Synthesis, physico-chemical properties and biomedical applications of poly(amido-amine)s

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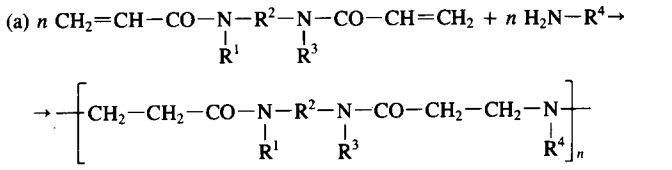
The aim of this article is to provide a comprehensive survey on synthesis, chemical and physico-chemical properties, and applications in several fields, including the biomedical field, of a family of tertiary amino polymers, the poly(amido-amine)s.

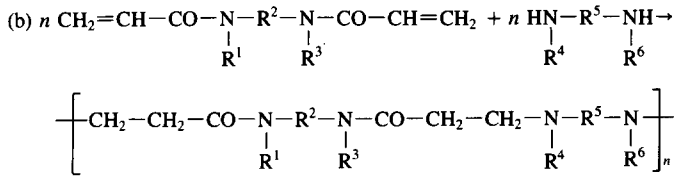
(Keywords: poly(amido-amine)s; synthesis; thermodynamic properties; chemical applications; biomedical applications)

SYNTHESIS AND STRUCTURE OF POLY(AMIDO-AMINE)S AND RELATED POLYMERS

Poly(amido-amine)s are a class of polymers characterized by the presence of amido and tertiary amino groups regularly arranged along the macromolecular chain. Additional functional groups may be introduced as side substituents.

Linear poly(amido-amine)s are obtained by poly addition of primary monoamines, or bis(secondary amines), to bis-acrylamides[1-4]:

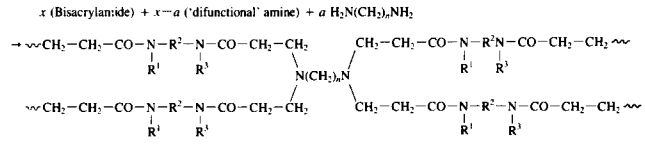




The reaction takes place readily in water or alcohols, at room temperature, and without added catalysts. Aprotic solvents are not recommended if high molecular weight polymers have to be obtained. The above method is a general one as far as aliphatic or cycloaliphatic amines are concerned. Under the same conditions, aromatic amines do not give high polymers. Many poly(amido-amine)s are highly crystalline[1,3] and some crystallinity is even observed, in some cases, where a certain degree of irregularity has been introduced[1]. Some examples of poly(amido-amine)s are reported in *Table 1*.

Poly(amido-amine)s carrying additional functions as side substituents can be easily obtained, starting from the appropriate monomers[1,5]. In fact, hydroxyl groups, tertiary amino groups, allyl groups, etc., if present in the monomers, do not interfere with the polymerization process. In the case of amino acids the polyaddition may be performed in the presence of triethylamine, which is then eliminated from the polymers using a carboxylic ion-exchange resin. In the absence of triethylamine, practically no reaction occurs between neutral amino acids, and bisacrylamides in water, at low temperature. Natural α-amino acids, other than glycine, give low molecular weight products[1]. Some examples of poly-(amido-amine)s bearing additional functional groups as side substituents are reported in *Table 2*.

Crosslinked resins of poly(aminoamine) structure can be prepared in the same way as the linear polymers by partly substituting α,ω-diaminoalkanes for the same quantity of difunctional aminic monomers in the polymerization process. According to the general scheme of poly(amido-amine) synthesis, bis(primary amine)s, having four mobile hydrogens, act as tetrafunctional monomers[1,2]:



An alternative method can be used[6]. By substituting in the polymerization process a part of the aminic monomer for allylamine, linear poly(amido-amine)s having pendant allyl groups are obtained. Subsequently, these polymers are treated with a hydrophilic monomer in the presence of radical initiators. Crosslinked products in which the polyamidoamine chains are connected by polyvinylic chains are finally obtained, as shown in the following case:

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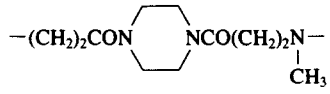
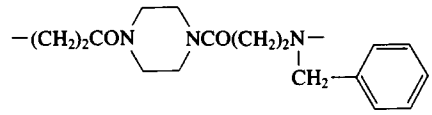
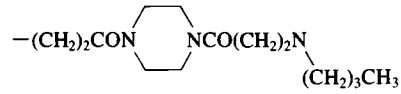
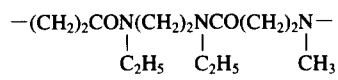
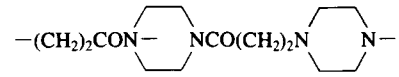
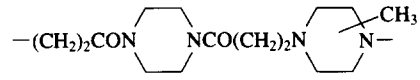
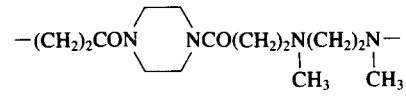
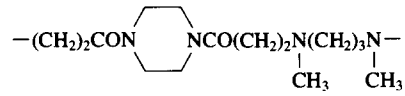
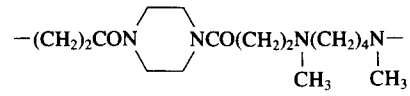
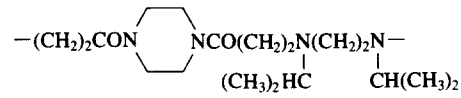
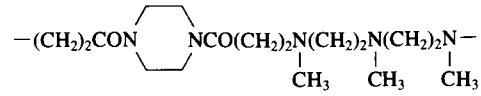
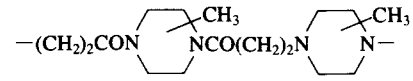
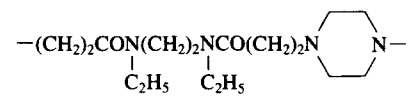
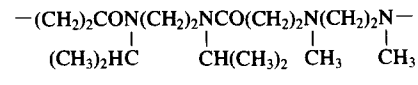
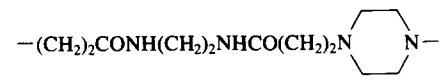
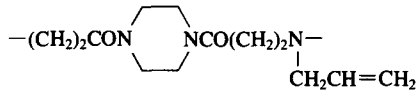
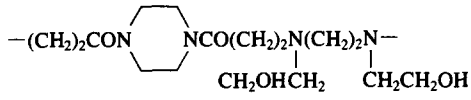
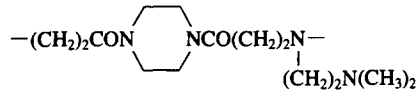
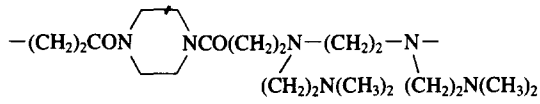
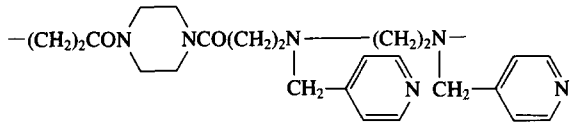
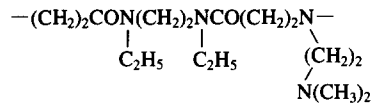
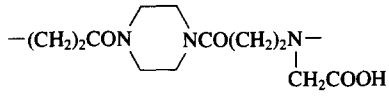
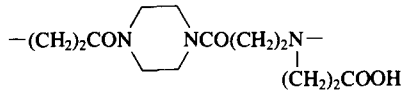
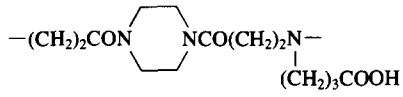
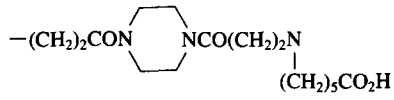
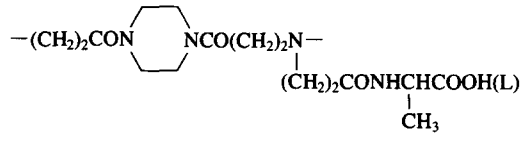
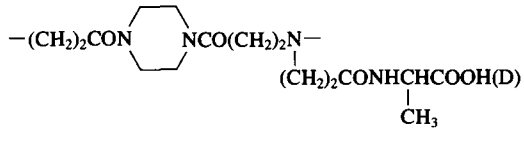
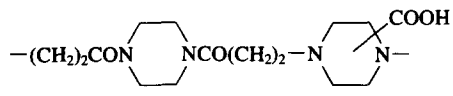
Table 1 Some examples of poly(amido-amine)s

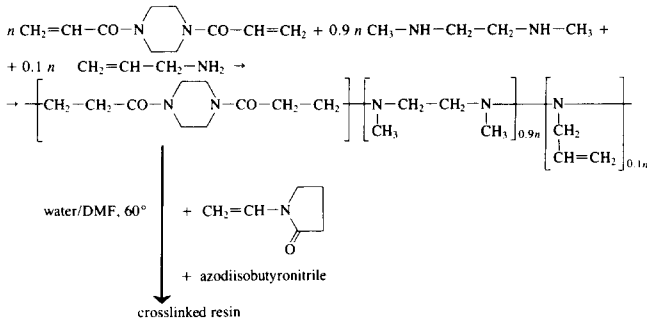
No.	Structure of the repeating unit	$[\eta]$ (dl/g)[a]	Ref.
1		0.40	1, 4
2		0.15	1, 4
3		0.27	1, 4
4		0.20	1, 3
5		0.46[b]	1, 2
6		0.81	1, 2
7		0.41	1, 3
8		0.43	19, 20
9		0.40	19, 20
10		0.12	1, 3
11		0.27	24
12		0.31	1, 3
13		0.20	21
14		0.17	1, 3
15		0.12[c]	1, 3

[a] in chloroform at 30°C where not otherwise indicated
[b] in aq. 0.1M HCl/1M NaCl
[c] in aq. 0.1M CH_3COOH/1M CH_3COONa

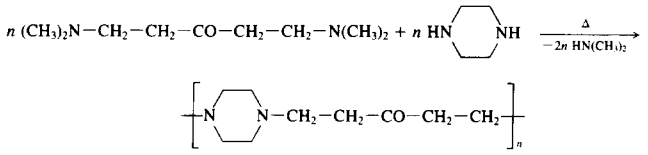
Table 2 Some examples of poly(amido-amine)s carrying functional groups as side substituents

No.	Structure of the repeating unit	$[\eta]^a$ (dl/g)	Ref.
1		0.33	Unpublished
2		0.37	39
3		0.19	17, 20
4		0.25	13, 17
5		0.18	13, 36
6		0.12	21
7		0.12[b]	1
8		0.15	27
9		0.16	27
10		0.23	27
11		0.10[b,c]	1
12		0.25[b,d]	1
13		0.28[b,c]	1

[a] in chloroform at 30° where not otherwise indicated
[b] η int (C=0.5 g/dl) in 90:10 methanol/water at 30°
[c] $[\alpha]_D^{25} = -9.8°$
[d] $[\alpha]_D^{25} = +10.4°$

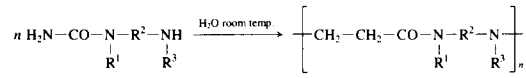


Crosslinked poly(amidoamine)s obtained by the former method are clearly insoluble in water. In aqueous media, however, they swell to a large extent, giving rather brittle gels. The latter method, as a rule, leads to products with better mechanical properties when swollen in aqueous media. Polymers structurally related with poly(amido-amine)s can be obtained by substituting either bis-(acrylic ester)s, on divinylsulphone, for bis-acrylamides[1,7] or hydrazines for amines[8]. Other polymers, of poly-(aminoketone) structure, can be obtained by poly-condensation of ketonic bis (Mannich base)s with bis-amines[9], as in the following example.



Some examples of polymers structurally related to poly-(amido-amine)s are reported in *Table 3*.

Poly(amido-amine)s having different distributions of amino and amido groups along the macromolecular chain have also been synthesized. For example, 'alternating' poly(amido-amine)s, in which amino and amido groups regularly alternate along the macromolecular chain, have been synthesized by self-polyaddition of *N*-acryloyl bis (sec.amine)s, obtained *in situ* by acidic removal of the triphenylmethyl group from the corresponding *N*-α-acryloly-*N*-ω-triphenylmethyl (bis(sec.amine)s[10]:



Other poly(amido-amine)s have been synthesized by poly(acylation–addition) of piperazine, and other bis(sec amine)s, with various activated derivatives of acrylic acid. In these polymers, amino and amido groups are not regularly along the macromolecular chain. It has been found that the disposition depend on the activated derivative used[11,12].

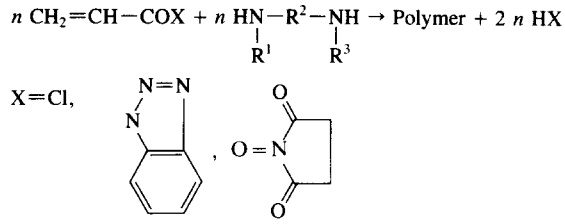
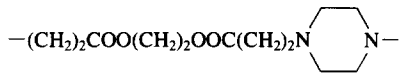
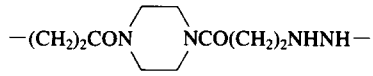
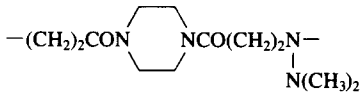
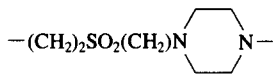
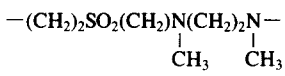
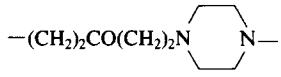
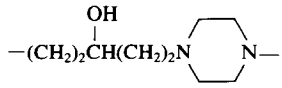


Table 3 Some polymers structurally related with poly(amido-amine)s

No	Structure of the repeating unit	$[\eta]^a$ (dl/g)	Ref.
1		0.41	1, 7
2		0.16	1, 8
3		0.10	1, 8
4		0.60[b]	1, 7
5		0.19	28
6		0.25	9
7[c]		0.18	9

[a] in chloroform at 30°C where not otherwise indicated
[b] in dimethylsulphoxide at 100°C
[c] by $LiAlH_4$ reduction of **6**

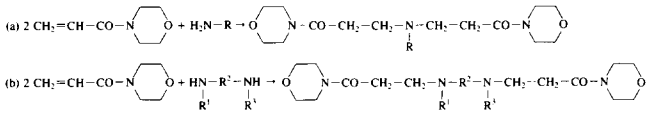
PROPERTIES OF POLY(AMIDO-AMINE)S

Poly(amido-amine)s are usually soluble in, or at least swollen by, water. They are also soluble in many organic solvents. Their intrinsic viscosities range from 0.15 to 0.9 dl/g, and molecular weights (number average) from 5×10^3 to 5×10^4. The protonation and complex formation of a number of poly(amido-amine)s in aqueous solution have been studied by potentiometric, calorimetric, viscosimetric, spectrophotometric, e.s.r., ^{13}C n.m.r. and quantum mechanical techniques.

Protonation studies

In all the poly(amido-amine)s studied, except those deriving from amino acids (see below), the results of the potentiometric titrations clearly show that the basicity of the aminic nitrogens —N— of each repeating unit does not depend on the degree of protonation of the whole macromolecule. Consequently, 'real' basicity constants can be determined. Because of the similarity with some branches of classical inorganic chemistry, these studies are known as 'Macroinorganics'[13-23].

The number of basicity constants is always equal to the number of the aminic nitrogens present in the repeating unit, and these are similar to those of the corresponding non-macromolecular models. The latter were purposely synthesized from the same amines used in the preparation of polyamodo-amine)s, and *N*-acryloylmorpholine[14-19]:



The above behaviour is quite unusual in the polyelectrolyte domain.

More particularly, by considering polymers **7**, **8**, **9** of *Table 1*, an increase in the number of methylenes between the two tertiary atoms leads to an increase of the basicity constants[19,20] and, within each polymer, the successive protonation constants decrease in the usual manner $\log k_1 > \log k_2$. The enthalpy values follow a trend comparable with that observed in the case of low molecular weight aliphatic amines, or the above models. The basicity constant of some representative poly(amido-amide)s are reported in *Table 4*.

The existence of a linear relationship between the protonation enthalpies and Q_N, i.e. the net charge of the nitrogen atom to be protonated for both polymers, and non-macromolecular models, means that no interaction exists between the monomeric units[13,16,18,20-22]. The substantial independence of the repeating units is possibly due to the presence of the diacylpiperazine groups sheltering the positive charges on the protonated nitrogens present in different units.

Viscosimetric titrations in 0.1M NaCl[19,20] prove that the conformational freedom is reduced on protonation. All the polymers in the neutral form are most likely in a random coil structure due to the great number of accessible conformations of about the same energy. For the polymer **7** in *Table 1*, for instance, the first protonation leads to the formation of a strong hydrogen bond between 'onium' ions and carbonyl groups belonging to the same monomeric unit.

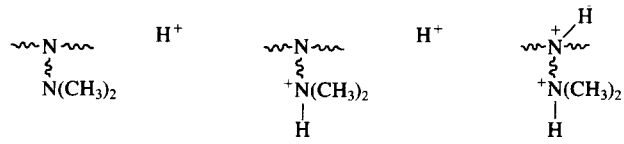
When the first protonation of all the monomeric units is complete, the above effect strongly reduces the conformational freedom of the whole polymer, which tends to assume a rigid structure. This is clearly shown by a pronounced jump of its reduced viscosity at $\alpha = 0.5$ (α = degree of protonation)[19,20].

The second protonation leads to an electrostatic repulsion between the positively charged onium ions belonging to the same monomeric unit, and this effect further compels the polymer to adopt a more rigid structure. Consequently, a second jump is observed at $\alpha = 1$.

The reduction of conformational freedom clearly decreases with the lengthening of the aliphatic chain in each repeatung unit. For instance, polymers **8** and **9** in *Table 1* show the same jumps, but they are somewhat less pronounced[19,20].

Also by considering poly(amido-amine)s with additional tertiary amino groups in the side chains (polymers **3** and **4** in *Table 2*), their thermodynamic values are similar to those of their non-macromolecular models. However, the models are slightly more basic, as already found for the other poly(amido-amine)s[17,19-21,23]. By comparing the basicity constants of polymers **3** and **4** of *Table 2*, and their corresponding models, with those of their isomeric counterparts (polymers **1** and **7** of *Table 1*, and their models), which contain tertiary amino groups only in the main chain, we observe that the log K_1's of the former compounds are larger, while the reverse was true for log K_2's[14-23].

On the basis of our results, we think that the mechanism of protonation of 'branched' poly(amido-amine)s involves the following steps[20]:



No effective hydrogen bridges can be formed between the very distant 'onium' ion and carbonyl groups in the first step of protonation. Both ^{13}C n.m.r. and quantum-chemical results agree with this explanation, and this explains the fact that in these polymers the first protonation has practically no influence on the reduced viscosity, whatever the number of methylenic groups between the amino nitrogens may be.

The second protonation involves the amino nitrogens of the main chain. The interactions between onium groups

Table 4 Some basicity constants of poly(amido-amine)s, and their non-macromolecular models, at 25°C in 0.1 M NaCl

	Polymers				Models			
No[b]	log K_1	log k_2	log k_3	log k_4	log k_1	log k_2	log k_3	log k_4
1,1	7.79	—	—	—	8.07	—	—	—
7,1	8.09	4.54	—	—	8.25	4.80	—	—
11,1	8.08	6.94	1.89	—	8.25	7.44	2.56	—
5,1	7.01	2.98	—	—	7.12	3.29	—	—
3,2	8.80	4.11	—	—	9.05	4.35	—	—
4,2	9.02	7.91	4.46	2.16	9.05	8.34	4.42	2.43

[a] for the structure of the models, see the text. Each model corresponds to the polymer located in the same row

[b] the furst number refers to the system, and the second to the Table in which the structure is reported

Table 5 Basicity constants of polymeric aminoacids at 25°C in0.1M NaCl

Compound[a]	Reaction	log K° [b]	log K_i [c]	
7	$L^- + H^+ = LH^{\pm}$	8.30(2)	1.07(3)	$\log K_1 = 8.30 + 0.07 \log[(1-\alpha)/\alpha]$
	$LH^{\pm} + H^+ = LH_2^+$	2.01(1)	0.80(7)	$\log K_2 = 2.01 - 0.20 \log[(1-\alpha)/\alpha]$
8	$L^- + H^+ = LH^{\pm}$	8.52(2)	1.14(2)	$\log K_1 = 8.52 + 0.14 \log[(1-\alpha)/\alpha]$
	$LH^{\pm} + H^+ = LH_2^+$	3.57(4)	1.23(1)	$\log K_2 = 3.57 + 0.23 \log[(1-\alpha)/\alpha]$
9	$L^- + H^+ = LH^{\pm}$	8.47(2)	1.10(1)	$\log K_1 = 8.47 + 0.10 \log[(1-\alpha)/\alpha]$
	$LH^{\pm} + H^+ = LH_2^+$	4.21(1)	1.12(1)	$\log K_2 = 4.21 + 0.12 \log[(1-\alpha)/\alpha]$
10	$L^- + H^+ = LH^{\pm}$	8.50(1)	1.16(1)	$\log K_1 = 8.50 + 0.16 \log[(1-\alpha)/\alpha]$
	$LH^{\pm} + H^+ = LH_2^+$	4.28(1)	1.08(1)	$\log K_2 = 4.28 + 0.08 \log[(1-\alpha)/\alpha]$

[a] see *Table 2*
[b] the values in parentheses are standard deviations
[c] $\log K_i = \log K^{\circ} + (n-1)\log[(\alpha-\alpha)/\alpha]$

are the same as for the 'linear' polymers, but there is only one 'onium' ion (that belonging to the main chain), which can interact with one or another of the two neighbouring carbonyl groups. This leads to a greater conformational freedom, and hence the viscosimetric titrations do not show any jump even after the second protonation step[19].

Poly(amido-amine)s of similar structure, in which the bis-acryloyl-piperazine) moieties had been replaced with non-cyclic, hence more flexible bis-acrylamidic moieties (polymer **13** of *Table 1* and polymer **6** of *Table 2*, for instance), on the whole gave very similar results[21].

Poly(amido-amine)s deriving from amino acids, and therefore carrying carboxylate groups as side substituents (polymers **7–10** of *Table 2*), behave in a different way with respect to all other poly(amido-amine)s[27]. In fact, they exhibit a typical polyelectrolyte behaviour inasmuch as their log K values depend on the degree of protonation of the whole macromolecule, i.e. they follow the modified Henderson–Hasselbach equation:

$$\log K_i = \log K_i^{\circ} + (n-1)\log[1-\alpha)/\alpha]$$

The basicity constants of polymeric amino-acids are reported in *Table 5*.

Heavy metal ion complexing behaviour

The coordinating ability of some poly(amido-amine)s, and their models, with respect to copper(II) ion in aqueous solution are summarized in *Table 6*[17,20,21,23–26]. 'Real' stability constants could be determined for these polymers also in the case of complex formation. For most other poly(amido-amine)s reported in *Tables 1* and *2*, insoluble hydroxides precipitate prior to the formation of a significant amount of complex. These results show that increasing the chelating ring size from five to six leads to a distinct reduction in the [CuL] (L = ligand) complex stability or even to the inability to chelate copper(II) to give stable complexes.

As regards the non-macromolecular models, the [CuL] stability constants are always slightly higher than those of the corresponding polymers (*Table 6*). This is presumably related to different entropy effects. The electronic and e.p.r. spectra of both of the two types of complexes are similar, and consistent with an octahedral tetragonally distorted structure[20,24]. The fact that the e.p.r. and electronic data of polymers and models are quite similar is in agreement with the substantial independence of the repeating units of the polymer in the complex formation process.

Viscosimetric studies have been carried out on the systems Cu–(polymer **7** of *Table 1*), and isomeric

Table 6 Stability constants of copper(II) complexes with some poly(amido-amine)s and their non-macromolecular models in 0.1 M NaCl at 25°C

Ligand[a]	Reaction[b]	Stability constant (log)
7, 1	$Cu^{2+} + L \rightleftharpoons CuL^{2+}$	8.96
	$CuL^{2+} + OH \rightleftharpoons Cu(OH)L^+$	5.52
8, 1	$Cu^{2+} + L \rightleftharpoons CuL^{2+}$	5.36
	$CuL^{2+} + OH^- \rightleftharpoons Cu(OH)L^+$	5.14
	$CuL^{2+} + 2OH^- \rightleftharpoons Cu(OH)_2L$	10.25
3, 2	$Cu^{2+} + L \rightleftharpoons CuL^{2+}$	8.47
	$CuL^{2+} + OH^- \rightleftharpoons Cu(OH)L^+$	6.12
M7, 1	$Cu^{2+} + L \rightleftharpoons CuL^{2+}$	9.10
	$CuL^{2+} + 2OH^- \rightleftharpoons Cu(OH)_2L$	8.40
M8, 1	$Cu^{2+} + L \rightleftharpoons L^{2+}$	6.45
	$CuL^{2+} + OH^- \rightleftharpoons Cu(OH)L^+$	5.09
	$CuL^{2+} + 2OH^- \rightleftharpoons Cu(OH)_2L$	9.90
M3, 2	$Cu^{2+} + L \rightleftharpoons CuL^{2+}$	8.61
	$CuL^{2+} + OH^- \rightleftharpoons Cu(OH)L^+$	5.7
	$CuL^{2+} + 2OH^- \rightleftharpoons Cu(OH)_2L$	8.7

[a] see footnote of *Table 4*. When the number is preceded by M, it refers to the corresponding model
[b] L = Ligand

Cu–(polymer **3** of *Table 2*)[20]. With the linear polymer, it was found that the viscosity monotonically decreases upon increasing the pH. This decrease is very pronounced, until the formation of the complex [CuL] (which of course depends on pH) is complete; after that, the viscosity remains relatively constant until complete deprotection of the ligand. A second slight jump occurs, correspomding to the maximum formation of HL_2^+ species, and is due to a conformational transition. To the contrary however, in the case of the 'branched' polymer, no noticeable variations in reduced viscosity were observed over the whole range[20]. This is unusual, and probably due to the fact that the conformational transition involved in complex formation, as in protonation (see above), only affects side substituents and does not alter to a large extent the characteristics of the main chain.

Of all the polymeric amino acids considered (polymers **7–10** of *Table 2*), only (**7**) and (**8**) are able to form stable complexes in aqueous solution[27]. The equilibrium constants can be calculated by taking into account the dependence of the protonation constant on α via the Henderson–Hasselbach equation. At pH > 7 for (**7**), and > 4 for (**8**), the stability constants of the CuL^+ species do not follow a regular trend. This means that beyond this point, new complex species are probably formed and therefore the computation cannot be carried out. The values of $[Cu(7)]^+$ stability constant is always larger than

those of $[Cu(\mathbf{8})]^+$, consistently with what happens in the case of the corresponding amino acids glycine and alanine, and of the non-macromolecular models of the polymers purposely synthesized by us. This indicates that chelation occurs between the carboxyl and the amino group. On the other hand, no appreciable conformational variation seems to occur during complex formation, as shown by viscometric and spectrophotometric measurements.

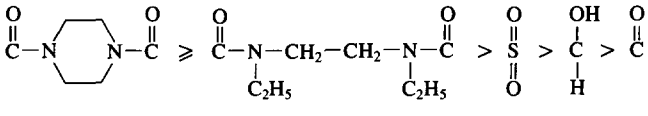
Some date on tertiary amino polymers structurally related with poly(amido-amine)s

The stepwise protonation of polymers **5**–**7** of *Table 3*, containing different shielding groups between the amine moieties of the repeating units has been studied from a thermodynamic standpoint[28,29].

This was done for comparison purposes with structurally related poly(amido-amine)s, whose repeating units behave independently toward protonation (see above). This study was performed by potentiometric and calorimetric techniques, and specific methods for the treatment of either 'real' or 'apparent' thermodynamic functions in polyelectrolytes having more than one basic group in the repeating unit have been developed[28]. It would be beyond the scope of this article to report these results in detail. From these results, however, it was possible to conclude that the three polymers behave in a quite different manner toward protonation, although the number of basicity constants is equal to the number of aminic nitrogens present in the repeating unit. In particular, both the basicity constants of (**6**) are undoubtedly 'apparent', while those of (**5**) and (**7**) can be considered on the borderline between 'real' and 'apparent', with the exception of the second basicity constant of (**5**), which is undoubtedly 'real'. This means that in all cases but one, the $(n-1)$ values in the modified Henderson–Hasselbach equation (see above), are greater than zero.

It may be further observed that the 'polyelectrolyte' behaviour of polymers (**5**) and (**7**) is mainly due to the entropy term. The protonation enthalpies of (**5**) and (**7**) are undoubtedly sharp, while $\Delta H_2^{\ominus}$ is constant over a significant pH range[28].

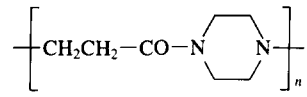
On the grounds of the results obtained for poly(amido-amine)s (see above), and for the polymers mentioned in this section, we can conclude[28] that the shielding effectiveness of different groups decreases in the order:



Thus, the shielding effect of the first two groups (those found in poly(amido-amine)s) is sufficiently high to prevent any detectable influence in the basic groups of a given unit by already charged groups in the remainder of the macromolecule. This is probably due to the size and stiffness of these groups, while the effectiveness of SO_2 is probably related to its quite large size. Finally, the difference found, in this respect, between alcoholic and carbonylic functions might be due to their different degrees of solvation, as also revealed by the much higher water solubility of (**7**). In fact, a strongly coordinated shell of water molecules is known to exist around the OH group, and this is probably responsible for its relatively high shielding ability.

Some data on poly(amido-amine)s with different sequences of amino and amido groups along the main chain

The protonation behaviour of poly(amido-amine)s in which amido- and tertiary amino groups have sequences different from those found in 'regular' poly(amido-amine)s has been studied[30]. For example, poly(1,4-piperazinediyl-1-oxotrimethylene)



shows a linear decrease of $\log K$ by increasing the overall degree of protonation of the macromolecule. In other words, its basicity constant is apparent, and the polymer exhibits typical polyelectrolyte behaviour. It is apparent that the shielding of one carbonyl group bound to a piperazine ring is not sufficient to minimize the interactions between neighbouring units.

CHEMICAL APPLICATIONS OF POLY(AMIDO-AMINE)S

Flocculating and dispersing activity on minerals

The possibility of utilizing the poly(amido-amine)s as flocculating and/or dispersing agents has been evaluated. In fact, these polymers contain different functional groups in their structure, endowed with chemical affinity toward the metal ions present in the mineral, and therefore may change the aggregation state of the mineral fine particles.

For this purpose, poly(amido-amine)s **7** of *Table 1*, and **2**, **7**, **8**–**10** of *Table 2*, have been studied with mineral chalcocite (Cu_2S)[31]. Sedimentation tests carried out at various reagent concentrations and pH's demonstrate that all polymers, which are capable of forming stable and soluble complexes with copper ions, have a strong dispersing activity on chalcocite. On the contrary, the polymers which are not able to form complexes, have a strong flocculant activity, which decreases with increasing pH. These results are summarized in *Table 7*.

The mechanism of action of these polymers can be understood by taking into account the properties of the poly(amido-amine)s in solution, and the conformations they assume at various pH's[20].

Catalytic activity

It has been shown previously that the basicities of the tertiary amino groups present in the repeating units of poly(amido-amine)s are not affected by the degree of protonation of the whole macromolecule, with the exception of those deriving from amino acids, while the opposite is true in the case of most of the polyelectrolytes described so far. As a consequence, poly(amido-amine)s exhibit as many basicity constants as aminic nitrogens in their repeating units, and these constants can be determined with accuracy. This prompted us to investigate the properties of these polymers also as catalysts, and to compare their behaviour with that of nonmacromolecular molecules.

In this respect, we have studied[32] the ionization rates of ethylnitroacetate in aqueous solution catalysed by poly(amido-amine) **7** of *Table 1*, having a number average

Table 7 Action of polymers on chalcocite

Polymers[a] (100 ppm)	Polymer in solution (ppm)	Adsorbed polymer (ppm)	Cu^{2+} (ppm)	Dispersing flocculating power (per cent)[b] F_p
pH = 7				
7, 2	1.4	98.6	0.3	−438
8, 2	7.3	92.7	0.0	+ 76
9, 2	2.6	97.4	0.0	+ 67
10, 2	0.4	99.6	0.2	+ 81
7, 1	90	10	23.0	−150
2, 2	45	55	11.0	−180
pH = 9				
7, 2	1.7	98.3	0.2	−134
8, 2	8.3	91.7	0.1	− 27
9, 2	3.4	96.6	0.0	= 12
10, 2	3.9	96.1	0.2	+ 38
7, 1	98	2	16.0	−350
2, 2	22	78	4.0	−360

[a] see footnote to *Table 4*

[b] $F_p = \frac{P_o - P}{P_o}$

where P_o = weight of the solid in the supernatant without polymer, and P is the weight of the solid in the supernatant after addition of the polymer. When F_p has a negative value, the polymer acts as dispersing agent; on the contrary, when F_p has a positive value, the polymer acts as flocculating agent.

molecular weight of 10300. The ionization of ethylnitroacetate (SH) in aqueous solution:

$$SH + B \underset{k_{BH^+}}{\overset{k_B}{\rightleftharpoons}} S^- + BH^+$$

has been chosen as a model reaction for our studies on the catalytic activity of poly(amido-amine) (B) because it offers several advantages. First of all, the formation of the conjugate base can be conveniently followed by observing its strong adsorption at 300 nm. Furthermore, the quantitative relationship between catalytic power ($\log k$) and basic strength of the catalyst (pKa) has been formally established for several non-macromolecular bases[33].

From our results, obtained by the stop flow technique, it was possible to conclude that the poly(amino-amine) acts as catalyst in the ionization reaction of ethyl nitroacetate in a way predictable by the Brønsted equation established for 'normal' bases (average $k_B = 8.13 \times 10^3$ mol$^-$ dm^3 s^{-1}).

This further confirms that the repeating units of this polymer behave independently, and that no significant effects due to macromolecularity are present. To our knowledge, this is the first instance in which the catalytic power of a synthetic polymer can be quantitatively related to a thermodynamic quantity of the same polymer, namely its basicity constant, which, owing to the peculiar nature of poly(amido-amine)s, had been accurately determined (see above).

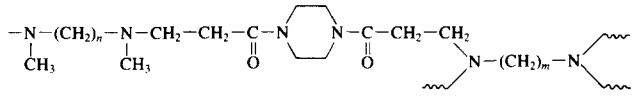
The same poly(amido-amine), and the isomeric poly(amido-amine) carrying one of the tertiary amino groups as a side substituent (polymer **3** of *Table 2*), were employed as polymeric ligands towards Cu(II) ions, for the oxidative coupling of 2,6-dimethylphenol (DMP), which is catalysed by aminic complexes of copper, in methanol as solvent[34]. The polymeric catalyst appeared to be about four times more active than the copper complexes of *N,N,N′,N′*-tetramethylethylenediamine (TMED), an already well known catalyst for this reaction[35].

From kinetic and spectrophotometric measurements it could be concluded that the most active polymeric catalysts are probably binuclear copper complexes with one bis-aminic unit per Cu ion, and only one bridging OH^- between two Cu ions. The kinetics demonstrated also that the rate of the electron transfer from substrate to Cu is so rapid in the case of the polymeric catalysts that prior complexation of DMP with the catalyst does not attain an equilibrium. Thus, no substrate saturation was observed and simple first order kinetics for both DMP and $CuCl_2$ were obtained instead of the usual Michaelis–Menten scheme.

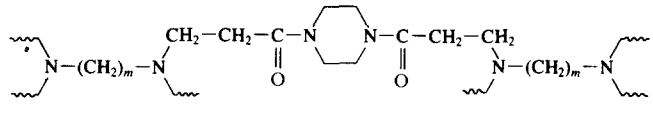
Selective ion-exchange resins

The ion-complexing of linear poly(amido-amine)s in aqueous solution, as pointed out before, is rather peculiar in the polyelectrolyte's domain. Their complexes have well defined stoichiometries: in particular, one metal ion is coordinated by each repeating unit[24]. Sharp stability constants have been determined in aqueous solution. In several instances, complexes could be isolated in the solid state, giving elemental analyses quite consistent with the proposed stoichiometries[17,20–21,23–26]. In order to investigate a possible application of poly(amido-amine)s in the field of ion-exchange resins, and, particularly, to obtain resins able to selectively remove, and recover, traces of heavy metal ions from solutions, we have prepared some poly(amido-amine)s in a crosslinked form. On these resins, we have also studied the influence of the nature of the crosslinking agent on the basicities and complexing abilities[24,36–39].

In a typical study[37,38], two groups of resins have been prepared and studied. In the first group, the tertiary amino groups belongs partly to the crosslinking agents (C), and partly to linear segments (L). In the second group, all the aminic groups belong to the crosslinking agents. The general structure of the resins of the first group is

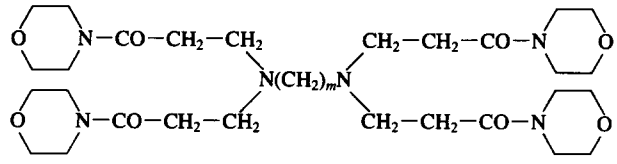


where the ratio L:C is 3:1 (mol/mol), and n and m can be $n = 2, m = 2$ (RN_2C_2); $n = 4, m = 2$ (RN_2LLC_2); $n = 2, m = 4$ (RN_2C_4); $n = 4, m = 4$ (RN_2LLC_4). The structure of resins of the second group is



As far as the protonation constants of the resins are concerned, the constants relative to the linear portions of the resins of the first group are very close to those of the corresponding linear polymers (polymers **7–9** of *Table 1*). This means that neither the nature of the crosslinking agent, nor the crosslinking itself significantly affects the basicity of the aminic nitrogens present in the linear part. On the other hand, by comparing the protonation constants relative to the crosslinking units in resins RN_2C_2 and RN_2LLC_2 with those of RC_2, it would appear that in these resins the basicity of the crosslinking units is considerably lower when an already protonated linear portion is present[38]. The basicities of the resins of the

second group, on the whole, are very similar to those of the corresponding non-macromolecular models



obtained by addition of *N*-acryloylmorpholine to α,ω-bisamino alkanes[37].

The behaviour of these resins in complex formation is very close to that of the corresponding linear polymers. As a matter of fact, only the resins whose linear portion (L) corresponds to a poly(amido-amine) which can form stable complexes with some metal ions, are able to retain the same metal ions[37].

In the case of Cu(II) complexes, their stability constants in the resins are usually lower than the corresponding constants previously obtained with the linear polymers[37]. The above difference is due to the fact that the complex formation in the case of the linear polymer occurs through a cyclic structure around the copper atom, as previously reported[26,40]. The formation of such cyclic structure is obviously biased in the case of tightly linked resins.

In order to evaluate the possibility of practical applications of the above resins, studies on the separation of Co(II), N(II) and Cu(II) ions in column operations have been performed[37,39]. The following example refers to a resin whose linear portion corresponds to polymer **2** of *Table 2*, crosslinked with 1,4-diaminobutane.

In this experiment[39], 51 mg of resin was brought in contact with 50 ml buffered solution (pH 4.8) containing Cu(II) ions. After equilibration, the amount of Cu(II) ions present in solution was determined polarographically. The quantity of Cu(II) ions adsorbed from the resin is equivalent to the theoretical capacity of 2 mmols per g of resin, corresponding to one Cu(II) ion for every pair of tertiary nitrogen atoms, i.e. for every repeating unit of the linear portion of the resin. This result is in agreement with that expected from previous considerations on the Cu(II) stability constant, and on spectrophotometric studies performed on the linear polymer[39]. In order to evaluate the possibility of practical applications of the resin, studies on the separation of Co(II), Ni(II) and Cu(II) ions in a column have been performed[39]. It was observed that the elution condition are sharply different for the three ions. In other words, the pH conditions at which each ion is eluted after adsorption show little or no superimposition.

In order to better demonstrate this point, a mixed solution containing 0.05 mg of each metal (Co(II), Cu(II) and Ni(II), was adsorbed on the resin at pH 5.75. After elution with 70 ml of buffer, all Co(II) ions were eluted, while both Ni(II) and Cu(II) ions were completely retained. Ni(II) ion was completely eluted with 60 ml of buffer at pH 4.20. Finally, the copper(II) ions could be quantitatively eluted with 0.1 M HCl. The recovery of adsorbed ions was in all cases practically quantitative, the average error being less than 2%.

Other resins gave similar results[24,38]. Therefore, it may be concluded that several poly(amido-amine)s, when in the form of crosslinked resins, have remarkable properties with a view to practical application in the separation of heavy metal ions in aqueous solution. A complete separation of Co(II), Cu(II) and Ni(II) may be reached simply by varying the pH of the solution. The resins have a high loading capacity, and may be easily regenerated. Therefore, they compare favourably with many commercial resins designed for similar purposes.

BIOMEDICAL APPLICATIONS OF POLY(AMIDO-AMINE)S

The possibility of biomedical applications of poly(amido-amine)s lies in their ability to form stable complexes with heparin. Heparin is a well known anticoagulant agent, widely used in clinical practice. It is a mucopolysaccharide containing carboxyl- and sulphonic groups. In aqueous solution, it behaves as a polyanion with a high density of negative charges[41].

In many cases, the anticoagulant activity of heparin must be inhibited when no longer needed. This is usually obtained by administering protamine sulphate. Protamine is a highly basic natural polymer of polypeptide structure. It is itself a powerful anticoagulant when administered alone, or in excess over heparin. Moreover, it has several untoward side effects, and the neutralization of heparin by protamine may be followed by the so-called heparin rebound[42].

Heparin adsorbing resins

In a previous study[43], we have found that several poly(amino-amine)s are able, in a linear form, to neutralize the antocoagulant activity of heparin, much as protamine sulphate does. This is apparently due to their ability to form complexes with heparin in aqueous solution, as later confirmed by n.m.r. and low-angle X-ray scattering. Consequently, we assumed that poly(amido-amine)s in a crosslinked, water-insoluble form, would act as heparin adsorbers. This proved to be true in several cases[5,6,44-46].

The structures of the repeating units of heparin-complexing poly(amido-amine) resins (referring to their linear portion), are given in *Table 8*. It may be noted that the linear portions of resins N_2, N_2L and N_2LL correspond to polymers **7**–**9** of *Table 1*, while resin E_2R is in fact a copolymeric product.

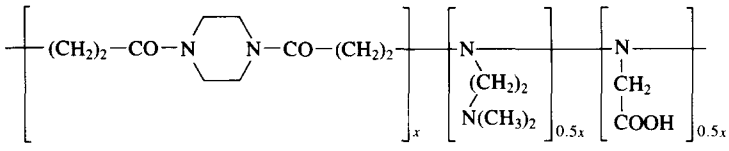
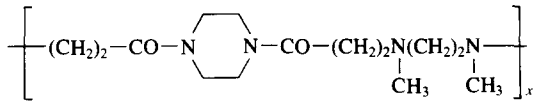
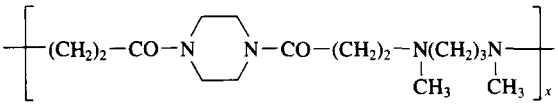
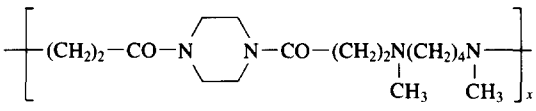
Resins of E_2R structure proved to be selective heparin adsorbers from plasma or blood[6,44]. The amount of heparin which could be adsorbed ranged from 30 to 100% by weight (calculated on dry resin), according to the crosslinking method employed. The adsorbed heparin could not be washed out, at least in the interval between pH 6 and 8. The resins apparently show no undesirable effects on blood.

In particular, they have not any hemolytic effect on red blood cells, nor they do alter recalcification time, prothrombin time, partial thromboplastin time, or fibrinogen content. They do not interfere with the macromolecules normally present in blood, and do not affect platelet count. Their biological effects and physical and chemical properties might allow them to be used to make molecular filters capable of de-heparizing blood.

Subsequently, resins of N_2, N_2L and N_2LL structure have been prepared and studied[46]. Their adsorbing capacities at pH 7.4 (phosphate buffer) was determined, and found to be related to their net average charge per repeating unit, i.e., with their basic strength. Results are given in *Table 9*.

In the case of N_2LL resin, the retentive power at various

Table 8 Some heparin-complexing poly(amido-amine) resins[a]

Resin	Structure of the repeating unit
E_2R	
N_2	
N_2L	
N_2LL	

[a] the resins were crosslinked by means of 1,4-diaminobutane (20% of the sum of aminic monomers) (see text)

Table 9 Heparin-adsorbing capacities of N_2, N_2L and N_2LL resins at pH 7.4 (phosphate buffer)

Resin	Adsorbing capacity (% by weight of dry resin)	Net average charge per unit
N_2	5.82	0.5
N_2L	7.71	1.0
N_2LL	22.2	1.5

pH's was also investigated[47]. The resin, after heparinization, was eluted with M/15 phosphate buffers at pH 4.8; 7.4; 8.5. No heparin was found in the eluates.. The resin was then eluted with carbonate buffers between pH's 10.4–12.8, increasing the pH by 0.2 units after each step. All the heparin was desorbed between pH 10.8 and 11.4, the elution peak being remarkably sharp, and centred around pH 11.0. These results show that heparin is very stably adsorbed on the resin, and, consequently, N_2LL is a good candidate for preparation of heparinizable devices.

SURFACE-GRAFTING OF POLY(AMIDO-AMINE)S ON VARIOUS MATERIALS

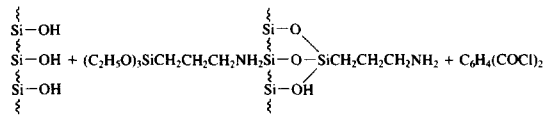
It is well known that most non-physiological materials induce thrombus formation when placed in contact with blood. An interesting approach to the development of non-thrombogenic surfaces is to coat synthetic polymers with heparin. This is usually achieved by first adsorbing quaternary ammonium salts on the surface to be heparinized, resulting in a positively charged surface. Heparin is then adsorbed on this surface by means of ionic bonds. Only in a few instances, is heparin directly adsorbed on cationic polymers.

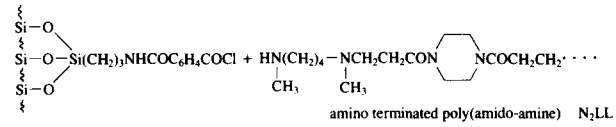
In all the above cases, the ability of adsorbing heparin, and to become non-thrombogenic, is a surface property. It follows that grafting heparin-complexing poly(amido-amine)s on a surface of a given material, not necessarily an organic polymer, would eventually lead to the same result.

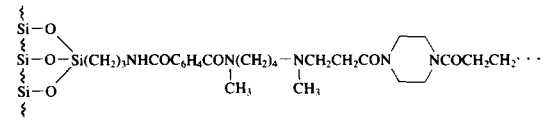
We have studied surface-grafting of poly(amido-amine)s on glass, plasticized poly(vinylchloride), and poly(ethylene terephthalate) (Dacron). The heparin adsorbing capacities and retentive power of the products have been also evaluated. The three cases will be dealt with separately.

Glass

The preferred method for grafting poly(amido-amine)s onto glass articles is as follows[46]:







Amino- and vinyl (see below) -terminated poly(amido-amine)s were easily obtained as previously described[48]. They had also been used as macromers to obtain block and graft copolymers[48–50]. The above reaction, as well as the grafting reactions on PVC and Dacron (see below) could be performed on articles irrespectively of their shape. Some results obtained with glass microspheres (0.05 mm diameter) are worth mentioning[46]. Heparin loading was 1.03 mg/g. Scanning electron micrographs, and a dot map of sulphur, showed that heparin adsorp-

Table 10 Desorption of heparin from heparinized grafted Dacron surfaces

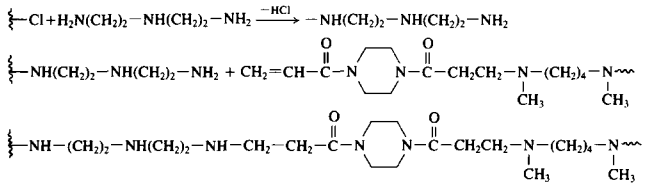
	Test	Standard time	Contact time saline[c]		Standard time	Contact time NaOH 1M[c,d]		
			30 min	15 h	30 min		30 min	30 min	30 min
Product **II**	APPT[a]	31.6	114.6	73.0	36.3	31.4	134.7	49.6	35.8
	TT[b]	13.7	82.9	23.2	14.1	11.6	116.4	14.6	12.2
Product **III**	APTT[a]	32.0	>360	178.0	37.0	33.5	>360	124.0	35.7
	TT[b]	14.1	>360	119.0	14.9	13.2	>360	60.3	13.2

[a] activated partial thromboplastic time (seconds)
[b] thrombin time (seconds)
[c] 5 ml of fresh solution added after each period of contact time. Experiment performed with 0.1 g samples of grafted material
[d] it was possible to calculate that the amount of heparin released only by 0.1M NaOH is about 2.5 mg/g of Product **II**, and 27.5 mg/g of Product **III**

tion was fairly uniform on the particles' surfaces. Desorption of heparin with saline was only 0.68%; and 1.21% with plasma. The bulk of adsorbed heparin could be desorbed only with 0.1M NaOH.

Plasticized PVC

Grafting of N_2LL on plasticized PVC surfaces was performed starting from a vinyl-terminated sample, according to the following scheme[51].

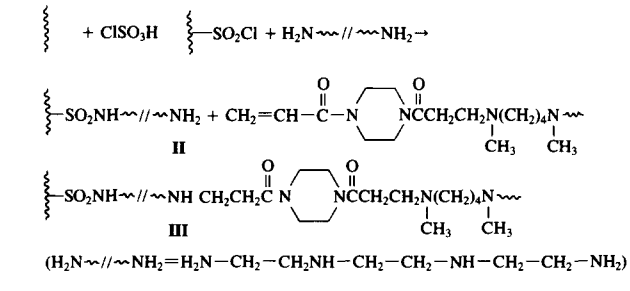


Both reaction steps may be performed in aqueous media; this means that the plasticizer is essentially retained during the process. The following results refer to PVC tubes of medical grade, having internal diameter of 0.8 cm.

The grafting reaction could be monitored by e.s.c.a. After grafting, the tubes were heparinized, and then extracted with saline, citrated plasma, and aqueous 0.1M NaOH. No heparin was extracted by saline or plasma. In the case of NaOH, heparin was gradually released over a 12 h period. The total amount was about 1.5×10^{-6} g/cm^2. This amount is not very high, if compared with that adsorbed on grafted glass microspheres (see above). Heparin, however, once adsorbed is very strongly retained[47,51].

Poly(ethylene terephthalate) (Dacron)

Grafting of N_2LL on Dacron was performed on medical grade fabrics as follows:

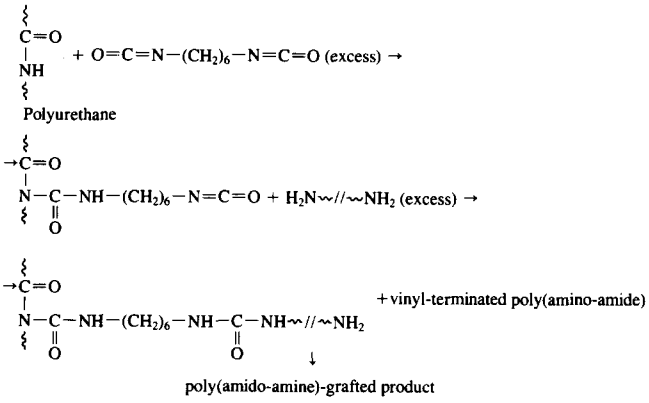


All reaction steps were monitored by e.s.c.a. and i.r.[52].

Heparinization studies were performed on both the end product **III** and the aminated intermediate product **II**. The heparinized surfaces were carefully mixed with saline until no heparin was found in the eluates. At this stage, a considerable amount of heparin is still present on the poly(amido-amine) grafted surface, which can be removed only by eluting at pH > 10, e.g. with 0.1M NaOH. This confirms that strong polymer–heparin interaction takes place. The heparin present on the intermediate product **II**, at the same stage, is less than one-tenth, even if contemporaneous titration of basic nitrogens indicated that only a 3-fold increase takes place passing from **II** to **III**. The last result is an indication that only a part of the aminic groups of **II** react in the last step. Heparinization results are shown in *Table 10*.

Polyurethanes

Grafting of poly(amido-amine)s on polyurethane surfaces can be performed according to the following scheme[53].



The last step is similar to those reported in the cases of PVC and Dacron. The amounts of aminic groups introduced was evaluated titrimetrically, and found to depend mainly on the contact time between diisocyanate and polyurethane.

Heparinization studies were performed as in the previous cases, and the results are shown in *Table 11*.

Use of activated derivatives of polymeric acids in grafting of polyaminic compounds on the surface of various materials

Another method of grafting aminic compounds, which is presently being studied in the case of Dacron, but could be reasonably applied in many other cases, including those described in this article, is the following[54].

In a previous study, it was found that poly(1-acryloylbenzotriazole) reacts rapidly and quantitatively with most

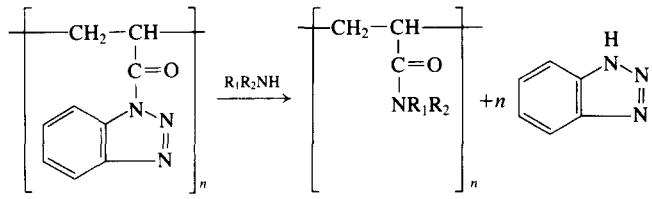
Table 11 Desorption of heparin from heparinized poly(amido-amine) grafted polyurethane films with 0.1M NaOH, after exhaustive soaking with saline

		Contact time[b,c]		
Test[a]	Standard time	30 min	30 min	30 min
APTT	37.0	>360	166.6	40.1
TT	10.8	>360	90.6	11.4

[a,b] see footnote to *Table 10*

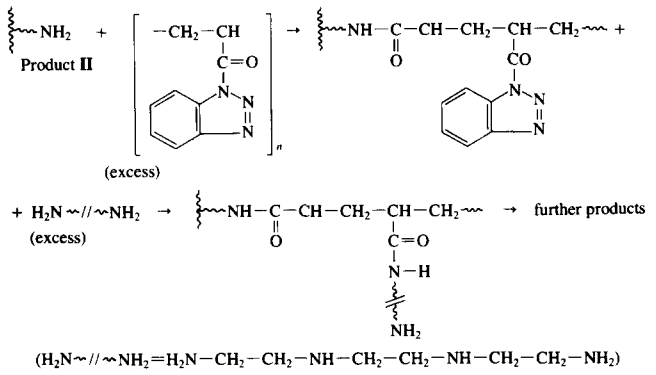
[c] it was possible to calculate that the heparin released only by 0.1M NaOH was about 4.7×10^{-5} g/cm^2

primary and secondary aliphatic amines giving amidic linkages[55].



It follows that treatment of product **II** of the Dacron-grafting scheme (see above) with a large excess of poly(1-acryloylbenzotriazole) results in grafting of the latter onto the surface. Only a minor proportion of the benzotriazolide groups are consumed at this stage, leaving the remaining groups available for further reactions. As a consequence, treatment with an excess of, for instance, triethylenetetramine, introduces a relatively large amount of primary and secondary amino groups, which, in turn, are available for further reactions, for instance with a vinyl terminated poly(amido-amine).

Thus the insertion of poly(1-acrylolybenzotriazole) chains as an intermediate step results in a kind of 'multiplying' effect. The overall reaction path is the following:



Only preliminary results are available on the amount of basic nitrogens introduced by this way, as well as on the final reaction with vinyl-terminated poly(amido-amine)s. It would appear that the amounts of basic nitrogens, with respect to the corresponding products in which the poly(1-acryloylbenzotriazole) step was omitted, have been multiplied by a factor of about five. Further studies on this point are presently in progress.

CONCLUSIONS

Heparin-complexing poly(amido-amine)s have been fairly easily grafted onto many different materials, and the treated surfaces proved to be able to adsorb heparin from aqueous solutions. Most of the adsorbed heparin is stably retained in saline, and can be removed only by eluting at pH > 10 with 0.1M NaOH solution.

The strength of this bond depends on electrostatic interaction between the protonated aminic nitrogens of poly(amido-amine)s and the negatively charged groups of heparin. In any case, it may be concluded that surface-grafting of poly(amido-amine)s can impart remarkable heparin-adsorbing ability to materials already widely used in medical practice. The *in vivo* performances of articles treated in this way are presently being investigated.

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