Semester Project

Measuring the mesh size of hydrogels

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Contents

1 Introduction

The importance of the hydrogels is tremendous. They can be used in multiple systems like scaffolds, controlled drug release systems or contact lenses. Hydrogels consists of polymers that are cross-linked in a 3D networks. They can either be natural or synthetic, homopolymers or copolymers. They have the ability to absorb and retain large amounts of water. This is known as the swelling of hydrogels and is due to the hydrophilic nature of some of the functional groups (alcohols, carboxyls, etc.) of the backbone chain. The 2 main techniques to create the cross-linking are the chemical method or the radiation method [1]. In this case, the radiation is preferred, because the initiator and the catalyst aren't required and the process leaves no residue in the environment. Cross-linking is performed under UV light radiation for the hydroxymethyl methacrylate (HEMA). HEMA has many advantages, it's inert to biological process and is not absorb by the body [2]. The aim of this study is to measure the mesh size of HEMA hydrogels under different concentrations of polymers and different water ratios.

2 Mehtod

2.1 HEMA cross-linking protocol

In order to do the cross-linking oh the hydrogel, some solutions are required

- Hydroxymethyl methycrylate (HEMA)

- EGDMA

- Demineralized Water

- Irgacure 600

- and a UV lamp

The first step is to mix the HEMA with EGDMA and then to add water and Irgacure. It's important to well mix the solution with the vortex to have an homogenous solution. After that, depending on the purpose of the hydrogel (tensile test or buoyancy), the solution is put in either small tubes or in the tensile test mold. Then, the solution is put under a UV lamp during 10 minutes and is recovered with a coverslip.

2.2 equations

To determine the mesh size, several equations are required [10]. The first one is useful to calculate the Young's modulus.

$$\tau = G * (\alpha - \frac{1}{\alpha^2}) \tag{2.1}$$

In this equation 2.1, τ is the the stress applied to the polymer sample, G is the tensile modulus and α is the deformation ratio (deformed length divided by the initial length).

$$\tau = \frac{\rho RT}{M_c} * (1 - \frac{2M_c}{M_n}) * (\alpha - \frac{1}{\alpha^2}) * (\frac{V_{2,s}}{V_{2,r}})^{(1/3)} \tag{2.2}$$

Where ρ is the density of the polymer, R is the gas constant, T the temperature in K, M_c is the molecular weight between the crosslinks, M_n the molecular weight of the polymer chains, $V_{2,r}$ the polymer volume fraction in the relaxed state and $V_{2,s}$ the polymer volume fraction in the swollen state.

From the equations 2.1 and 2.2 :

$$G = \frac{\rho RT}{M_c} * (1 - \frac{2M_c}{M_n}) * (\frac{V_{2,s}}{V_{2,r}})^{(1/3)} \tag{2.3}$$

Since the hydrogels are highly cross-linked $\frac{2M_c}{M_n}$ tends to 0. Therefore, the desired molecular weight between crosslinks can be determined. To find the mesh size of the hydrogels, the density of the hydrogels is needed and obtained from the buoyancy method. The density is used to find the polymer volume fraction in the relaxed state $V_{2,r}$ and the polymer volume fraction in the swollen state $V_{2,s}$. Finally, the mesh size is found using the following equation:

$$\epsilon = V_{2,s}^{(-1/3)} * \frac{2M_c C_n}{M_r} * l \tag{2.4}$$

Where ϵ is the mesh size, M_r is the molecular weight of HEMA monomer, C_n is the Flory characteristic ratio and l the length of the bond along the polymer backbone

2.3 Buoyancy protocol

The cross-linked hydrogels are taken out of their containers and placed in a multi-wells plate. The buoyancy technique is used to determine the density of the hydrogels. For this purpose, a special balance is required. The first step is to weight the hydrogel in the air, and then weight it again, but this time in water, because the density of water is known and is equal to 1000 $\frac{kg}{m^3}$. The hydrogel is weighted a last time in absolute ethanol (density = 789 $\frac{kg}{m^3}$). The equation for the buoyancy is the following:

$$\frac{\rho_o}{\rho_f} = \frac{m_o}{m_o - m_i} \tag{2.5}$$

where:

- ρ_o is the density of the object.

- ρ_f is the density of the fluid.

- m_o is the weight of the object in the air.

- m_i is the apparent immersed weight.

It's important to measure the weight right after the polymerization but before the swelling. Then, the hydrogels are put in water in order to swell. The weight are measured after some days only in the air. At the end, the hydrogels are dried during 2 days just by removing water. These measurements are useful to determine the polymerization volume and the dried volume. The tables 2.1 and 2.2 report the values obtain from the measurements of the mass by the buoyancy method for both 40% and 50% water with the different concentrations of EGDMA. From these tables and with the equation 2.5, the densities of the hydrogels can be determined.

40% water	weight in air in [mg]	weight in water in [mg]	weight in ethanol in [mg]
	91.4	12	29.7
	92.7	9.3	30.2
2% EGDMA	69.9	9.7	23.7
	98.9	13.9	32.5
	81.6	11.1	25.5
	95.2	9.9	31.2
	124.2	16.2	39.4
4% EGDMA	88.3	15.1	29.2
	125.7	6.9	40.2
	120.8	16.4	39.5
	74.7	9.1	23.9
	95.4	12.3	30.7
6% EGDMA	104.2	14.2	38.8
	147.7	21.3	46.8
	140.5	21.9	48

Figure 2.1: Mass obtained in different media for 40% water.

50% water	weight in air in [mg]	weight in water in [mg]	weight in ethanol in [mg]
	127.2	12.9	39.4
	128.1	14.2	40.9
4% EGDMA	124.7	14.5	39.2
	131.3	15.6	41.5
	132.5	13.8	40.7
	131.2	18.4	41.5
	134.4	16.1	43.2
	132.1	17	41.7
6% EGDMA	130.4	16	41.2
	132	16.8	42
	131.3	16.4	41.2
	132.5	15.7	40.4

Figure 2.2: Mass obtained in different media for 50% water.

The densities are obtained by dividing the first column of the tables 2.1 and 2.2 by the addition of the first and second columns of the same tables and then multiplied by the density of water for the first column of table 2.3. For the second column of table 2.3, the first column of tables 2.1 and 2.2 is divided by the addition of the first and second columns of the same tables and then multiplied by the density of ethanol. Then, the third column is obtained by taking the mean value of the 2 first columns and finally for

each concentration of EGDMA, the average of all the densities is taken for the following calculations. An example might be useful, so for the first density of the 40% water and 2% EGDMA is found by doing $\frac{91.4}{91.4+12} * 1000 = 1151.133501$.

density 40% Water in [kg/m^3]	ratio air-water	ratio air-ethanol	mean
	1151.133501	1168.794165	1159.963833
	1111.510791	1170.2448	1140.877796
2% EGDMA	1161.129568	1193.746753	1177.438161
	1163.529412	1175.182229	1169.35582
	1157.446809	1147.636364	1152.541586
		mean1	1160.035439
	1116.060961	1173.6375	1144.849231
	1150	1155.587264	1152.793632
4% EGDMA	1206.284153	1178.827411	1192.555782
	1058.080808	1159.968421	1109.024615
	1157.088123	1172.339483	1164.713803
		mean2	1152.787412
	1138.719512	1160.202756	1149.461134
	1148.01444	1163.378671	1155.696556
6% EGDMA	1157.777778	1257.091743	1207.43476
	1168.512658	1154.958375	1161.735516
	1184.6543	1198.427027	1191.540664
		mean3	1173.173726

Figure 2.3: density obtained in different media for 40% water.

density 40% Water in [kg/m3]	ratio air-water	ratio air-ethanol	mean
	1112.860892	1143.061503	1127.9612
	1124.670764	1159.069954	1141.87036
	1131.578947	1150.740351	1141.15965
4% EGDMA	1134.831461	1153.626949	1144.2292
	1116.259478	1138.80719	1127.53333
	1163.120567	1154.033445	1158.57701
		mean1	1140.22179
	1136.094675	1162.736842	1149.41576
	1147.697654	1152.952434	1150.32504
	1139.86014	1153.426009	1146.64307
6% EGDMA	1145.833333	1157.2	1151.51667
	1142.732811	1149.785794	1146.2593
	1134.417808	1135.09772	1134.75776
		mean2	1146.48627

Figure 2.4: density obtained in different media for 50% water.

Swelling mass 40% Water in [mg]	24h	48h	120h	144h	168h	192h	mean	dried
	98	96	100	95	103	96	98	61
	93	97	108	96	98	94	97.6666667	62
2% EGDMA	81	84	77	77	76	76	78.5	46
	105	106	110	109	110	108	108	69
	90	88	96	90	92	92	91.3333333	52
	97	101	99	106	102	98	100.5	64
	120	119	121	148	124	123	125.833333	84
4% EGDMA	97	94	106	110	104	95	101	63
	128	126	126	126	130	130	127.666667	80
	119	124	120	122	125	122	122	80
	81	73	76	80	75	82	77.8333333	48
	111	96	99	101	100	84	98.5	59
6% EGDMA	129	151	139	137	139	140	139.166667	84
	142	148	143	145	146	145	144.833333	101
	144	139	141	144	147	142	142.833333	105

Figure 2.5: Swelling mass obtained at different time and dried mass for 40% water.

Swelling mass 50% Water in [mg]	24h	48h	72h	96h	mean	dried
	124	127	124	127	125.5	73
	132	140	143	141	139	75
4% EGDMA	122	121	134	142	129.75	73
	132	128	132	135	131.75	76
	134	132	136	134	134	78
	131	133	137	138	134.75	77
	140	140	145	146	142.75	80
	131	134	135	138	134.5	79
6% EGDMA	129	131	133	136	132.25	75
	127	130	130	134	130.25	78
	134	137	136	138	136.25	77
	134	135	138	140	136.75	76

Figure 2.6: Swelling mass obtained at different time and dried mass for 50% water.

Then with the mean values of the swelling mass and the dried mass reported in tables 2.5 and 2.6, the volume of the hydrogels can be calculated. Indeed, the volume is the mass in kg divided by the density in $\frac{kg}{m^3}$. To get the volume in μl, the results are multiplied by 10^9.

Volume in [ul]	after polymerization	after swelling	after drying
40% Water	78.79556015	84.4853927	52.5878465
	81.2532248	85.6065979	54.3441201
2% EGDMA	59.36617509	66.670168	39.0678692
	84.57648073	92.3585432	59.006847
	70.80004833	79.2451521	45.1176779
	83.15505435	87.7844849	55.9025575
	107.7382773	109.155125	72.8664677
4% EGDMA	74.04265807	84.6920551	52.8277175
	113.3428405	115.116171	72.1354593
	103.7164664	104.746762	68.6864016
	64.98697328	67.7128883	41.7586977
	82.54761991	85.2299849	51.051463
6% EGDMA	86.29865846	115.258125	69.5689761
	127.1373716	124.669799	86.9388932
	117.9145658	119.872815	88.1212058

Figure 2.7: Volume of hydrogels in μl for 40% water.

Volume in [ul]	after polymerization	after swelling	after drying
50% Water	112.7698366	111.262693	64.7185383
	112.184364	121.730106	65.6817119
4% EGDMA	109.2748066	113.70013	63.9700151
	114.7497367	115.143015	66.4202589
	117.5131555	118.843493	69.1775557
	113.2423648	116.306468	66.4608391
	116.9289694	124.19353	69.600577
	114.8371069	116.923474	68.676241
6% EGDMA	113.72327	115.336675	65.4083225
	114.6314281	113.111693	67.736753
	114.5465077	118.864902	67.1750274
	116.7650085	120.510301	66.9746464

Figure 2.8: Volume of hydrogels in μl for 50% water.

Finally, the polymer volume fraction in the relaxed state $V_{2,r}$ and the polymer volume fraction in the swollen state $V_{2,s}$ can be found. They are calculated by dividing the polymerization volume or the swelling volume by the dried volume respectively.

Volume ratio	$V_{2,r}$	$V_{2,s}$
	1.49836066	1.60655738
	1.49516129	1.57526882
2% EGDMA	1.51956522	1.70652174
	1.43333333	1.56521739
	1.56923077	1.75641026
mean	1.50313025	1.64199512
	1.4875	1.5703125
	1.47857143	1.49801587
4% EGDMA	1.4015873	1.6031746
	1.57125	1.59583333
	1.51	1.525
mean	1.48978175	1.55846726
	1.55625	1.62152778
	1.61694915	1.66949153
6% EGDMA	1.24047619	1.65674603
	1.46237624	1.4339934
	1.33809524	1.36031746
mean	1.44282936	1.54841524

Figure 2.9: Polymer volume fractions for 40% water.

Volume ratio	$V_{2,r}$	$V_{2,s}$
	1.74246575	1.71917808
	1.708	1.85333333
4% EGDMA	1.70821918	1.77739726
	1.72763158	1.73355263
	1.69871795	1.71794872
	1.7038961	1.75
mean	1.71482176	1.75856834
	1.68	1.784375
	1.6721519	1.70253165
6% EGDMA	1.73866667	1.76333333
	1.69230769	1.66987179
	1.70519481	1.76948052
	1.74342105	1.79934211
mean	1.70529035	1.74815573

Figure 2.10: Polymer volume fractions for 50% water.

2.4 Tensile test

For the tensile test, the hydrogels were taken out of the mold and put into the Instron machine (pictures of the system can be seen in the annexes part). The hydrogel is put as shown in figure 2.11 between the big pieces that act like tweezers. Then, the machine begin to move further apart the 2 "tweezers", this will cause the tension of the hydrogel. The Instron allows to adjust not only the rate of elongation of the hydrogel but also the maximal displacement of the 2 pieces. These pieces are recovered with white rubber in order to enable the camera to visualize properly the 2 spots on the hydrogel. Therefore, the elongation and the deformation ratio can be recorded precisely. A second system of recording is used. It's directly linked to the Instron machine and saved data about: time, position, load, displacement,... It's really important to keep the hydrogels in water, otherwise they become dry and have an higher tendency to break.

Figure 2.11: Set up of the hydrogel in the Instron machine

3 Results

3.1 Young's modulus

Since the Instron machine apply a preload of 1N, the load recorded in the files should be subtracted by 1N. Then, to calculate the strain applied to the hydrogel, the cross section on the middle part of the hydrogels 3.1 is calculated and is equal to $0.000008m^2$. The strain is thus simply the division from the load by the cross section. A second set of values is recorded by the camera and is useful to determine the Young's modulus. By using the time of the elongation and the percentage of elongation, the rate of elongation can be found. A tendency curve 3.2 is used to ascertain that the rate is linear and to obtain the value of the equation. After that, this value is used to synchronize the 2 set of recorded data. The synchronization is basically the elongation rate times 1000, divided by 100 and times the time of the first set of values.The multiplication by 1000 is due to the fact that the second set is recorded in milliseconds and seconds are required and the division by 100 is simply due to the fact that the elongation is recorded in percentage. And then to find the Young's modulus, the stress is divided by the synchronized time as seen in the right part of 3.2.
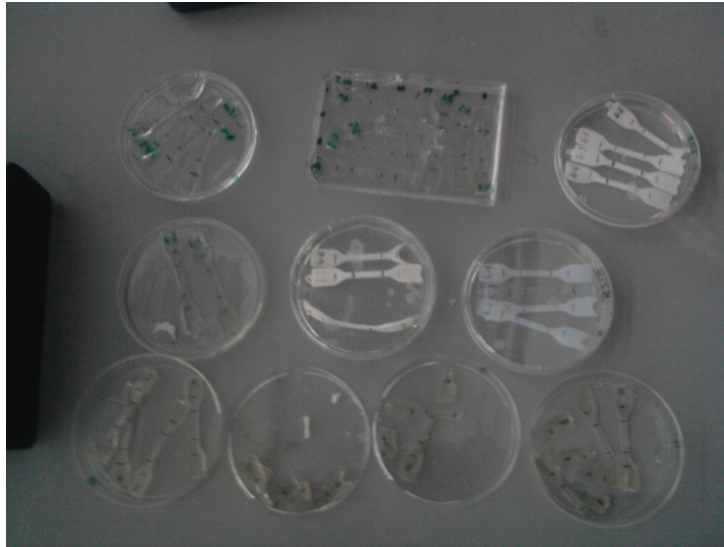
Figure 3.1: Overview of all the samples used to determine the Young's modulus and mesh size.

The following images will show the different Young's modulus obtained from the different conditions of the experiment. The last-but-one image is simply the mean value of the Young's modulus for each condition. The last picture is an example of what the Young's modulus looks like when the polymer breaks during the tensile test.
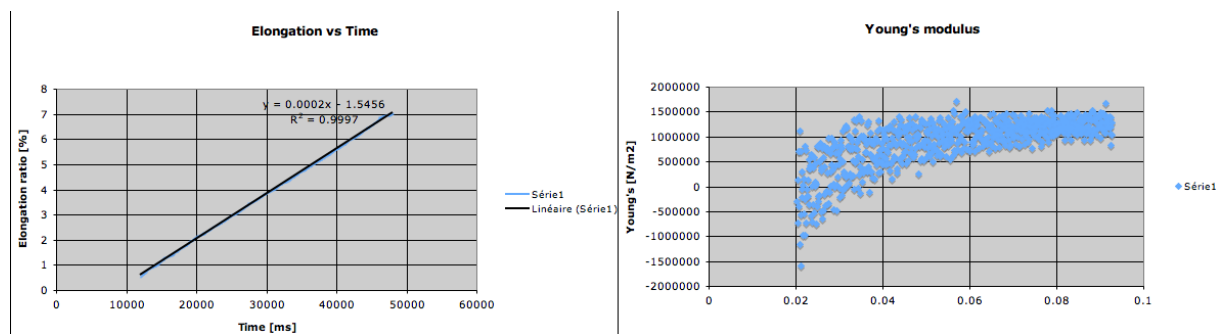
Figure 3.2: Rate of elongation and evolution of Young's modulus for a 2% EGDMA, 40% water sample
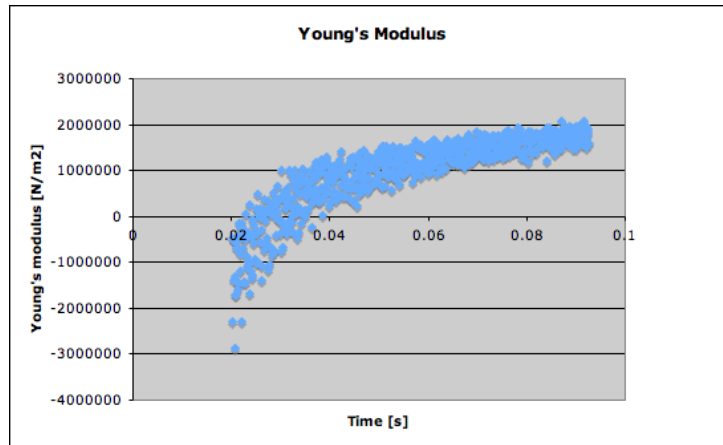
Figure 3.3: Evolution of Young's modulus for a 4% EGDMA, 40% water sample
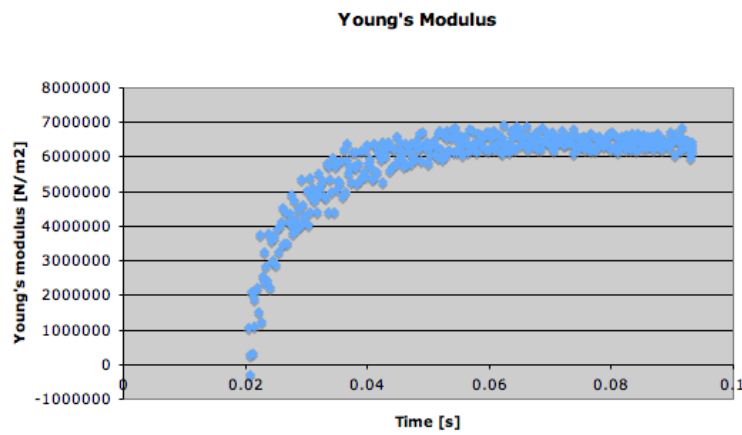
Figure 3.4: Evolution of Young's modulus for a 6% EGDMA, 40% water sample
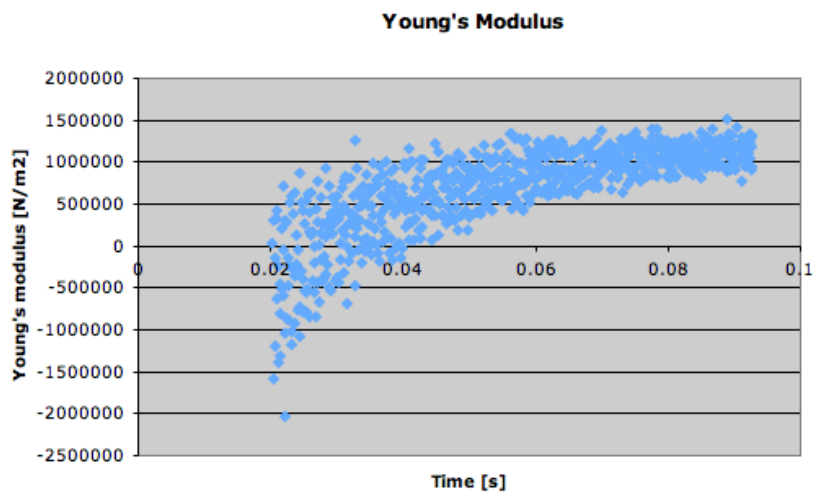
Figure 3.5: Evolution of Young's modulus for a 4% EGDMA, 50% water sample
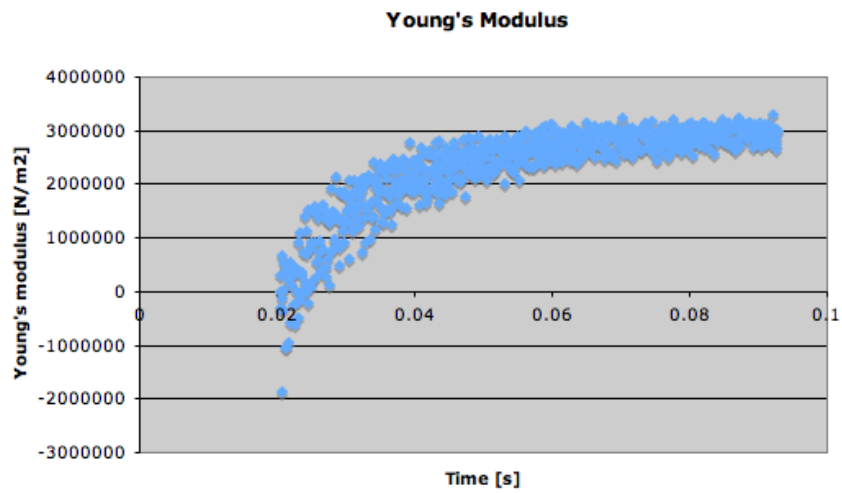
Figure 3.6: Evolution of Young's modulus for a 6% EGDMA, 50% water sample
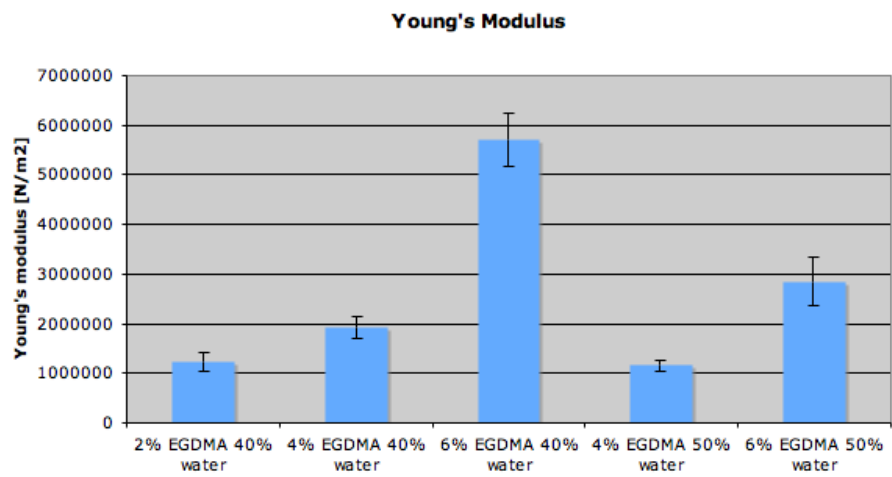
Figure 3.7: Young's modulus for each condition
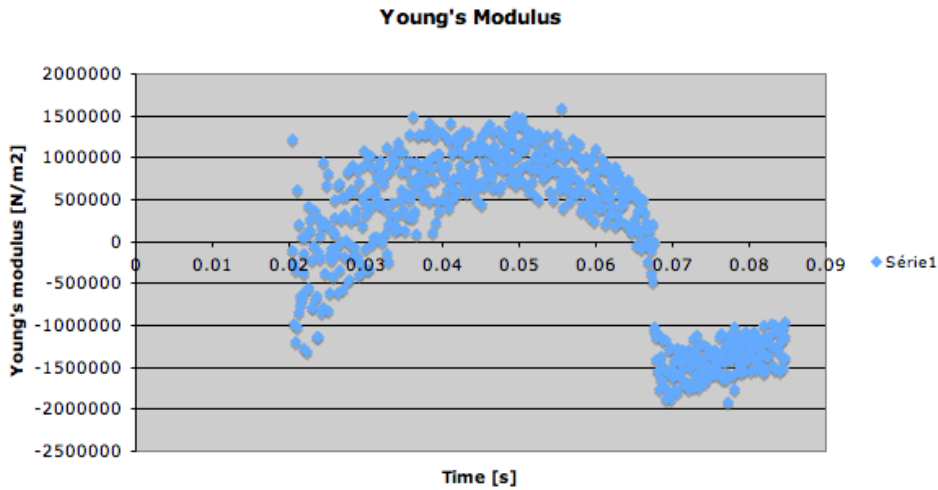
Figure 3.8: Example of the Young's modulus of a breaking polymer

With the figure 3.7, we can see that there some differences in the Young's modulus for the different conditions. For the same percentage of water, if the concentration of the polymer increases, the Young's modulus increases too. This mean that the polymer can resist to higher stresses before breaking. So it is less susceptible to changes and will be less prone to shatter. When there is an higher concentration of EGDMA, the polymer can do more crosslinks during the polymerization phase under the UV lamp. Therefore, the network is more tight and the polymer is more resistant to stresses. For the same percentage of EGDMA, if the percentage of water is increased, then the Young's modulus of the polymer is reduced. This is due to the fact that if there is more water in the solution, there is like an higher dilution of HEMA and EGDMA, which implies a lower possibility of making connections and crosslinks during the polymerization phase. Hence, the polymer has a lower Young's modulus and has higher chances of breaking when stresses are applied.

3.2 Mesh size measurement

To determine the mesh size, the equation 2.3 is required. The only unknown in this equation is M_c, since the Young's modulus was determined with the tensile tests and the density was found with the buoyancy technique, R is the gas constant (8.314 J/mol K), T is the ambient temperature (298 K), $V_{2,r}$ and $V_{2,s}$ the different polymer volume fractions. For each calculation of M_c, the mean value of the density, the mean value of $V_{2,r}$ and the mean value of $V_{2,s}$ were used. Then, the results is multiplied by 1000 to convert the kg/mol in g/mol. After that, the equation 2.4 is use to determine the mesh size of the different hydrogels. In the case of HEMA hydrogels, C_n which is the Flory characteristic

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ratio is equal to 6.2, l is the distance between the bond of the backbone chain and is equal to 1.54 Angstrom and M_r is the molecular weight of the HEMA monomer which is equal to 130.14 g/mol. The other elements of the equation were found as said before by the buoyancy measurements and the tensile tests. Therefore, the mesh size of the polymers under the different conditions can be assessed and are reported in the following table 3.9:

	Mesh size in nm			
	40% Water		50% Water	
2% EGDMA	4% EGDMA	6% EGDMA	4% EGDMA	6% EGDMA
1.792046328	1.6738447	5.75850091	2.00223089	1.23924191
1.962509706	1.52280133	5.26456834	1.83019249	1.47020826
2.143992485	1.56805511	6.81153195	2.03142359	1.27157056
2.053362487	1.59628033	6.87641516	1.98702648	1.22963378
8.047434457	1.47552751	6.64493402		1.29838682
	1.72080082	7.62174289		1.15813771
	2.57557495	7.14416649		1.10821196
	2.69287852	7.30926266		1.33220623
		7.52680664		
		6.80904052		
		6.92886485		

Figure 3.9: Assessment of the mesh size for the different polymer conditions

Finally, for each case, the mean value of the mesh size was computed and then reported on the graph 3.10, so the comparison between the various conditions can be achieved. As we can see, when the concentration of EGDMA is increased for the same percentage of water, the mesh size of the polymer is reduced very drastically. Indeed with only a difference of 2% of the EGDMA concentration, the mesh size is reduce by a coefficient 2. If the water percentage increases and the concentration of EGDMA reminds constant, then the mesh size of the polymer increases a lot from 1.5nm to 2nm in the case of 4% EGDMA. With a change of only 10% in the percentage of water, to keep on constant mesh size, the percentage of EGDMA must be doubled. So, the water and EGDMA added are of a crucial importance to determine the mesh size of the polymer and a small change in one concentration has an high impact on the network of the polymer. There is also a strong relation between the Young's modulus and the mesh size of the polymer.
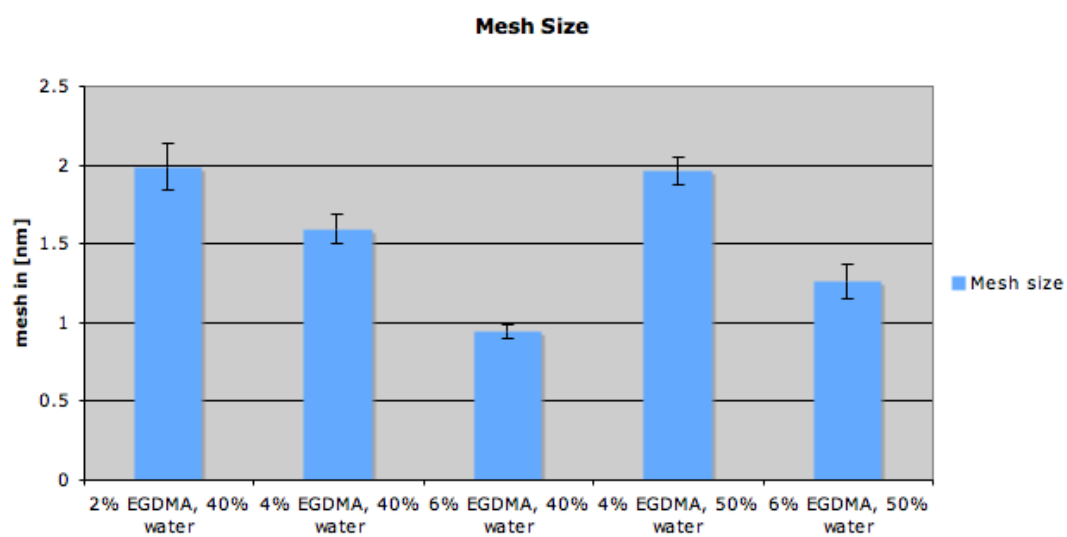
Figure 3.10: Comparison of the mesh size for the different polymer conditions

4 Conclusion

There are many difficulties to obtain good results. The first one is during the polymeriza-tion phase. When the solution is placed in the mold and then covered with the coverslip, bubbles may form at the interface between the solution and the coverslip which leads to the formation of holes in the polymer. Thus, the polymer will not resist to the tensile tests or will cause false results for the buoyancy. An other difficulty appears during the tensile test. When the polymer is placed between the "tweezers", it needs to be sprayed with water. Otherwise, it will dry and it will be more susceptible to break during the elongation phase of the tensile test. As it can be observe in the results section, the more water is added to the solution, the more the polymer network is loose cross linked and the higher is the mesh size. On the contrary, the more EGDMA is added to the solution, the more the polymer network is tighten and therefore the smaller is the mesh size. Hence, there is an high impact of the concentration of water and of the concentration of EGDMA on the polymer network. If the cross linking is high, the polymer is more resistant to stress and thus the Young's modulus is higher resulting in a polymer with small mesh size. In contrast, if the cross linking is low, the polymer is more susceptible to changes and thus the Young's modulus is lower resulting in a polymer with a high mesh size.
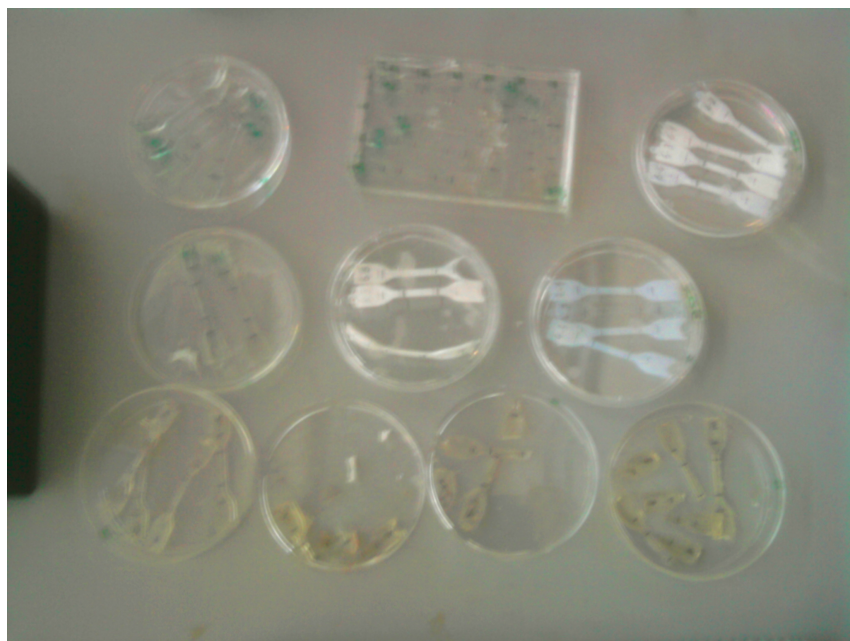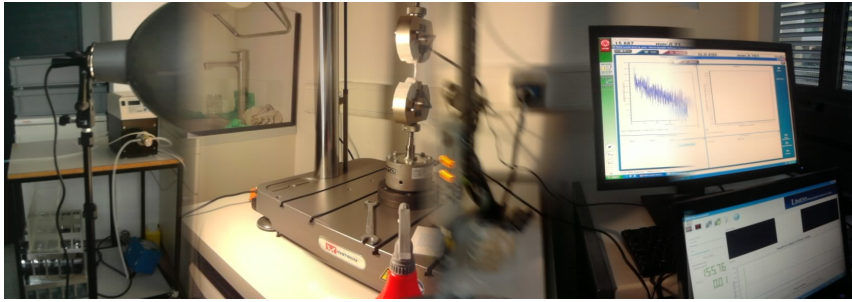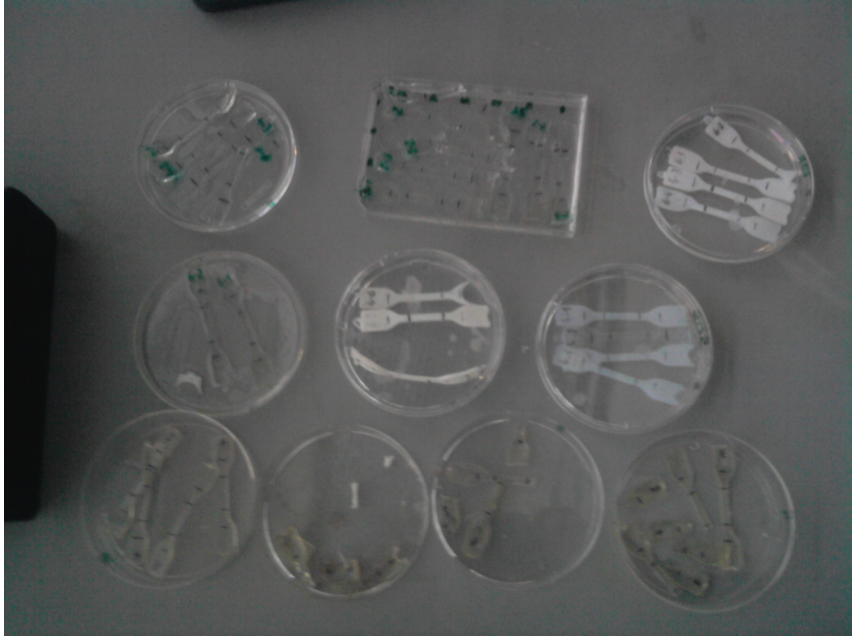
5 Annexes

5.1 Tensile test setup
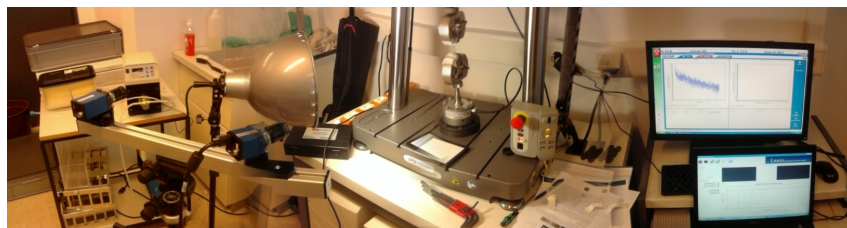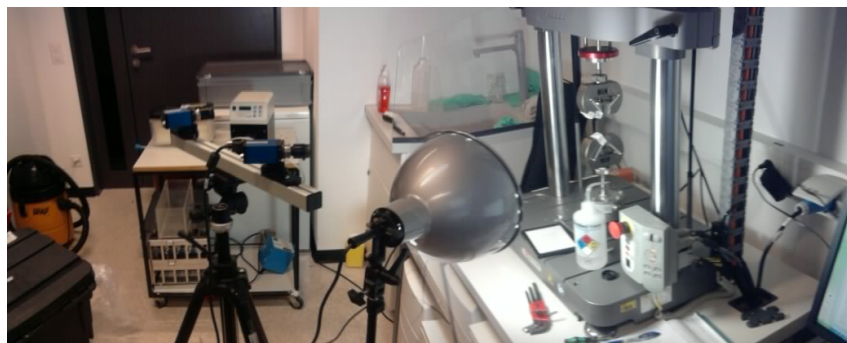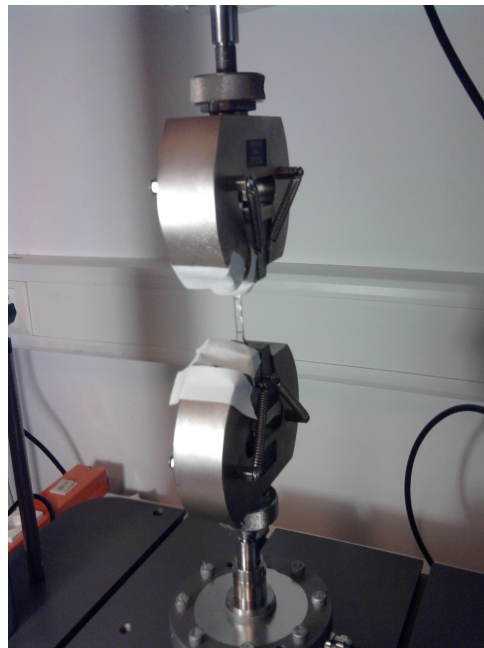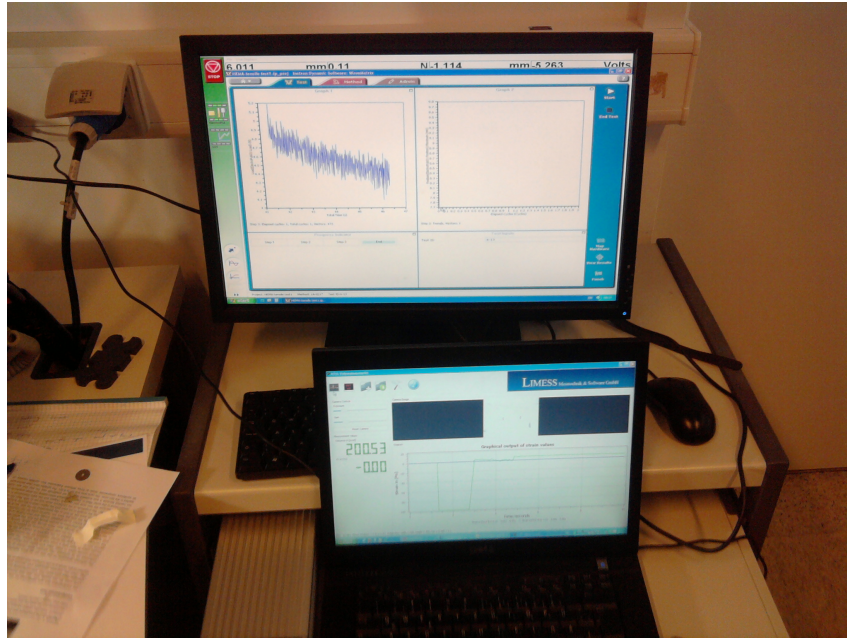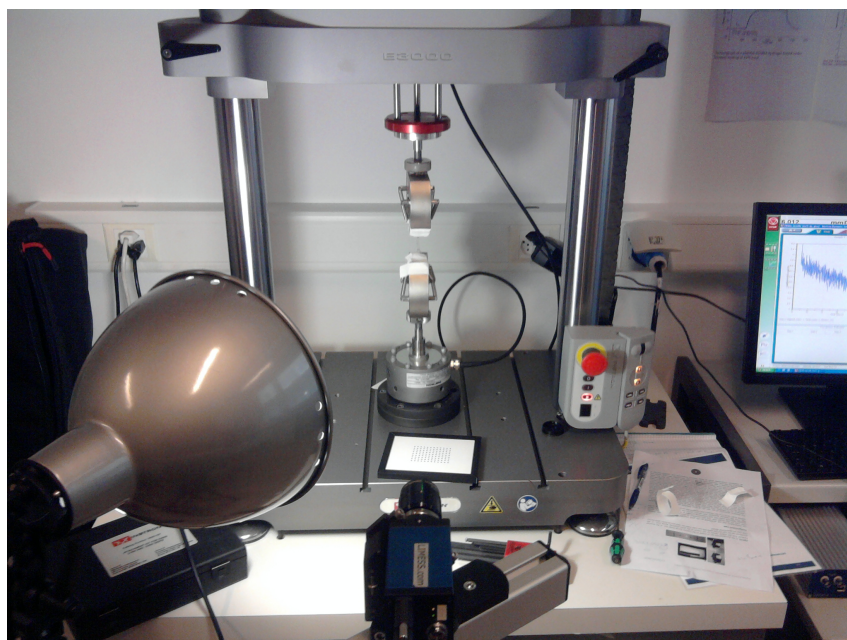
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