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Polymeric Reagents in the Synthesis of Peptides

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The essential features of the method of polymeric reagents in relation to peptide synthesis are described. The polymer-activators have been classified in terms of the type of activating groups. The procedures used in the synthesis of polymer-activators and polymeric reagents are given and the principal aspects of the employment of the method of polymeric reagents in the synthesis of peptides of various structure as well as the trends and prospects for the development of the method are discussed.
The bibliography includes 201 references.

CONTENTS

I. Introduction 366
II. The essential features of the method of polymeric reagents in relation to peptide synthesis 366
III. Synthesis of polymer-activators 367
IV. Polymeric activated esters 371
V. Synthesis of peptides with the aid of polymeric reagents 372
VI. Polymeric condensing agents 376
VII. Conclusion 377

I. INTRODUCTION

The application of polymers in organic syntheses, and biotechnology has vigorously increased in recent times. For example, recently published monographs[1-7] have been devoted to these questions. Thus the employment of polymers as various supports, in particular for the immobilisation of enzymes[1,3,7,8] and the cells of micro-organisms[1,9-11] and for the affinity chromatography of proteins[5,12,13] is well known. Studies are being prosecuted in order to synthesise polymeric therapeutic agents,[1,14-16] in particular, artificial antigens[17] and synthetic models of enzymes.[3,18,19] Polymeric catalysts[2,20,21] and high-molecular-weight protecting groups are widely used.[2,22] However, the most vigorously developing field is that involving the application of polymers as chemical reagents.[2,4,24,25] The main advantage of this approach is associated primarily with an increase of the yields and the facilitation of the purification of the desired product; in many cases the specificity of the reactions carried out is greatly increased. The application of polymers in multistage monotonic operations, for example, in the synthesis of polypeptides,[26,27] polynucleotides,[28] polysaccharides,[29] and polycondensation polymers with exactly specified numbers of units[30] proved to be especially valuable. Polymers can serve under these conditions as separators of the reacting groups, imitating the conditions of high dilution. The reduction to a minimum of competing intermolecular† interactions makes it possible to employ this principle effectively for the synthesis of cyclic peptides,[7,31] macrocyclic compounds,[32] asymmetric ketones and α-substituted carboxylic acids,[33-35] the Dieckmann cyclisation,[36] the conversion of bifunctional compounds into the monoblocked form,[37,38] etc. The direction of the reaction and the selectivity can change significantly on passing from the usual low-molecular-weight reagents to polymeric reagents.[39,40]

Polymeric reagents are finding useful applications in virtually all fields of organic synthesis. In peptide synthesis the tendency has arisen to refer to polymers which fulfil acylating functions as polymeric reagents.[27,42-46]

†The term "intrapolymer" reactions is also used for such systems.[41]

The methods of synthesis of polymeric reagents, the use of the latter in peptide synthesis, and the trends and prospects in the development of this approach are discussed in the present review.

II. THE ESSENTIAL FEATURES OF THE METHOD OF POLYMERIC REAGENTS IN RELATION TO PEPTIDE SYNTHESIS

The search for new approaches to the synthesis of biologically active compounds which are protein in nature occupies a special place among the problems of peptide chemistry. "Classical" peptide chemistry, which deals with reactions in solution, has encountered many problems difficult to solve, which include the reduction of the yields with increase in the size of the peptide synthesised, the difficulty of isolating the desired product in a homogeneous state as a consequence of the occurrence of side reactions, and the reduction of the solubility of peptides with increase in the length of their chain.

In the attempts to overcome these difficulties, new ideas have appeared, which are associated mainly with the employment of synthetic polymers. At the beginning of the 1960's Merrifield[26,50] suggested that insoluble polymers be used as carriers of the peptide chain, serving at the same time as the C-terminal protecting group. It then became possible to use a large excess of the activated low-molecular-weight component, whose separation together with soluble side products can be readily achieved by the filtration of the peptide–polymer complex. This "solid phase" method of synthesis gained wide popularity, which was promoted to an appreciable extent by studies on its mechanisation and automation.[51,52] However, in the course of the development of this method, some disadvantages also appeared,[53,54] being associated mainly with the difficulty of eliminating the insoluble side products, accumulated in the course of the synthesis, from the final products.

If a soluble polymer is used as the C-terminal protecting group of the growing peptide, it is possible to diminish the influence of diffusion factors on the course of the reaction. This is the so called "liquid phase" method[55] of peptide synthesis. An obstacle to the wide-scale employment of the liquid phase method, especially in the synthesis of higher

peptides, is apparently the difficulty of purifying the reaction product owing to the similarity of the solubilities and molecular weights of the polymer and the peptide synthesised.

In the middle 1960's a methodologically new trend arose, also associated with the employment of polymers but this time as activators of the carboxy-groups of aminoacids or peptides.[27,56] In the general case the covalent polymer–low-molecular-weight reagent complex, which has the free energy necessary for the occurrence of the reaction, effects the transfer of activated groups to the molecules of the other reactant present in solution (reaction (1)]. Here one can also include the reactions between groups present in the polymer phase, i.e. intramolecular (or intrapolymer) interactions [reaction (2)]. Polymeric reagents of the types indicated are also referred to as polymeric transferring agents.[42]

$$\textcircled{P}\text{—A} + \text{B} \longrightarrow \text{A—B} + \textcircled{P}\,, \qquad (1)$$

$$\textcircled{P}\text{—A—B} \longrightarrow \text{—A—B—} + \textcircled{P}\,,$$

Ⓟ - polymer-activator. (2)

Polymeric condensing agents[43] fit the above definition because the reaction takes place via an intermediate stage—the activation of component A:

$$\textcircled{P} + \text{A} + \text{B} \longrightarrow \textcircled{P}\text{—A} + \text{B} \longrightarrow \text{A—B} + \textcircled{P}\,.$$

In peptide synthesis component A is usually an *N*-substituted aminoacid, whose carboxy-group must be activated before the formation of the peptide bond. Component B is an aminoacid or a peptide with a free N-terminal amino-group. The polymer-activator carries electron-accepting groups for the generation of the positive charge on the carbonyl carbon atom of the aminoacid sufficient for effective attack by the nucleophile. In classical peptide chemistry, activated esters, anhydrides, imides, etc. are usually employed for this purpose.[47–49] In order to accelerate the aminolysis and to ensure that it goes to completion, an excess of the polymeric reagent, which is separated in the last stage of the synthesis, is used as a rule.

The physicochemical properties of the polymer play a very important role both in the synthesis and in the isolation of the product. It is possible to formulate certain general requirements which must be met by polymeric reagents: (1) the polymeric skeleton must be readily permeable to the peptide molecules over a wide range of molecular weights. In the case of polymers of the gel type, the latter must exhibit a satisfactory swellability in the solvents employed; (2) the physical properties of the polymeric species must ensure their ready separation by filtration from the soluble products and their mechanical and osmotic stabilities must be satisfactory; (3) polymeric derivatives of aminoacids must be highly reactive and resistant to racemisation; (4) polymeric reagents must be stable on storage also to the solvolytic action of solvents; (5) polymers must not absorb the soluble components; (6) it is desirable that the polymeric components should be capable of being used many times, i.e. that there should be a possibility of their regeneration without reducing their initial capacity and reactivity and without changing the physicochemical parameters of the polymer.

Among the polymeric reagents used at the present time, polymeric activated esters are most often employed for peptide synthesis:

$$\text{Z—NH—CHR}^1\text{COOH} + \text{HX}\textcircled{P} \longrightarrow \text{Z—NHCHR}^1\text{—CO—X}\textcircled{P} \xrightarrow{\text{H(NHCHRCO)}_n\text{NHCHRCOO—Y}}$$

$$\longrightarrow \text{Z—NH—CHR}^1\text{CO(NHCHR)}_n\text{NHCHRCOOY} + \text{HX}\textcircled{P}\,;$$

Ⓟ = polymeric skeleton; X = activating group;
Y, Z = *N*- and *C*-protecting groups; n = 0, 1, 2...

The condensation of *N*-protecting aminoacids with a polymer-activator leads to the activation of the carboxy-group. The polymeric reagent obtained is introduced into the aminolysis reaction with the corresponding derivative of the aminoacid or peptide containing a free amino-group. The reaction product passes to the solution. After the removal of the *N*-protecting group, the resulting peptide can be made to react again with a second activated aminoacid, effecting thereby the growth of the peptide chain. An important factor in the synthesis of a polymeric aminoacid derivative is the absence of insoluble side products which might contaminate the required product in subsequent aminolysis.

III. SYNTHESIS OF POLYMER-ACTIVATORS

There exist various ways of introducing activating groups into the polymeric skeleton. In some cases the initial polymer is subjected to a series of chemical transformations, while in others a molecule with the functional group already present is attached to a suitable polymeric carrier. The functional group can also be introduced by copolymerising the corresponding monomers or by the ionic binding of the "functional" compound to a strong ion-exchange resin.

In the vast majority of studies, high-molecular-weight nitrophenols and polymers with the *N*-hydroxy-group are most successfully used for the activation of *N*-protected aminoacids.

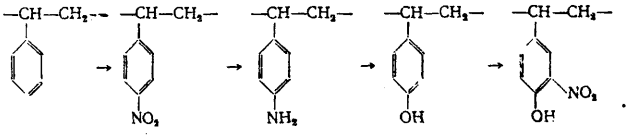
There exists at the present time a fairly extensive literature on high-molecular-weight phenols[57,58] of different structure, whose nitration is potentially capable of ensuring the formation of the required nitrophenol polymer-activator. However, in peptide synthesis the higher requirements which must be met by the physicochemical properties of the polymer significantly restrict the choice of such polymers. For example, the widely familiar phenol-formaldehyde resins of the "resite" and "novolak" types[59] are apparently relatively unsuitable for such purposes owing to the difficulties arising in the regulation of the steric structure, the content of functional groups, and the physical shape of the polymer particles. There exist brief reports about the use of such polymers,[56,60] but experimental data on their application are virtually lacking. Amorphous powdered polymers of this type have been obtained by the polycondensation of di(4-hydroxyphenyl) sulphone with formaldehyde[61] or of salicylic acid with anisole and formaldehyde.[62] Many phenol-formaldehyde polymers have also been used in the solid phase synthesis.[31,63–65] The yields of peptides when such a resin was employed were appreciably lower compared with a hydroxylated polymer having a polystyrene skeleton. This effect has been attributed by the authors to steric hindrance—the methylene bridges were located next to the functional groups.

In one of the first studies on the use of polymeric reagents in peptide synthesis,[27] it was suggested that one should use poly-4-hydroxy-3-nitrostyrene, obtained by the hydrolysis of the copolymer of *p*-acetoxystyrene with 4% of divinylbenzene and subsequent nitration with concentrated acid at 0 °C.[66] This polymer was used successfully in the synthesis of low-molecular-weight model peptides bradykinin and luliberin,[67] but in the course of its application disadvantages were revealed—the decomposition of the polymeric species during the aminolysis reaction and mechanical manipulations which might have led to the contamination of the desired products.[68,69]

Williams[70] suggested that poly-*o*-nitrophenol be synthesised by the block copolymerisation of *p*-methoxystyrene with divinylbenzene, subsequent elimination of the methyl group using boron tribromide and then nitration. The effectiveness of the acylation by *N*-protected aminoacids of both the

given polymer and the preceding one[27] was approximately the same (1–1.5 mmol per gramme of polymer).

A similar polymer-activator was obtained in accordance with the following scheme using polymer-analogue reactions:[56]

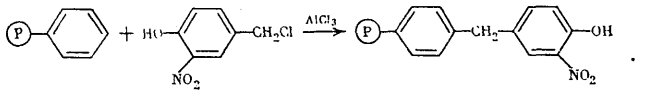


The content of reactive hydroxy-groups was 0.05–0.5 mmol per gramme of polymer, while their theoretical content was 5–7 mmol g^{-1}. Evidently the above scheme is relatively unsuitable for the synthesis of the activator owing to the occurrence of side processes leading to a sharp reduction of the reactivity of the polymer.

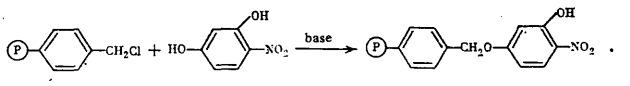
An insoluble phenolic polymer in the form of spherical grains has been obtained.[71] The authors used the copolymerisation in suspension of *p*-acetoxystyrene with styrene and divinylbenzene and the acetate group was split off by hydrazinolysis under mild conditions.

With one exception,[71] in the cases examined the polymers consisted of particles of irregular shape, which was bound to hinder their use owing to cracking and mechanical wear. The occurrence of side reactions in the synthesis of certain polymer entails, together with a decrease of the content of functional groups, also significant changes in the steric structure, for example, owing to additional cross-linking. In order to eliminate the above negative phenomena, it was suggested[72] that the complete functional group be introduced into the polymer-carrier with the required set of physical properties. The authors synthesised a polymeric *o*-nitrophenol using as the carrier spherical grains of a copolymer of styrene with 2% of divinylbenzene, which has found extensive application in the solid phase synthesis of peptides in view of satisfactory physicochemical parameters.

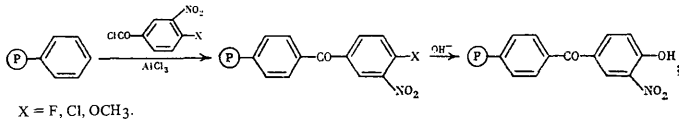
An analogous approach has been used by other workers,[69] who modified the styrene grains with 4-hydroxy-3-nitrobenzyl chloride by means of the Friedel–Crafts reaction:



A polymer-activator of similar structure has been obtained by means of the following scheme:[68]

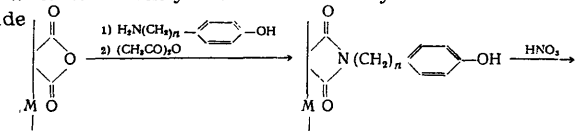


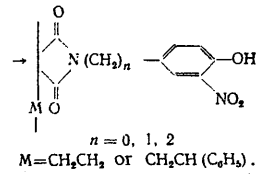
Finally, a polymeric 4-hydroxy-3-nitrobenzophenone has been proposed quite recently for acyl-activation in peptide synthesis:[73]



X = F, Cl, OCH_3.

Low-molecular-weight activated ethers of 4-hydroxy-3-nitrobenzophenone proved to be 40 times more reactive than the corresponding *o*-nitrophenyl ethers. A polymeric *o*-nitrophenol has been synthesised in the laboratory of the authors of the review on the basis of a cross-linked copolymer of styrene with maleic anhydride[74] and of ethylene with maleic anhydride





$n = 0, 1, 2$

M=CH_2CH_2 or $CH_2CH(C_6H_5)$.

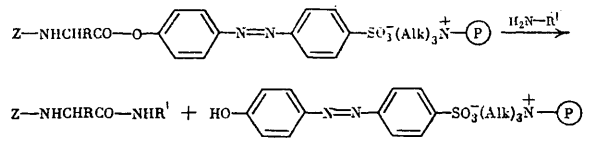
The polymer-carrier consists of spherical grains obtained by cross-linking the initial linear copolymers by aromatic diamines (3–5 mole %) in an emulsion system.[75] Their modification by aminophenols with subsequent nitration led to poly-4-hydroxy-3-nitrophenylmaleimides with a degree of conversion close to 100%.

Practically the same polymer-activator has been obtained by Japanese investigators,[76] who also synthesised a soluble polymeric nitrophenol. However, the method of synthesis of cross-linked polymers differed from that given above;[74] the authors[76] copolymerised *p*-acetoxyphenylmaleimide with styrene in the presence of divinylbenzene with subsequent elimination of the acetyl group and nitration. Both the polymeric mononitro-derivative and the dinitro-derivative—poly-4-hydroxy-3,5-dinitrophenylmaleimide—were obtained.

The polymer-activators obtained by a procedure involving the attachment of a complete activating group to the polymer-carrier were distinguished by a high mechanical strength and swellability in organic solvents of different polarity: in dimethylformamide (DMF), dioxan, methylene chloride, etc. One cannot rule out the possibility that such polymers exhibit a smaller steric effect on the aminolysis reaction, because the reaction centre in the polymeric activated ester is remote from the polymer chain.

A soluble polymer-activator with a nitrophenol group has been obtained[27] from a copolymer of DL-lysine and L-tyrosine[77] and, whilst being insoluble in solvents of low polarity, it dissolves satisfactorily in DMF, ethanol, and water at basic pH. However, this polymer has been used only for the cyclisation of linear peptides.[27] A soluble polynitrophenol obtained from poly(ethylene glycol) is also known;[78] it has been used in the synthesis of model dipeptides.[79]

Mention should be made of a study[80] where the reagent was used in the form of an electrostatic complex:



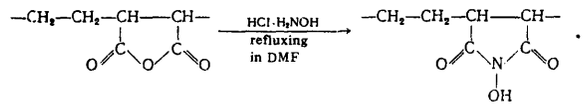
The activated ester of an *N*-protected aminoacid and 4-hydroxyazobenzene-4-sulphonic acid was attached to an ion-exchange resin (Dowex 1X2) by salt formation.[81,82] The aminolysis of the insoluble ester obtained in this way was carried out in formamide. The relatively high swellability of this polymer in highly polar media should favour the use of free aminoacids and peptides as the amino-components. The advantage of this approach consists in the fact that the activated ester was synthesised in solution and it could therefore be purified before being attached to the polymer. A disadvantage of the reagent is the risk of ion exchange and the consequent contamination of the aminolysis products. An acetylated insoluble polystyrene ion exchanger has been proposed[83] for the synthesis of the amide bond in an aqueous medium. However, under the conditions indicated, the acylation of aniline was insufficiently effective—the yield of acetanilide did not exceed 26%.

Attempts have been made to use polymeric thiophenols in peptide synthesis.[56,84,85] Among these, only poly-*o*-nitrothiophenol[85] and poly-2-mercaptopyridine[85] have been used in the synthesis of two peptides, the reagents based on the

mercaptopyridine being more reactive (the yield in the condensation reaction was 90–98%). Poly-2-mercaptopyridine also proved an effective thiolytic agent—it has been used for this purpose in order to eliminate the Nps# protecting group at intermediate stages in the synthesis of leucine-enkephalin.[85]

The *O*-acylation of *N*-substituted hydroxylamines attracted much attention by investigators, because the activated esters of this type are highly reactive and are resistant to racemisation.[48] *N*-Hydroxysuccinimide esters[86] derived from *N*-hydroxybenzotriazole[87] have gained much popularity in the classical peptide synthesis and benzotriazole has been frequently used as the catalyst in peptide formation reactions.[88] These particular derivatives have served as prototypes of polymeric reagents with an *N*-hydroxy-group.

A linear copolymer of ethylene with *N*-hydroxymaleimide has been synthesised as follows:[89]

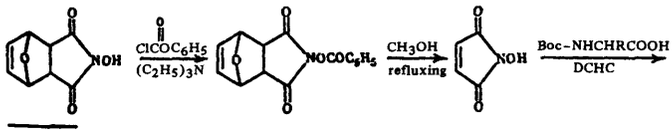


This polymer and its *O*-acyl derivatives dissolved only in polar solvents—DMF and dimethyl sulphoxide (DMSO). For this reason aminoacids were activated by this polymer in the above solvents and the resulting esters were subjected to aminolysis in dimethoxyethane or ethyl acetate in which the polymer does not dissolve. Despite the satisfactory results obtained in the synthesis of low-molecular-weight peptides, its application is limited because many peptides dissolve only in the same solvents as the given polymer. For this reason, the authors apparently used subsequently in the synthesis of higher peptides (from hexa- to octa-peptides) a highly cross-linked polymer, which was obtained by irradiating the above poly-*N*-hydroxysuccinimide with high-energy electrons.[89] The content of active hydroxy-groups then fell to 2%, i.e. the polymer virtually "worked" only in the surface layer.

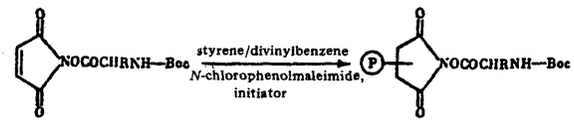
In contrast to the previous workers,[89] Japanese investigators proposed different methods for the introduction of *N*-hydroxy-groups into the polymer, involving the treatment of the styrene–maleic anhydride copolymer in pyridine under milder conditions—at room temperature.[90–94] The polymer was obtained in the form of a colourless powder with an *N*-hydroxy-group content close to theoretical.[90] It was used for the acetylation and benzoylation of certain amines in a homogeneous system; it was not employed for peptide synthesis.

It has been suggested that *O*-substituted maleimides can be used for the synthesis of cross-linked spherically granulated poly-*N*-hydroxysuccinimides, provided that they are subjected to emulsion copolymerisation with styrene and divinylbenzene.[91] However, spherical grains could not be obtained under the conditions indicated. It is of interest that the maleamic acid (an aminoacid) units present in the polymer chain were readily converted into a five-membered imide ring by dehydrocondensation on treatment with aqueous hydrochloric acid,[90,91] while dehydrating agents or heat were required for the low-molecular-weight analogues.

A novel method of synthesis of the polymeric reagent has been proposed by Akiyama et al.[94]:



#o-Nitrophenylsulphenyl (Ed.of Translation).



Boc = t-butoxycarbonyl
DCHC = dicyclohexylcarbodiimide .

According to the authors' idea, this procedure guaranteed a higher purity of the polymeric activated esters. On the other hand, the introduction of chlorophenylmaleimide into the polymer chain entailed smaller changes in the swellability of the polymer and the solvent was altered during aminolysis.

The copolymerisation of benzyloxymaleimide (or acetoxymaleimide) with styrene and 0.6% of divinylbenzene followed by acid elimination of the *O*-protecting group led to a polymer containing 3–3.5 mmol g^{-1} of *N*-hydroxy-groups.[93]

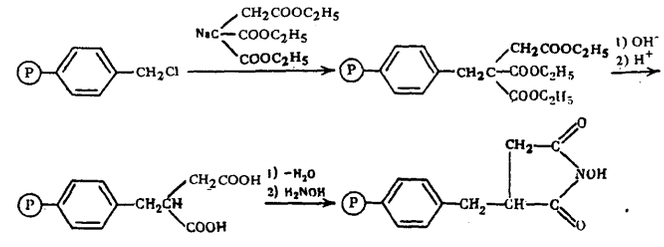
However, it is evident that the schemes for the synthesis of polymeric *N*-hydroxysuccinimides providing for the use of *O*-substituted maleimides are fairly cumbersome and require the expenditure of much time; the synthesis of the initial monomer does not take place with a high yield in all the stages. Furthermore, in the polymerisation of optically active monomers, one cannot rule out the possibility of racemisation, because the process is carried out at an elevated temperature (Akiyama et al.[94] used only simple aliphatic aminoacids).

A simpler and rapid method of synthesis of poly-*N*-hydroxysuccinimide involves the copolymerisation of maleic anhydride with a vinyl monomer (usually styrene) in the presence of divinylbenzene and subsequent modification of the anhydride groups. The introduction of *N*-hydroxy-groups by treating the anhydride polymer with hydroxylamine hydrochloride in boiling DMF has been described,[95] the degree of conversion reaching 88%. Such polymers swell satisfactorily in solvents with a high dielectric constant.

A polymer with an analogous functional group has been obtained by treating the soluble copolymer of ethylene and maleic anhydride with a mixture of hydroxylamine and a polyamine as the cross-linking agent (hydrazine, spermine, and spermidine) in an aqueous pyridine medium. The polymer was distinguished by a high permeability to large molecules, which is indicated by the high yield in the peptide formation reactions and by the quantitative acylation of the terminal amino-groups of insulin.[96]

All the polymer-activators of the *N*-hydroxysuccinimide type examined consisted outwardly of amorphous powders. Repeated use of these polymers is difficult, since the polymer particles of irregular shape are rapidly comminuted, which impairs their capacity for filtration in the swollen state.[96] It is therefore not surprising that only one publication has been devoted to each polymer.

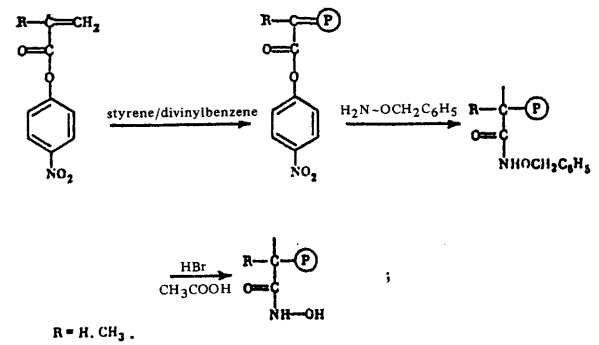
A scheme for the synthesis of the polymer-activator, in which a cross-linked chloromethylated polystyrene in the form of spherical grains is subjected to a series of chemical transformations, has been presented in a review article:[43]



Cross-linked copolymers of maleic anhydride in the form of grains having a regular spherical shape have been obtained by Soviet workers[75,97–100] by cross-linking the initial copolymers by aromatic diamines with a rigid extended molecular structure [benzidine, di(aminophenyl), ether, etc.] in DMF dispersed in a liquid polysiloxane. *N*-Hydroxy-groups were introduced by treating the anhydride polymer with hydroxylamine hydrochloride in pyridine; their content in the final polymers reached about 4 mmol per gramme of the polymer. The grains of all the poly-*N*-hydroxysuccinimides obtained swelled satisfactorily in polar organic solvents, while the polymers into the composition of which *N*-vinylpyrrolidone has been introduced[97–100] also swelled satisfactorily in an aqueous medium. The particles of the polymeric *N*-hydroxysuccinimide had a satisfactory mechanical strength and osmotic stability and were easily filtered in the swollen state. The acylation of the hydroxy-groups in the polymer was almost quantitative.[97–102] These polymers have a macronetwork isoporous skeleton[103] and it has been shown[104] that polymers with this structure are satisfactorily permeable to large molecules.

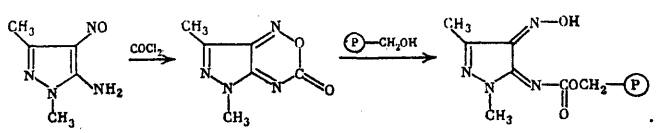
As can be seen, there exists a fairly large selection of methods for the synthesis of polymeric *N*-hydroxysuccinimides. Evidently, the simplest and cheapest versions are those which begin with copolymers of maleic anhydride. One should bear in mind that such copolymers contain rigorously alternating units[105,106] and that by selecting the appropriate comonomers it is possible to alter significantly some of their properties, for example their hydrophilic/hydrophobic properties,[98,100] and the microenvironment of the reaction centre.[94]

An attempt has been made[93] to use derivatives of hydroxamic acid for activation:



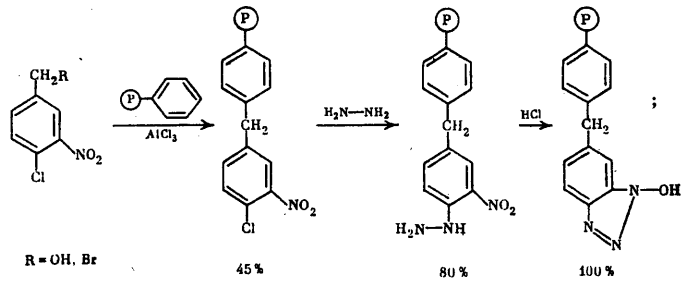
In the case of a polymer containing acrylic acid residues, the above reactions occurred with a higher yield than for a copolymer of methacrylic acid. The attempt to copolymerise methacryloylhydroxylamine acid with styrene was unsuccessful.[93] The aminolysis of *O*-acyl derivatives of the resulting polymer (R = H) by certain aliphatic aminoacids and amines led to fairly low yields of amides, which can be attributed to the low reactivity of this polymeric reagent[107] and steric hindrance.

Guarneri et al.[108] suggested that a polymeric derivative of 5-amino-4-nitrosopyrazole be used as the activator:



It had been shown earlier that low-molecular-weight activated esters based on 5-aminopyrazolone oxime exhibit a very high acylating capacity in the virtual absence of racemisation.[109] An interesting feature of this oxime is that, by virtue of the presence of conjugated double bonds, it is coloured green, while its *O*-acyl form is red, as a result of which it is possible to follow visually the occurrence of aminolysis. The amount of the *N*-protected aminoacids applied to the polymer (condensation with the aid of DCCD) was 0.25–1 mmol g^{-1}. The introduction of aminoacids with a bulky side group into the polymer involves certain difficulties.

Among the activated esters known at the present time, the acyl derivatives of *N*-hydroxybenzotriazole are some of the most powerful acylating agents[88] and the *N*-hydroxy-derivative itself effectively accelerates the aminolysis reactions of other activated esters[88] and is frequently used in combination with dicyclohexyl carbodiimide (DCCD) for the condensation of peptide fragments in order to reduce the extent of racemisation. An insoluble polyhydroxybenzotriazole has been obtained in accordance with the following scheme:[110]

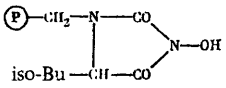


A macroporous spherically granulated copolymer of styrene with 2% of divinylbenzene (XE-305), alkylated with a chloronitrobenzyl derivative by the Friedel–Crafts reaction, was used as the carrier. Best results were obtained when a substituted benzyl alcohol (R = OH) was employed. Further treatment of the resulting derivative with hydrazine in boiling methylcellosolve with subsequent dehydration by boiling concentrated HCl afforded a polymer-substituted *N*-hydroxybenzotriazole (up to 1.4 mmol g^{-1} [111]). The polymer swelled satisfactorily in DMF and methylene chloride and reactions involving it occurred extremely rapidly. The authors[111] regard this support as almost ideal for the method of polymeric reagents in peptide synthesis.

It has been shown[73] that the reactivity of polymeric derivatives of *N*-hydroxybenzotriazole is higher than that of the corresponding derivative of *o*-nitrophenol by a factor of 100, while that of the low-molecular-weight analogue is higher by a factor of 8000. This difference has been attributed to the low rate of mass transfer within the polymer pores.[73]

When a soluble polymeric form of *N*-hydroxybenzotriazole was used,[78,79] no effect of the polymeric activating group on the rate of aminolysis was noted. However, it has been concluded[79] that such soluble polyoxyethylene-bound reagents are useful only in the synthesis of peptides which are satisfactorily soluble (only in this case is the separation of the polymer from the peptide at the end of the reaction sufficiently complete—by precipitation, recrystallisation, or ultrafiltration).

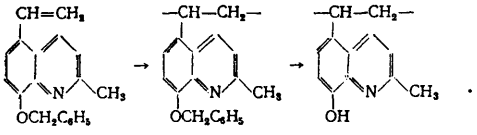
There are apparently definite prospects for the development of new polymer-activators based on substituted hydroxylamines. For example, it has been shown[112,113] that the esters of certain *NN*-diacylhydroxylamines and a series of oximes with electron-accepting substituents are highly reactive in aminolysis reactions. There exists also a brief communication[114] about the use of a polymer containing 3-hydroxyhydantoin groups in peptide synthesis:



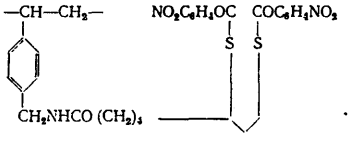
Ⓟ = polystyrene cross-linked with 2% of divinylbenzene

This polymer has been used either to activate Z-Gly in the synthesis of the peptide ZpGly-Ala-OC_2H_5 or as an additive in the synthesis of the same peptide by the DCHC method. In the second case the yield and optical purity of the products were higher.

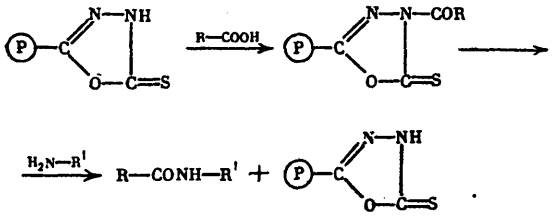
Other types of polymer-activators have been described in the literature. For example, the polymeric analogue of 8-hydroxyquinaldine has been described.[115] It had been used earlier for peptide synthesis in solution.[116] It was obtained by the copolymerisation of 8-benzyloxy-5-vinylquinaldine with 20 mol % of divinylbenzene and subsequent debenzylation with concentrated HCl:



The polymer was tested in the synthesis of a simple dipeptide. The limited amount of data available makes it impossible to assess its advantages, but it apparently possesses no advantages compared with the compounds discussed above. However, this instance (in addition to the polymeric hydroxybenzotriazole) is an example of a polymeric reagent where the effect of intramolecular catalysis has been used in the aminolysis reaction. A further two communications appeared subsequently[117,118] in which the synthesis of polymeric reagents operating by the same principle was described. These were polymeric derivatives of lipoic acid[117] (the coenzyme of acyltransferases) and 1,3,4-oxadiazoline-5-thione.[118] The first derivative[117] was used to activate only *p*-nitrobenzoic acid and the diacyl and not the monoacyl form obtained:



Examples of peptide synthesis were not described. Polymeric 1,3,4-oxadiazoline-5-thiones (cross-linked and soluble modifications of polymers) have been used for the acyl-activation of *N*-protected aminoacids (alanine, phenylalanine, and leucine), the yield amounting to 25–100%; the polymeric reagents employed were made to react only with the ethyl ester of glycine (the yield of dipeptides was 72–97%).



It has been suggested that the polymeric reagent based on polyacrylamide with thiol functional groups be used in an aqueous medium for the synthesis of the amide bond.[119] The acylation of this polymer was sufficiently effective, the content of acetyl groups reaching 2.2 mmol per gramme of the polymer. The data on the application of the resulting polythioester in an aqueous medium are discussed in Section V.

An attempt has also been made to use polymeric mixed anhydrides as acylating agents.[68,83] The anhydride of an *N*-protected aminoacid and a sulphonated polystyrene was employed for the acylation of certain simple aminoacids and peptides.[68,83] An analogous reagent has been proposed[120] for the esterification of alcohols; the anhydride of benzoic acid and a carboxylated polymer has been used for the acylation of aromatic amines.[121,122] The employment of such reagents is problematic, because the nucleophilic attack is not always selective—is not directed to the required carbonyl, its direction depending to a large extent on the steric hindrance. The degree of racemisation occurring when this method is employed is greatly influenced by different factors.[123]

IV. POLYMERIC ACTIVATED ESTERS

In the general case the rate of aminolysis depends on the concentration of the reactants. Since the reaction proceeds within the polymer grain, the effective concentration of the carboxy-component (*N*-protected aminoacid) in the polymeric ester should be determined by the content of the component in the dry polymer, the swellability of the polymeric ester, and the accessibility of the reaction centres to the amino-component. The amount of solvent should be sufficient for the complete swelling of the polymer particles and for their suspension in the solution of the amino-component. Since swellability is a constant parameter of the given polymer and solvent, in order to increase the rate of the reaction it is essential to endeavour to achieve the maximum possible content of the activated aminoacids in the polymer. It is noteworthy that in the case of soluble polymers the creation of a high concentration of the carboxy-component is prevented by the increasing viscosity of the polymer solution.

The effect of the application of polymeric reagents in peptide synthesis depends to a large extent on the yields of the aminoacid derivatives and the ease with which they can be introduced into the polymer. For example, in certain studies a large excess (up to a ninefold excess[72]) of expensive amino-derivatives has been used for condensation with the polymer and, if one takes into account the fact that an excess of the carboxy-component is also used in aminolysis, then the useful employment of the aminoacid derivative then amounts to only several per cent.

The degree of acylation of the polymer-activator by *N*-protected aminoacids is determined by many factors—the method of esterification, the nature of the activating groups and their steric environment, the homogeneity of the polymer network, and the structure of the aminoacid. In order to assess the effectiveness of the methods of synthesis of the polymeric esters, one should evidently employ not the content of the aminoacid residues in the polymer but the yields of the carboxy-component introduced into the polymer and the degree of its utilisation. Here one should bear in mind that in most cases the polymer can be regenerated while the isolation of the aminoacid derivatives which have not reacted with the polymer is extremely difficult. For such assessment, it is essential to know the content of the functional groups in the polymer-activator. In certain cases the investigators restrict themselves to elemental analysis, which does not allow inferences about the content of the reactive groups or groups accessible to acylation. The occurrence of side reactions during the synthesis of the polymer-activator and also the slight changes in the architecture of the polymer skeleton can nevertheless influence significantly the effectiveness of the esterification.

An important condition for the synthesis of polymeric esters of any type is a high initial concentration of the reactants in solvents inducing an effective swelling of the polymer. In this sense, DMF, methylene chloride, and their mixtures can be well recommended.[69,101,124]

DCCD has been used as the condensing agent in the activation reaction by most investigators. For polymeric phenols, the method proved to be the most successful.

Condensation via the *p*-nitrophenyl ether or azide[60] methods yielded worse results and has been hardly used. Acylation of the polymers by the Schotten–Baumann method is also relatively unpromising from the standpoint of racemisation.[62,73]

There exist data concerning the use of symmetric anhydrides of *N*-acylaminoacids,[73,78] but best results (81–100%) have been obtained in this instance by employing a soluble polymer-activator[78] and a comparatively large excess (by a factor of 2–4) of symmetrical anhydrides.

It must be emphasised that in the case of polymeric phenols, obtained by the addition of the activating group to the polymer-carrier, a higher degree of acylation has been achieved in contrast to the polymers where the phenol ring enters into the composition of the polymer chain; it approached 100%.[69,72] When the functional group is remote from the polymer chain, more complete condensation is evidently favoured.

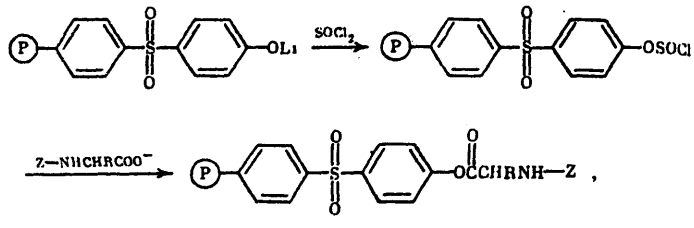
Data have been published[69] on the kinetics of the condensation of benzyloxycarbonyl- and t-butoxycarbonyl-phenylalanine with 4-hydroxy-4-nitrobenzylpolystyrene (cross-linked) in DMF and methylene chloride. It was shown that in the case of the second derivative the rate of reaction was somewhat higher. The solvent did not exert a major influence, some difference being observed only in the initial rates; after 5 h have elapsed, the degree of acylation of both aminoacid derivatives became identical and reached a maximum. In the same study it was noted[69] that the esterification of *N*-protected proline used DCCD was unsatisfactory, the method of mixed anhydride yielding much better results.

DCCD has also been used preferentially for the acylation of polymers with an *N*-hydroxy group; the condensation usually occurred with a satisfactory yield. The difficulties associated with the removal by washing from the polymeric ester of the dicyclohexylurea produced in the course of the reaction must be included among the disadvantages of the DCCD method. A case has been described[43] where, even after repeated washing of the polymer by various solvents, the urea derivative could not be fully eliminated from the polymer. It is apparently undesirable to use DCCD for the introduction of *N*-protected asparagine and glutamine into the polymer in view of the possible formation of nitrile derivatives,[125,126] which may be incorporated into the polymer. A side reaction of DCCD with *N*-hydroxysuccinimide, which can occur also in the polymer, has been described.[127]

The synthesis of polymeric aminoacid esters via mixed anhydrides has certain advantages, because the side products formed are readily soluble in organic solvents. This method has been used to acylate polyphenols,[60,61] poly(5-vinyl-8-quinaldine),[115] and also *N*-hydroxysuccinimide polymers.[89,102,126]

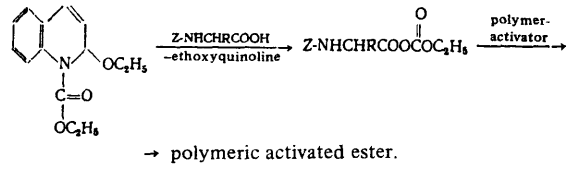
It has been shown[128,129] that high yields of low-molecular-weight *N*-hydroxysuccinimide esters of acyl aminoacids can be obtained in the virtual absence of racemisation if *N*-methylmorpholine is used as the base in the formation reaction of the mixed anhydride. This method has been employed successfully[126] also for the synthesis of polymeric *N*-hydroxysuccinimide esters using DMF and tetrahydrofuran (THF) as the solvents, the polymer being acylated by the mixed anhydride at 40–50 °C. The entire synthesis occupied about 1 h and the yields were 70–90%.

For the condensation of an *N*-protected aminoacid with a polymer having a phenol-formaldehyde structure, the latter was subjected to preliminary activation with $SOCl_2$ or $COCl_2$:[61]



where Z is an *N*-protecting group and R a side group of the aminoacid. For this polymer, the yield in the acylation reaction by the above method was virtually the same as in the carbodiimide and mixed anhydride methods (the aminoacid content in the polymer was 0.9–1.1 mmol g^{-1}).

The method based on the application of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline can also be included among the mixed anhydride methods:[130]



→ polymeric activated ester.

Although this reagent has been used exclusively for the formation of the amide bond, especially in solid phase synthesis,[131] the authors tested it in the synthesis of polymeric *N*-hydroxysuccinimide esters.[126] It was found that the reaction proceeds fairly slowly (in DMF or THF) and the yields do not exceed 40–50%.

The use of transesterifying agents is one of the most effective methods of synthesis of activated esters.[132] The method involves the acylation of the corresponding hydroxy-component (*N*-hydroxysuccinimide, phenol derivatives) by the anhydride of a strong organic acid (trifluoroacetic, trichloroacetic) with subsequent transesterification of the resulting ester with an *N*-protected aminoacid in the presence of a base in an aprotic solvent. Despite the possibility of the occurrence of side reactions[133] in the transacylation of *N*-trifluoroacetoxysuccinimide with an *N*-acylaminoacid in the presence of a tertiary amine, this method has been used fairly successfully also for the synthesis of polymeric *N*-hydroxysuccinimide esters.[126] It is noteworthy that extremely high yields have been noted in the condensation of acylpeptides by this method, although in the presence of an optically labile *C*-terminal aminoacid there is a possibility of racemisation.[126]

Different types of polymeric activated esters of virtually all natural aminoacids have now been obtained. The benzyloxycarbonyl and t-butoxy carbonyl,[60,89,101] nitrobenzenesulphenyl,[102,124] and trifluoroacetyl[126] groups have been used as N^{α}-protecting groups.

The simplest method for the determination of the content of the activated aminoacid in the polymeric ester is the gravimetric method, which is fairly accurate if the amount of aminoacid applied to the polymer is large (in excess of 0.5 mmol per gramme). In a number of studies[90,91,93,101] the capacity was determined from the yields of the cyclohexylamide derivatives obtained as a result of the interaction of the isolated polymer with an excess of cyclohexylamine. In an alternative version,[69,73] the polymeric ester was treated with a

solution of benzylamine in toluene and the excess benzylamine which failed to react was titrated with 0.1 *N* $HClO_4$ in acetic acid using Methyl Violet as the indicator. Aminoacid analysis after the exhaustive hydrolysis of the sample of the polymeric ester with a 1:1 mixture of 12 *N* HCl and CH_3COOH has been used in two investigations.[69,124] In one of them,[124] the benzyloxycarbonyl group was determined spectrophotometrically (at 257 nm) after the alkaline hydrolysis of the polymeric nitrophenyl ester of the *N*-protected aminoacid. In certain cases it is possible to use elemental analysis, for example, when the acyl derivative contains sulphur (the Nps group, methionine, or cysteine).[102,124] All the methods indicated yield approximately identical results and one must also bear in mind that in practice a high accuracy in the determination of the capacity of the polymeric reagent is not required because it is as a rule used in excess in the aminolysis reaction. In addition, one may mention that methods for the non-aqueous titration of activated esters have been examined in two communications,[134,135] but these apparently can also be applied to the corresponding polymeric derivatives.

V. SYNTHESIS OF PEPTIDES WITH THE AID OF POLYMERIC REAGENTS

The aminolysis of a polymeric ester, leading to the formation of a peptide bond, proceeds in the solvated polymer–solvent heterogeneous system. The rate of the process should depend on many factors: the nature of the activated ester, the rate of diffusion of the amino-component in the polymer, the accessibility of the reacting group of the polymer, the capacity of the polymeric reagent, and the partition coefficient of the amino-component between the solvent and the swollen polymer, as well as the changes in the swellability of the polymer during the reaction. The reaction kinetics can change significantly as a function of the size of the amino-component, because the rate-limiting stage of the process can change. In one case this is the chemical stage and in another the diffusion stage. In order to reduce the contribution of diffusion factors, the reaction is usually performed in solvents causing a satisfactory swelling of the polymer (extending its polymer network), or when used as carriers which are weakly swelling and have a large surface area and a large pore diameter.[110] In all cases the necessary condition for the successful aminolysis is the solvation of the reaction centres of the polymer. As the reaction proceeds and the polymeric reagent is gradually deacylated, the character of the interaction of the solvent with the polymer can change. This is expressed as a rule by the polymer becoming less hydrophobic and by a change of swellability. For example, in the case of *N*-hydroxysuccinimide hydrophobic polymers, some decrease of swellability was observed during aminolysis[75,101] (in DMF and methylene chloride) or its increase (in water)[97,98]—for hydrophilic poly-*N*-hydroxysuccinimide reagents. The magnitude of this change should depend on the reactant ratio and the capacity of the polymeric reagent. This effect should in its turn influence the permeability and the partition coefficient.

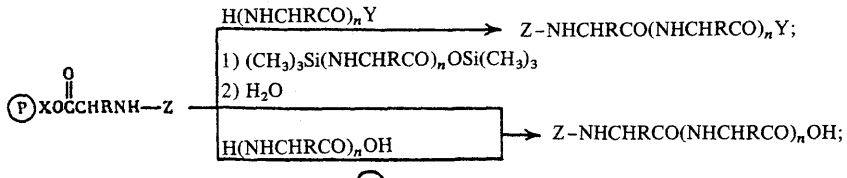
The study of the kinetics of the solid-phase synthesis[136,137] and the aminolysis of polymeric esters[138] showed that the course of such reactions differs significantly from that of reactions in solution. With increase of the degree of reaction, a sharp fall of its rate is usually observed. For example, a study of the rate of aminolysis of polymeric *N*-hydroxysuccinimide esters of Nps-aminoacids[138] showed that the rate constants calculated assuming the first order of the reaction differed at the beginning and at the end of the reaction by 1–3 orders of magnitude. The cause of this difference is apparently the fact that the reacting groups fixed in different compartments of the polymeric species exist under non-equivalent conditions owing to the different microenvironments ("clones" of the reaction centres). This leads to different rates of diffusion of the amino-component to the reaction centres ("microheterotropism"). The swellability of the polymeric reagent, varying in the course of the reaction, may exert an additional influence on the diffusion.

The dependence of the change in the rate of reaction on solvent polarity need not necessarily be correlated with the analogous dependence for reactions in solution, because the change in swellability may have a greater influence on the rate than chemical causes. Thus Kemp[139] found that the reactivity of *N*-hydroxysuccinimide esters in solution decreases in the sequence toluene–methylene chloride–DMF. In the solid phase synthesis the sequence was the same for the *N*-hydroxysuccinimide ester–aminoacyl-polymer (cross-linked polystyrene) system.[137] However, in the aminolysis of polymeric *N*-hydroxysuccinimide esters,[138] the opposite dependence was observed: in DMF the rate of reaction was a maximum and the degree of swelling of the polymer-activator was also greatest in the same solvent.[75,137]

Evidently, as regards the preparative aspect, the application of an excess of the polymeric ester should lead not only to a greater degree of completion of the reaction but should also appreciably improve its kinetic indices, because in this case the most accessible clones of the activated groups of the polymeric esters enter into the reaction while changes in the volume of the polymer are small. A graphical representation of the time variation of the degree of acylation of the methyl ester of leucine by the soluble *p*-nitrophenyl and the polymeric nitrophenyl ester of carbobenzoxyphenylalanine in chloroform in the presence of a twofold excess of the carboxy-component has been published.[69] When the initial concentrations were identical, the curves virtually coincided and the degree of conversion reached 100%.

Comparative estimation of the reactivities of the polymeric reagents is not simple in view of the absence of clear-cut criteria. The yield is in many cases almost quantitative. A "direct" kinetic comparison of two polymeric reagents has been achieved in a study[110] where the degrees of acylation of an alanine ester by t-butoxycarbonylalanine esters were compared as a function of time; the insoluble 4-hydroxy-3-nitrobenzylpolystyrene[69] and *N*-hydroxybenzotriazolylpolystyrene[110] were used as the polymer-activators. The rate of reaction for the first polymer was appreciably lower. The ratio of the reaction half-lives for these polymers was 20:1 and the 100% conversion times were 10 and 0.2 h respectively.

With increase of the length of the peptide synthesised, the role of the diffusion factor increases as a result of which the rate of acylation and the yields can decrease significantly. The size of the peptides synthesised hitherto with the aid of polymeric reagents has not exceeded 10 aminoacid residues in the chain. Within these limits, no clear retardation of the reaction or decrease of the yields as the peptide grows have been observed. The nature of the *N*-terminal aminoacid[110] and the character of the activating group[96] played a much more important role than the size of the amino-component. Thus it has been shown[140] that the method of polymeric reagents can be combined fairly successfully with the liquid phase method for the synthesis of peptides. In particular, the reaction involving the condensation of the polyhydroxybenzotriazole ester of t-butoxycarbonylalanine (taken in threefold excess) with the high-molecular-weight component H-Val-O-POE (POE = polyoxyethylene M-6000) during a period of 2 h occurred almost quantitatively; the yield in the analogous reaction with the ester based on polynitrophenol was 30% after 24 h.



X = activating group of the polymer (P)
Z = *N*-protecting group
R, R^1 = side group of aminoacid
Y = OAlk, NH$_2$
n = 1, 2, 3, ...

High-molecular-weight amino-components, namely insulin and poly-ε-benzyloxycarbonyl-L-lysine (M = 10000), have also been acylated with the aid of the poly-*N*-hydroxysuccinimide reagent.[96] Analysis of the reaction products showed that the free amino-groups were almost wholly blocked. Admittedly, a considerable molar excess (50 to 100-fold) of the polymeric t-butoxycarbonylalanine ester was used in the reaction and for this reason one cannot rule out the possibility that the reaction proceeded mainly in the surface layer of the polymer particles.

The majority of the known polymeric reagents swell satisfactorily only in organic solvents and for this reason esters or amides of aminoacids and peptides, which are soluble in these media, were used as the amino-components. The solubility of the intermediate peptide fragments depends not only on their molecular weight but also on the character of the functional side groups and their blocking.[54] When polymeric reagents are used, the set of compatible protecting groups and the strategy used in constructing the peptide chain are essentially the same as in the classical peptide synthesis. However, the problem of the solubility of the growing amino-component is particularly acute in this method owing to the heterogeneity of the reaction system. An increase of its solubility can be achieved only by reducing to a minimum the protecting side groups, which should enhance its hydrophilic properties,[54,140,141] or special protecting groups increasing the affinity of the peptide for organic solvents, both low-molecular-weight[142] and high-molecular-weight,[140] should be used.

The application of free aminoacids and peptides as nucleophiles in combination with organic solvents is limited owing to their low solubility. However, it has been shown[143,144] that, when certain peptides are suspended in a solution of an activated ester in DMSO, the reaction can proceed at an appreciable rate and with a high yield (the equilibrium shifts towards the soluble reaction product in the course of the reaction). The above method has been used to synthesise peptides with the aid of polymeric macronetwork *N*-hydroxysuccinimide esters[101] and model tetra- and penta-peptides as well as a whole series of diastereoisomeric dipeptides have been obtained.[145] The presence of quaternary ammonium bases increases significantly the rate of reaction.[143,145] It is noteworthy that activated esters are not altogether stable in polar organic solvents[144,146] and, since in this case the rate of aminolysis is nevertheless comparatively low and depends on the solubility of the peptide, the method can prove to be unsuitable for the synthesis of complex peptides.

There have been a number of studies[101,147–149] where trimethylsilyl (TMS) derivatives of aminoacids and peptides were used in the reaction with polymeric *N*-hydroxysuccinimide esters. Such derivatives exhibit an increased solubility in aprotic solvents, but they are hydrolytically unstable. The deblocking of the TMS-derivatives is almost instantaneous on treatment with water or alcohol[150] and the TMS group is for this reason suitable as a temporary protecting group for OH, COOH, and in certain cases SH functional groups.[148,151] There exists a fairly large selection of silylating agents at the present time[152,153] and mild methods have been devised for the introduction of the TMS group into aminoacids and peptides.[148] We may note that the amino-group is usually also silylated (although not always[153]), whereupon its nucleophilic properties can be appreciably weakened but the deacylated hydroxy-component evolved in the reaction desilylates it. For this reason, in the case of trifunctional aminoacids entering into the composition of the peptide chain, a stable protecting group is required for the NH$_2$ and SH side groups. For reaction to occur, total silylation of all the functional groups, sufficient to ensure the dissolution of the amino-components, is necessary. If a non-nucleophilic silylating agent is added to the suspension of an insoluble peptide or aminoacid and the activated ester, the equilibrium shifts in the required direction—the aminocomponent is converted into the soluble reactive form. TMS-amides are usually employed as silylating agents.[153,155] However, one should bear in mind that such TMS-amides can themselves interact with the activated esters,[156] i.e. they can initiate competing reactions in the system. This can be avoided if the amino-component is silylated separately and is then introduced into the aminolysis reaction after the removal of the excess of the TMS-agent.

Aminolysis in aqueous and aqueous organic media has definite advantages. Firstly, there is no need for the blocking of the weakly nucleophilic functional groups of the amino-component, secondly, the problem of the solubility of the peptides is in many cases solved, since they usually lose their ability to dissolve in organic solvents as the chain grows, and, thirdly, an aqueous medium at the physiological pH has the least denaturing effect on biologically active compounds. Intense research has been conducted recently on these lines. This concerns the use of enzymes for the formation of the peptide bond, for the introduction and removal of the protecting groups, and in chemical methods of synthesis of the peptide bond. A recently published review has been devoted to the problems enumerated above.[157]

Three types of hydrophilic polymeric reagents, to some extent suitable for the formation of the peptide bond in aqueous media, are known: acylated polymeric sulphonic acid[83] and polymeric thiol[119,158] as well as poly-*N*-hydroxysuccinimide reagents.[97–100]

A mixed anhydride of a polymeric sulphonic acid cation exchange resin and acetic acid[83] has been used in the reaction with aniline in an aqueous medium at pH 3.5–5.5; the low yield of acetanilide (it did not exceed 26%) was explained by the hydrolytic loss of the acylating agent and the low concentration of aniline in the polymer phase (3% relative to the concentration in the external solution).

When an acetylated polymeric thiol[119,158] was used in the aminolysis of amines and aminoacids, best results (yield up to 95%) were achieved at pH 10–12 and the lowest yield of amines was obtained at pH 7.5. This type of dependence is a

consequence of the catalysis of the aminolysis by OH^- ions and the positive influence of imidazole $H_2BO_2^-$ and Ag^+ ions was also noted. Best results were also obtained using sterically unhindered amines taken in excess (two equivalents).

In contrast to the polymeric reagents examined above,[83,119,158] which were in fact used only as acetylating agents (for the introduction of the *N*-acetyl group), hydrophilic poly-*N*-hydroxysuccinimide reagents have found practical applications in the synthesis of taftsin,[98,99] 26–33 fragment of cholecystokinin,[98,99] and diastereoisomeric dipeptides.[97] Poly-*N*-hydroxysuccinimide esters of *N*-acylaminoacids exhibited a high reaction capacity (up to 1.8 mmol g^{-1}) and swelled satisfactorily in aqueous media.[99] The rate constants for the competing aminolysis and hydrolysis reactions in heterogeneous system poly-*N*-hydroxysuccinimide reagent–amino-component–aqueous medium have been calculated at different pH for two peptide formation reactions.[159,160] The time variation of the calculated rate constants confirmed the idea of the clonal distribution of the reaction centres.[138] The optimum conditions for the aminolysis were discovered from the kinetic data obtained, in particular the optimum pH was in the region of the p*K* of the amino-group of the amino-component other conditions being equal.

When hydrophilic poly-*N*-hydroxysuccinimide reagents were used to analyse the optical purity of the aminoacids,[97] a polymeric ester of an *N*-protected L-aminoacid was introduced into the reaction with the aminoacid to be analysed, taken in a free form; the aminolysis was carried out at pH 8.5–9.0 (yield 55–98%). The removal of the *N*-protecting (t-butoxycarbonyl) group from the dipeptide obtained was also achieved in an aqueous medium *in situ*. Racemisation and stereoselectivity were not observed when this method was used.

Biologically active peptides—taftsin and the 26–33 fragment of cholecystokinin[98,99]—were synthesised in an aqueous medium at pH 8–9 as a rule under pH-static conditions (arginine was employed in a free state and the hydroxy-groups of tyrosine and threonine were not protected); the 26–33 fragment of cholecystokinin was obtained in an organic solvent (DMF) as a control for the method. The yields in the condensation reaction amounted to 60–86% in both aqueous and organic media.

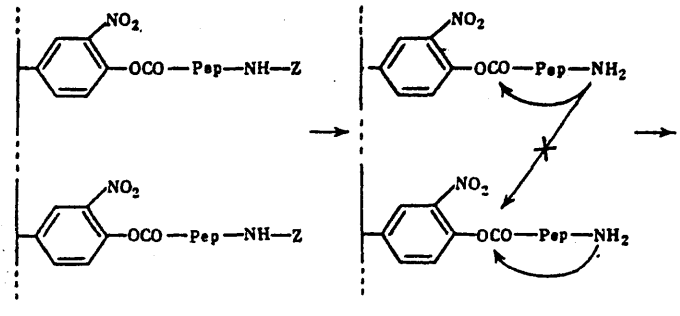
It was noted[98,99] that, when polymeric reagents are used for peptide synthesis in an aqueous medium, there is a possibility of difficulties in the elimination of the hydrolysed carboxy-components from the aminolysis products.

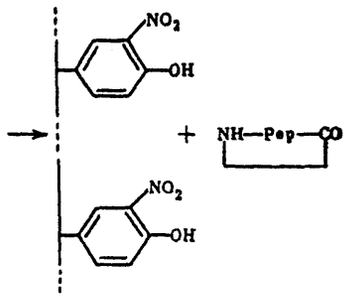
None of the versions of the aminolysis examined above eliminate the necessity for protection also of the functional side group as well as the α-aminogroup in the trifunctional aminoacid in the synthesis of the polymeric activated ester. However, in this case the group may be blocked by protecting groups which are removed simultaneously with the N^{α}-protecting group, for example the *O*-t-Bu group combined with the N^{α}-Boc group. Such a strategy has been used in the synthesis of pentagastrin.[149]

Both in the classical peptide synthesis and in the method of polymeric reagents, the aminolysis can be usually followed qualitatively with the aid of thin-layer chromatography by tracing the disappearance of the amino-component. When polymeric esters containing a chromophoric group in the acyl component are used, there is a possibility of the direct quantitative estimation of the degree of reaction by spectrophotometric measurements on the solution. The kinetics of the aminolysis of polymeric insoluble esters of Nps-aminoacids (λ = 380 nm) [138] and the interaction of the polymeric esters Boc-Ala with the *p*-nitrobenzyl ester of alanine (λ = 280 nm) were investigated in this way.[110] In a study[159] of the kinetics of the aminolysis reaction, the increase of the concentration of the peptide synthesis was followed by studying samples of the supernatant solution with the aid of liquid chromatography after the removal of the *N*-protecting group. Titrimetric determination of the amine which failed to react with the aid of $HClO_4$ in dioxan was used.[69]

In its practical aspect, the method of polymeric reagents has not so far found extensive application in the solution of specific synthetic problems. Peptides synthesised in this way served, with some exceptions, only to test the usefulness of a particular polymer-activator and to develop a methodology. As a rule, all the peptide derivatives were obtained by the stepwise growth of the peptide chain from the C-end. Apart from numerous model peptides, certain natural biologically active compounds and their fragments were obtained. One of the first known peptide hormones—bradykinin Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg—has been synthesised with the aid of the nitrophenol polymer[124] in an overall yield of 39% (65.3% relative to the protected nonapeptide) and exhibited the full biological activity. Satisfactory results were obtained in the synthesis of pentadecapeptides using insoluble *N*-hydroxysuccinimide polymers,[89,96,101] 4-hydroxy-3-nitrobenzylpolystyrene,[69,161] and polymeric *N*-hydroxybenzotriazole.[67,84,110,111] Fragments of oxytocin and pentagastrin,[162,163] thyroliberin,[110] the substrates for pig elastase,[164] the pentapeptide fragment of ox carboxypeptidase A,[96] taftsin and its analogues,[98,165] luliberin,[67,166] fragments of ACTH,[161] the *C*-terminal half of α-thymosin,[111] the 26–33 fragment of cholecystokinin,[98] and leucine-enkephalin [84] were synthesised in this way. The use of polymeric *N*-hydroxysuccinimide esters of t-butoxycarbonyl-L-aminoacids proved convenient in the synthesis of the diastereoisomeric dipeptides used in the enantiomeric analysis of aminoacids.[97, 145,147,167] The possibility of the repeated employment of the polymeric reagent, the ease of standardising all the mechanical operations, and also their simplicity are significant advantages in routine analysis. Polymeric activated esters have also been used to acylate biogenic and synthetic amines. The high yields and ease of purification of the resulting amides made it possible to employ them successfully in the synthesis of physiologically active aminoacid derivatives of β-phenylethylamine,[168] penicillins and cephalosporins,[166] aminoacyl- and peptidyl-nucleotides,[169] and acylaminophosphonic acid.[170]

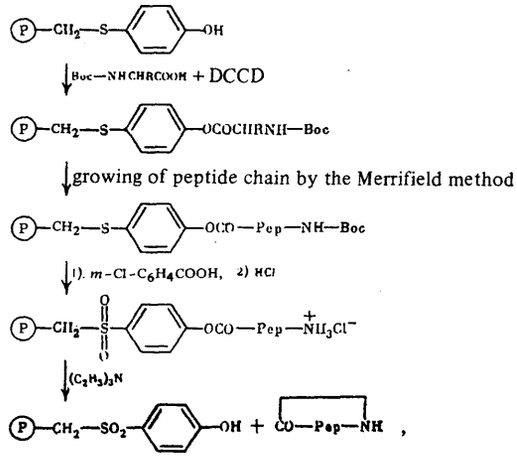
Cyclic peptides are usually obtained by the intramolecular condensation of linear peptides and, in order to suppress the competing intermolecular interactions, the reaction is carried out under the conditions of high dilution.[47] However, as a rule, even under these conditions, an appreciable amount of oligomeric side products is formed. In order to solve this problem, it has been suggested that peptide chains be fixed to an insoluble carrier after which intermolecular aminolysis is to be carried out.[27] The polymer serves in this case as a separator for the peptide chains, so that the unimolecular reaction should become the dominant process. Two versions of the application of monomers for this purpose are possible. In the first version the peptide chain was added to the polymer via a functional side group.[171] In the second version a polymer-activator was used for cyclisation:





Pep = peptide, Z-N = protecting group .

The first stage included the formation of the polymeric activated ester of the *N*-protected peptide. Nitrophenol polymers were used exclusively for this purpose,[27,84] while the condensing agent was DCCD. The yields were fairly low at this stage. However, one should bear in mind that, in order to reduce side reactions to a minimum, the content of acyl residues in the polymer should not be high. In the subsequent stages the *N*-protecting group was removed under acid conditions and the amino-group was deprotonated by an organic base, after which the polymer was maintained in the swollen state for the required time.[27] A series of simple cyclic di-, tri-, and tetra-peptides have been obtained in this way in a yield of 60–80%.[60] In order to obtain cyclic peptides, a linear peptide was initially synthesised by the solid phase method,[31] the ester bond in the peptide-polymer was then activated, and the cyclisation was finally carried out by the method indicated above[27] (yield 40–60%):



where Boc = t-butoxycarbonyl, DCCD = dicyclohexylcarbodiimide, R = side group of the aminoacid, and Pep = peptide.

The "selective activation" principle§ has been used in a number of other studies for the synthesis of both cyclic and linear peptides.[172–175] The application of oxidants for the labilisation of the peptidyl–polymer bond limits the choice of peptides for the synthesis, because in this case they must not contain a readily oxidisable aminoacid (methionine, cysteine, or tryptophan).

The idea that peptide chains and the reacting groups in the polymer are fully isolated from one another in space and that their interaction is extremely limited has been criticised in a number of investigations.[43,176] Many facts indicated that, even in the case of polymers with a relatively high degree of cross-linking, the internal mobility of the polymeric segments cannot be eliminated.[177]

The question of the influence of the condensation method and of the nature of the polymer-activator on the degree of racemisation of the peptide synthesised has been hardly touched upon in the literature.[178] One should distinguish racemisation proceeding during the activation of the carboxy-component by the polymer-activator and the racemisation in the aminolysis of the activated ester.[139,179] The methods for the formation of the peptide bond have come to be evaluated in this respect by the synthesis and analysis (liquid chromatography[76]) of a "test peptide", where readily racemised acylaminoacids are used as the carboxy-component.[180] The degree of racemisation in the synthesis of the model peptide Z-Gly-L-Ala-L-Leu-OBzl has been estimated[178] (the Izumiya test[181]) by the method of polymeric reagents, which consisted of polymeric esters based on 4-hydroxy-3-nitropolystyrene[27,124] and polymeric *N*-hydroxysuccinimide.[89] The authors compared this with the racemisation occurring when the usual low-molecular-weight condensing agents are employed. Polymeric Z-Gly-L-Ala esters have been obtained with the aid of DCCD and the aminolysis reactions carried out in dimethoxymethane. Diastereoisomeric analysis of the deblocked tripeptide showed that the content of the D-isomer was 6.4% for the *N*-hydroxysuccinimide polymer and 37.4% for the nitrophenol polymer. On the other hand, when DCCD or DCCD with *N*-hydroxysuccinimide were used as condensing agents for the formation of the peptide bond in solution, the amounts of the D-isomer were 24.6 and 2.8% respectively. The data obtained were of the integral type as regards the polymers and the differential estimation of the influence of various factors on the racemisation from experimental data is difficult. The above results[178] are therefore valuable only in the practical sense. It is evident, for example, that the *N*-hydroxysuccinimide polymer is preferable to the nitrophenol polymer from the standpoint of racemisation. When polymer-activated aminoacids with protecting groups of the urethane type are used, the probability of racemisation is minimal, with certain exceptions.[182] For this reason, in most studies where such derivatives were employed, the racemisation was either not estimated during the synthesis or the peptides synthesised were compared with optically pure authentic specimens. Racemisation was not usually observed. This factor made it possible to use the *N*-hydroxysuccinimide polymeric reagents for the enantiomeric analysis of aminoacids.[97,145,147,168]

The attempt to employ the optically active polymeric 3-hydroxyhydroxyhydrantoin for asymmetrically selective peptide synthesis is noteworthy.[114] Two versions of the synthesis of the dipeptide Z-Gly-Ala-OC_2H_5 from DL-Ala-OC_2H_5 were achieved in the above study. In the first version the corresponding activated Z-Gly ester was obtained in the preliminary step by the DCCD method and in the second the polymer was used as an additive in condensation by the same method. In the first case the dipeptide obtained contained 54.6% of the L-aminoacid and in the second case 72.6%. This effect was accounted for by the interaction of the D-isomer with the 3-hydroxyhydantoin group in the polymer, which increased the amount of the L-isomer in the solution.

VI. POLYMERIC CONDENSING AGENTS

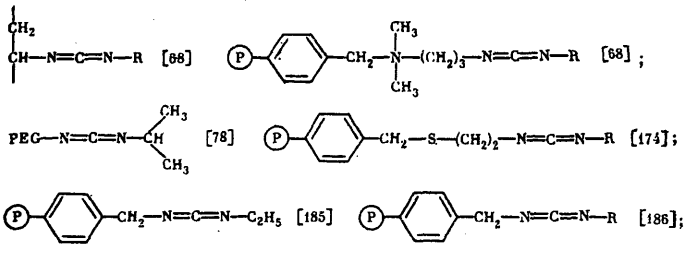
The use of dehydrating agents makes it possible to simplify greatly the working procedures associated with the formation of the peptide bond. Carbodiimides are usually employed

§ In non-Soviet literature different terms are used for the principle: the "safety-catch principle" [176] and "*a posteriori* activation".[177]

for this purpose.[183] The polymeric reagent with the functional group indicated has been known for a comparatively long time:[184]

$$[-(CH_2)_6-N=C=N-]_n \ .$$

Data for a series of cross-linked[68,174,185,186] and soluble[78] polymeric carbodiimides having the following structures appeared:



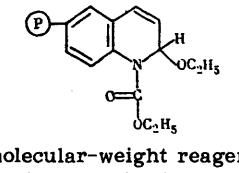
PEG = poly(ethylene glycol), R = alkyl.

For this synthesis, the polymer containing primarily amino-groups was usually treated with the corresponding isocyanate and the resulting polymer-bound urea was dehydrated. Cross-linked polystyrenes have been used as carriers as a rule.

The advantage achieved when such polymers are used may be imagined to consist in a more complete activation of the carboxy-component if the condensing agent is taken in excess and both this agent and its conversion products can be readily removed from the peptide in solution.

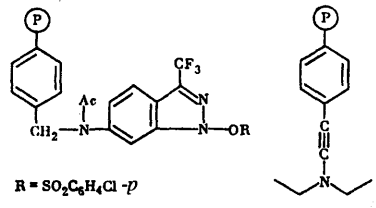
The practical application of classical peptide synthesis and also studies on the kinetics and mechanism of peptide condensation with the aid of DCCD have shown that such reactions have complex mechanisms.[187,188] A side reaction involving the formation of *N*-acylurea, leading to the loss of the carboxy-component, has been frequently observed under these conditions. This side reaction occurred also in the case of a polymeric analogue,[186] which precluded its complete regeneration. The formation of symmetrical anhydrides[188] can also hinder the purification of the final product.

A condensing agent based on polyvinylquinoline[189,190] activates selectively the carboxy-component, converting it into a mixed anhydride of ethoxycarbonic acid:



An analogous low-molecular-weight reagent, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, had been used earlier in the classical and solid phase methods of synthesis.[130,131]

A further two polymeric condensing agents have been obtained from chloromethylated polystyrene, namely polymer-bound indazole[191] and aynamine:[192]



R = $SO_2C_6H_4Cl$-*p*

There is little information about peptide synthesis with the aid of polymeric condensing agents and only several di- and tri-peptides were synthesised in the above studies. Tests for racemisation[189,190] showed that it occurs at the level of low-molecular-weight analogues. The advantages of these reagents became more clearly defined when they were used in other fields of organic synthesis, for example, for the oxidation of alcohols,[193] for the preparation of symmetrical anhydrides[186] and esters, including activated esters.[184]

VII. CONCLUSION

In essence, in any approaches to peptide synthesis one of the reactants is used in excess to ensure the completion of the reaction. The method of polymeric reagents has the advantage that it makes it possible to simplify the purification of the reaction product—the excess polymeric reagent and the deacylated polymer-activator can be readily separated from the low-molecular-weight reactant. In addition, the majority of the known polymer-activators can be used repeatedly, since they are readily regenerated. In particular, the inclusion of the activating components in the polymer makes it involatile and removes its toxicity and unpleasant small. By taking intc account the specific features of the microenvironment of the reaction groups and the possibility of modelling high dilution conditions by their separation, it is possible to solve a series of problems which are difficult to solve with the aid of other methods of peptide synthesis.

In the practical application of any method, the prospect of its automation is always attractive. As regards the method of polymeric reagents, the column version of the automation of peptide synthesis has been most frequently forecast.[194] In this case each stage in the formation of the peptide bond requires a separate column with a polymer-activated carboxy-component.

The main purpose of the column is to obtain at the outlet the pure product in a quantitative yield. However, studies in the authors' laboratory[195] showed that a (2-4)-fold excess of poly-*N*-hydroxysuccinimide reagents relative to the amino-component and low rates of elution did not lead to the required required result: the product required additional purification. This is apparently associated with the inadequately high rate of reaction and the absence of fractionation in the case of comparatively low and similar molecular weights of the dissolved components of the reaction mixture. A higher excess of the polymeric reagent in trivial aminolysis reactions is undesirable. Furthermore, the automated method should include a further two stages involving the concentration of the solutions and *N*-deblocking, which are fairly difficult to automate. However, attempts have been made, for example, to employ polymers for the removal of a series of *N*-protecting groups: Nps,[85] Boc,[196] and Fmoc.[197,198] The above consierations apparently complicate the automation of the method of polymeric reagents; virtually no studies on this subject have been described in the literature. A complicating factor is also the fact that industrial manufacture of the polymeric reagents has not so far been initiated, although these are as a rule fairly stable compounds which are convenient to store and transport.

The limits of the applicability of the method of polymeric reagents in relation to the synthesis of high-molecular-weight peptides have not so far been determined. However, it is apparently more most useful to obtain by this method a comparatively small peptide containing 15–20 aminoacid residues (in order to avoid diffusional complications) with subsequent combination of the resulting fragments in solution using either chemical or enzymic methods.

During the preparation of the review for publication, the communication of Patchornik and co-workers appeared in which they describe approaches to the methodology of peptide synthesis using a "mediator" (Mediator Methodology) in a

two-polymer system.[199] Also published was a review by the same authors on polymeric transferring agents[200] and polymeric activated esters based on a high-molecular-weight 1-hydroxy-2-pyrrolidinone[201] were proposed.

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