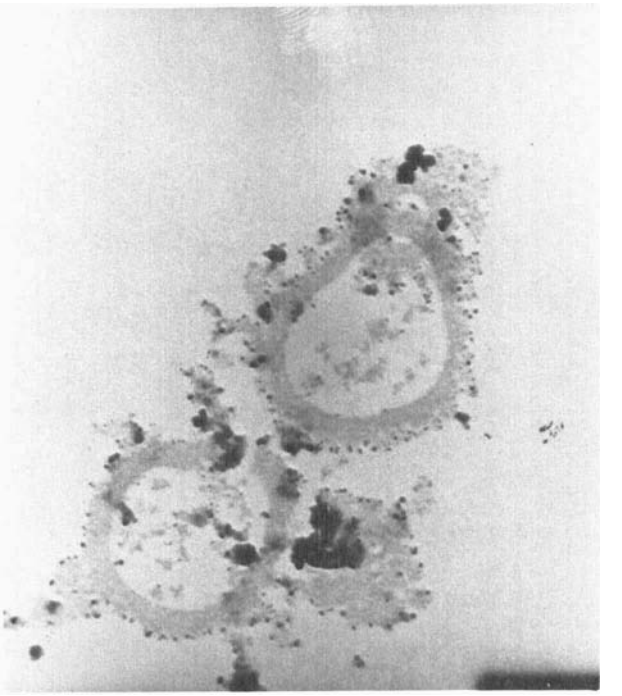
prepared by micell polymerization had a porous structure and that they could not find any capsules in their preparations [8]. In order to see whether the interfacial polymerization process led to an altered inner structure of the products the cut sections of nanoparticles were investigated by TEM. From these investigations it was shown that a mixture of particles and capsules were for-

med during the preparation (Fig. 3), but the particles were observed more frequently.

Madan et al. [9] who investigated the mechanism of polymerization for the preparation of microcapsules reported that in the case of the reaction between hydrophilic diamines and lipophilic diacid halides the polymer was formed in the organic phase, because the partition of the diamine into the organic phase was quicker than the diffusion and subsequent hydrolyzation of the diacid halide in the water. The products were thought to be capsules.

In the described process there existed only one diffusable and polymerizable substance, the monomeric methylcyanoacrylate. Due to its hydrophilic nature it will become enriched in the small hydrophilic drops. The formed polymer will precipitate after reaching a critical molecular weight. Experiments in an unstirred isooctan/methanol system showed that the precipitated polymer became enriched in the hydrophilic phase. During the preparation of nanoparticles the solvated polymer will accumulate in the drops forming new uncoated surfaces where further monomeric methylcyanoacrylate can polymerize. This may be the explanation for the favourable formation of particles instead of capsules.

Size Distributions In order to check whether the described process allowed the manufacture of nanoparticles



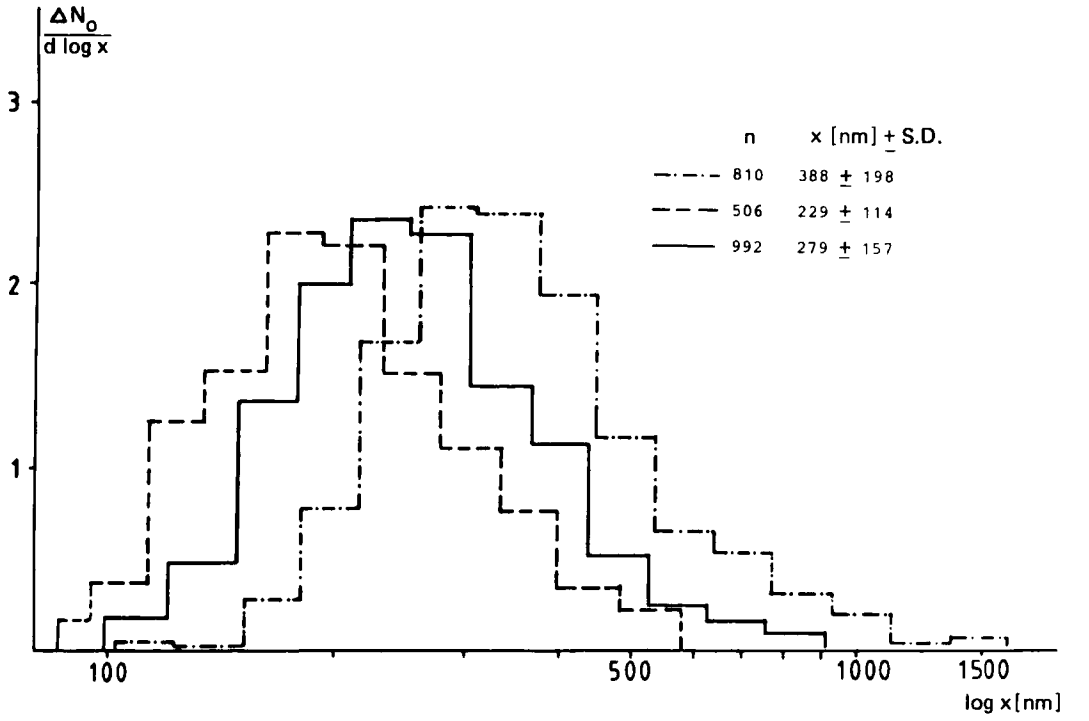
F I G U R E 3

1,1 µm

TEM picture of nanoparticles prepared by interfacial polymerization, showing capsules and particle, black spots are due to uranyl acetate used for contrasting, magnification: 40 000 x

in a reproducible way the size distributions of three separate batches were determined. Fig. 4 shows the size variation and the corresponding mean diameters. Transformation of the data into a logit/log diagram revealed that the particle sizes were log normal distributed with a correlation coefficient of $r > 0{,}995$.

The size distributions of the batches prepared with different monomer concentrations were shifted to greater



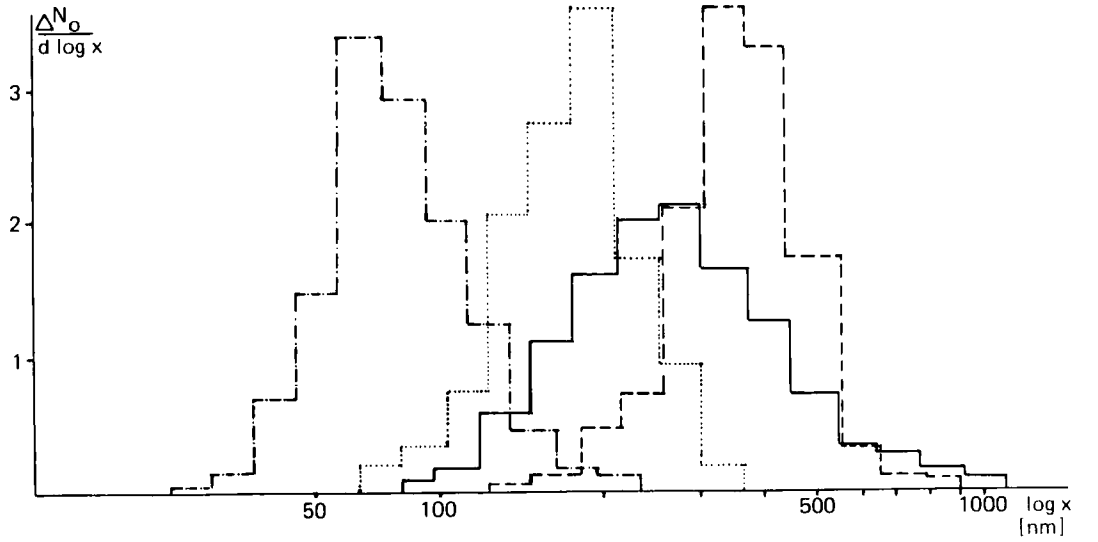
F I G U R E 4

Size distribution of three batches of nanoparticles
n= measured particles
x= mean diameter ± S.D.

diameters with increasing monomer concentrations. Furthermore, the distribution became broader which may be due to thicker particles or thicker capsule walls (Fig. 5 and Tab. 1).

The mean diameter of the nanoparticles prepared according to Couvreur [5] are in accordance with the values reported from this group [6].

Drug Content If nanoparticles should be used as a drug carrier their payload has to be high enough to minimize



F I G U R E 5

Influence of monomer concentration on size distribution

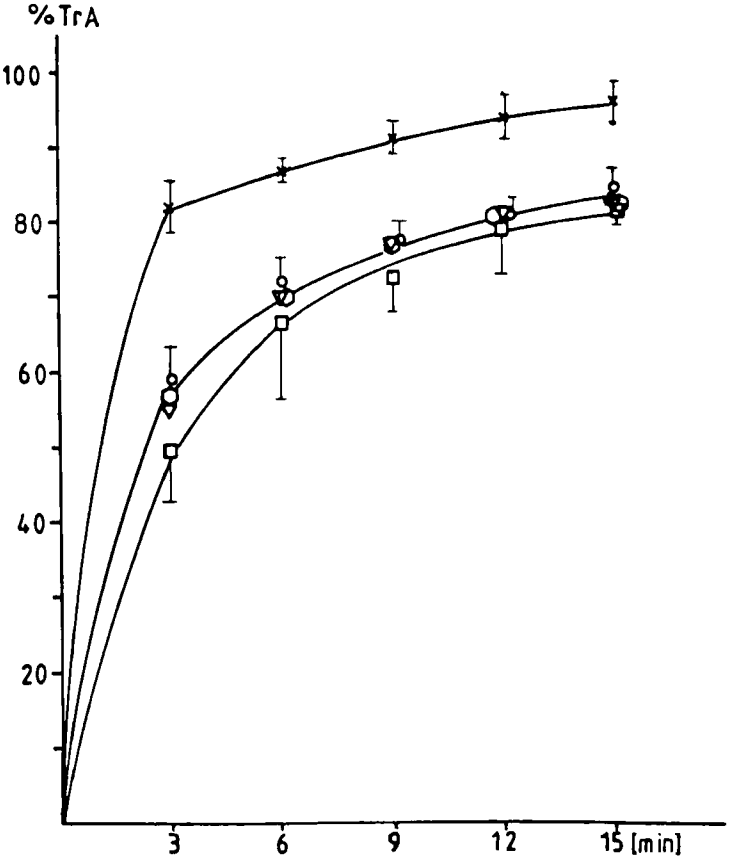
PMCA 100
PMCA 200
PMCA 400
PMCA Couv.[5]

the amount of carrier material which has to be administered Tab. 1 shows that the nanoparticles prepared by interfacial polymerization have a much higher drug content. In the micell polymerization process the low water solubility of the drug restricted a higher payload whereas in the interfacial polymerization process it was possible to select an appropriate solvent system so that drops of a highly concentrated drug solution were coated individually by the polymer.

Using higher monomer concentrations the drug content of the particles decreased because a higher amount of wall material encapsulated a constant amount of drug. Consequently the encapsulation efficiency was in a similar range for all monomer concentrations and for the nanoparticles prepared according to Couvreur et al. [5]. (Tab. 1).

Intense ultra sonic waves are known to induce degradation or chemical reactions of substances [10]. For this reason the encapsulated triamcinolone acetonide was investigated by TLC on silica gel (cyclohexane : ethylacetate : water 25 : 75 : 1) [11] after dissolving the particles in acetonitrile, but no degradation products could be detected.

Drug Release The results of the drug release experiments obtained by membrane filtration are shown in Fig. 6. All nanoparticles released the drug in an identical fashion with no significant difference. In comparison to micronized triamcinolone acetonide crystals (mean diameter 1,9 µm) the nanoparticles liberated the drug with a slower velocity than the crystals dissolved. Furthermore, there was no difference between batches of nanoparticles prepared with different monomer concentrations. Therefore, it was concluded that the quick drug release was caused by drug adsorbed onto the surface of the nanoparticles.



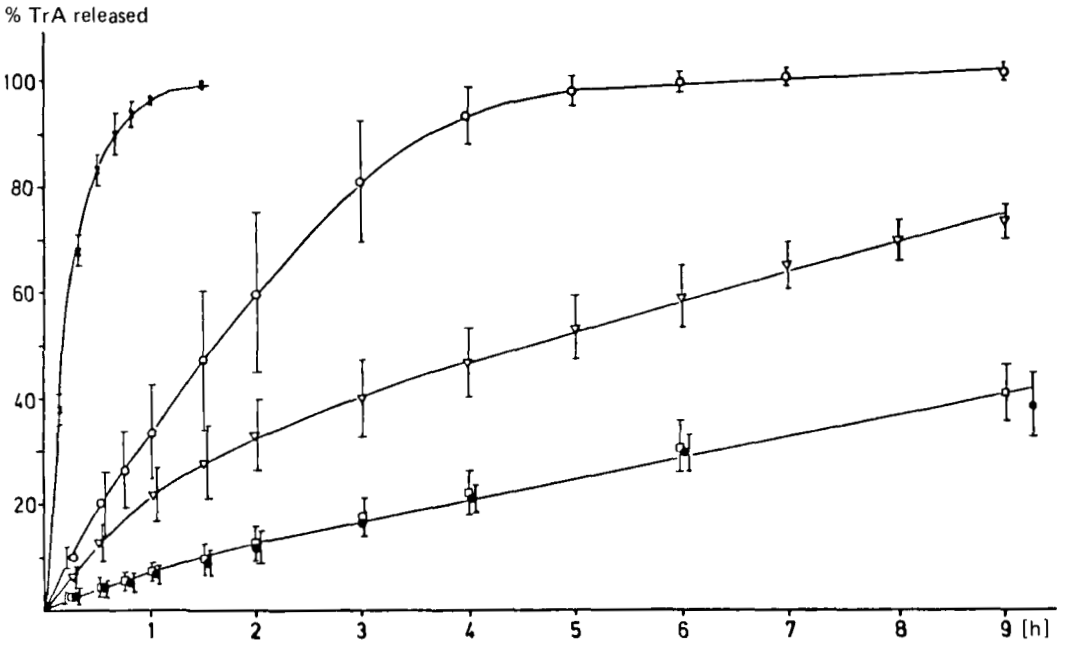
F I G U R E 6

Drug release of PMCA nanoparticles determined by membrane filtration (mean of 3 experiments ± S.D.)

PMCA 100
PMCA 200
PMCA 400
PMCA Couv.[5]
TrA crystals

In an attempt to eliminate this drug amount the PMCA nanoparticles were suspended in absolute ethanol, separated by centrifugation, again suspended in 50 % w/v ethanol, separated and freeze-dried. Using this approach the drug content dropped to about 0,2 % w/w (Tab. 2). Nevertheless, the nanoparticles prepared by interfacial polymerization had a drug content about 12 times higher than the nanoparticles produced by the procedure of Couvreur [5]. The results of the drug release experiments which were performed by dialysis are shown in Fig. 7. PMCA 100 nanoparticles released TrA more slowly than the nanoparticles produced according to Couvreur [5] but faster than the particles with higher monomer concentrations. Between PMCA 200 and PMCA 400 there was no difference concerning drug release. Furthermore, the drug release curves of PMCA 200 and PMCA 400 approached straight lines with $r_{PMCA\ 200}$ = 0,991 and $r_{PMCA\ 400}$ = 0,99.

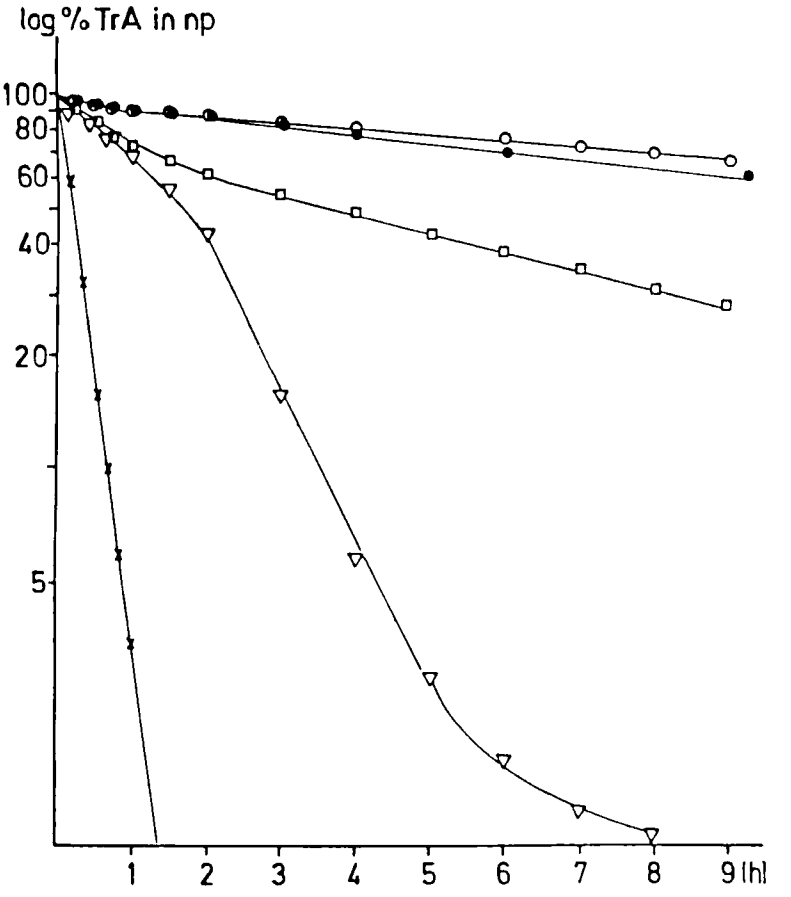
Further evaluation of the release data revealed that the release curves could be described more appropiate by first order kinetics (Fig. 8 and Tab. 2) as jugded from the correlation coefficients of the corresponding plots (Fig. 8 and Tab. 2). In order to investigate the mechanism of drug release the nanoparticles were examined by SEM after finishing drug release. The contents of the dialysis bags were prepared for SEM as des-



F I G U R E 7

Influence of monomer concentration on drug release of nanoparticles without surface adherent drug determined by dialysis (mean of 9 experiments ± S.D.)

cribed under materials and methods. No marked changes in the outer appearance of the nanoparticles prepared by interfacial polymerization could be observed (data not shown) in contrast to the nanoparticles prepared according to Couvreur [5] (Fig. 9). These particles were almost completly degraded, leaving large aggregates of polymer with an irregular shape. This rapid degradation



F I G U R E 8

First order plot of drug release from nanoparticles without surface adherent drug

PMCA 100
PMCA 200
PMCA 400
PMCA Couv. [5]
TrA crystals

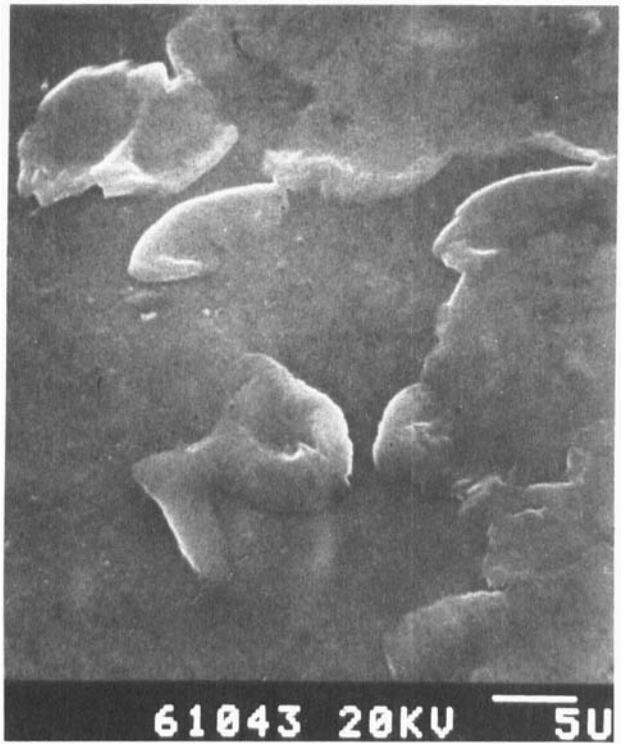
T A B L E 2

Drug Content and Drug Release of Nanoparticles without Surface adherent Drug

nanoparticle batch	drug content[1] % w/w ± rel. S.D.	time interval [h]	$k_{1.order}$ x 10^{-4} [$min.^{-1}$]	$r_{1.order}$[2]
TrA crystals	——	0 - 1,5	441	0,996
PMCA 100	0,225 ± 27	0 - 9	22	0,996
PMCA 200	0,258 ± 4,8	0 - 9	8,8	0,998
PMCA 400	0,197 ± 14	0 - 9	8,3	0,995
$PMCA_{Couv.}$[5]	0,016 ± 22,9	0 - 2	77	0,998
		2 - 5	134	0,999

(1) n = 9

(2) r = correlation coefficient



F I G U R E 9

SEM picture of nanoparticles prepared by micell polymerization after finishing drug release, bar represents 0,5 µm, magnification: 4 000 x

of the particles explained the rapid drug release which could be seen after 2 h (Fig. 8). Couvreur et al. [8] reported a porous structure for particles prepared by micell polymerization. Therefore, the surface for hydrolytic degradation, which the alkylcyanoacrylate polymers are known to undergo [12], is much larger than one would expect from the diameter of the particles. The reason for the slower degradation of PMCA 100, 200 and 400 nano-

T A B L E 3

Organ Distribution of ^{99m}Tc labeled PMCA Nanoparticles in Rats, 2 h after intravenous Injection

organ (whole)	% of injected dose
liver	48,8
spleen	0,55
kidney	4,7
lung	0,72
thyroid glands	0,045
bone marrow[1]	0,97
blood[2]	0,78
muscle[1]	0,042
bone[1]	0,82

(1) ,1 g of organ measured

(2) 1 ml of blood measured

particles may be due to the less porous structure restricting the hydrolytic degradation to the outer surface of the particles.

In Vivo Distribution Davis et al. [13] showed that the alteration of surface features of particles can change the organ distribution. Grislain [14] investigated the distribution of isobutylcyanoacrylate nanoparticles, a more stable and more hydrophobic polymer. For the determination of the in vivo distribution ^{99m}Tc labeled PMCA nanoparticles were administered intravenously to rats and the radioactivity of different organs was measured (Tab. 3). Like other colloidal particles these

nanoparticles accumulated predominantly in liver and kidneys (Tab. 3). Their in vivo distribution corresponded to the pattern found by Grislain [14] for isobutylcyanoacrylate nanoparticles. Thus nanoparticles may be useful drug carriers for targeting to the liver, particularly in the therapy of parasitic diseases or liver cancer.

CONCLUSIONS

Interfacial polymerization is a useful method for the preparation of polymethylcyanoacrylate nanoparticles. These particles, which have a similar size to those prepared by micell polymerization [5], are capable of carrying a high drug amount. Furthermore, drug release was substantially retarded in comparison to the other nanoparticles. In vivo distribution of PMCA nanoparticles confirmed that they could be valuable carriers for drugs in the therapy of liver diseases.

REFERENCES

(1) S. S. Davis, in "Optimization of Drug Delivery", Bundgaard, Hans, ed., Munksgaard, Copenhagen, 1983, p. 333

(2) R. L. Juliano, in "Drug Delivery Systems", R. L. Juliano, ed., Oxford University Press, New York, Oxford, 1980, p. 189

(3) G. L. Dale, in "Drug Delivery Systems", R. L. Juliano, ed., Oxford University Press, New York, Oxford, 1980, p. 237

(4) T. Yoshioka, M. Hashida, S. Muramish, et al., Int. J. Pharm., 81, 131, 1981

(5) P. Couvreur, M. Roland, P. Speiser, Belg. Patent, No. 869,107, 1978

(6) P. Couvreur, B. Kante, V. Lenaerts, et al., J. Pharm. Sci., 69, 199, 1980

(7) D. A. Wood, T. L. Whately, A. T. Florence, Int. J. Pharm., 8, 35, 1981

(8) P. Couvreur, B. Kante, M. Roland, et al., J. Pharm. Pharmacol., 31, 331, 1979

(9) P. L. Madan, Drug Develop. Ind. Pharm., 4, 289, 1978

(10) A. Weissler, The Journal of the Acoustical Society of America, 25, 651, 1953

(11) K. Florey, in "Analytical Profiles of Drug Substances", K. Florey, ed., Academic Press, New York, London, 1972, p. 397

(12) F. Leonard, R. K. Kulkarni, G. Brandes, et al., J. Appl. Polym. Sci., 10, 259, 1966

(13) S. S. Davis, P. K. Hansrani, in "Radionuclide Imaging in Drug Research", C. Wilson, ed., Croom Helm, London, 1982, p. 217

(14) L. Grislain, P. Couvreur, V. Lenaerts, et al., Int. J. Pharm., 15, 335, 1983