HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF BOVINE SERUM ALBUMIN NANOPARTICLES

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Nanoparticles derived from natural macromolecules (Marty et al 1978) are possible biodegradable drug delivery systems. The combination of drug or protein substrates with pre-formulated nanoparticles, or their inclusion during formulation, may present an adaptable carrier device.

The binding capacity of un-substituted nanoparticles will be influenced by the availability of surface ionic and hydrophobic areas. Investigation of the effective surface hydrophobicity would seem essential to the prediction of ligand binding mechanisms.

One method studies the hydrophobic characteristics of proteins via their interaction with hydrophobic ligands immobilised on inert media. Hydrophobic interaction chromatography was therefore performed on epoxy-activated 4% agarose conjugated with ligands from n-propyl, n-butyl, n-pentyl and phenyl glycidyl ethers (Ellingboe et al 1970). Column equilibration and elution was in 2M NaCl, 2mM KH_2PO_4, pH 6.8; effluent was monitored continuously at 280nm. Bovine serum albumin (BSA) nanoparticles were prepared by ethanol titration in a modified method of Marty et al (1978), fixed, and purified by sequential gel filtration. Glutaraldehyde crosslinkage times were at 2, 4, 6 and 10 min. with fresh samples applied to the columns in 0.01M phosphate buffer pH 7.0.

Table 1. Ratio of mean elution volumes (n = 3 - 5) to column bed volumes for nanoparticles and a BSA standard on four hydrophobic media.

Reaction time	Propyl	Butyl	Pentyl	Phenyl
2 min	0.82, 1.29	0.62, 0.99	0.64, 1.13	1.23, 1.79
4 min	0.76, 1.08	0.56, 0.91	0.58, 1.05	1.17, 1.63
6 min	0.87, 1.32	0.57, 0.92	0.60, 1.04	1.20, 1.69
10 min	0.96, 1.33	0.55, 0.95	0.62, 1.01	1.25, 1.74
Standard BSA	1.01	0.71	1.75	1.26

Three features may be observed:

i. The single high molecular weight fraction obtained from gel filtration on 1% agarose may be separated into two hydrophobic species.

ii. Independent of the ligand type, nanoparticles produced at 4 min crosslinkage time exhibited minimum hydrophobic interaction, with a trend to increased retention with increased time, and

iii. For each crosslinkage time, one of the two nanoparticle components was more hydrophobic than BSA, the other less. The ratio of the area of the first peak to that of the second increased on storage, suggesting that the first fraction may be an agglomerate of the more hydrophobic particles of the second.

Nanoparticles studied on butylepoxy-agarose at different ionic strengths produced a range of salting-out parameters (Table 2). This coefficient is directly proportional to hydrophobic interaction area (Melander and Horvath 1977), and confirmed that the particles produced after 4 min crosslinkage had the smallest hydrophobic area, but also indicated a plateau at 10 - 14 min crosslinkage time.

Table 2. Effect of crosslinkage time on salting-out parameter for nanoparticles on butylepoxy-agarose.

Reaction time (min)	2	4	6	10	14
Salting-out parameter	0.1099	0.204	0.398	0.990	0.888

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