

hexafunctional crosslinkers generate more rigidly crosslinked polymer networks, compared with tetrafunctional or bifunctional crosslinkers. When a hydrophilic cross-linking agent is employed, the hydrogel behaves with a high affinity towards water and when the crosslinker is replaced with a more hydrophilic one, the swelling of the hydrogel is enhanced. For instance by replacing bisacrylamide with more hydrophilic glyoxalbis (diallyl acetal) (GLY) as crosslinker, the swelling of the crosslinked N-isopropylacrylamide (NIPAAm) hydrogel is enhanced (Xue and Hamley 2002). Of course a pronounced increase of the polymer–water interaction parameter is also observed with increasing GLY content. The swelling degree of the hydrogel, here considered a parameter to measure the hydrophilicity of hydrogel, is obviously reduced as the amount of crosslinker increases. The degree of swelling is observed to decrease with the crosslink density at exponent of 0.5 (Alvarez-Lorenzo and Concheiro 2002). However, at low crosslink density, the increase in amount of hydrophilic crosslinker may increase the degree of swelling. The poly(2-hydroxyethyl methacrylate) hydrogels show an exceptionally large increase of swelling with increasing the amount of crosslinker tripropylene glycoldiacrylate at pH 12.0 (Ferreira et al. 2000). Moreover the structure and elasticity of the hydrogels depend on the nature of cross-linking agent as well as on the cross-linking degree. The polymeric network differs from that of the less-crosslinked one so that we can say the two hydrogels are completely different despite having the same cross-linking agent and the same polymer.

Another parameter to be taken into account is represented by the different cross-linking methods, which can distinctly influence the polymer network structure (Martens and Anseth 2000). Cross-linking by  $\gamma$ -ray irradiation randomly introduces the crosslinks in the hydrogels, whereas generally the chemically crosslinked hydrogels exhibit inhomogeneous distribution of cross-linking points due to the difference in the reactivity ratios of monomers and crosslinkers (Pradas et al. 2001). The small angle neutron scattering studies demonstrate that the poly(NIPPAm) hydrogels crosslinked by  $\gamma$ -ray irradiation are more homogeneous than the hydrogels crosslinked by conventional polymerization (Norisuye et al. 2002). Nevertheless with polysaccharides, specific reactive groups in the polymer skeleton have been utilized to be crosslinked and homogeneous stoichiometry hydrogels were obtained. Hyaluronane is a straight chain polymer consisting of alternating N-acetyl-D-glucosamine and beta-D-glucuronic acid residues. The hydrogel was synthesized by using 2-chloro-1-methyl pyridinium iodide (CMP-I) as carboxylate groups activating. The CMP-I was added in a stoichiometric amount to activate 50% of the carboxylic groups of the polysaccharide. The cross-linking agent was an alkyldiamine, which underwent a nucleophilic attack on the carboxylic group (Fig. 7) (Barbucci et al. 2000).

The hydrogel was then analyzed by potentiometric and NMR techniques. Both the results were close to the theoretical result—those obtainable from the amount of CMP-I added to the solution, i.e., 50% of the carboxylic groups present in the polysaccharide. By NMR studies a homogeneous structure of the Hyal hydrogel was obtained with a regular alternated crosslinked arm in the backbone (Barbucci et al. 2006a) (Fig. 8).