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Characterization of Colloidal Drug Carriers

Determination of surface hydrophobicity by hydrophobic interaction chromatography

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Summary

Hydrophobic interaction chromatography (HIC) is presented as a suitable method to determine the surface hydrophobicity of colloidal drug carriers. The experimental design is described in great detail. Results obtained with surface-modified polystyrene particles as model carriers and parenteral fat emulsions are discussed as examples. HIC was able to distinguish between particles chemically modified by the introduction of functional groups. Polystyrene particles of various size were surface-modified by adsorption of the block-polymer Poloxamine 908. HIC distinguished between the hydrophobicities of these adsorption layers. The measured surface hydrophobicities of particles and fat emulsions were well in agreement with the in vivo organ distribution data obtained after i.v. injection of the carriers.

Zusammenfassung

Charakterisierung kolloidaler Arzneistoffträger / Bestimmung der Oberflächenhydrophobie durch hydrophobe Interaktions-Chromatographie.

Hydrophobe Interaktions-Chromatographie (HIC) wird als eine sinnvolle Methode präsentiert, um die Oberflächenhydrophobie von kolloidalen Arzneistoffträgern zu ermitteln. Das experimentelle Design wird detailliert beschrieben. Resultate von oberflächenmodifizierten Polystyrol-Partikeln und parenteralen Fettemulsionen werden beispielhaft diskutiert. HIC konnte zwischen Partikeln unterscheiden, die durch Einführung funktionaler Gruppen chemisch modifiziert waren. Die Oberflächen von Polystyrol-Partikeln unterschiedlicher Größe wurden durch die Adsorption des Blockpolymers Poloxamine 908 modifiziert. HIC unterschied die Hydrophobien dieser Adsorptionsschichten. Die gemessenen Oberflächenhydrophobien der Partikel und Fettemulsionen stimmten gut mit der In-vivo-Organverteilung nach i.v. Injektion der Arzneistoffträger überein.

Key words: *Colloidal drug carriers, surface hydrophobicity determination · Hydrophobic interaction chromatography · Surface hydrophobicity*

1. Introduction

The surface hydrophobicity is an important parameter for the development of new colloidal drug carriers. To create the desired properties of the carriers, the surface can be modified by the adsorption of polymers. The hydrophobicity of the adsorbent surface is one of the parameters determining the adsorption process [1] and therefore the resulting thickness of the adsorbed layer. The surface hydrophobicity is also a relevant factor for the interaction of carriers with cells in vitro and for their in vivo organ distribution [2]. For bacteria [3, 4], yeast particles [5], and albumin particles [6] it has been shown that a high surface hydrophobicity leads to a rapid phagocytosis in in vitro cell cultures. By lowering the hydrophobicity the phagocytotic uptake could be reduced significantly [6]. The same applies to model drug carriers made of polystyrene latex surface modified with poloxamer and poloxamine polymers in culture [7] and in vivo [8, 9]. Reduced uptake by the reticuloendothelial system was reported for hydrophilic fat emulsions used in parenteral nutrition [10, 11]. At the interface between blood and artifical surfaces (carrier surface) the complement system is activated [12]. The hydrophobicity of the surface determines the amount of blood proteins (e.g. opsonins) adsorbed [13].

Thus a sensible experimental design for controlled production and testing of new drug carriers necessarily has to include the determination of the surface hydrophobicity. The method of contact angle measurement traditionally applied is limited to uncoated particles, and even they cannot be measured in their original dispersion medium [14]. The polymers used for the particle coating can only be investigated on cast films of the carrier material [15]. The application of rose bengal isotherms or, less time consuming, the rose bengal partitioning method again is restricted to particles not coated with hydrophilic polymers. Furthermore these binding methods do not provide any information about possible subpopulations, because they only give average measures of the hydrophobicity [2].

Recently we have introduced hydrophobic interaction chromatography (HIC) as a suitable characterization method for both uncoated and coated drug carriers [16]. Genuinely developed for the resolution of proteins [17], HIC has been successfully applied to the determination of hydrophobicity of bacteria [18]. Drug carriers can be examined by HIC in their original dispersion medium preserving their real surface properties, e.g. hydration and conformation of adsorbed polymers [2]. In addition, detection of subpopulations concerning hydrophobicity is possible within one sample [2].

2. Experimental

2.1. Materials

Polystyrene microspheres (mean diameter 60 nm (PS-0.06), 140 nm (PS-0.14), 140 nm surface modified by introduction of additional hydroxyl groups (PS-0.14-OH)) used as model carrier particles were purchased from Polysciences, Northampton, U.K. Poloxamine 908 was obtained from Ugine Kuhlman Ltd., Bolton, U.K. and used as received. Intralipid 10 % fat emulsion was provided by KaBi Vitrum, Warrington, U.K.

For the use as HIC-matrices Sepharose CL-4B (cross-linked agarose) and Agethane (ethyl-agarose) were purchased from Pharmacia, Uppsala, Sweden, and propyl-, pentyl- and hexyl-agarose from Sigma Chemical Co., Dorset, U.K.

$Na_2HPO_4 \times 2\ H_2O$ and $NaH_2PO_4 \times 2\ H_2O$ were purchased from Sigma and used for a phosphate buffered solution (PBS), pH 6.8, after Sørensen. Triton-X 100 was obtained from Sigma.

2.2. Sample preparation

The surface of 60 nm and 140 nm polystyrene particles was modified by the adsorption (coating) of Poloxamine 908 (PS-0.06-908, PS-0.14-908). The particle suspension (2.5 % w/v) was

mixed with an equal volume of polymer solution (2 % w/v) and incubated overnight [2]. The adsorption process was followed by measuring the coating layer thickness using photon correlation spectroscopy (PCS). The coating layers of Poloxamine 908 were 14 nm on PS-0.06 and 8 nm on PS-0.14.

2.3. *Hydrophobic interaction chromatography*

Hydrophobic interaction chromatography is a column chromatography which can distinguish between substances or particulates by the differing strengths of their hydrophobic interactions with a hydrophobic gel matrix. The matrices should not show any ion exchange or charge effects [19]. Therefore the neutral gels mentioned above were used. The strength of the interaction between particles and matrix depends on the hydrophobicity of the particles, the hydrophobicity of the matrix and the interactions with and between the dispersion medium's water molecules.

Bound carriers can be eluted by altering the elution conditions in different ways. For example, lowering the ionic strength decreases the hydrophobic interactions, and the change to an ion with a lower "salting-out" effect in the elution medium (e.g. PO_4^{3-}, SO_4^{2-}) leads to "chaotropic" effects and consequently to similar results [20]. Alternatively, non-ionic detergents can be applied [19]. The hydrophobic region of the detergent molecule binds to the matrix and displaces the particulates. In the presented study Triton X-100 was used.

For the investigation of the uncoated polystyrene particles the apparatus used for HIC consisted of a column (bed volume 10 ml, diameter 1.0 cm, height 12.7 cm), an arrangement of two solvent pumps and a UV spectrometer connected to a chart recorder for detection of the particles (at 400 nm) (Fig. 1). Before starting the experiment, the column was equilibrated with elution buffer (0.02 mol/l PBS, pH 6.8, in 0.3 mol/l NaCl). Higher salt concentrations led to flocculation of uncoated particles on the column as shown by PCS determination of the eluted particles. Latex particles (15 µl of a 2.5 % w/v suspension) were loaded onto the column and eluted at a flow rate of 0.3 ml/min.. To create a surfactant gradient, 0.1 % Triton X-100 in elution buffer was pumped from the reservoir to the mixing container at the same flow rate. Between two experiments regeneration of the column was performed using a washing sequence of different solutions: 10 ml of 0.1 % Triton X-100 in water, then 10 ml of distilled water, followed by 10 ml of 10 % ethanol, 20 ml of 5 % butanol, again 10 ml of 10 % ethanol, 10 ml of distilled water and finally reequlibration of the gel with elution buffer.

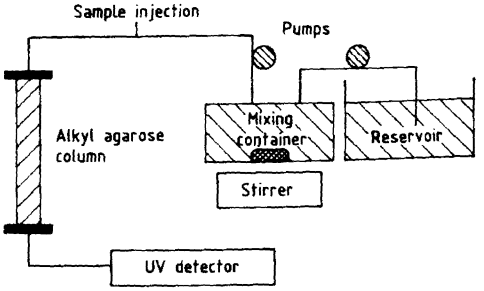

Fig. 1: Hydrophobic interaction chromatography apparatus. In order to create a Triton X-100 gradient, 0.1 % surfactant solution was pumped from the reservoir to the mixing container [2].

Coated latex particles and fat emulsions naturally showed a weaker interaction with the hydrophobic matrix and were eluted with buffered saline only (elution peak, EP). Strongly bound particles and droplets were washed off the column by 0.1 % Triton X-100 in elution buffer without application of a gradient (wash peak, WP). Whereas for the hydrophobic uncoated particles the ethyl-agarose was sufficient, the hydrophobicity of the agarose's alkyl-chain had to be increased (propyl-, pentyl-, hexyl-agarose) to distinguish between the more hydrophilic carriers.

To compare the hydrophobicities of coated drug carriers, we calculated retention coefficients (void volume/elution volume) and the ratios of the areas under the curve (AUC) of the elution peak to the wash peak. The elution volume was measured at the peak's maximum intensity. To quantify the shape of the elution peaks obtained with buffered saline and to compensate for a strong tailing, a shape quotient was calculated: area of symmetrical peak / (area of symmetrical peak + tail). If on very hydrophobic columns (pentyl-, hexyl-agarose) a fraction of particles is irreversibly bound and cannot be washed off with Triton X-100, this portion will not appear in the wash peak. The particles as a whole appear falsely hydrophilic. To detect such effects the fraction retained on the column can be calculated by comparing the AUCs of the elution and wash peaks of the sample with a control.

3. *Results and discussion*

3.1. *Uncoated latex particles*

As an example for the range of uncoated standard latices analyzed [21], PS-0.14 and PS-0.14-OH particles are presented. The chromatograms were both obtained on ethyl-agarose. The one from PS-0.14 showed a broad peak with an elution volume of 28–40 ml. The broadness of the peak demonstrates the heterogeneity in surface hydrophobicity of the particles. A small particle fraction less hydrophobic than the main population could be eluted even earlier (Fig. 2).

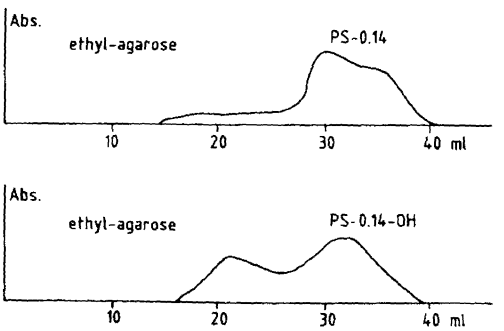

Fig. 2: Chromatograms of uncoated latex particles on ethyl-agarose gel: polystyrene (PS-0.14) and hydroxylated polystyrene (PS-0.14-OH) particles. Elution was performed using a Triton X-100 gradient.

The hydroxylated particles (PS-0.14-OH) gave a broad distribution. A distinct peak already appeared around 20 ml, but the major peak was detected at 28–39 ml similar to PS-0.14, except that this second peak was smaller (Fig. 2). That means that within the particle population of PS-0.14-OH there was a relatively hydrophilic fraction and a second fraction similar in surface hydrophobicity to the unmodified PS-0.14. These results can be easily explained by the production method of the particles, as employed by Polysciences. First of all the polystyrene latices are polymerized and then the surface is modified by the chemical introduction of hydroxyl groups, for example. Of course, this reaction will not lead to surface modification of all the particles to the same extent, so the second peak can be attributed to particles with little or no surface modification.

This example already demonstrates the importance of the characterization by HIC, because the efficiency of an applied surface modification becomes controllable and possible batch to batch variations detectable. The latter especially is absolutely necessary in the sensitive field of phagocytosis studies using cell cultures.

3.2. *Coated latex particles*

A series of poloxamer and poloxamine coatings on equally sized particles were investigated by using alkyl-sepharoses with increasing alkyl-chain length. Particles coated with Poloxamer 407 and Poloxamine 908 proved to be the least hydrophobic ones [22, 23]. Subsequently these two polymers were employed as coatings for differently sized polystyrene particles.

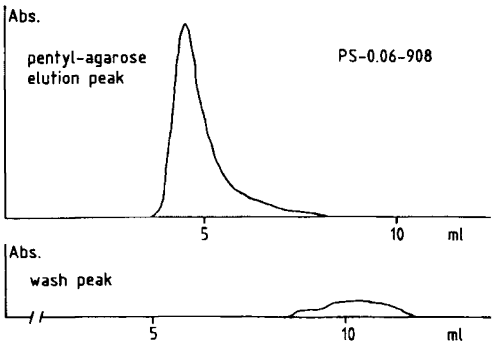

Fig. 3: Chromatogram of Poloxamine 908-coated 60 nm latex particles (PS-0.06-908) on pentyl-agarose gel. Elution peak with PBS buffer (upper) and wash peak with Triton X-100 0.1 % in buffer (lower) [2].

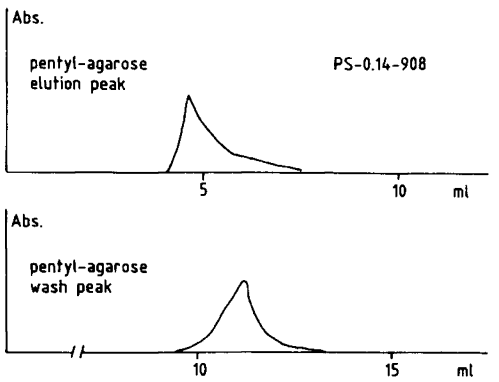

Fig. 4: Chromatogram of Poloxamine 908-coated 140 nm latex particles (PS-0.14-908) on pentyl-agarose gel. Elution peak with PBS buffer (upper) and wash peak with Triton X-100 0.1 % in buffer (lower) [2].

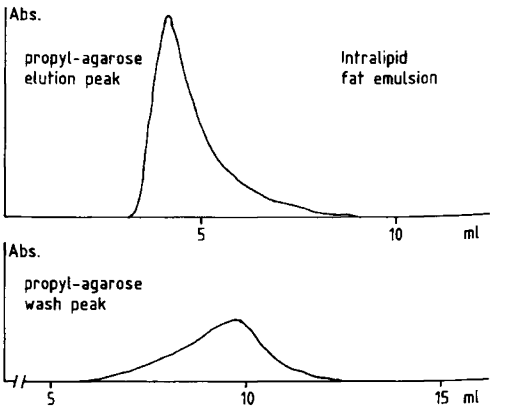

Fig. 5: Chromatogram of Intralipid 10 % fat emulsion on propyl-agarose gel. Elution peak with PBS buffer (upper) and wash peak with Triton X-100 0.1 % in buffer (lower) [2].

Table 1: Retention coefficients, AUC ratios and shape quotients of elution peaks of Poloxamine 908-coated particles and Intralipid fat emulsion (EP, WP – elution, wash peak only) [2].

Sample	Alkyl-agarose	Retention coefficient	AUC ratio elution/ wash peak	Shape quotient of elution peak
PS-0.06–908	propyl	0.93	EP –	0.54
	pentyl	0.99	9.0 : 1	0.61
	hexyl	0.91	1 : 1.3	0.80
PS-0.14-908	propyl	0.98	11.1 : 1	0.64
	pentyl	0.97	1.2 : 1	0.43
	hexyl	–	– WP	–
Intra-lipid 10 %	propyl	0.91	2.1 : 1	0.53
	pentyl	–	– WP	–

Chromatograms of PS-0.06-908 and PS-0.14-908 are shown as examples. After the propyl-agarose had given similar results, the pentyl-agarose provided interesting information concerning the differences of the two particles: A large fraction of the PS-0.06-908 (Fig. 3) passed down the column without interaction, only a small amount of particles could be washed off the column by Triton X-100 (wash peak). In contrast, for the PS-0.14-908 (Fig. 4) the ratio of elution peak to wash peak was significantly shifted to the wash peak. It indicated that the more hydrophobic fraction within the PS-0.14-908, which was retained on the column and could only be washed off with the surfactant, was much larger than the one within the PS-0.06-908 (Table 1). This observation was confirmed by experiments using hexyl-agarose. The portion retained on the column increased for the PS-0.06-908, but there was still a distinct amount eluted with the void volume. PS-0.14-908 could not be eluted with buffer at all, but only by using Triton X-100.

Thus HIC can distinguish between the Poloxamine 908 coatings on 60 nm and 140 nm latex particles, proving the coated 140 nm particles the more hydrophobic. Furthermore it is obvious that the coating is not homogeneous throughout the particles. These results are well in agreement with in vivo data obtained from gamma-scintigraphy studies in NZW rabbits. The uptake of i.v. injected Poloxamine 908-coated particles by liver/spleen increased from about 25 % for 60 nm particles [8, 24] to 30–35 % for particles of about 140 nm [1].

3.3. Fat emulsions

Similar to the presented coated polystyrene latices, Intralipid 10 %, an egg lecithin-based fat emulsion, proved to be hydrophilic enough to be eluted from propyl-agarose. However, in contrast to the particles a distinct wash peak was observed. Furthermore the chromatogram from Intralipid 10 % obtained on pentyl-agarose showed no elution peak but only a large wash peak (Fig. 5). A small fraction even seemed to be irreversibly bound to the column. This was indicated by the reduced AUC of the wash peak obtained with Triton X-100. The HIC data place the fat emulsion between the uncoated and the Poloxamine 908-coated particles on the hydrophobicity scale. Again this correlates with the gamma-scintigraphy data. The RES uptake of egg lecithin-based fat emulsion is significantly lower than the uptake of the uncoated particles, but increased compared to the least hydrophobic Poloxamine 908-coated latices [10, 11].

4. Conclusions

Hydrophobic interaction chromatography is a suitable method for the determination of the surface hydrophobicity of drug carriers. Apart from size and charge measurements HIC will be a useful complementary device for in vitro screening of biodegradable carriers, especially within the concept of differential opsonization [25]. Since the correlation to the in vivo distribution is satisfying, highly promising carriers can be selected to be tested in animal studies.

5. References

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