

Functionalization of Preformed Microspheres -An Overview

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INTRODUCTION

All of these microspheres (MISs) used for chemical and biomedical applications are functional materials in the general sense of the world "functional" but the term "functionalization" means chemical modifications of MISs for the purpose of introduction chemically reactive groups such as OH, COOH or NH₂ into the surface or bulk of the particles.

However, many laboratories using polymer MISs may not have the necessary provisions or staff with sufficient training in basic polymer and heterophase polymerization processes needed for the formation of well-defined functional MISs.

Thus, during the past three decades a vast arsenal of procedures for the functionalization of preformed MISs (both inorganic and organic) has been introduced. Many of these procedures are indeed technically satisfactory for routine applications, but the fact that functionalized preformed MISs can seldom be fully characterized in terms of MIS composition and macromolecular structure. This means that many functional MISs obtained by post polymerization functionalization, and hence the use of these MISs, may not be amenable to rational design and optimization. However, when the target MISs are required for exploratory work, then functionalization of preformed MISs provides a relatively convenient route to a wide range of functional MISs from commercially available materials or readily accessible in-house preparations.

The discussion is on the basis of the main polymers types, including inorganics (mainly silica), polysaccharides polystyrenes and polyacrylamides. Functionalization of less commonly available polymer MISs based on polyacrolein, copoly(dimethylacrylamide-formamidoalkyl acrylate)s, poly(glycidyl methacrylate), poly[methyl α -(hydroxymethyl)acrylate] and polypyrrole latexes are also covered for the sake of reference. A new functionalization approach, termed active ester synthesis, is also

discussed for producing well-defined amphiphilic functional MISs based on copoly(styrene-acrylamide)s with general solvent compatibility.

The basic chemistry of functionalization is of course independent of particle size, but experimental procedures required for modifying small particles (nanospheres, colloidal particles) are substantially different from those for larger particles (microbeads). This is mainly due to particles handling and the relative difficulty or ease by which the functionalized particles can be recovered for the reaction mixture.

It should be evident that once the basic macromolecular structure of a given microsphere type is functionalized. This applies equally to functional MISs obtained by direct (co)polymerization of functional monomers. Typical examples of such second step derivatizations are those of silica and polychloromethylstyrene MISs. But once again it should be borne in mind that difficulties of characterization and rationalization of MISs become correspondingly more and more intractable when dealing with multi-step reactions.

Another important question in the functionalization of preformed MISs is whether the desired chemical reaction proceeds on the particle surface or throughout the bulk of the particles (Figure).

INORGANIC MICROSPHERES

Silica is the main type of inorganic MISs in common use, but related inorganic oxides such as alumina, zirconia and glass beads are also used.

Functionalization of siliceous particles is based on the chemistry of surface silanol groups. The silanol function can be derivatized via several reaction pathways [3], including condensation with alcohols, trichloro- and trialkoxysilanes (Figure). In particles, reaction with triethoxysilanes [3,4-8] provides a highly versatile route for the production of stable functionalized silica particles in a single step. Silylating reactions according to Fig 2 are

carried out by refluxing the silica particles in a solution of the reagent in pure toluene [5,5], in toluene containing (trace of) water [6,7], are in aqueous media[8].

In either case, the chemistry of the reaction is complicated because the OH groups may be free or H-bonded, depending on the terminal history of the particles[6,9]. The silylation reaction shown in Fig 2 are also applicable to other inorganic particles such as titania and zirconia(10). Thus modification of colloidal

silica particles was performed according to Figure 2 by reaction with hydroxyl bearing reagents(e.g. octanol) to form their bonds, or trichloro- or trialkoxy silanes carrying various functions (Figure 3). Colloidal silica particles containing OH or NH₂ groups were also prepared by diborane reduction of the corresponding particles bearing ester or nitrile functional groups.

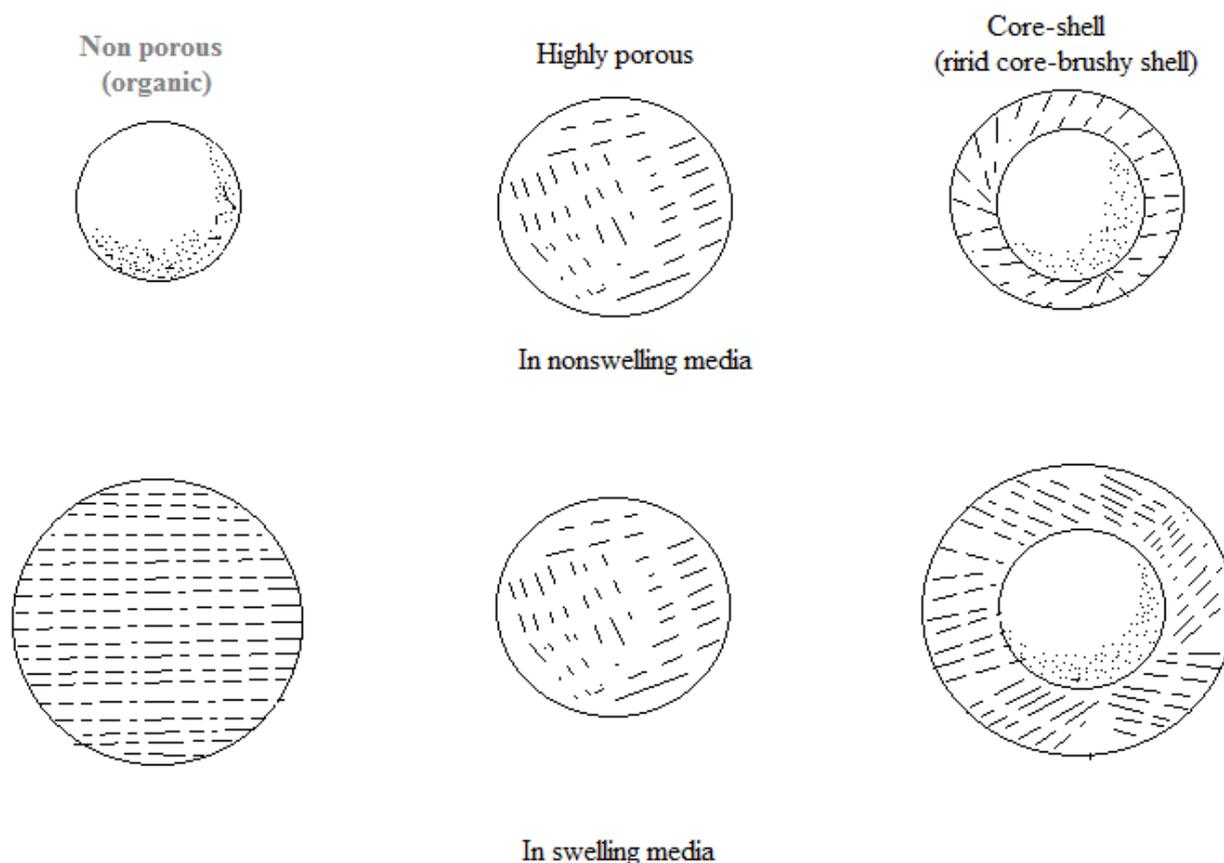
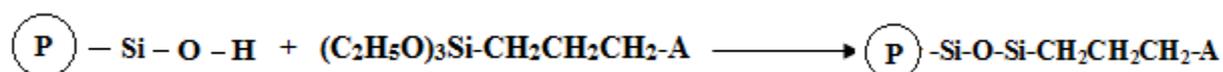
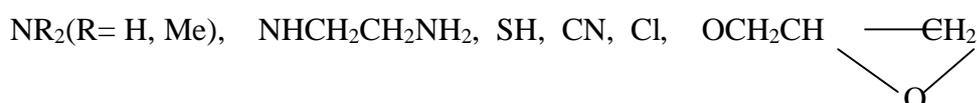


Figure 1: Idealized particle morphologies in nonswelling and swelling media, which determine the course of functionalization on the particle surface or through the bulk.



Examples of A:



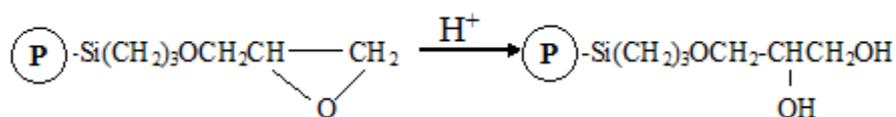
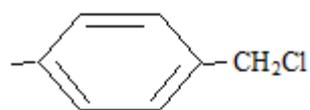


Fig. 2: General scheme for functionalization of silica particles by commercially available triethoxysilane reagents.

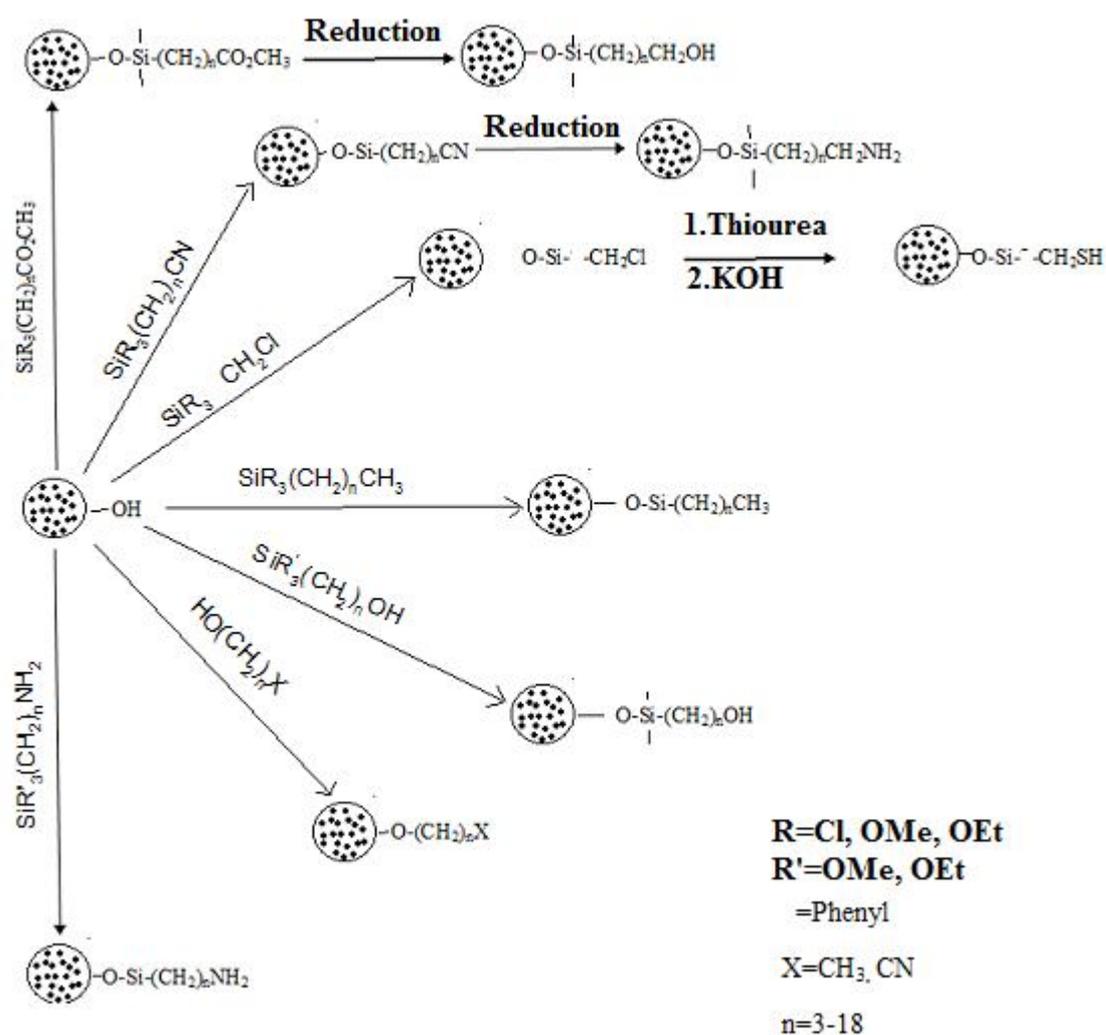


Fig. 3: Functionalization of colloidal silica particles by alcohols, trichloro- and triethoxysilanes carrying various functions

Thiol functionality on the particles was formed by reaction of thiourea with chlorohexyl particles, followed by hydrolysis. Characterisation of the modified particles was accomplished by FTIR, TGA, electron microscopy for chemical analysis [ESCA], contact angle measurement, wettability and elemental analysis. Experimental conditions for reproducible particle modification include the removal of surfactant and the organic contaminants from the particles by (burning) heating at 300-400.

POLYSACCHARIDE MICROSPHERES

Polysaccharides based MISs (cellulose, sepharose, sephacryl and others) generally carry primary and secondary hydroxyl groups, and those crosslinked by epichlorohydrin (e.g. sepharose) also contain tertiary OH on the crosslinking units. The earliest examples of functionalized polysaccharides MISs are cellulose ion exchangers (Figure 4).

The use of polysaccharides based polymer supports for affinity chromatography and enzyme immobilization involves the preparation of "activated" gels, followed by the attachment of the affinity ligand or enzyme via its NH_2 , SH and/or OH groups. One generally useful activated intermediate for this purpose is the "active ester derivative" obtained by succinylation, followed by reaction with a phenolic or N-hydroxy compound in the presence of a carbodiimide (Figure 5). A wide range of other commonly used activation methods are also outlined in Figure 5.

POLYSTYRENE MICROSPHERES

Beaded copolymers of styrene and divinylbenzene are the common materials used for the manufacture of strongly acidic and strongly basic ion exchange resins. Commercially important polystyrene ion exchangers are produced in one or two steps, as depicted in Figure 6. A variety of related chelating agents can also be produced from the chloromethylated polystyrene by processes basically similar to that of the ammonium resins shown in Figure 6.

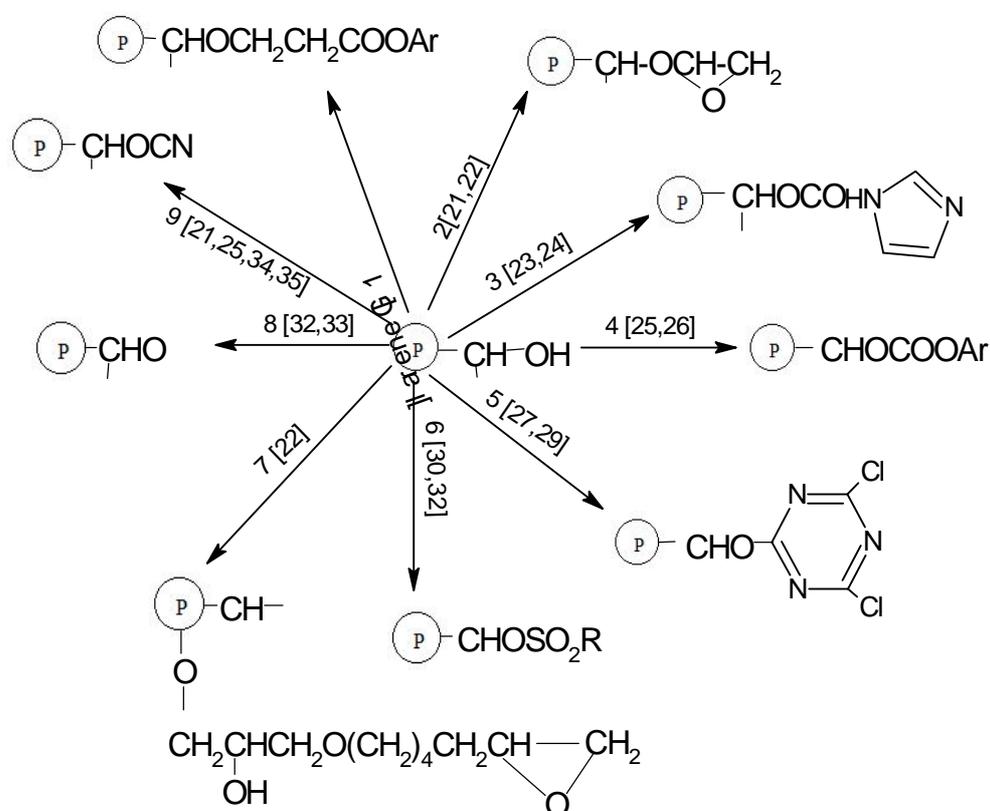
On the basis of its chemical structure, copoly(styrene-divinylbenzene) is relatively

more inert than other commonly available polymer supports. Largely for this reason, as well as for their compatibility with organic solvents, styrene based polymer supports have been generally adapted for solid phase peptide synthesis. As mentioned above, numerous derivatives of polystyrene MISs (Figure 6) are also being studied for a wide range of other analytical, catalytic and synthetic uses. Chloromethylation (Figure 6) and bromination of beaded polystyrene provide two of the most useful intermediates for the synthesis of styrene based polymer supports. It must be borne in mind, however, that both chloromethylation and bromination involve a variety of side reactions and complications, depending on experimental conditions and the desired level of functionalization.

Another frequently reported derivatization of polystyrene nano- and microspheres is diazotization (formation of $-\text{N}=\text{N}^+\text{X}^-$). This is accomplished by reaction of the particles with fuming nitric acid, reduction of the resulting nitro groups on the particles by sodium dithionite, followed by reaction with sodium nitrite in acidic media.

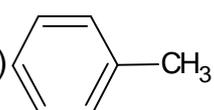
Polystyrene MISs and monospheres carrying chloromethyl or a variety of other functional groups have also been produced by (co)polymerization of the corresponding functional monomers. The chloromethyl nanospheres obtained in this way have been converted to a wide range of derivatives by reaction with amines, as shown in Figure 7. These and related modifications have also a relatively long history in the case of chloromethylated polystyrene MISs, and are frequently reported by different groups for different potential applications.

One particularly interesting modification of chloromethylated polystyrene MISs is the formation of CH_2NH_2 groups on the polymer for use in solid phase peptide synthesis, and a number of different methods has been reported for this purpose. Resin beads with amino groups at the end of a spacer arm have attracted considerable interest for use in solid phase synthesis and related chemical applications.



1. $(\text{CH}_2\text{CO})_2$, then HOAr with EDC 2. $\text{ClCH}_2\text{CH}-\text{CH}_2$ 3. Carbonyldiimidazole

4. ClCOOAr 5. Cyanuric chloride;

6. ClSO_2R ($\text{R}=\text{CH}_2\text{CF}_3$ or )

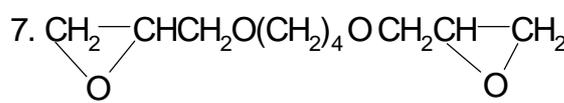
7.  8. IO_4^- ; 9. CNBr , ArOCN

Fig. 5: Activation and derivatization of polysaccharide microspheres

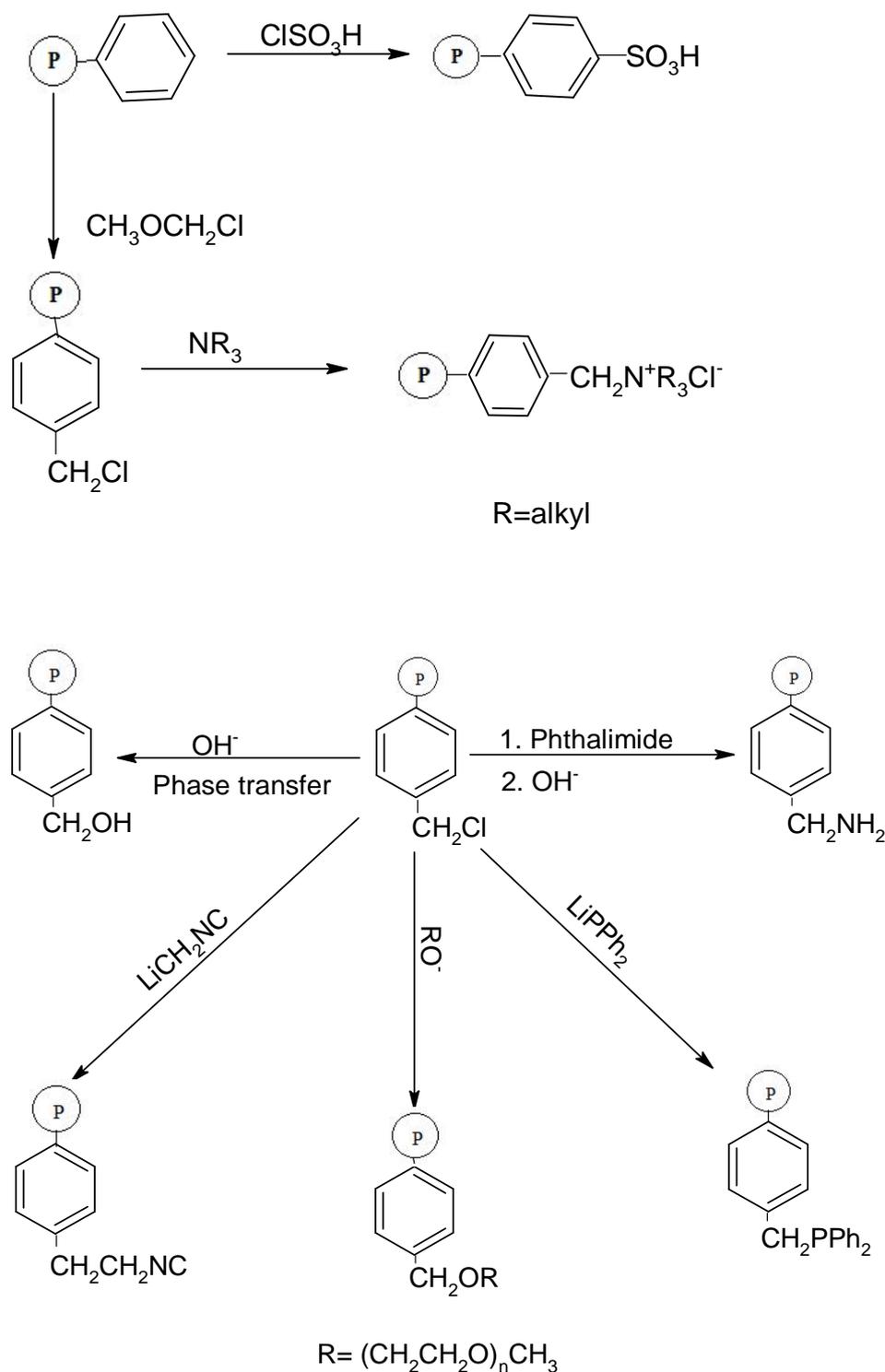


Fig. 6: Sulfonation and chloromethylation of polystyrene microspheres, and the conversion of the chloromethyl groups to other functions

POLYACRYLAMIDE MICROSPHERES

Functionalization of micron size particles of different types of polyacrylamide (polymer supports, sorbents), including polyacrylamide (Biogel), polyacryloyl aminomethyl

dimethylacetal, polyacryloylmorpholine (also known as Enzacryls), poly[(trishydroxymethyl)methylacrylamide](Tris acryl) and polydimethylacrylamide

(PepSyn), are well documented. The basic structures of these polymers are shown in Figure. Reports on the functionalization of nanometer size polyacrylamide particles.

Derivatization of Biogel as reported by Inman and Dintzis is outlined in Figure. The functionalized gels obtained in this way can be further derivatized and activated (i.e. for example and ligand attachment) by reagents such as nitrous acid, carbodiimide and glutaraldehyde.

AMPHIPHILIC MICROSPHERES

Among conventionally available polymer MISs, those based on silica, polysaccharides or polyacrylamides have hydrophilic backbone structures, and hence are compatible with aqueous (and strongly polar) solvents and substrates; whereas those based on polystyrene or polymethacrylates have hydrophobic backbone structures and are compatible with nonaqueous and relatively nonpolar solvents and substrates. Our work on the development of hydrophobic and hydrophilic polymer supports of potential interest for solid phase synthesis and catalysis during the past 20 years has led to the idea that copolymer structures incorporating both hydrophobic and hydrophilic groups in the polymer backbone should have general solvent and substrate compatibility, and should be more generally useful. However, synthesis of well-defined copolymer MISs incorporating both hydrophobic and hydrophilic monomers (e.g. styrene and acrylamide) is impractical for reasons of copolymerization reactivity ratios and differential monomer solubility.

To this end, a new synthetic approach has been elaborated for the development of amphiphilic MISs based on an indirect route and chemistry of active esters (active ester synthesis or leaving group substitution.) According to this method, a well-defined beaded copolymer of styrene and an activated

acrylate with approximately alternating comonomer units in the backbone is produced by suspension polymerization, followed by displacement of the activating (or leaving) groups on the polymer by one or more suitable chosen nucleophiles carrying the desired structure "A¹" or functionally "A".

As can be seen in Figure, the functional residue "A" can be positioned at the end of a spacer arm "B". In addition, the choice of the structural residue (A¹) offers the possibility of "tailoring" the composition of the copolymer support as may be desired for various specific applications. However, it should be noted that when A¹=NH₂ in Figure, i.e. the generation of unsubstituted amide residues (CONH₂) on the polymer, aminolysis leads to the formation of highly rigid matrixes, probably due to very strong H-bonding within the hydrophobic environment of styrene residues.

Typical examples of amphiphilic functional MISs readily available by the new synthetic approach and the range of functional groups which can be introduced into the polymer is virtually unlimited. In the case of polymer support with low degrees of functionality (< 1 mmol/g), the reaction sequence (1,HA¹; 2,HBA) is more convenient, except when HA¹ is a volatile compound (e.g. dimethylamine). There is a possibility that a fraction of the reactive sites generated on the resin initially may be relatively more accessible than those introduced at the end. Accordingly, the reaction sequence (1,HBA; 2,HA¹) is preferable in principle. However, the practical significance of this differential accessibility may depend on the particular application involved. The possibility of generating reacting sites with relatively low accessibility is of special interest in the design and study of site isolation on the polymer support.

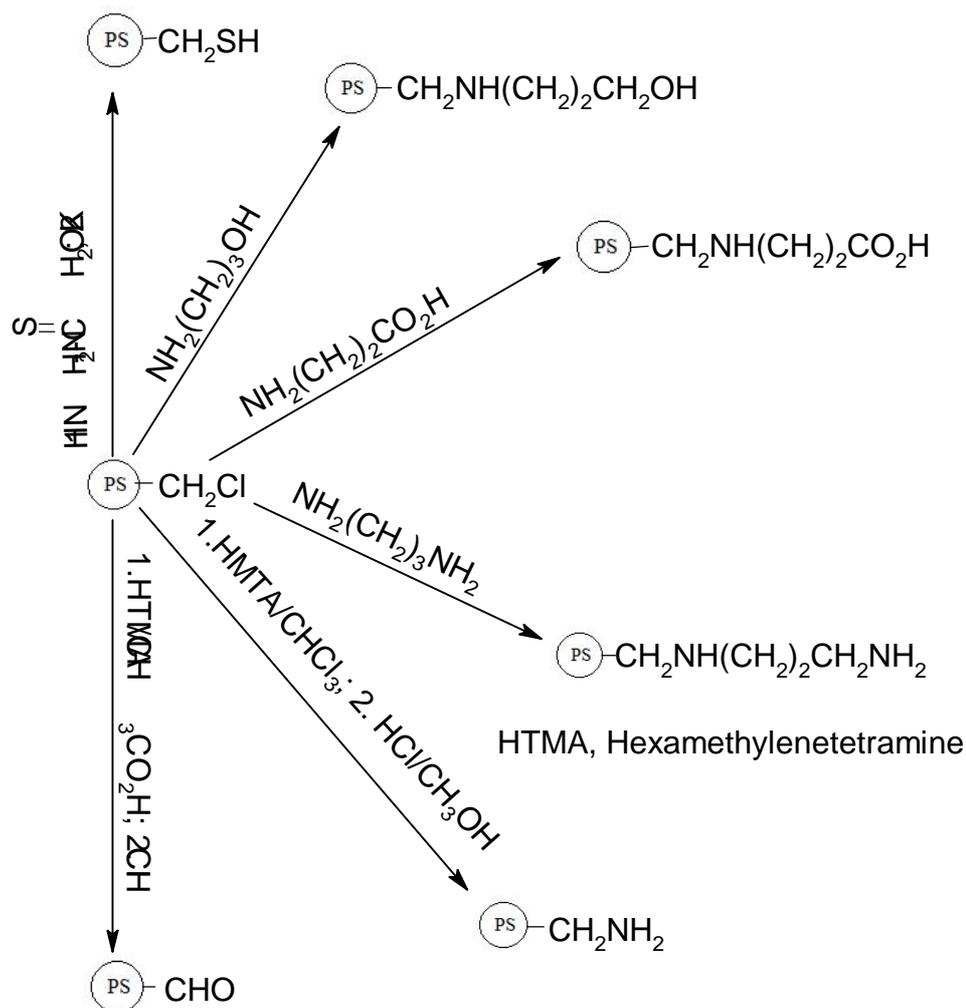


Fig.7: Functionalization of chloromethylated polystyrene nanospheres by amines

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