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# Poly (acrylamide) hydrogels with improved thermal, morphological properties and swelling behaviour: Influence of lipase immobilization onto hydrogel

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***Abstract:*** *Presenting study have been focused on redesigning poly(acrylamide) superabsorbents with high swelling performance via organic structure additions, interpenetrating network formation and hydrophilic reactive hydrogel formation as well as enzyme addition. Poly(acrylamide) hydrogels (PAAm) prepared by immobilization of the lipase enzyme in the form of reversibly bound material, were studied in terms of swelling, degree of loading and releasing enzyme, enzyme activity properties, thermal stability and surface morphology. The swelling behaviors of lipase-immobilized and lipase-removed hydrogels (LIRHs) were investigated as a function of time. Swelling data was fitted to various diffusion models and model parameters were evaluated using a regression technique. Model analysis indicated that the swelling transport followed Fickian and non-Fickian mechanisms as a function of time. The activity recovery of immobilized lipase into the PAAm hydrogel network reached 78% for the 3.0% lipase enzyme and also, more than 7.0% lipase loading into the network structure caused a decrease not only to the bound enzyme activity but also to the immobilization yield of the enzyme.*

***Keywords*:** *hydrogel, enzyme, characterization, kinetics, diffusion coefficient*

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## I. INTRODUCTION

Hydrophilic polymers constitute three-dimensional networks when they are cross-linked in terms of chemical and physical aspect. They swell in the aquatic media but do not dissolve. Hydrogels can absorb much water as well as water solutions, making them unique materials for a wide range of applications such as personal hygienic products, agricultural and other specialty fields as well as controlled drug delivery systems [1-3].

Polyacrylamide-based hydrogels have various chemical and physical characteristics. They have been studied as smart polymers for applications including immobilization of biocatalysts, biomedical applications, and bioseparators. Recent studies in the field of industrial applications including cosmetic, agricultural and pharmaceutical applications mainly deal with systems which include PAAm hydrogels because they have high swelling capacity, high swelling rate, and good strength of the swollen gel [4].

The hydrogels' high absorbance capacity and design with good mechanical performance have great significance in the potential application fields of various materials. For instance, dissolution of inorganic materials, enzymes and proteins in the polymers is a technique enabling superabsorbent hydrogels to be obtained [5,6]. In order to overcome this drawback, studies have focused on redesigning the superabsorbents with high swelling performance via organic structure addition, interpenatrating network formation and hydrophilic reactive hydrogel formation as well as protein or enzyme addition. Among them, enzymes in polymer structure are most extensively studied due to their inherent good absorbance and high physical resistance properties. Lipases are pervasive enzymes and have been found in most organisms from microbial, plant, and animal metabolism and they are a subclass of esterases which can hydrolyze esters to glycerols and fatty acids at a water–oil interface. Lipases perform essential roles in the digestion, transport and processing of trigliserides, fats and oils in living organisms. They also find therapeutic applications against digestive disorders and diseases of the pancreas, as detergent additives for removal of fat stains and as catalysts for the manufacture of specialty chemicals and for organic synthesis [7]. Swelling is a continuous process of transition from initial and the final state of the hydrogel in the swelling region. It is well known that solvent sorption operation into the hydrogel structure do not integrate to the classical theory of diffusion[7]. Penetrant sorption by hydrogels can be characterized by Fickian transport with a concentration gradient and diffusion coefficient. In one dimension, Fick's second law may used to achieve the time-dependent concentration profile of a diffusing species in a gel when the concentration of the diffusion species, c, and the diffusion coefficient D are known.

$$dc/dt = -D(d^2c/dx^2)$$

According to Bajpai classification, two essential groups can be investigated for hydroles swelling processes [8], the first is Fickian transport phenomena, which appears below the equilibrium swelling time and in this category, the polymer chains of the hydrogel have high mobility and water enters easily into the hydrogel network. The second is non-Fickian diffusion, which appears above the equilibrium swelling time and during non-Fickian mass transfer phenomena, the polymer chains are not appropriately mobile to allowance the penetration of water into the polymer [9,10,11]. Various mathematical models have been recommended characterizing the kinetics of hydrogel swelling processes. The Fickian diffusion models, which apply Fick's laws to the penetration of the solvent in hydrogel structure, envision the fractional approach to equilibrium increases linearly with the square root of time up to roughly 0.4 and that the swelling curve is not sigmoidal. The collective diffusion models, established by Tanaka et al., treat the swelling of a gel as the expansion of a network driven by a gradient of stress [12,13,14].

In the present study, unlike conventional additives or comonomer systems, incorporation of the lipase enzyme to the PAAm hydrogel was thought to be an original and alternative method to get superabsorbent hydrogels. For this purpose, PAAm (polyacrylamide)-lipase hydrogels of various compositions were synthesized by using an in situ free radical polymerization technique in the presence of lipase and BAAm (N,N-methylene bisacrylamide) as crosslinker. The lipase enzyme immobilized in the hydrogel system was expected to dissolve in water during the swelling test, thereby leaving big pores behind. It was thought this may lead to highly swollen structures due to an increased hydrophilic character with the enzyme addition, and probable further accommodation of water in those pores while the enzyme dissolves. The water absorbency, thermal stability, enzyme activity, morphology of the resultant lipase-immobilized hydrogels and the swelling kinetics parameter of the system are all discussed here. Special attention is paid to the effect of both lipase enzyme and its loading degree on the above mentioned properties.

## II. EXPERIMENTAL

### 2.1. Materials

Acrylamide (99.0% AAm), ammonium persulfate (98.0% APS) and sodium metabisulfite (98.0% SMBS) were purchased from Merck (Darmstad, Germany) and N, N'-methylenebisacrylamide (99.0% BAAm, Sigma Aldrich) and p-nitrophenyl palmitate (pNPP, Sigma, USA) were used without further purification. $NaH_2PO_4$ and $Na_2HPO_4$ were obtained from Sigma Aldrich Chemicals. Lipase from porcine pancrease (activity: 16.5 $Umg^{-1}$; molecular mass: 56 kDa; source: Porcine pancrease; form: lyophilized from saline and calcium chloride, pH 7.0, powder) was purchased from AppliChem, Turkey and used without further purification.

### 2.2. Synthesis of Neat PAAm Hydrogels

Entire free radical polymerization of the AAm monomer was carried out in the presence of N, N' methylenebisacrylamide as the crosslinker (2.0 w/w %, with respect to monomer weight) and ammonium persulphate/sodium methabisulphite ($(NH_4)_2S_2O_8/Na_2S_2O_5$) as the initiators (1.214 w/w%, with respect to total monomer weight) based on the monomer). 0.244 g of AAm monomer, crosslinker and initiators were dissolved in 5.0 mL distilled water and poured into glass test tubes. Polymerization was held in a water bath at 37°C for 24 hours to complete the crosslinking reaction. Resultant hydrogels were removed from the tubes and cut into discs. Disc shaped hydrogels were purified by extraction with excess water in order to remove any possible residual monomers and crosslinking agents for a day and then further dried in the freeze dryer.

### 2.3. Synthesis of Lipase-Immobilized and Removed Hydrogels (LIRHs)

Appropriate amounts of monomer (AAm) and crosslinker (BAAm) were dissolved in 5.0 mL distilled water and poured into glass test tubes and then various amounts of lipase (0.0-10.0 % by weight of monomer) were added. After mixing of solution for 30 minutes, initiators were added to this mixture. Polymerization was held in a water bath at 37°C for 24 hours to complete the crosslinking reaction. At the end of the period, the hydrogels were removed from the tubes and cut into discs. Resultant hydrogels were purified by extraction with excess water in order to remove the lipase enzyme, any possible residual monomers and crosslinking agents, for a day and then further dried in the freeze dryer. Lipase was removed from the dried hydrogels by immersion in deionized water and whole hydrogels were kept in distilled water for 3 days. During the removal of the enzyme from the hydrogel network, the enzyme releasing behavior of the hydrogels was monitored over 3 days (Scheme 1).

### 2.4. Characterization

Swelling behavior of all hydrogels was followed by gravimetric measurements. The freeze-dried hydrogel sample was weighed and immersed in deionized water and taken out at regular intervals of time to measure change in weight. The measurements were conducted at room temperature in a water bath. The percent of swelling was calculated with the following equation:

$$S\ \% = \frac{m_t - m_o}{} \times 100$$

$$m_o$$

where $m_t$ is the mass of the swollen gel at time t and $m_0$ is the mass of the dry gel.

The swelling percentages at the end of 3 days for all the hydrogels are given as an average of three measurements in Fig. 1&2. Thermal characterization of the hydrogels was carried out by thermogravimetric analysis (TGA) on 10-20 mg samples by heating from room temperature to 700°C at 10°C/min under nitrogen atmosphere using a TGA by Perkin Elmer, Diamond DSC. SEM micrographs were obtained after being sputter-coated with gold. SEM analyses were carried out by using an ESEM-FEG/EDAX Philips XL-30.

### 2.5. Free enzyme activity assay

Lipase activity was determined by using p-nitrophenyl palmitate (pNPP) as substrate [18]. The substrate solution was prepared by freshly mixing the solution. A (30 mg of pNPP in 10.0 mL of isopropanol) with solution B (0.1 g of gum Arabic and 0.4 mL Triton X-100 in 90.0 mL of 50 mM Tris–HCl buffer, pH 8.0) while stirring until all was dissolved. The mixture of 9.0 mL of substrate solution and 1.0 mL of enzyme solution was incubated at 40±0.1°C for 15 min and absorbance was measured at λ = 410 nm. Each reported value was the mean of three experiments at least, and the standard deviation was within ca. ±5%. Also all results are reproducible.

### 2.6. Immobilized enzyme activity assay

Instead of the enzyme solution, 100 mg of immobilized lipase was used. The other experimental procedures were similar to those described above [15]. Each reported value was the mean of three experiments at least, and the standard deviation was within ca. ±5%. Also all results are reproducible.

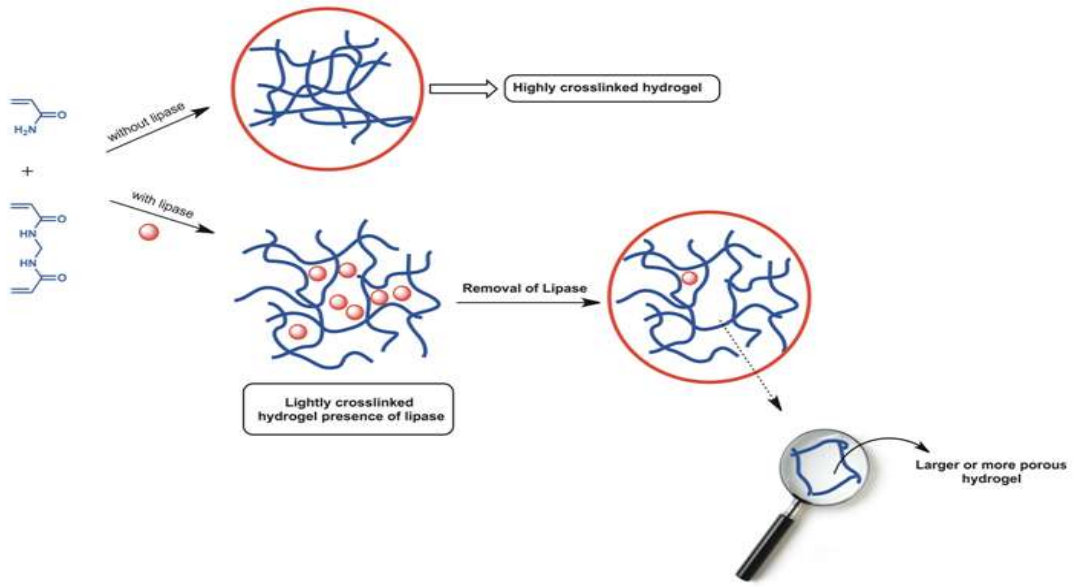
### 2.7. Protein determination

Dissolved protein concentration was determined according to the Lowry method, using bovine serum albumin (BSA) as the standard. The amount of bound protein was determined by the difference between the loaded protein amount and the protein amount present in the supernatants [16,17]. Each reported value was the mean of three experiments at least, and the standard deviation was within ca. ±5%. Also all results are reproducible.

## III. RESULTS AND DISCUSSION

### 3.1. Immobilization Efficiencies of Lipase in PAAm Hydrogel

Neat and LIRHs were prepared by polymerization of the AAm/BAAm monomer/crosslinker solution in aqueous medium in the presence of the APS/SMBS initiator system. Neat hydrogel polymerization reactions were initiated with the APS/SMBS redox initiator system at 37°C in water. Also several amounts of lipase (1.0%-10.0%) were dissolved in AAm/BAAm/APS/SMBS aqueous mixture and lipase trapped hydrogels were obtained at the end of the 24 h. After the crosslinking reaction in aqueous medium for all hydrogels, samples were immersed in deionized water and kept in fresh water for 3 days to remove the lipase from the PAAm based hydrogels (Scheme 1).




**Scheme 1.** General mechanism of Lipase-immobilized and lipase-removed hydrogels (LIRHs).

Table 1 shows that several amounts of lipase enzyme were immobilized by entrapment method into the polymer network and showed that the highest immobilization yield is 78.0% with 3.0% lipase loading (bound enzyme activity 93.6 $Umg^{-1}$ support).

**Table 1.** Immobilization efficiencies of lipase enzyme in PAAm hydrogels

| Lipase loading (g lipase/ g monomer, %) | Lipase activity ($Umg^{-1}$ support) | Bounded lipase activity ($Umg^{-1}$ support) | Immobilization yield (%) |
|---|---|---|---|
| 1.0 | 40 | 28.8 | 72.0 |
| 3.0 | 120 | 93.6 | 78.0 |
| 5.0 | 200 | 149.4 | 74.7 |
| 7.0 | 280 | 229.6 | 82.1 |
| 10.0 | 400 | 168.6 | 42.1 |

These results show that enzyme entrapment methodology was an essential technique for immobilization of a high degree of enzyme into the polymer network. Under these circumstances, a lipase loading of 120.0 $Umg^{-1}$ into the polymer network by entrapment method was achieved and bound enzyme activity of 93.6 $Umg^{-1}$ (78.0% immobilization yield) was obtained.

It can be seen from the immobilization efficiency results of lipase (Table 1), that more than 7.0% lipase loading into the network structure decreases not only the bound enzyme activity but also the immobilization yield of lipase. As can be seen from Table 1, the lipase immobilization efficiency as well as the activity of the bound enzyme was found to be dependent on the enzyme loading ratio. With entrapment of the enzyme into the PAAm hydrogel network with a 10.0% enzyme loading ratio, the binding of the lipase was not very efficient and resulted in an immobilization yield of 42.1%. Through the use of a lower amount of lipase, the bound lipase efficiency was increased almost two-fold.

After reaching swelling equilibrium, lipase activity assays were performed on the swelling solutions to obtain an amount of unbound lipase (Table 2).

**Table 2.** Releasing yield of the lipase from the hydrogel

| Lipase loading (g lipase / g monomer, %) | Lipase activity ($Umg^{-1}$ support) | Bounded lipase activity ($Umg^{-1}$ support) | Lipase activity in swelling solution ($UmL^{-1}$) | Releasing yield (%) |
|---|---|---|---|---|
| 1.0 | 40 | 28.8 | 10.8 | 37.5 |
| 3.0 | 120 | 93.6 | 22.7 | 24.2 |
| 5.0 | 200 | 149.4 | 38.7 | 26.0 |
| 7.0 | 280 | 229.6 | 51.0 | 22.2 |
| 10.0 | 400 | 168.6 | 32.8 | 18.9 |

After immersion of lipase and PAAm hydrogels in water, the release of lipase occurred as shown in Table 2. As can be seen, a very high amount of lipase (10.0%) was released due to the large porosity of the resulting hydrogels. In this case of swelling polymers, the diffusivities/releases of the loaded enzymes were strongly affected by the degree of swelling within the hydrogel matrix.

### 3.2. Swelling Behavior of Lipase Immobilized PAAm Hydrogel

Hydrogel swelling properties are quietly affected by several factors such as the properties of the monomer nature (hydrophilic, hydrophobic or ionic), the conditions of polymerization and the swelling medium. The percentage of swelling is the most important parameter in swelling studies. The swelling behavior of all hydrogels was evaluated using Equation 1. Fig. 1 exhibits the swelling behaviors of pure and enzyme removed hydrogels in deionized water.

According to Fig. 1, 3.0%, 5.0% and 7.0% LIRHs have the highest degree of swelling (~5800%). However, the maximum water swelling value of pure hydrogel was 4600% at the end of 3 days.

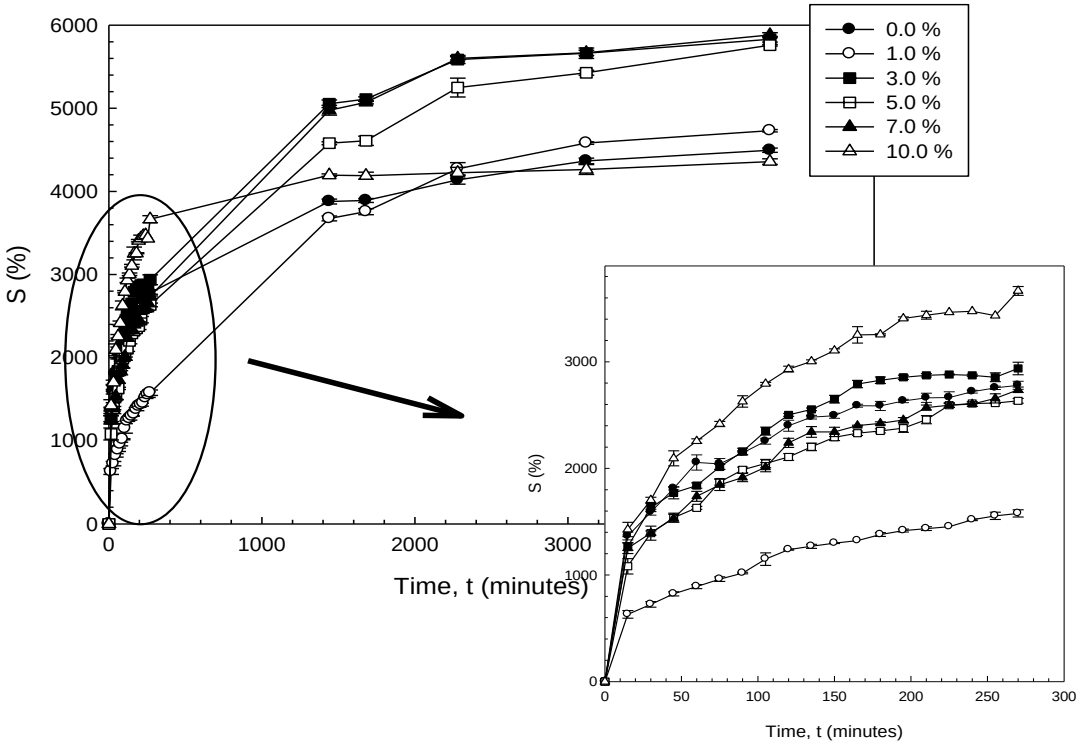



**Fig. 1.** Swelling behavior of lipase LIRHs in deionized water

Also, 10.0% LIRHs had a rapid swelling ratio during the early stage of the swelling experiment, but its water absorption rate decreased dramatically after 16 hours and the hydrogel then showed its lowest absorption rate. This result can be ascribed to insufficient crosslinking reaction with high amounts of lipase leading to more flexible polymer chains and breakage of the secondary interactions, with time. This most probably created more space for water within the matrix of the hydrogel resulting in high swelling in 16 hours. This relatively low amount of crosslinking may cause 10.0% lipase loaded hydrogels to develop weak interactions between the polymer chains, and the hydrogels were found to disintegrate in water after 16 hours.

Fig. 2 obviously shows that the amount of enzyme loading directly affected the maximum swelling degree of the hydrogels, and more than 7.0% LIRHs in the monomer mixture decreased the water absorption character of the corresponding hydrogel.

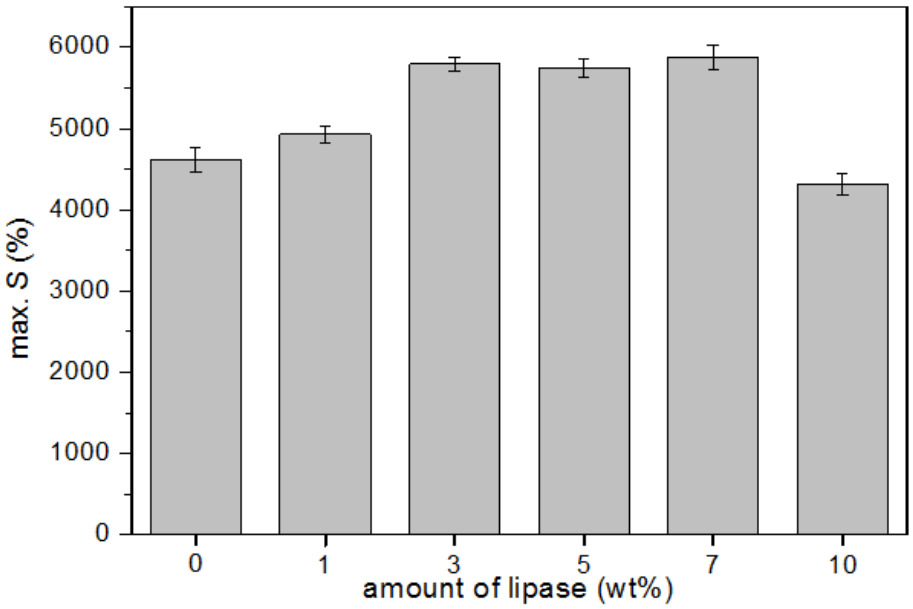



**Fig.2 .** Effect of enzyme loading on the maximum swelling degree of the LIRHs

The inset of Fig. 1 & 2 shows that the proportion of the polymerization reaction directly affected the swelling behavior of the hydrogels after removing the lipase. Entire hydrogels had a rapid swelling rate in the first 4 h; however 10.0% LIRHs showed maximum swelling behavior by the first 16 h. In contrast, 3.0% and 7.0% LIRHs were in maximum swelling behavior at the end of the 3 days due to the lower cross-linked density, compared with neat hydrogel. It was also found that as the swelling time of the LIRHs increased, the swelling ratio of the hydrogel samples increased too. However, while the lipase loaded hydrogels prepared with 3.0% and 7.0% enzyme showed pronounced increases in the swelling ratio in three days, the increase in the swelling ratio of the hydrogels prepared with 1.0% and 10.0% lipase were not so significant as the time increased.

**3.3 Thermal and Morphological Characterization of Lipase Immobilized PAAm Hydrogel**

The TGA curves of neat PAAm and enzyme loading PAAm hydrogels are given in Fig. 3. As can be seen from the TGA trace, 1.0% lipase containing hydrogels degrade at a slightly faster rate in the temperature range of 0–400°C compared with neat PAAm hydrogel and thereafter the situation reverses. For this hydrogel, the weight loss in the above mentioned temperature range most probably resulted from the removal of water and degradation of some weakly attached enzyme. The 1.0% and 7.0% loaded enzymes & PAAm hydrogels display retardation of thermal degradation above 400°C. On the other hand, as is clear from the figure, the char yield of the 1.0% and 7.0% enzyme containing hydrogels was found to be higher than that of the neat PAAm hydrogel.

Higher thermal stability for the enzyme & PAAm hydrogels might be attributed to extensive interaction between the enzyme and the PAAm network which resulted from the availability of a larger surface area of the enzyme for the hydrogel matrix as well as formation of sub-networks. This contribution of the enzyme to the PAAm network system can lead to restricted molecular mobility of the polymer chains, resulting in inhibition of diffusion of the decomposed product in the hydrogel matrix.

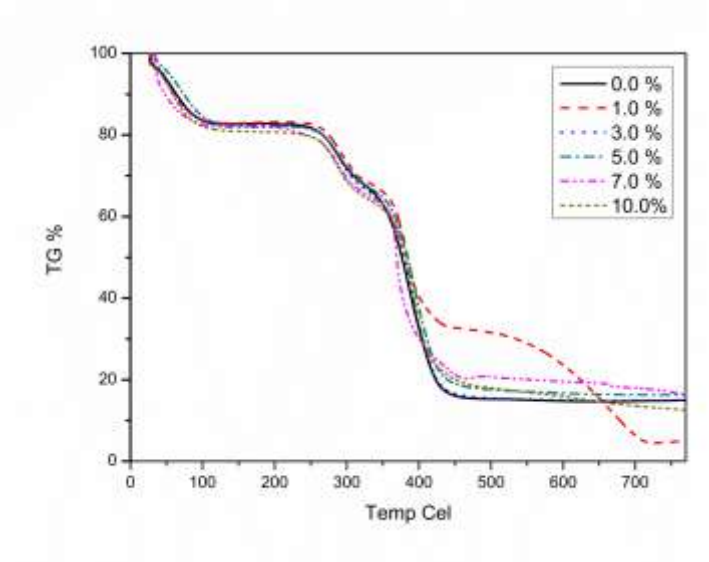



**Fig. 3.** TGA curves of neat hydrogel and LIRHs

Fig. 4 presents low magnification images of neat and enzyme loaded hydrogel samples. It can be seen that 1.0%, 3.0% and 5.0% lipase removed hydrogel samples had almost similar morphologies except for the neat PAAm hydrogel which had a thicker cell wall. On the other hand, 7.0% and 10.0% lipase removed hydrogels have distinctively different morphologies: 7.0% lipase removed hydrogel has pores in a pore structure and the 10.0% lipase removed sample shows no regular porous structure. It is quite clear that the neat PAAm hydrogel (Fig. 4a) exhibited a somewhat porous structure but mostly had closed cells with few ca 200-300 micrometer sized open cells. In the case of 3.0% lipase, loaded and removed from the hydrogel structure, a number of dispersed and enzyme directed open cell structures dominated, with almost-open cells. Moreover, with the enzyme loaded and removed from the hydrogel, the cell size increased from ca 500 micrometers at 3.0% lipase loading ratio. The higher sizes in the open cells for both hydrogels with a 3.0% lipase loaded ratio may result from the formation of sub-networks having extended crosslinked chains by leaving much larger pores behind [18].

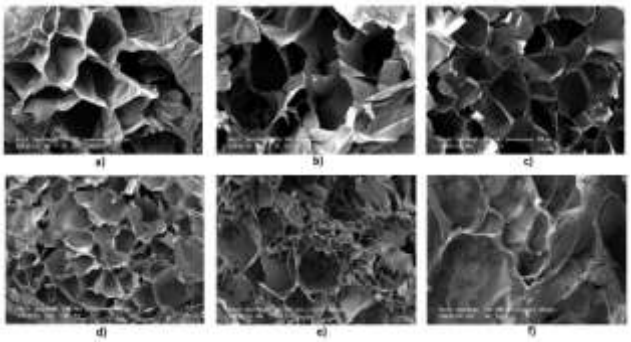


**Fig. 4.** a) SEM images of neat hydrogel, b) 1.0% lipase removed hydrogel, c) 3.0 % lipase removed hydrogel, d) 5.0 % lipase removed hydrogel, e) 7.0% lipase removed hydrogel and f) 10.0% lipase removed hydrogel.

Especially in 7.0% lipase, loaded and removed from the hydrogel structure, this morphology had the narrowest cells and it additionally contained, in one big cell, lots of interconnected small rooms (Fig. 4d&e) which made it more attractive for water accommodation and helped also in sharing the applied compressive forces effectively.

According to the high magnification SEM images, the 7.0% lipase removed hydrogel has a sub-porous structure (Fig. 4e). The 10.0% lipase removed sample does not show a porous structure, but very small pore structures occur in higher magnification of the SEM images (Fig. 4f). On the other hand, as demonstrated in Fig. 5, when focused on the hydrogel surface area of 7.0% and 10.0% loading and removing enzymes, irregular rooms and pores, pores in pores at the rod ends, and spaces in between, were significantly different compared to the neat PAAm hydrogel surface. This indicated a change in the morphological structure of the neat hydrogel as a result of the loading enzyme. After the enzyme loading and removing process for the 3.0% and 7.0% enzymes, there was a significant increase in microfractures. Fractures covering the entire surface and providing a uniform area were believed to be the result of the formation of sub-networks .These results may be attributed to the fact that 3.0% and 7.0% hydrogels have the highest swollen network structures. The 10% enzyme removed hydrogel is the lowest swollen gel sample (Fig. 5).

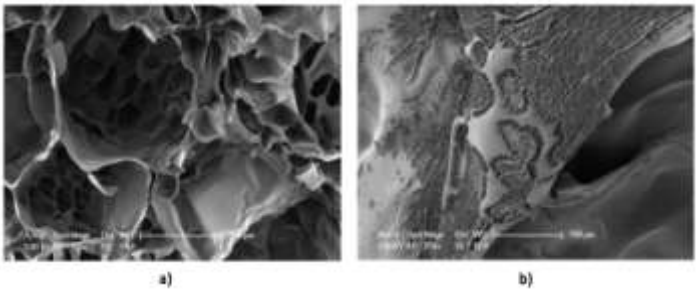


**Fig. 5.** High magnification SEM images of a) 7.0% and b) 10.0% lipase removed hydrogel.

### 3.4. Swelling Kinetics of Lipase Immobilized PAAm Hydrogel

A simple and useful empirical equation, the so-called power law equation, is commonly used to determine the mechanism of diffusion in cylindrical shape polymeric networks:

$$S/Seq = kt^n$$

The constants, k and n, are characteristics of the solvent (deionized water) and cylindrical shape polymer system. The diffusional exponent, n, is dependent on the geometry of the device as well as the physical mechanism of solute uptake. Peppas et al. were the first to give an introduction to the use and limitations of these equations [19]. In the model, S is the degree of swelling at any time t, $S_{eq}$ is the degree of swelling when equilibrium is reached and t is the swelling time. By determining the diffusional exponent, n, one can gain information about the physical mechanism controlling the solvent uptake from the hydrogel network.

For a hydrogel network, n = < 0.5 indicates Fickian diffusion, n > 0.5 indicates non-Fickian transport and n = 1 implies relaxation-controlled transport. The previously discussed power law equation, even though it effectively describes the major portion of the swelling behaviour, fails to give a precise analysis above  S/Seq = 0.60.

From Table 3, the plots of log (S/Seq) vs time (logt) for all lipase & PAAm hydrogels were drawn and it is obvious that the diffusional exponent n value was obtained at n < 0.5 for all lipase-loading ratios.

**Table 3**. Effect of lipase loading ratio on the swelling kinetics parameters of lipase&PAAm hydrogels

| t (min) | $S/S_{eq}$ (0.0% lipase) | $S/S_{eq}$ (1.0% lipase) | $S/S_{eq}$ (3.0% lipase) | $S/S_{eq}$ (5.0% lipase) | $S/S_{eq}$ (7.0% lipase) | $S/S_{eq}$ (10.0% lipase) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60 | 0.4494 | 0.1764 | 0.3213 | 0.2840 | 0.3046 | 0.5168 |
| 120 | 0.5133 | 0.2523 | 0.4320 | 0.3727 | 0.3846 | 0.6792 |
| 180 | 0.5584 | 0.2788 | 0.4841 | 0.4071 | 0.4105 | 0.7540 |
| 240 | 0.5861 | 0.3090 | 0.4970 | 0.4533 | 0.4393 | 0.8043 |
| 270 | 0.5925 | 0.3126 | 0.5117 | 0.4551 | 0.5655 | 0.8517 |
| 1440 | 0.8383 | 0.7388 | 0.8652 | 0.7933 | 0.8434 | 0.9678 |
| 1680 | 0.8395 | 0.7544 | 0.8789 | 0.8017 | 0.8624 | 0.9585 |
| 2280 | 0.8882 | 0.8605 | 0.9737 | 0.8963 | 0.9552 | 0.9609 |
| 3120 | 0.9497 | 0.9273 | 0.9807 | 0.9407 | 0.9600 | 0.9709 |
| 4320 | 1 | 1 | 1 | 1 | 1 | 1 |

For Fickian diffusion, the n values close to 0.5 or over 0.5 have been reported in most published articles, while fewer articles have reported the case of n < 0.5. Fickian diffusion actually refers to the situation where the water penetration rate in the hydrogels is less than the polymer chain relaxation rate. Therefore, n = 0.5 indicates a perfect Fickian process. Nevertheless, when the water penetration rate is much lower than the polymer chain relaxation rate, it is possible to record n values below 0.5 (Fig. 6). [20].

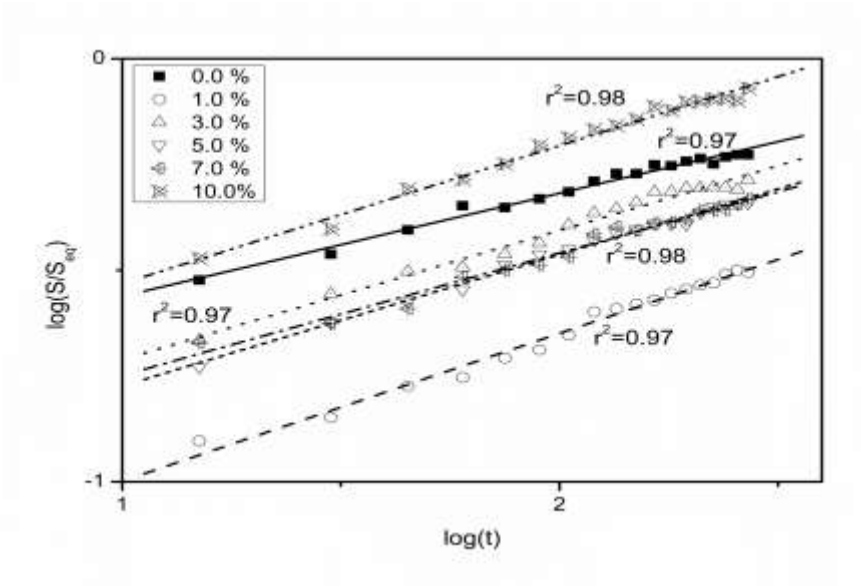



**Fig. 6.** Plot of log ($S/S_{eq}$) vs log (t) for lipase immobilized PAAm hydrogels in distilled water (0-270 minutes and 25°C)

Lipase immobilized PAAm hydrogels have shown a high degree of swelling in distilled water in comparison to neat PAAm hydrogels due to the presence of hydrophilic groups which ionize in this media. Equilibrium swelling data were used for evaluation of the swelling parameters like the swelling exponent (n) and swelling coefficient (k). In all the experiments, the swelling exponent n, was observed to be less than 0.5 indicating the presence of the Fickian diffusion process in these gels (Table 4).

**Table 4.** n and k values of lipase&PAAm hydrogels for 0-270 minutes

| Enzyme loading (g lipase /g monomer, %) | n | k |
|---|---|---|
| 0.0 | 0.2438 | 0.1564 |
| 1.0 | 0.3492 | 0.0449 |
| 3.0 | 0.3056 | 0.0961 |
| 5.0 | 0.3106 | 0.0832 |
| 7.0 | 0.2892 | 0.0916 |
| 10.0 | 0.3261 | 0.1387 |

## IV. CONCLUSION

PAAm hydrogels were prepared in the present study. Porcine pancreatic lipase was used as enzyme structure in different loading degrees and the optimum amount of enzyme loading ratio was determined. The effect of enzyme loading on hydrogel properties was discussed interms of swelling, thermal and morphological properties. Moreover, insertion of enzyme into a gel network of 3.0%, 5.0% and 7.0% loading ratios caused a peak in swelling values up to nearly 5800%. It was also found that LIRHs exhibited larger-sized open cells with interconnected rooms inside, compared to the neat PAAm hydrogel, which made it more attractive for water accommodation as a result Fickian diffusion process. Finally it can be safely concluded that lipase immobilized PAAm hydrogels can be tuned to serve as good super absorbers and thermally stable hydrogels by using functionally. Resulting macroporous hydrogels can be used as a protein, enzyme or drug releasing systems for biotechnological or future medical applications.

## REFERENCES


[1]. S Francis, M. Kumar and L. Varshney, Radiation synthesis of superabsorbent poly(acrylic acid)–carrageenan hydrogels, Radiation Physics and Chemistry, 69, 2004, 481–486.
[2]. AS Hoffman, Hydrogels for biomedical applications, Advanced Drug Delivery Reviews, 54(1), 2002, 3-12.
[3]. Y Qiu and K. Park, Environment-sensitive hydrogels for drug delivery, Advanced Drug Delivery Reviews, 53(3), 2001, 321-339.
[4]. P Akay, D. Omay, Synthesis and characterization of chitosan & amino acid superabsorbent hydrogels, International Polymer Processing, 29(2), 2014, 1-9.
[5]. J Kost and R. Langer, Responsive polymeric delivery systems. Advanced Drug Delivery Reviews, 46(1-3), 2001, 125-148.
[6]. S Jing, C. Xin and S. Zhengzhong, The electric-field-sensitive hydrogels. Progress in Chemistry, 19(9), 2007, 1393-1399.
[7]. WC Lin, D.G. Yu and M.C. Yang, pH-sensitive polyelectrolyte complex gel microspheres composed of chitosan/sodium tripolyphosphate/dextran sulfate: swelling kinetics and drug delivery properties. Colloids and Surfaces B: Biointerfaces. 44(2-3), 2005, 143-151.
[8]. AK Bajpai, S.K. Shukla, S. Bhanu and S. Kankane, Responsive polymers in controlled drug delivery. Progress in Polymer Science, 33(11), 2008, 1088-1118.
[9]. H Li, T.Y. Ng, Y.K. Yew and Y.K. Lam, Modeling and simulation of the swelling behavior of pH-stimulus-responsive hydrogels, Biomacromolecules, 6(1), 2006, 109-120.
[10]. Q Liu, X. Wang and D.D. Kee, Mass transport through swelling membranes. International Journal of Engineering Science, 43, 2005, 1464-1470.
[11]. KR Rajagopal, Diffusion through polymeric solids undergoing large deformations. Journal of Materials Science & Technology, 19, 2003, 1175-1180.
[12]. J Singh and M.E. Weber, Kinetics of one-dimensional gel swelling and collapse for large volume change, Chemical Engineering Science, 51, 1996, 4499-4508.
[13]. Y Li and T. Tanaka, Kinetics of swelling and shrinking of gels, Journal of Chemical Physics, 92(2), 1990, 1365-1371.
[14]. N Karaca N, G. Temel, D.K. Balta, M. Aydin and N. Arsu, Preparation of hydrogels by photopolymerization of acrylates in the presence of type I and one-component type II photoinitiators, Journal of Photochemistry and Photobiology A:Chemistry, 209, 2010, 1-6.
[15]. S Ertugrul, G. Donmez and S. Takac, Isolation of lipase producing Bacillus sp. from olive mill wastewater and improving its enzyme activity, Journal of Hazardous Materials, 149(3), 2007, 720-724.
[16]. OH Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry, 193(1), 1951, 265–275.
[17]. D Omay and Y. Güvenilir, Synthesis and characterization of poly(d,l-lactic acid) via enzymatic ring opening polymerization by using free and immobilized lipase. Biocatalysis and Biotransformations, 31(3), 2013, 132-140.
[18]. N Milasinovic, N. Milosavlijevic, J. Filipovic, Z.K. Jugovic and M.K. Krusic, Efficient immobilization of lipase from Candida rugosa by entrapment into poly(N-isopropylacrylamide-co-itaconic acid) hydrogels under mild conditions. Polymer Bulletin, 69, 2012, 347-361.
[19]. J Siepmann and N.A. Peppas, Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC), Advanced Drug Delivery Reviews, 48(2-3), 2001,139-157.
[20]. HJ Naghash and O. Okay, Formation and structure of polyacrylamide gels. Journal of Applied Polymer Science, 60(7), 1996, 971-979.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