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Uptake of PMMA nanoparticles from the gastrointestinal tract after oral administration to rats: modification of the body distribution after suspension in surfactant solutions and in oil vehicles

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Abstract

Polymethyl (2-^{14}C) methacrylate nanoparticles of a diameter of 130 ± 30 nm were administered to Wistar rats as a single dose by oral gavage either in form of a suspension in saline, in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or with addition of 5% oleic acid. The animals were sacrificed after 30 min, 1, 2, 4, 8 h, 1, and 4 days, the blood was collected, and different organs and tissues were removed. The gastrointestinal (GI)-tract was separated into stomach, small intestine, and colon. The contents of those parts were collected and the remaining GI-tract sections thoroughly rinsed. The radioactivity in the above organs, tissues, and GI-tract contents were determined using a scintillation counter. The radioactivity concentrations were highest in the GI-tract content and decreased rather rapidly (between 2 h and 1 day). Rather high concentrations (up to 10% of the administered dose at a given time point) also were seen in the GI-tract walls. These concentration did not correlate totally to those in the GI-tract contents. The concentration in the residual body reached 1–3% of the administered dose at a given time point. The highest concentrations in the body were observed between 15 and 60 min but remained at considerable levels for 4 days. By far the highest uptake (about 200–300% of the other preparations) was seen with the saline preparation containing 5% polysorbate 80. No significant difference appeared between saline without surfactants and peanut oil. The addition of oleic acid to the peanut oil increased the uptake of the nanoparticles by about 50%.

Keywords: Body distribution; Nanoparticles; Oil and surfactant coating; Peroral administration

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1. Introduction

Oral drug administration represents the most convenient and common route of drug delivery. However, the bioavailability of a large number of drugs after oral administration is very low due to various reasons: too short gastric residences times, drug instability in the gastrointestinal tract, or lack of intestinal permeation of the drug. One possibility to improve the gastrointestinal uptake of perorally poorly absorbed drugs is their binding to colloidal particles, nanoparticles. Nanoparticles can protect labile molecules from degradation in the gastrointestinal tract and might be able to transport non-absorbable molecules into the systemic circulation. Drugs whose bioavailability was improved by binding to the particles include vincamine (Maincent et al., 1986), avarol (Beck et al., 1994), insulin (Damgé et al., 1988), azidothymidine (Löbenberg et al., 1997), and an HIV-protease inhibitor, CGP 578 13 (Leroux et al., 1995).

There are several reasons why nanoparticles can improve the bioavailability. One possibility is a simple stabilisation of the drug by the nanoparticles by preventing its precipitation in the intestinal interior. Another possibility is the bioadhesion and provision of direct contact with the absorbing membranes of the gut. The third possibility is uptake of the drug together with the particles across the gastrointestinal walls.

For a long time, the wall of the gastrointestinal tract was generally assumed to be an impermeable barrier to the passage of inert particulates. However, as early as 1844 the passage of large undissolved food particles from the intestinal lumen into the organism was reported (Herbst, 1844). At the beginning of this century intact starch particles were observed in the blood and the urine after peroral administration (Hirsch, 1906; Verzár, 1911). Later, a number of investigators showed that the passage of colloidal particles across the intestinal mucosa indeed is possible (Sanders and Ashworth, 1961; Volkheimer and Schulz, 1968; Volkheimer, 1977; LeFevre et al., 1978).

Three possibilities of uptake have been suggested: (1) uptake by a paracellular pathway; (2) intracellular uptake and transport via epithelial cells lining the intestinal mucosa; and (3) a lymphatic uptake via the M-cells and the Peyer's patches (Kreuter, 1991). Volkheimer (1977) reported that paracellular passage was the major mechanism of intestinal uptake of large particles (greater than 1 μm). The appearance of particles in the blood stream, 10 min after peroral dosing was extremely rapid. Sanders and Ashworth (1961) reported a paracellular uptake of polystyrene and Aprahamian et al. (1987) of polyalkylcyanoacrylate nanoparticles. Intracellular uptake involves an endocytotic uptake mechanism. This mechanism was proposed by Jani et al. (1989) as a second pathway for the intestinal uptake of 100 nm polystyrene nanoparticles. These results may be supported by findings of Kreuter et al. (1989) who observed a nanoparticle uptake by cells lining the intestinal mucosa.

The principal route of entrance for particulates, however, seems to be uptake by the gut-associated lymphoid tissues (GALT), represented by Peyer's patches. Uptake through the GALT was men-

Table 1
Percentage of organ and tissue weight of rats compared to total weight

Rat organs	Percent of whole body weight female/male
Eyes (−)	0.11
Small intestine and colon (*)	5.54
Subcutane fat (−)	5.0
Brain	0.9
Gonads	1.2/0.03
Skin (−)	18.0
Heart	0.34/0.43
Bones (−)	10.9
Bone marrow (*)	1.3
Liver	4.9/5.4
Lungs	0.74/0.62
Lymph nodes	ca. 0.02
Stomach (*)	0.65
Spleen	0.27
Muscles (smooth) (*)	5.0
Muscles (skeletal) (*)	40.0
Adrenal glands (−)	0.02
Kidney	0.92/0.88
Kidney fad (−)	4.0
Thyroid (−)	0.017
Blood (*)	5.0

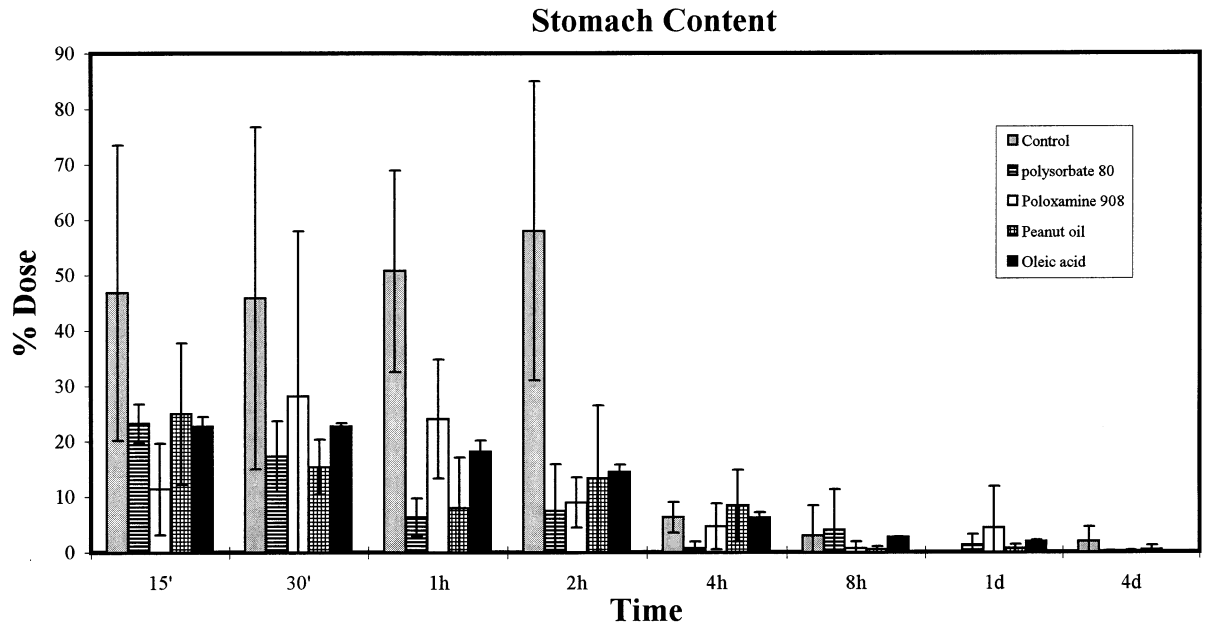

Fig. 1. Percentage of administered nanoparticle dose vs time in the stomach contents after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

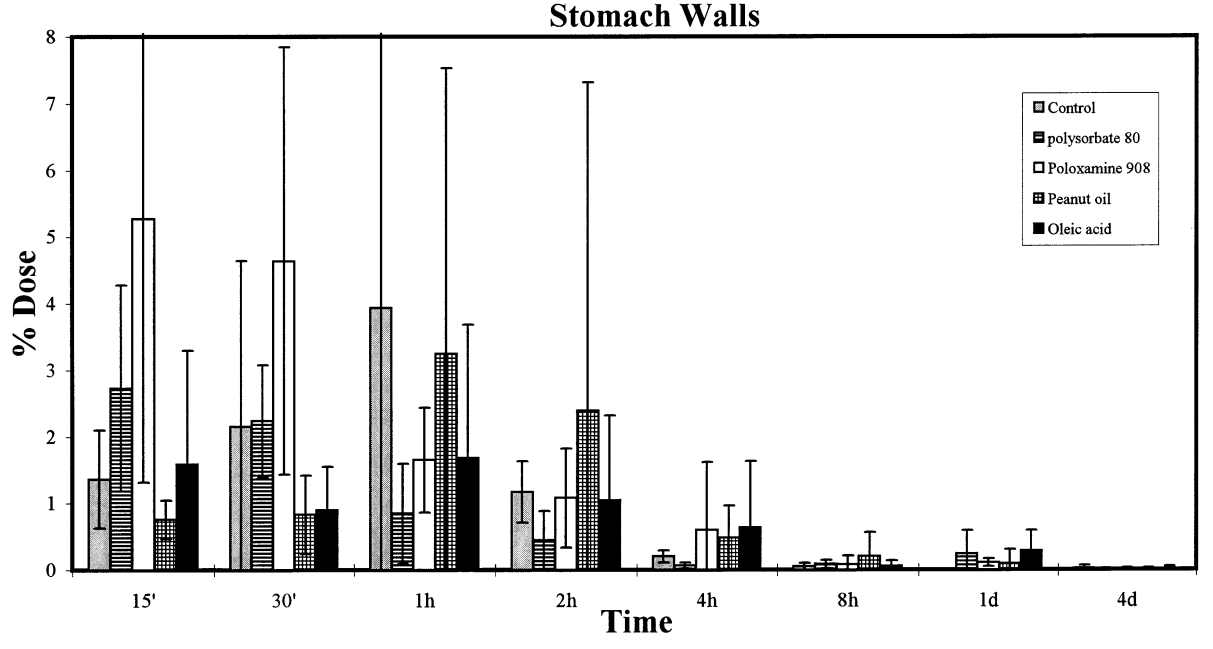

Fig. 2. Percentage of administered nanoparticle dose vs time in the stomach wall after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

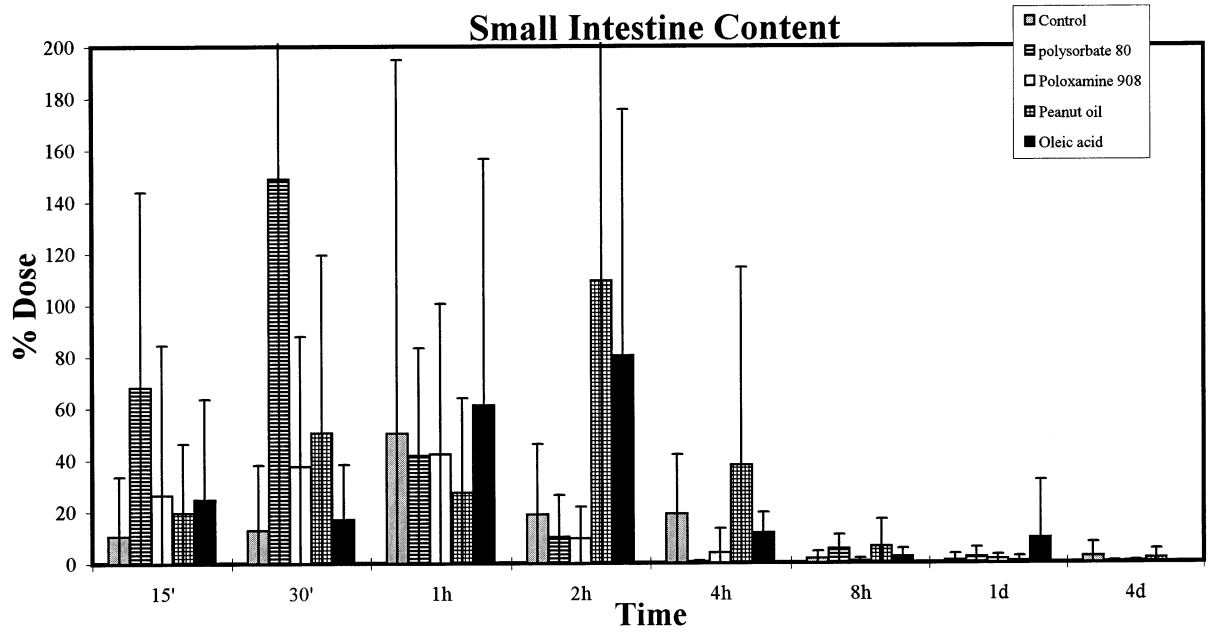

Fig. 3. Percentage of administered nanoparticle dose vs time in the small intestine after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

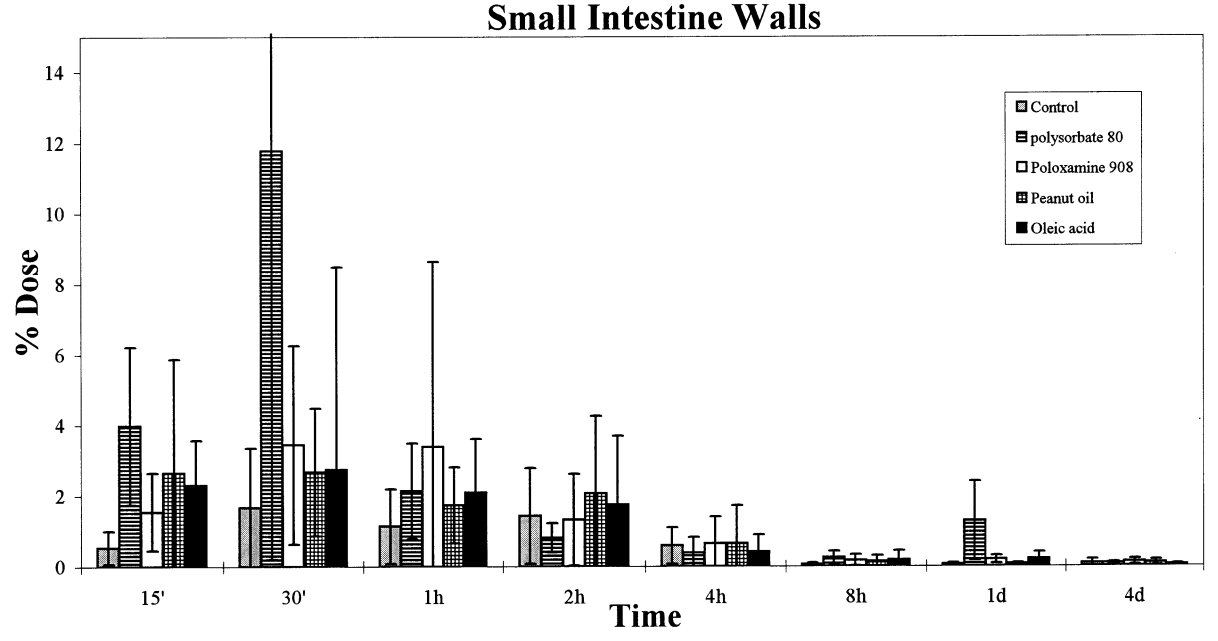

Fig. 4. Percentage of administered nanoparticle dose vs time in the small intestine walls contents after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

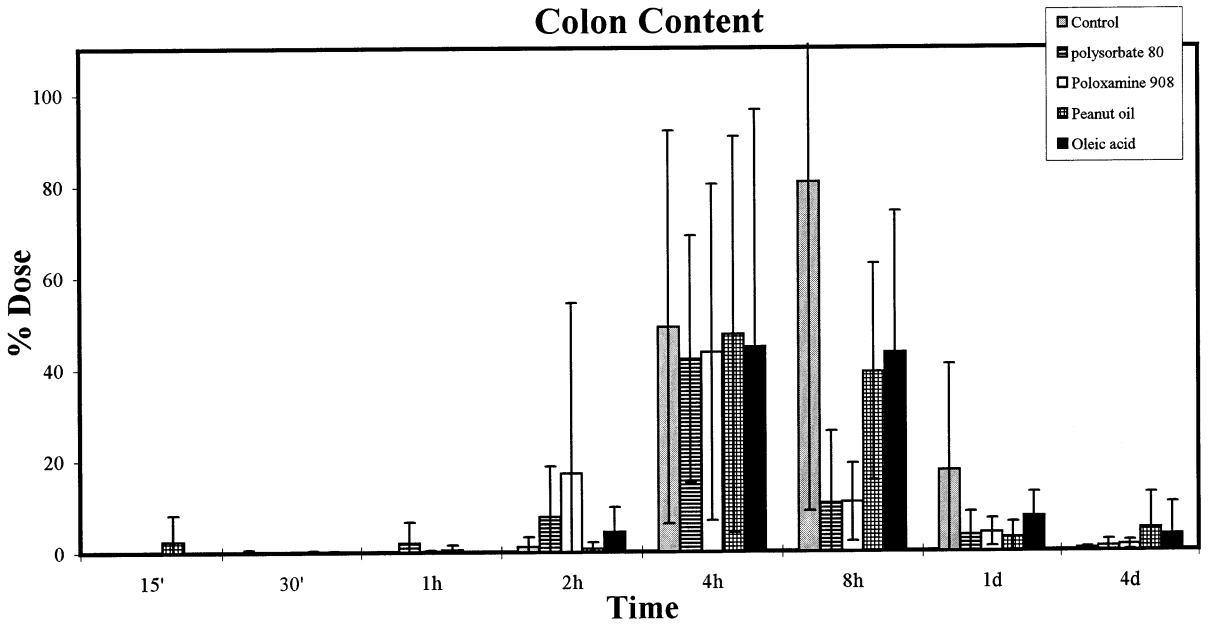

Fig. 5. Percentage of administered nanoparticle dose vs time in the colon contents after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

tioned by LeFevre et al. (LeFevre et al., 1978, 1980), Eldridge et al. (1990) and Jani et al. (Jani et al., 1989, 1990). The lymphoid tissue of these Peyer's patches are usually located on the antimesenteric border. Each patch typically comprises of 40–50 nodules which are separated from the gut lumen by a layer of epithelial cells, the M-cells. There is a thin layer of vascularised connective tissue between the nodules and the serosa. The M-cells (microfold cells) are also called follicle-associated membranous cells and are intimately associated with leukocytes. Once absorbed, nanoparticles cross the mesentery via the mesentery lymph vessel in direction of the mesentery lymph nodes and from the lymphatic circulation through the venous circulation to organs such as liver and spleen (Jani et al., 1997).

The most detailed oral quantitative body distribution study so far was performed by Nefzger et al. (1984) with ^{14}C-labelled poly(methyl methacrylate) (PMMA) nanoparticles of a size of about 130 nm. The present study attempts to investigate the influence of different suspension media and surfactants on the extent of absorption of nanoparticles from the gut with the same type of particles. Oleic acid, peanut oil as well as a 5% aqueous solution of two surfactants, poloxamine 908 and polysorbate 80 were selected as vehicles. These surfactants were chosen after a study of Tröster et al. (1990). In this study the body distribution of nanoparticles after i.v. injection and the influence of surfactants on this distribution were investigated. The authors identified two main substances with different body distribution characteristics, poloxamine 908 and polysorbate 80. Poloxamine 908 was very effective in increasing blood circulation time and reducing liver uptake, whereas polysorbate 80 was the overall most potent substance to target the particles to organs that do not belong to the reticuloendothelial system (RES, also frequently called MPS – mononuclear phagocytic system). PMMA is rather hydrophobic but becomes slightly more hydrophilic after contact with water as indicated by a decrease in contact angle and a pronounced contact angle hysteresis (difference between advancing and receding contact angles) (Johnson et al., 1986). Surfactants such as poloxamine 908 and polysorbate 80 increase the surface

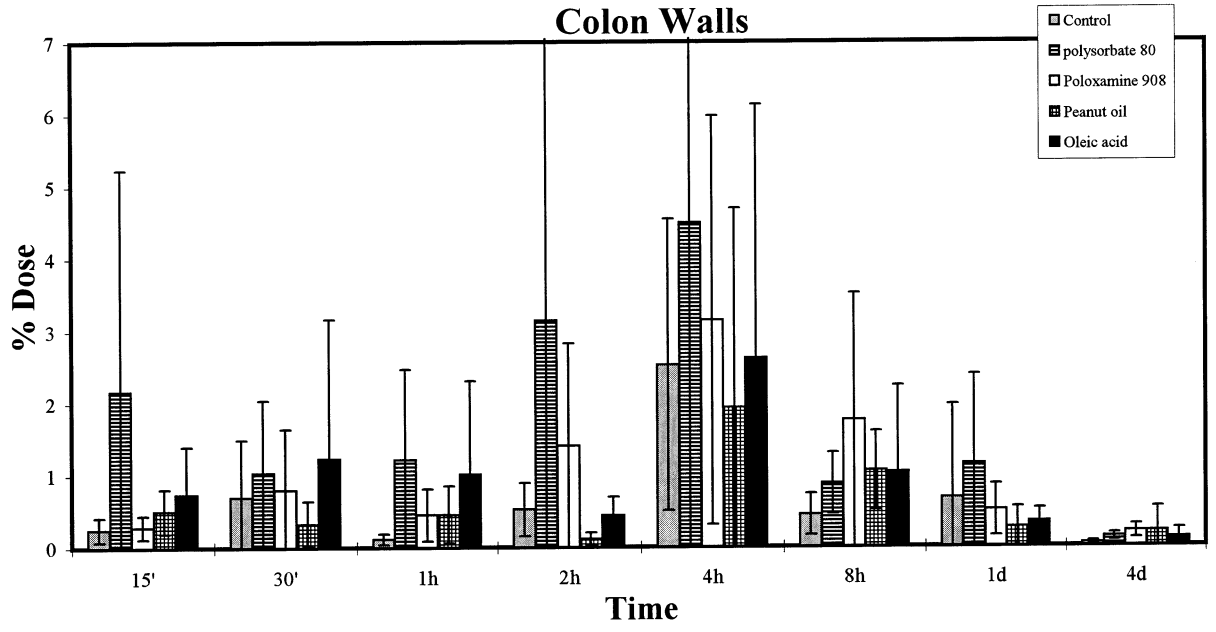

Fig. 6. Percentage of administered nanoparticle dose vs time in the colon walls after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

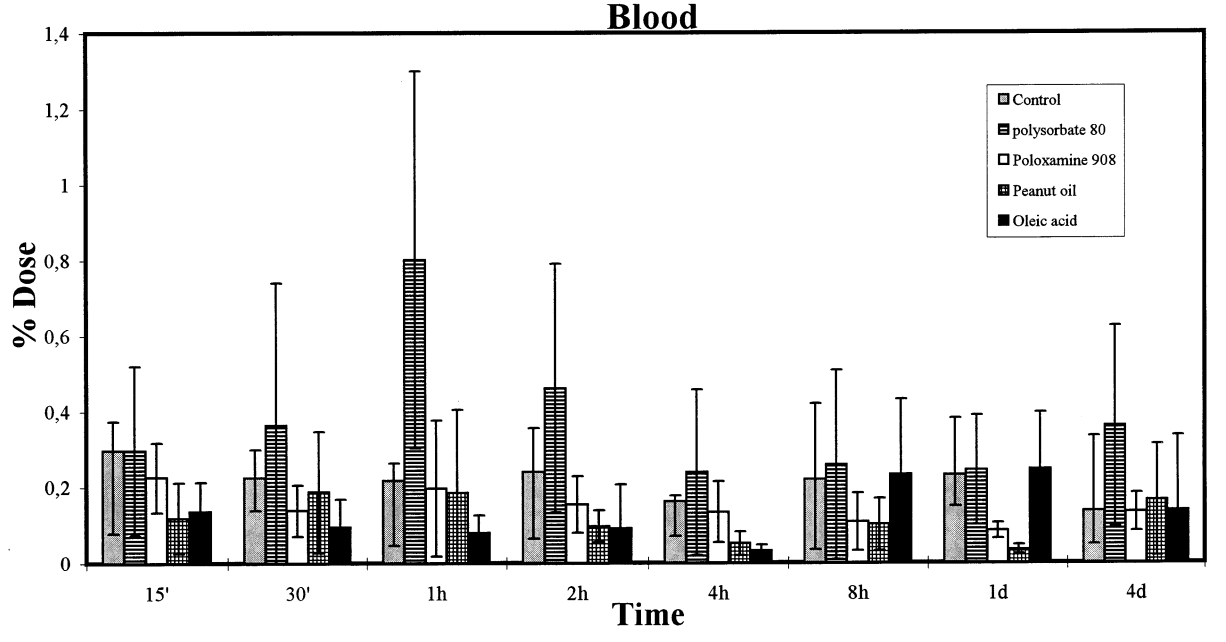

Fig. 7. Percentage of administered nanoparticle dose vs time in the blood after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

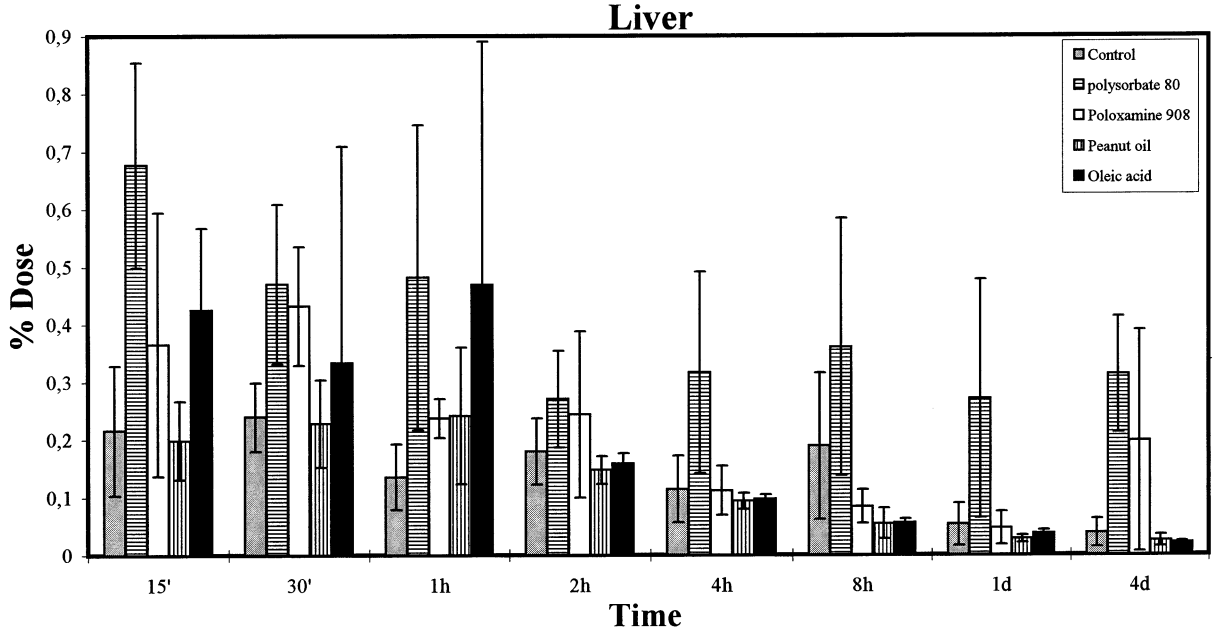

Fig. 8. Percentage of administered nanoparticle dose vs time in the liver after peroral administration of nanoparticles in the form of a suspension in saline (control), in saline with an additional content of 5% of polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid.

hydrophilicity considerably (Tröster and Kreuter, 1988), whereas oils such as peanut oil would maintain or render the PMMA surface hydrophobic.

As a consequence, by the choice of the above vehicles a variation in the surface hydrophilicity/hydrophobicity of the same nanoparticle core is achieved. The PMMA nanoparticles in the present study were administered to rats by gavage either in form of a suspension in saline, in saline with an additional content of 5% of one of the two surfactants, or suspended in peanut oil without or after addition of 5% oleic acid.

2. Materials and methods

2.1. Materials

Poloxamine 908 was obtained from C.H. Erbslöh (Krefeld, Germany), polysorbate 80 from ICI Surfactants (Essen, Germany), and peanut oil and oleic acid from Wasserfuhr GmbH (Bonn, Germany).

2.2. Preparation of nanoparticles

For the production of ^{14}C-labelled nanoparticles methyl(2-^{14}C)methacrylate synthesised by Amersham Radiochemical Center (Bucks, UK) was used as labelled monomer. Methyl(2-^{14}C)methacrylate (0.95 ml) was dissolved in phosphate buffered saline to give 95 ml of a 1% solution. This solution was irradiated with 5 kGy at a rate of 22 Gy/min in a ^{60}Co-source leading to the polymerisation and formation of nanoparticles. The polymer was freeze-dried as a suspension in 0.15 M phosphate buffered saline. The powder consisting of 43.7% polymethyl(2-^{14}C)methacrylate and 56.3% of buffer salts (dibasic sodium phosphate dihydrate, monobasic potassium phosphate, sodium chloride (7.6:1.45:4.8)) was obtained. The specific activity of the resulting polymer was 150 MBq/g.

Table 2
Percentage of the injected nanoparticles dose in various organs and tissues after peroral administration using suspension in saline, in saline with 5% polysorbate 80 or poloxamine 908 or suspended in peanut oil without or after addition of 5% oleic acid

% Dose	Percent of the administered dose									
	Control		Polysorbate 80		Poloxamine 908		Peanut oil		Oleic acid	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
Time: 15 min										
Lymph nodes	0.00037	0.000123	0.00110	0.000502	0.00043	0.00037	0.00028	0.00005	0.00034	0.00006
Heart	0.00518	0.004366	0.01527	0.008184	0.01729	0.01504	0.00365	0.00153	0.00552	0.00293
Lungs	0.01187	0.009234	0.03909	0.017231	0.01506	0.00694	0.01615	0.01393	0.01198	0.00796
Spleen	0.00201	0.000586	0.01555	0.007981	0.00384	0.00229	0.00494	0.00386	0.02546	0.01836
Testicles	0.00599	0.001119	0.01273	0.004716	0.02924	0.02006	0.00658	0.00129	0.02694	0.02364
Ovaries	0.00420	0.000432	0.02324	0.018225	0.00487	0.00294	0.00690	0.00002	0.03108	0.01633
Kidney	0.02220	0.004629	0.07541	0.021357	0.03238	0.00977	0.02442	0.00842	0.06816	0.03173
Brain	0.00634	0.009144	0.00941	0.003786	0.00313	0.00072	0.00378	0.00052	0.01217	0.01015
Muscles	0.18812	0.015525	1.57019	1.601157	0.34772	0.13624	0.27787	0.13715	0.79909	0.57552
Bone marrow	0.01312	0.010988	0.04073	0.015622	0.00916	0.00391	0.00558	0.00059	0.01707	0.01148
Time: 30 min										
Lymph nodes	0.00026	0.000030	0.00091	0.000594	0.00087	0.00051	0.00032	0.00010	0.00037	0.00017
Heart	0.00945	0.009557	0.02238	0.017934	0.00730	0.00159	0.00366	0.00100	0.00672	0.00683
Lungs	0.04780	0.061861	0.03288	0.022578	0.01610	0.00516	0.01161	0.00651	0.01918	0.02894
Spleen	0.00476	0.003844	0.00870	0.008350	0.00859	0.00678	0.00230	0.00143	0.01159	0.01020
Testicles	0.00751	0.000857	0.04185	0.014272	0.01018	0.00530	0.04131	0.04200	0.01363	0.01388
Ovaries	0.00769	0.002323	0.04543	0.043147	0.01070	0.00547	0.00888	0.00158	0.02402	0.01821
Kidney	0.03631	0.003999	0.10306	0.090456	0.04229	0.00716	0.02499	0.00737	0.03910	0.02182
Brain	0.00283	0.000387	0.01201	0.004610	0.00401	0.00053	0.00275	0.00039	0.01140	0.01672
Muscles	0.26795	0.063070	1.09730	1.011725	0.61074	0.46221	0.23488	0.04463	0.71783	0.39309
Bone marrow	0.01115	0.008530	0.03673	0.016572	0.03112	0.02171	0.01425	0.01735	0.01134	0.00992
Time: 60 min										
Lymph nodes	0.00023	0.000032	0.00071	0.000521	0.00120	0.00087	0.00046	0.00007	0.00031	0.00009
Heart	0.00250	0.000722	0.01876	0.009824	0.00502	0.00175	0.00403	0.00264	0.00381	0.00333
Lungs	0.00893	0.006779	0.03906	0.013782	0.00822	0.00300	0.00803	0.00235	0.00719	0.00275
Spleen	0.00253	0.000872	0.01473	0.010778	0.00242	0.00052	0.00372	0.00208	0.00298	0.00223
Testicles	0.00400	0.001592	0.02277	0.014309	0.01437	0.00942	0.00965	0.00297	0.00777	0.00070
Ovaries	0.00263	0.000355	0.00713	0.003377	0.00595	0.00066	0.05202	0.03381	0.05152	0.02062
Kidney	0.01987	0.004911	0.04046	0.009202	0.02929	0.00280	0.03038	0.00818	0.04134	0.02426
Brain	0.00307	0.000856	0.00966	0.003997	0.00334	0.00052	0.00511	0.00119	0.00341	0.00123
Muscles	0.20846	0.064675	1.04710	0.304559	0.38540	0.20080	0.53836	0.37719	0.35342	0.12799
Bone marrow	0.00678	0.002282	0.01474	0.006031	0.01208	0.00697	0.01371	0.00892	0.01830	0.02023
Time: 2 h										
Lymph nodes	0.00029	0.000075	0.00038	0.000140	0.00029	0.00006	0.00067	0.00057	0.00028	0.00007
Heart	0.01089	0.009099	0.01862	0.004690	0.00576	0.00272	0.00228	0.00070	0.00282	0.00028
Lungs	0.01701	0.008236	0.02826	0.009674	0.00962	0.00247	0.00496	0.00132	0.00658	0.00067
Spleen	0.00546	0.007287	0.01031	0.005872	0.00310	0.00167	0.00209	0.00069	0.00368	0.00336
Testicles	0.01176	0.008575	0.06187	0.059253	0.00679	0.00119	0.00845	0.00165	0.00731	0.00114
Ovaries	0.00412	0.001535	0.00563	0.002176	0.00430	0.00101	0.00457	0.00242	0.00575	0.00350
Kidney	0.03364	0.032757	0.03787	0.008537	0.02942	0.01262	0.01907	0.00848	0.02027	0.00163
Brain	0.00433	0.002648	0.00627	0.001683	0.00491	0.00503	0.00352	0.00115	0.00301	0.00080
Muscles	0.47861	0.217611	0.71528	0.303863	0.32525	0.07940	0.27214	0.06217	0.33271	0.15697
Bone marrow	0.01244	0.005577	0.04356	0.014242	0.00702	0.00232	0.05976	0.06485	0.00782	0.00587

Table 2 (*Continued*)

% Dose	Percent of the administered dose									
	Control		Polysorbate 80		Poloxamine 908		Peanut oil		Oleic acid	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
Time: 4 h										
Lymph nodes	0.00043	0.000280	0.00032	0.000035	0.00022	0.00003	0.00030	0.00014	0.00032	0.00016
Heart	0.00629	0.004793	0.02495	0.010136	0.00517	0.00181	0.00306	0.00092	0.00221	0.00043
Lungs	0.00611	0.003087	0.04346	0.036793	0.01185	0.00759	0.00585	0.00132	0.00591	0.00148
Spleen	0.00443	0.005375	0.01070	0.010516	0.00422	0.00380	0.00251	0.00121	0.00271	0.00136
Testicles	0.00738	0.000721	0.03035	0.017468	0.00555	0.00025	0.00524	0.00255	0.00877	0.00071
Ovaries	0.00586	0.003026	0.00722	0.001311	0.00210	0.00042	0.00689	0.00190	0.00437	0.00094
Kidney	0.01291	0.002521	0.04239	0.03891	0.01432	0.00439	0.01516	0.00596	0.01545	0.00282
Brain	0.00450	0.002439	0.01982	0.022627	0.00258	0.00063	0.00334	0.00105	0.00303	0.00043
Muscles	0.22410	0.047412	0.93388	0.299098	0.23142	0.07232	0.24096	0.05336	0.23183	0.03297
Bone marrow	0.01849	0.018416	0.03162	0.032843	0.02420	0.01471	0.00618	0.00370	0.00369	0.00088
Time: 8 h										
Lymph nodes	0.00039	0.000098	0.00061	0.000527	0.00025	0.00003	0.00033	0.00021	0.00022	0.00002
Heart	0.01477	0.014074	0.02204	0.009890	0.00733	0.00493	0.00181	0.00051	0.00180	0.00018
Lungs	0.02020	0.026316	0.03991	0.021917	0.01022	0.00346	0.00405	0.00119	0.00367	0.00042
Spleen	0.00646	0.007665	0.01699	0.010226	0.00435	0.00424	0.00293	0.00208	0.00159	0.00056
Testicles	0.00655	0.000871	0.01294	0.004293	0.00507	0.00066	0.00699	0.00075	0.00764	0.00070
Ovaries	0.00396	0.002483	0.00398	0.000209	0.00414	0.00242	0.00244	0.00048	0.00434	0.00185
Kidney	0.01804	0.026141	0.04069	0.024336	0.01058	0.00157	0.00840	0.00297	0.00945	0.00309
Brain	0.00318	0.000370	0.00768	0.006305	0.00285	0.00027	0.00261	0.00024	0.00324	0.00046
Muscles	0.17548	0.020640	0.60250	0.360431	0.27517	0.05570	0.20947	0.04411	0.19781	0.03609
Bone marrow	0.01397	0.010204	0.03530	0.025934	0.02602	0.01969	0.00403	0.00131	0.00370	0.00058
Time: 1 day										
Lymph nodes	0.00028	0.000071	0.00030	0.000077	0.00023	0.00002	0.00037	0.00021	0.00022	0.00001
Heart	0.01406	0.012465	0.02765	0.021783	0.00573	0.00258	0.00205	0.00105	0.00196	0.00060
Lungs	0.00408	0.002718	0.04022	0.030212	0.00782	0.00370	0.00346	0.00090	0.00377	0.00039
Spleen	0.00860	0.010195	0.02440	0.010611	0.00343	0.00216	0.00140	0.00036	0.00268	0.00171
Testicles	0.00487	0.000598	0.02004	0.012828	0.00518	0.00071	0.00663	0.00140	0.00727	0.00091
Ovaries	0.00212	0.000385	0.00812	0.006111	0.01122	0.01081	0.00250	0.00068	0.00244	0.00053
Kidney	0.00443	0.001171	0.02686	0.024240	0.01062	0.00604	0.00470	0.00067	0.00647	0.00135
Brain	0.00269	0.000413	0.00701	0.002693	0.00296	0.00058	0.00290	0.00119	0.00280	0.00046
Muscles	0.18839	0.021403	0.69084	0.256256	0.26154	0.05829	0.20248	0.03015	0.22218	0.04563
Bone marrow	0.01026	0.002360	0.04491	0.026773	0.01651	0.01427	0.00605	0.00398	0.01667	0.01571
Time: 4 days										
Lymph nodes	0.00023	0.000009	0.00027	0.000034	0.00041	0.00008	0.00023	0.00002	0.00022	0.00001
Heart	0.00125	0.000189	0.02340	0.008893	0.00554	0.00594	0.00155	0.00030	0.00134	0.00054
Lungs	0.00537	0.006282	0.03947	0.010958	0.00449	0.00262	0.00262	0.00033	0.00247	0.00083
Spleen	0.00184	0.000719	0.01196	0.006088	0.00191	0.00059	0.00125	0.00037	0.00098	0.00030
Testicles	0.00527	0.000496	0.00884	0.001110	0.00555	0.00040	0.00687	0.00117	0.00497	0.00065
Ovaries	0.00149	0.000071	0.00701	0.002471	0.00398	0.00264	0.00194	0.00021	0.00144	0.00022
Kidney	0.00583	0.004455	0.03480	0.017256	0.01063	0.00475	0.00425	0.00167	0.00372	0.00052
Brain	0.00276	0.000268	0.00731	0.002274	0.00311	0.00038	0.00283	0.00043	0.00304	0.00033
Muscles	0.18269	0.061220	0.74662	0.300328	0.27960	0.20957	0.20048	0.05065	0.16801	0.03503
Bone marrow	0.00582	0.002039	0.07228	0.030341	0.01482	0.01342	0.00262	0.00033	0.00361	0.00087

2.3. Preparation of the non-coated nanoparticle suspension

The nanoparticle preparation (205.95 mg) was suspended in 9 ml distilled water and 9 ml phosphate buffer (see above). The resulting suspension of 5 mg nanoparticles/ml was ultrasonicated for 5 min at 50 kHz in a bath type ultrasonicator (Brasonic 12, Branson Europa B.V, Soest, Netherlands).

2.4. Preparation of the surfactant-coated nanoparticle suspensions

The nanoparticles were suspended in phosphate buffer, and polysorbate 80 or poloxamine 908 were added to obtain a 5% surfactant concentration. Then the suspension was sonicated as described above.

2.5. Preparation of a nanoparticle suspension in peanut oil

The nanoparticles were suspended in peanut oil to give a suspension of 5 mg nanoparticles/ml. This suspension was homogenised by ultrasonicating for about 5 min at 50 kHz in a bath type ultrasonicator (Brasonic 12, Branson Europa B.V, Soest, Netherlands).

2.6. Preparation of a peanut oil–oleic acid nanoparticles suspension

The nanoparticles were dispersed in oleic acid and peanut oil was added to obtain a 5% oleic acid solution in peanut oil. This suspension was then homogenised as described above.

2.7. Particle size determination

The particle size was measured by laser light scattering using a BI 200 SM Goniometer with a digital correlator and PC (Brookhaven Instruments Corporation, Holtsville, NY, USA).

The average diameter of the particles was 130 ± 30 nm (Kreuter, 1983).

2.8. Oral administration of particles to rats

The above described nanoparticle preparations were administered to eight groups of rats (Wistar Unilever rats, Harlan Winkelmann, Borchen, Germany) consisting of 16 male and 16 female rats of a body weight between 220 and 280 g, by oral gavage using a plastic stomach tube. The volume administered per rat was 0.5 ml. The rats were kept at a constant temperature of 24 ± 2°C and a relative humidity of 55 ± 10%. They had free access to water and were fed a standard rat diet (Altromin-Haltungsdiät 1324, Samen Schmidt Jacobi, Frankfurt, Germany).

2.9. Organ distribution and determination of the ^{14}C radioactivity

Four animals per group (two male and two female) were sacrificed after 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 1 day, and 4 days by placing them in carbon dioxide followed by subsequent decapitation. The blood was collected, and the heart, lungs, liver, spleen, gastrointestinal tract, kidney,

Table 3
Total percentage of the administered dose in all organs and tissues (without GI-tract)

Time	Control	Polysorbate 80	Poloxamine 908	Peanut oil	Oleic acid
15 min	0.77	2.78	1.05	0.67	1.56
30 min	0.86	2.24	1.31	0.76	1.28
60 min	0.61	2.50	0.90	1.09	1.04
2 h	1.00	1.66	0.79	0.62	0.64
4 h	0.57	1.70	0.55	0.43	0.41
8 h	0.67	1.40	0.54	0.40	0.52
1 day	0.52	1.41	0.46	0.30	0.55
4 days	0.39	1.63	0.66	0.41	0.35

gonads, bone marrow, muscles, lymph nodes, and brain were removed. The whole organs were weighed immediately after removal, and two samples of each organ were exactly weighed into scintillation vials and dissolved in tissue solubilizer BTS 450 (Beckman, München, Germany). In the case of the GI-tract the whole tract was excised and separated into stomach, small intestine, and colon. Small intestine and colon were further cut into smaller sections. The contents of these parts were collected and the GI-tract sections were rinsed thoroughly with saline 4–5 times. These samples were than treated as described above. The vials were stored at 50°C until all material was solubilized. One millilitre of 30% superoxide was added to remove the colour. In order to decrease the chemolumineszence glacial acetic acid was added. This treatment yielded clear colourless solutions. After addition of 10 ml scintillation cocktail (Ready Organic, Beckman, München, Germany) the samples were stored for about 1 week in darkness and the radioactivity was counted in a scintillation counter (model LS 1801 Beckman, München, Germany).

The radioactivity content of the organs was calculated as percent of the administered dose. For the calculation of the contents in the bone marrow, muscles, and blood average weights were used. These data were obtained from the Hoechst AG (Dr Maas, Hoechst AG) (Table 1).

2.10. Statistics

Statistical differences were estimated using the Student's t-test and the Mann-Whitney U-test.

3. Results

In the present study the uptake of PMMA nanoparticle suspensions via the gut as well as the modification of the body distribution based on the use of different coating materials were determined. PMMA nanoparticles with a diameter of 130 ± 30 nm were fed by oral gavage either in form of a suspension in saline, in saline with an additional content of 5% polysorbate 80 or poloxamine 908, or suspended in peanut oil without or after addition of 5% oleic acid as a single dose to Wistar rats. After 30 min, 1, 2, 4, 8 h, 1, and 4 days the animals were sacrificed. The following concentrations of nanoparticle radioactivity were found in the different organs, blood, tissues, and gut contents.

3.1. Stomach contents

High nanoparticle radioactivity levels were observed in the stomach contents up to 2 h after administration which then dropped considerable after this time (Fig. 1). The highest levels up to a nanoparticle concentration of 50–60% were seen with the aqueous suspension of uncoated nanoparticles (control). All other vehicles reached only one half or less of this level. This difference between control and the other preparations was especially pronounced after 2 h.

3.2. Stomach wall

Nanoparticle radioactivity associated with the stomach wall was low, below 5% of the adminis-

Table 4
Total percentage of the administered dose in all organs and tissues (without GI-tract content)

Time	Control	Polysorbate 80	Poloxamine 908	Peanut oil	Oleic acid
15 min	2.92	11.66	8.16	4.58	6.18
30 min	5.37	17.29	10.20	4.60	6.16
60 min	5.80	6.70	6.40	6.53	5.83
2 h	4.12	6.06	4.60	5.20	3.88
4 h	3.88	6.65	4.93	3.50	4.07
8 h	1.22	2.62	2.54	1.80	1.79
1 day	1.26	4.08	1.27	0.72	1.38
4 days	0.53	1.85	1.00	0.73	0.52

tered dose, and disappeared rapidly (Fig. 2). After 2 h only low levels of radioactivity were observed that disappeared almost totally after 4 days. This general kinetical behaviour reflects that of the stomach contents although on a much lower level. In contrast to the latter, especially high concentrations with the uncoated control nanoparticle preparation were seen in the stomach wall only after 1 h. Instead, the highest radioactivities were initially observed with poloxamine 908 and later, between 60 and 120 min, with peanut oil. After 4 h and longer times only low levels persisted with all preparations. No preference for a certain preparation was detectable after these times.

3.3. Small intestine contents

The highest amount of radioactivity were found in the small intestine contents (Fig. 3).

High variations appeared between neighbouring sections, so that it was difficult to calculate exactly the total nanoparticle quantity in the small intestinal contents. The problem of irregular gut distribution was already observed earlier (Kreuter et al., 1989). In general the highest radioactivity levels of about 40% of the dose were seen in the small intestine between 30 min and 4 h. Very high levels of radioactivity were seen after 15 and 30 min with the nanoparticles suspended in 5% polysorbate 80. In addition, exceptionally high levels also were seen with both peanut oil vehicles, without and with oleic acid after 2 and 4 h or 1 and 2 h, respectively. The rather late appearance of the control preparation in the small intestine content mirrored the high concentrations that still remained in the stomach (see above).

3.4. Small intestine walls

The quantitative determination of the orally administered ^{14}C-labelled PMMA nanoparticles in the walls of the small intestine indicated a similar irregular distribution as with the intestine content (Fig. 4). However, with some exceptions the radioactivity levels in the intestinal content and the walls did not totally correlate. This latter finding was not only true for single sections but also was seen for the extrapolated total small intestinal content and wall levels and was reflected by the high standard deviations (Figs. 3 and 4). Only in the case of the 5% polysorbate 80 preparation after 15 and 30 min comparably high concentrations of radioactivity to the small intestinal content again on a much lower total level (4 vs 70% and 12 vs 150%, respectively) were found.

With the other nanoparticles preparation total concentrations of about 2% of the dose were detectable already 15 min after administration. The concentration showed a maximum at 1 h with an average about 3% of the administered dose. After 4 h the concentration decreased to very low levels.

3.5. Colon contents

Significant radioactivity appeared in the colon contents after 2 h (Fig. 5), with most preparations reaching a maximum of about 50% of the administered dose after 4 h. With the exception of the uncoated control nanoparticles the radioactivity decreased after this time. The disappearance was more rapid with the surfactant containing aqueous nanoparticle suspensions. After 4 days very low levels of about 3–4% of the dose still remained in the colon content.

3.6. Colon walls

In the colon walls (Fig. 6) the radioactivity, surprisingly, appeared much earlier than in the colon contents, and with the polysorbate 80 preparation 2% of the administered dose were already seen after 15 min. The main quantity of radioactivity, however, appeared much later.

3.7. Blood

The appearance of the radioactivity of the orally administered coated and non-coated nanoparticles in the blood is shown in Fig. 7. After 15 min the non-coated nanoparticles and those coated with surfactants produced levels of 0.2% of the administered dose. In contrast, both oils at the same time point showed lower levels (0.1%).

The radioactivity concentration with the non-coated nanoparticles stayed constant over the whole time period investigated (0.16–0.22%). The

5% polysorbate 80 preparation was the only one that led to higher blood levels than the non-coated nanoparticles. Statistically significant different values ($P < 0.05$) were found with this preparation at the time points above 1 and 2 h. Peanut oil (2 h, 4 h and 1 day) and oleic acid in peanut oil (1 h, 2 h and 4 h) yielded statistically significant lower concentrations than the non-coated nanoparticles (factor 0.2).

3.8. Liver

Non-coated nanoparticles showed a liver uptake of 0.2% at 15 min (Fig. 8). After 1 day the levels declined to 0.05%. Polysorbate 80 showed the highest levels of 0.6% after 15 min (2.5-fold increase compared to the non-coated particles). At all time points higher levels compared to the non-coated nanoparticles were found (factor 1.2–6). A statistically significant difference ($P < 0.05$) was observed during the time period 15 min–2 h. Poloxamine 908 also led to higher levels up to 2 h. However, these levels were lower than with polysorbate 80. Peanut oil had almost no influence compared to the non-coated particles, but addition of oleic acid increased the concentrations by 50–100% between 15 and 60 min.

3.9. Residual organs and tissues

In the residual body very low levels of radioactivity appeared (Table 2). The single exception to the other preparations was the 5% polysorbate 80 nanoparticle suspension, that reached the highest levels in all organs and at all time points with a few single exceptions. The levels obtained with the polysorbate 80 preparation were about 0.025% of the dose in the spleen, 0.04% in the lungs, 0.001% in the lymph nodes, 0.04% in the bone marrow, 0.025% in the heart, 0.05–0.1% in the kidneys, 0.02–0.01% in brain, 1% in the muscles, 0.06% in the testicles and 0.05% in the ovaries. These levels were reached quite rapidly after 15–30 min and stayed at this level for up to 4 days.

An exception to the other organs and tissues were spleen and the endocrinal organs. In the spleen the highest levels appeared after 1 day, and in the endocrinal organs the elevated levels with polysorbate 80 dropped to those of the other preparation after a few hours. In addition, in the ovaries rather high levels between 0.03 and 0.05% were seen with peanut oil and oleic acid between 15 min and 1 h.

4. Discussion

The mechanism of gastrointestinal uptake of particles is still not totally understood. Three possibilities of uptake exist: an intracellular uptake, a paracellular uptake and an uptake via the M-cells, and the Peyer's patches (Kreuter, 1991). Possibly a simultaneous uptake by more than one pathway occurs. It seems, that the quantitative contribution of each uptake pathway may be different at different sites of the intestine. Nanoparticle uptake via intercellular spaces between the enterocytes in the jejunum seems to be the prominent mechanism 10 min after administration (Volkheimer, 1977; Alpar et al., 1989). In the opinion of these authors this paracellular pathways could explain the fast uptake and appearance in the blood and the other organs. In the ileum the particles mainly seem to pass through the M-cells and Peyer's patches, both belonging to the GALT, in relatively large quantities and were found in the intercellular space around the lymph nodes (Jani et al., 1989, 1990, 1992a,b; Florence, 1997). To a lesser extent, normal intestinal tissue also may be involved in particle uptake (Hillery et al., 1994).

Besides particles size (Jani et al., 1989, 1990) the surfaces properties of the particles seem to influence their uptake (Florence et al., 1995; Hillery and Florence, 1996). In addition, the surface properties also influence the bioadhesion (Diepold et al., 1989) and thus the gastric transit time of small particles (Kreuter et al., 1989).

For this reason in the present paper, the surface properties of the nanoparticles were altered by coating with surfactants. In addition, besides water other vehicles, peanut oil without and with addition of 5% oleic acid, a known absorption promoter, were used.

PMMA was chosen as the model nanoparticle material for the following reason: It is of interme-

diate lipophilicity, it can be labelled stably with ^{14}C within the polymer chain, and it is very slowly biodegradable. Therefore, radioactivity found in the tissues and the organs during the time period of the present study can be attributed to undegraded nanoparticles. The two surfactants employed, polysorbate 80 and poloxamine 908, behaved totally different in previous body distribution studies in rats (Tröster et al., 1990).

The present study confirms that nanoparticles indeed can be taken up by the gastrointestinal tract. The total radioactivity concentration after administration of uncoated particles (Table 3) in the body without the GI-tract reached a peak level of around 1% of the dose after 2 h. This level appears to be rather low compared to a total uptake of about 10% of the orally administered dose observed by Nefzger et al. (1984) using the same particles (different batch). However, the amount observed in that study was quantified by measuring the total amount excreted by bile and urine within 48 h, whereas the present study measures concentrations at given time points. Therefore, for comparing the results it has to be considered that biliary and urinary excretion were quite rapid (Nefzger et al., 1984). As a result of this rapid elimination the total uptake may be much higher than suggested by the present organ and tissue concentrations.

The present study contrasts the observation of Florence et al. (1995) and Hillery and Florence (1996) that surfactants decrease the uptake of nanoparticles. However, it is important to consider that in our study not only the core nanoparticle polymer was different, but also that Florence and Hillery used different surfactants, i.e. poloxamer 188 and 407. Our present study clearly demonstrates the importance of the coating material in that coating with different surfactants, polysorbate 80 and poloxamine 908, led to significantly and importantly different quantities in uptake. Polysorbate 80 reached comparatively high levels with a peak total concentration of 2.8% of the administered dose already after 15 min and maintained more than 100% higher total levels compared to all other preparations for over 4 days (Table 3). In all organs of the body except the GI-tract the radioactivity concentration were higher with this preparations for almost all time points. Deviations from this general picture probably were due to biological variations. Although with the other preparations at most time points also higher radioactivity levels compared to the control were seen, this increase was not statistically significant. Interestingly, the addition of oleic acid to peanut oil yielded higher total concentrations than peanut oil alone up to 30 min mainly by achieving significantly ($P < 0.05$ compared to peanut oil alone) higher radioactivity concentrations in the liver, spleen and bone marrow.

In addition to the total concentrations in the body without the GI-tract (Table 3), the total concentrations in the body including the GI-tract walls but without the GI-tract content were calculated (Table 4). This exhibited an inproportionate difference between the two total concentrations. Some of this additional overproportionate radioactivity in the GI-tract walls may be due to nanoparticles adhering to these walls that could not be removed by thorough washing. However, especially the early appearance of radioactivity in the colon walls demonstrates that nanoparticles taken up by and remaining in the GI-tract walls as well as radioactivity distributed by blood or lymph to these walls play an important role for the total levels observed in these tissues.

The present study clearly demonstrates that nanoparticles can be taken up in the GI-tract after peroral administration although the uptake is rather low ($\leq 10\%$ at the particle size used in this study, i.e. 130 nm). It confirms earlier studies that besides particle size the surface properties play an important role. In contrast to these earlier studies (Florence et al., 1995; Hillery and Florence, 1996) addition of surfactants, especially polysorbate 80, increased the nanoparticle uptake significantly and importantly. On the other hand, another quite different, hydrophobic vehicle, peanut oil, did not increase the nanoparticle uptake compared to saline.

This result that particles with a more hydrophilic surface at many time points were taken up in the gastrointestinal tract to a higher degree than the hydrophobic ones is rather surprising.

Earlier studies by Eldridge et al. (1990) and Jepson et al. (1993) would have suggested the opposite. At present, we have no explanation for this. It is possible that the surfactants induce a specific interaction with the cells lining the gastrointestinal tract similar to the interaction with endothelial cells after i.v. injection.

References

Alpar, H.O., Field, W.N., Hyde, R., Lewis, D.A., 1989. The transport of microspheres from the gastrointestinal tract to inflammatory air pouches in the rat. J. Pharm. Pharmacol. 41, 194–196.

Aprahamian, M., Michel, C., Humbert, W., Devissaguet, J.P., Damgé, C., 1987. Transmucosal passage of polyalkylcyanoacrylate nanocapsules as a new drug carrier in the small intestine. Biol. Cell. 61, 69–76.

Beck, P.H., Kreuter, J., Müller, W.E.G., Shatton, W., 1994. Improved peroral delivery of avarol with polybutylcyanoacrylate nanoparticles. Eur. J. Biopharm. 40, 134–137.

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

Diepold, R., Kreuter, J., Guggenbühl, P., Robinson, J.R., 1989. Distribution of poly-hexyl-2-cyano[3-^{14}C] acrylate nanoparticles in healthy and chronically inflamed rabbit eyes. Int. J. Pharm. 54, 149–153.

Eldridge, J.H., Hammond, C.J., Meulbroek, J.A., Staas, J.K., Gilley, R.M., Tice, T.R., 1990. Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. J. Controlled Rel. 11, 205–214.

Florence, A.T., 1997. The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. Pharm. Res. 14, 259–266.

Florence, A.T., Hillery, A.M., Hussain, N., Jani, P.U., 1995. Factors affecting the oral uptake and translocation of polystyrene nanoparticles: histological and analytical evidence. J. Drug Target. 3, 65–70.

Herbst, E.F.G., 1844. Das Lymphgefäßsystem und seine Verrichtungen. Vandenhoeck and Ruprecht, Göttingen, pp. 333–337.

Hillery, A.M., Jani, P.U., Florence, A.T., 1994. Comparative quantitative study of lymphoid and non-lymphoid uptake of 50 nm polystyrene particles. J. Drug Target. 2, 151–154.

Hillery, A.M., Florence, A.T., 1996. The effect of adsorbed poloxamer 188 and 407 surfactants on the intestinal uptake of 60 nm polystyrene particles after oral administration in rat. Int. J. Pharm. 132, 123–130.

Hirsch, R., 1906. Das Vorkommen von Stärkekörnern. Blut Urin. Exp. Pathol. Ther. 3, 390–392.

Jani, P.U., Florence, A.T., McCarthy, D.E., 1992a. Further histological evidence of the gastrointestinal absorption of polystyrene nanospheres in the rat. Int. J. Pharm. 84, 245–252.

Jani, P.U., McCarthy, D.E., Florence, A.T., 1992b. Nanosphere and microsphere uptake via Peyer's patches: observation of the rate of uptake in the rat after a single oral dose. Int. J. Pharm. 86, 239–246.

Jani, P., Halbert, G.W., Langridge, J., Florence, A.T., 1989. The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. J. Pharm. Pharmacol. 41, 809–812.

Jani, P.U., Halbert, G.W., Langdrige, J., Florence, A.T., 1990. Nanoparticle uptake by the rat gastrointestinal mucosa: Quantification and particle size dependency. J. Pharm. Pharmacol. 42, 821–826.

Jani, P.U., Noruma, T., Yamashita, F., Takakura, Y., Florence, A.T., Hashida, M., 1997. Biliary excretion of polystyrene microspheres with covalently linked FITC fluorescence after oral and parenteral administration to male wistar rats. J. Drug Target. 4, 87–93.

Jepson, M.A., Simmons, N.L., O'Hagan, D.T., Hirst, B.H., 1993. Comparison of poly(DL-lactide-co-glycolyde) and polystyrene microsphere targeting to intestinal M cells. J. Drug Target. 1, 245–249.

Johnson, B.A., Kreuter, J., Zografi, G., 1986. Effects of surfactants and polymers on advancing and receding contact angles. Colloid Surfaces 17, 325–342.

Kreuter, 1983. Physicochemical characterization of polyacrylic nanoparticles. Int. J. Pharm. 14, 43–58.

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

Kreuter, J., Müller, U., Munz, K., 1989. Quantitative and microautoradiographic study on mouse intestinal distribution of polycyanoacrylate nanoparticles. Int. J. Pharm. 55, 39–45.

LeFevre, M.E., Vanderhoff, J.W., Laissue, J.A., Joel, D.D., 1978. Accumulation of 2 mm latex particles in mouse Peyer's patches during chronic latex feeding. Experientia 34, 120–122.

LeFevre, M.E., Hancock, D.C., Joel, D.D., 1980. Intestinal barrier to large particles in mice. J. Tox. Env. Health. 6, 691–704.

Leroux, J.C., Cozens, R., Roesel, J.L., Galli, B., Kubel, F., Doelker, E., Gurny, R., 1995. Pharmacokinetics of a novel HIV-1 protease inhibitor incorporated into biodegradable or enteric nanoparticles following intravenous and oral administration to mice. J. Pharm. Sci. 84, 1387–1391.

Löbenberg, R., Araujo, L., Kreuter, J., 1997. Body distribution of azidothymidine bound to nanoparticles after oral administration. Eur. J. Pharm. Biopharm. 44, 127–132.

Maincent, P., Devissaguet, J.P., Le Verge, R., Sado, P., Couvreur, P., 1986. Disposition kinetics and oral bioavailability of vincamine loaded polyalkylcyanoacrylate. J. Pharm. Sci. 75, 955–958.

Nefzger, M., Kreuter, J., Voges, R., Liehl, E., Czok, R., 1984. Distribution and elimination of polymethyl methacrylate nanoparticles after peroral administration to rats. J. Pharm. Sci. 73, 1309–1311.

Sanders, E., Ashworth, C.T., 1961. A study of particulate intestinal absorption and hepatocellular uptake. Use polystyrene latex particles. Exp. Cell. Res. 22, 137–145.

Tröster, S.D., Kreuter, J., 1988. Contact angles of surfactants with a potential to alter the body distribution of colloidal drug carries on poly(methyl methacrylate) surfaces. Int. J. Pharm. 45, 91–100.

Tröster, S.D., Müller, U., Kreuter, J., 1990. Modification of the body distribution of poly(methyl methacrylate) nanoparticles in rats by coating with surfactants. Int. J. Pharm. 61, 85–100.

Verzár, F., 1911. Aufsaugung und Ausscheidung von Stärkekörnern. Biochem. Z. 34, 86.

Volkheimer, G., 1977. Persorption of particles: physiology and pharmacology. Adv. Pharmacol. Chemother. 14, 163–187.

Volkheimer, G., Schulz, F.H., 1968. The phenomenon of persorption. Digestion 1, 213–218.