This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Synthesis and Characterization of Biosuperabsorbent Based on Ovalbumin Protein

Sara Majdejabbari[a]; Hamidreza Barghi[a]; Mohammad J. Taherzadeh[a]
[a] School of Engineering, University of Borås, Borås, Sweden

Online publication date: 09 June 2010

To cite this Article Majdejabbari, Sara , Barghi, Hamidreza and Taherzadeh, Mohammad J.(2010) 'Synthesis and Characterization of Biosuperabsorbent Based on Ovalbumin Protein', Journal of Macromolecular Science, Part A, 47: 7, 708 — 715

To link to this Article: DOI: 10.1080/10601325.2010.483399

URL: http://dx.doi.org/10.1080/10601325.2010.483399

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Macromolecular Science, Part A: Pure and Applied Chemistry (2010) **47**, 708–715
Copyright © Taylor & Francis Group, LLC
ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601325.2010.483399

Taylor & Francis
Taylor & Francis Group

Synthesis and Characterization of Biosuperabsorbent Based on Ovalbumin Protein

SARA MAJDEJABBARI, HAMIDREZA BARGHI* and MOHAMMAD J. TAHERZADEH

School of Engineering, University of Borås, Borås, Sweden

Received October 2009, Accepted January 2010

A biosuperabsorbent (Bio-SAP) hydrogel from ovalbumin (egg protein) was synthesized via modification with an acylating reagent and a bifunctional crosslinker, and its swelling behavior was investigated. The protein was acylated using ethylenediaminetetraacetic dianhydride (EDTAD), and then crosslinked by glutaraldehyde and dried. Bio-SAP provided through this method includes modification of lysyl residues in the unfolded protein by adding one or more hydrophilic carboxyl groups to increase the hydrophilicity of protein. The water binding capacity was measured in deionized water, 0.9% NaCl solution and synthetic urine, which under the best conditions were 296, 64 and 56 g/g after 24 h, respectively. In addition, the effects of EDTAD/protein ratio on the chemical modification of the protein, the various chemical neutralization agents, pH sensitivity and ionic strength, as well as temperature and particle size on the water absorption capacity with and without load and its kinetic were also investigated.

Keywords: Biosuperabsorbent, ovalbumin protein, chemical modification, crosslinking, swelling

Downloaded At: 08:58 24 January 2011

1 Introduction

Superabsorbent polymer (SAP) hydrogels are crosslinked hydrophilic polymers which can absorb and retain a large amount of water or biological fluids in their polymeric structures and shrink after de-swelling (1). These materials have several applications in different branches such as cosmetic and toiletry formulations (1), bio-matrix for drug delivery systems (2), immobilization of enzyme (3), contact lens materials (4), sensors (1), water retention in agricultural and horticultural soil (1), flocculation agents (1), and liquid radioactive wastes treatment (5). However, hygienic products (1) such as diapers and feminine sanitary napkins might be the dominant market for the superabsorbents.

Polyacrylates, which are oil-based polymers, are the backbones of most of the superabsorbents on the market, although polyvinylalcohol, polymethylmetacrylates, polyvinylpyrrolidone, polyacrylamide and poly N-isopropylacrylamide are also used. However, there have been efforts in recent decades to develop biodegradable SAPs as well as replacing the oil-based materials with renewable resources. Proteins, chitosan, polylactic acid (PLA), polyglutamic acid, hyaluronic acid and some cellulose derivatives have been developed for this purpose. The current work has been dedicated to developing a biological superabsorbent or biosuperabsorbent (Bio-SAP) from proteins. Proteins are the major components of all the animal bodies as well as the microorganisms. In the previous studies, soy and fish proteins were chemically modified using ethylenediaminetetraacetic dianhydride (EDTAD) followed by crosslinking agent, and resulted in a hydrogel with high water-binding characteristic (6, 7).

Ovalbumin or egg albumin, which is a commercial source of protein, was used in this work. This protein is one of the important animal protein sources, with a high amount of lysine residue which makes it suitable to extend the modification by ethylenediaminetetraacetic dianhydride. To our knowledge, there is no previous publication on producing superabsorbents from this protein.

The objectives of the present work consists of synthesis of a novel protein-based Bio-SAP from pure ovalbumin protein and optimizing the amounts of EDTAD and glutaraldehyde to improve the rate of swelling and swelling capacity of the resultant biosuperabsorbent in deionized water, normal saline and synthetic urine. Furthermore, comparison of the effects of swelling properties at the various conditions, and synthesizing of different salts of polyanionic Bio-SAP, were in focus in this work.

*Address correspondence to: Hamidreza Barghi, School of Engineering, University of Borås, SE-50190 Borås, Sweden. Tel: +46-33-435 4674; Fax: +46-33-435 4008; E-mail: hamidreza.barghi@hb.se

2 Experimental

2.1 Modification of Ovalbumin Protein

Ovalbumin protein solution (1%) was prepared in deionized water; its pH was adjusted to 12.0 by the addition of 2M NaOH and heated for 60 min at 60°C to dissolve the protein while continuously stirred. The solution was then cooled to room temperature and modified by the addition of 0.05–0.35 g EDTAD per gram of protein, while the pH of the protein solution during the reaction was kept at 12.0 by adding 1M NaOH. After a 3 h reaction period, the pH of the protein solution was adjusted to 7.0 by the addition of 1M HCl. This solution was then thoroughly dialyzed against deionized water overnight using dialysis membranes (Mw 6000–8000, Fisher Scientific Co., PA, USA).

2.2 Determination of the Extent of Modification

The degree of modification of the protein is defined as the percentage of the total available amine groups. The extent of modification includes the total modified lysyl residue. The unmodified lysyl content and acylated ovalbumin protein can be determined by 2,4,6-trinitrobenzenesulfunic acid (TNBS) (8). In this method, one ml of 4% sodium bicarbonate ($NaHCO_3$) solution was added to 0.8 mL of the solution including less than 5 mg modified protein. Then, 0.2 mL of a 12.5 g/L water solution of TNBS was added and kept at 40°C for 2 h, followed by the addition of 3.5 ml HCl (35%). This solution was then incubated at 110°C for 3 h, cooled down at 25°C, and diluted with water up to 10 mL. The solution was then extracted by pure diethyl ether two times. The residue of diethyl ether in the solution was then evaporated at 40°C in water bath for 15 min. The resultant solution which contained ¥ -TNP lysine was measured by a UV-spectrophotometer at 415 nm. The quantity of reacted lysyl residues in the acylated and unacylated of ovalbumin protein by EDTAD was determined on the lysine standard curve (8).

Downloaded At: 08:58 24 January 2011

2.3 Preparation of Hydrogel

The pH of acylated protein aqueous solution 10% w/w was adjusted at 9.0. Then, 10 ml of this solution was added to 150 μL of 0.01–0.08 M of glutaraldehyde, and cured overnight at room temperature. It was then dried in an oven at 40°C before milling to 0.25–0.42 mm (40–60 mesh). The procedure is summarized in Figure 2.

2.4 Determination of Water Uptake

The samples of 30 mg of the dried Bio-SAP were placed in nonwoven heat-sealable pouches (similar to tea bags). The sealed pouches were then immersed in 200 mL deionized water, various $NaCl_{(aq)}$ concentrations or synthetic urine including 970 mL deionized water, 19.4 g urea, 8 g NaCl, 0.6 g $CaCl_2$ anhydrous, 2.05 g $MgSO_4.7H_2O$ (9) for 24 h at room temperature (25 ± 2°C), followed by centrifugation at 214 × g in order to drain excess water from the swollen gel (Vivaspin 20-ml centrifugal concentrators from Sartorius, Germany). The samples were then weighed instantly and the wet weights of swollen gel were determined. The wet pouches with swollen gels were then dried in oven at 104°C until constant weights were obtained. The maximum water uptake of gel was measured by the difference between the swollen and dried weights divided by the weight of dried gel according to the equation (1):

$$\text{Water uptake (g/g)} = \frac{W - W_0}{W_0} \quad (1)$$

Where W is the weight of swollen gel and W_0 is the weigh of dried matrix.

When the swelling kinetic was desired, this procedure was repeated, except the immersing time which was between 5 min and 24 h.

2.5 Swelling at Different pH Values

In order to measure the water uptake capacity of the Bio-SAP at different pH from 3.0 to 12.0, buffer solutions were prepared and a procedure similar to that mentioned before it was repeated. The buffers solutions were provided using 50 ml of 0.1 M potassium hydrogen phthalate for pH 3-5, 0.1 M potassium dihydrogen phosphate for pH 6-8, 0.1 M tris(hydroxymethyl)aminomethane for pH 9, 0.025 M borax for pH 10, 0.05 M sodium bicarbonate for pH 11, and 0.1 M disodium hydrogen phosphate for pH 12, in various amounts of HCl or NaOH to obtain the desired pH (10).

2.6 Measurement of Swelling Capacity Under Load

Two experimental techniques have been applied to measure the uptake capacity of the Bio-SAP under load: the centrifuge retention capacity (CRC) and absorbency under load (AUL).

In order to determine CRC, 0.03 g of the dried Bio-SAPs were placed in non-woven pouches (6 × 6 cm), and exposed to deionized water or 0.9% $NaCl_{(aq)}$ for 30 min at 37°C and allowed to dip into liquids. The swollen pouches were removed from the water and saline solution and placed on a sieve 0.15 mm (100 mesh) in order to remove the excess water for 5 min and then centrifuged in the centrifuge tube (Viva spin 20, Sartorius) at 214 × g for 5 min. The retention capacity of the Bio-SAP was calculated by measuring the absorbed water per initial dried Bio-SAP used.

The AUL was measured by using a piston to apply additional weights on top of the Bio-SAP sample. The test system consisted of a stainless steel sieve 0.08 mm (200 mesh) located on a Petri dish (Fig. 1), and 0.9% NaCl was added to the top of the filter plate. A filter paper was located on the filter plate, and the weighed and dried Bio-SAP

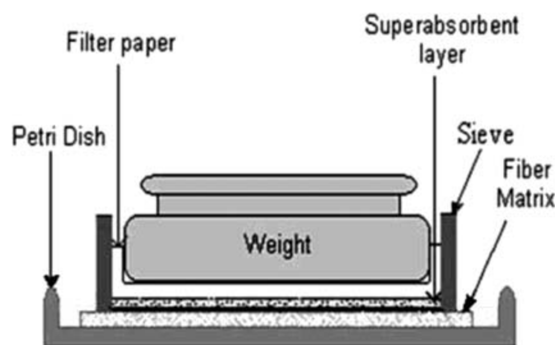

Fig. 1. Scheme of the device used for measuring absorbency under load (AUL).

(ca 0.5 g) was placed in contact with a saline solution onto to the filter screen of the test device (a cylinder 60 mm in diameter and 50 mm in height). A standard weight to attain a load of 2.07 kPa or 4.14 kPa was placed on top of the Bio-SAP sample. After 60 min, the swollen hydrogel sample was weighed, and absorbency under load was calculated by using Equation 1.

2.7 Infrared Analysis (FT- IR)

The Fourier Transform Infrared spectroscopy (Impact 410, Nicolet Instrument Inc, WI) was used to confirm the various functional groups and chemical structures of unmodified, EDTAD-modified ovalbumin protein, as well as ovalbumin protein-based superabsorbent hydrogel. The dried samples' powder was examined by infrared light ranging between 600 and 4000 cm^{-1}, and the absorption band in atmospheric conditions was determined.

3 Results and Discussion

The lysyl residues of ovalbumin proteins were chemically modified using EDTAD, crosslinked by glutaraldehyde, and dried to Bio-SAP (Fig. 2). EDTAD is a bifunctional reagent which is able to acylate polypeptides either inter- or intramolecularly. Theoretically, one molecule of EDTAD reacts with one lysyl residue and one water molecule (Fig. 3). In this reaction, three carboxyl groups can be incorporated, for each lysyl residue modified, into the protein molecule (11). This completely improves the net anionic charge of the protein with various sites for water binding, which results in unfolding the protein structure. Crosslinking of EDTAD-modified protein is rather performed in aqueous solution in order to develop both intra- and intermolecular bonding for immobilizing the modified protein in the aqueous solutions. The effect of several factors in this process was investigated, and is discussed here in detail.

3.1 Extent of Chemical Modification

Considering the molecular weight and lysyl groups of ovalbumin and the molecular weight of EDTAD, and the theoretical stoichiometry of one molecule EDTAD per one lysyl residue, we calculated 0.24 g/g EDTAD/protein. However, this ratio was experimentally investigated and the result is presented in Figure 4. The figure shows that the extent of modification increases when increasing the EDTAD to protein ratio. Increasing this ration up to 0.2 g/g resulted in increasing the modified lysyl residues to more than 90%. No further improvement was achieved by higher amount of EDTAD.

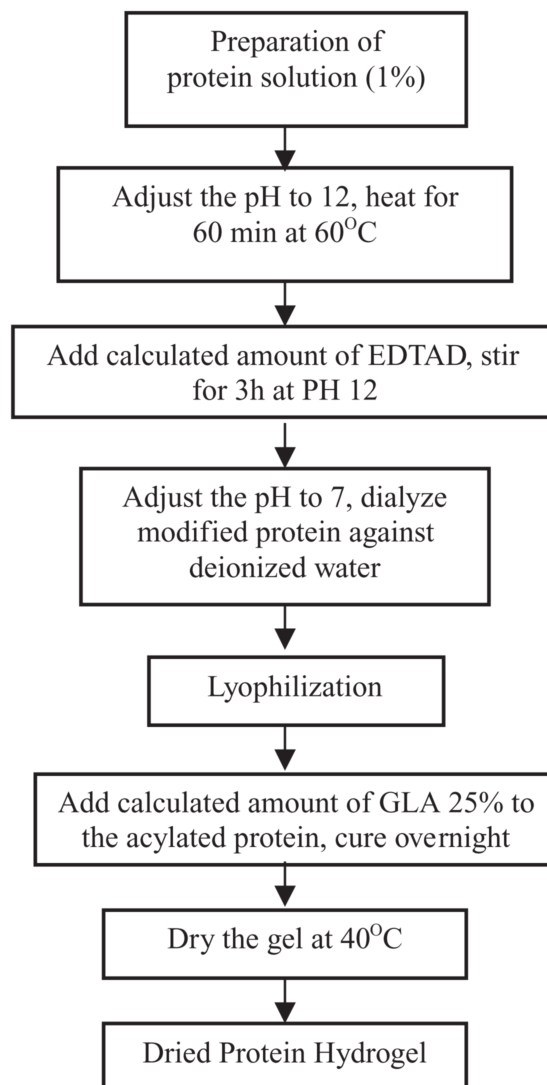

Fig. 2. Process flow chart for preparation of Bio-SAP from ovalbumin.

3.2 Effect of Glutaraldehyde

The effect of glutaraldehyde concentration as a crosslinker on the swelling behavior of the OVA protein hydrogel is shown in Figure 5. In this experiment, the concentration of glutaraldehyde increased from 0.01 to 0.08 M, while preserving the acylated ovalbumin protein concentration at

Downloaded At: 08:58 24 January 2011

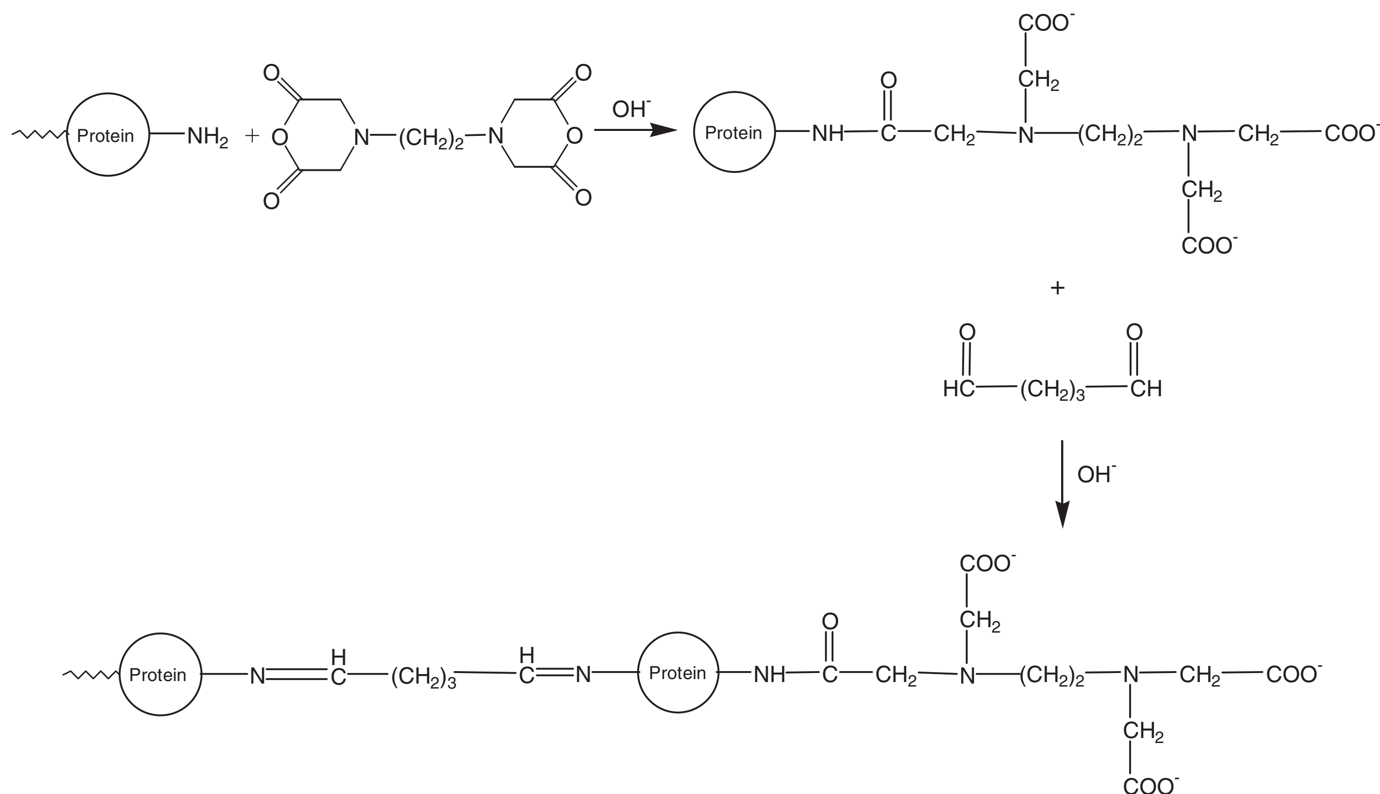

Fig. 3. Schematic reactions of protein with EDTAD and glutaraldehyde.

Downloaded At: 08:58 24 January 2011

1% w/w. The swelling capacity of the Bio-SAP against deionized water was achieved the best at 0.04 M glutaraldehyde concentration. The higher concentration of the crosslinker probably results in locating the extra glutaraldehyde between the peptide chains, which makes it less flexible with poor elastic structure so that it cannot enlarge and retain a large quantity of water. It should also be noticed that the color of the solution was changed from white to brown when increasing the concentration of glutaraldehyde.

3.3 Effect of Buffer Solution

The change of the equilibrium swelling degree of the Bio-SAP in different pH of the buffer solution is illustrated in Figure 6. It should be noticed that the presence of buffering chemicals and ions resulted in generally lower swelling capacity of the Bio-SAP compared to the previous results in deionized water (cf. Figs. 6 vs. 7). In the range of pH 3.0 to 6.0, the equilibrium swelling capacity was low (45.7 ± 3.2 g/g), which was slightly increased at higher pH. However,

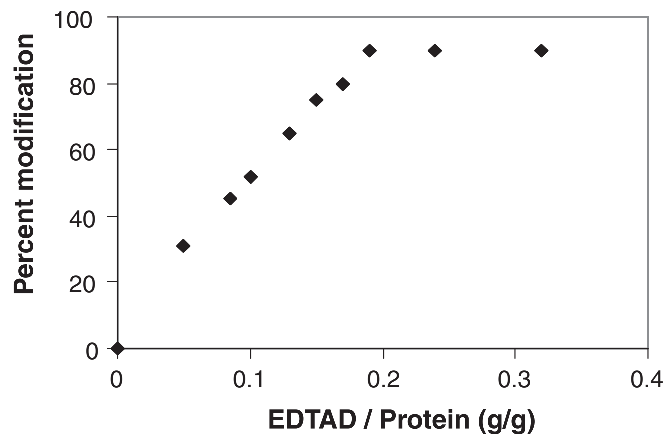

Fig. 4. The effects of EDTAD per ovalbumin ratio on the modification of lysyl residues.

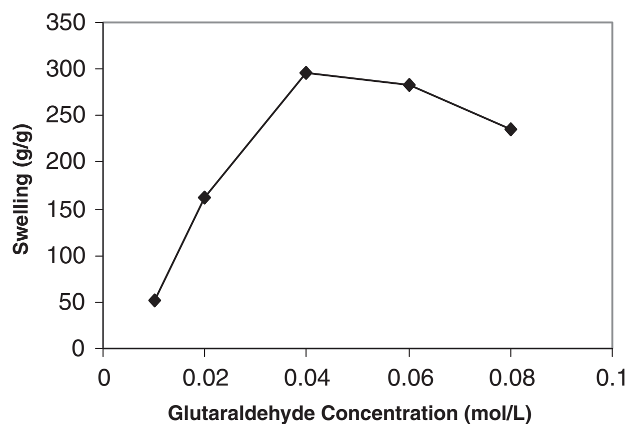

Fig. 5. Effect of glutaraldehyde concentration on the swelling capacity of the Bio-SAP.

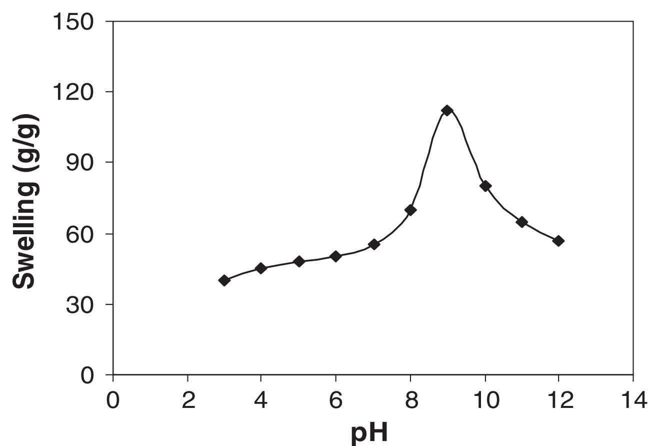

Fig. 6. Effect of pH (in buffer solutions) on swelling capacity of the Bio-SAP.

this capacity increased dramatically at higher pH and was at its maximum capacity of 115 g/g at pH 9.0 (Fig. 6). The increase in water uptake at pH 9.0 might be due to no net electrical charge at the surface of the Bio-SAP, so it should be the isoelectric point (pI) of ovalbumin Bio-SAP (12). It should be mentioned that increasing swelling capacity of hydrogel is not directly related to the binding of water molecules to ionized carboxyl groups, but related to untangling of the protein chain due to electrostatic repulsive force inside the Bio-SAP network.

3.4 Kinetics of Swelling

The swelling kinetic of the Bio-SAP in deionized water as a function of time is shown in Figure 7. For this purpose, the swelling capacity of the Bio-SAP was measured after different times of exposure to water up to 24 h. The capacity increased with longer time and was at most 296 g water/g dry gel after 24 h at room temperature. The maximum

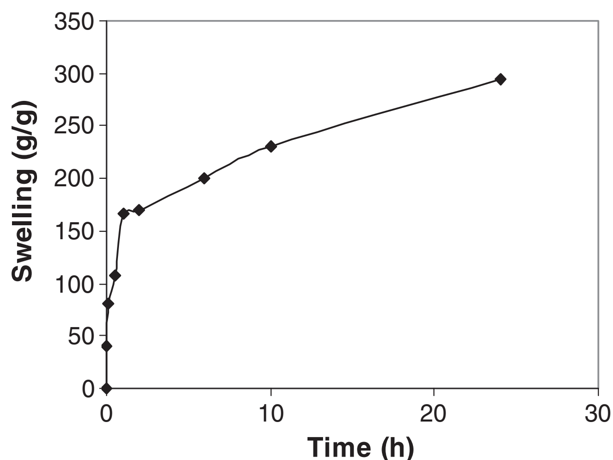

Fig. 7. Swelling kinetics of the Bio-SAP at 25°C.

Downloaded At: 08:58 24 January 2011

Table 1. Comparison of swelling rates of the Bio-SAP in deionized water, normal saline and synthetic urine (values represent mean ± standard error)

Superabsorbent	*Exposing time*		
	1 min	*1 h*	*24 h*
	Absorption in deionized water (g/g)		
Bio-SAP	51.0 ± 8.3	137.3 ± 29.6	276.3 ± 19.6
SAP L 520	70.1 ± 9.6	266.4 ± 24.2	279.8 ± 10.8
	Absorption in normal Saline (g/g)		
Bio-SAP	29.5 ± 3.9	47.5 ± 4.4	56.7 ± 7.1
SAP L 520	38.3 ± 5.4	63.5 ± 15.1	58.2 ± 3.1
	Absorption in synthetic urine (g/g)		
Bio-SAP	26.6 ± 2.0	39.2 ± 4.8	51.4 ± 4.9
SAP L 520	32.7 ± 3.2	45.6 ± 11.2	30.2 ± 3.6

rate of water uptake was obtained during the first hour; afterward the slope increased very slowly up to 24 h.

We have compared the swelling rate and capacity of the Bio-SAP with commercial polyacrylate-based SAPs, named ALCOGUM L520 (Alco Chemical Co., USA), and the results are presented in Table 1. The swelling rate of the Bio-SAP was comparable with the SAP after 24 h exposure to deionized water, normal saline and synthetic urine. However, the swelling rate of the Bio-SAP was slightly lower than that of the SAP (Table 1). This distinction might be due to the presence of a significant amount of residual folded structures, such as α-helix and β-sheet structure, in the Bio-SAP.

3.5 Effect of Ionic Strength

Reduction of swelling capacity by increasing ion concentration is a general behavior of the polyanionic hydrogels. It was therefore important to consider the swelling behavior of the Bio-SAP in this context by increasing the concentration of $NaCl_{(aq)}$ and charge of cations in the solution. The Bio-SAP showed a sharp decrease in the water uptake from 167 g water per g dry gel in deionized water to 52 g/g when it was exposed to 0.15 M NaCl solution for 1 h at 37°C (Fig. 8). This effect might be due to the different osmotic pressure between the hydrogel network and the external solution. Hence, the increasing ionic strength of the solution leads to reducing the equilibrium swelling ratio. The absorbency of Bio-SAP was also estimated in synthetic urine. According to results in Table 1, the Bio-SAP water uptake decreased from 167 g/g dry gel in deionized water to 44 g/g dry gel when it was exposed to synthetic urine after 1 h at 37°C.

3.6 Chemical Neutralization with various Alkaline Solutions

In this approach, the influence of various alkaline solutions on water uptake of the Bio-SAP was investigated. The

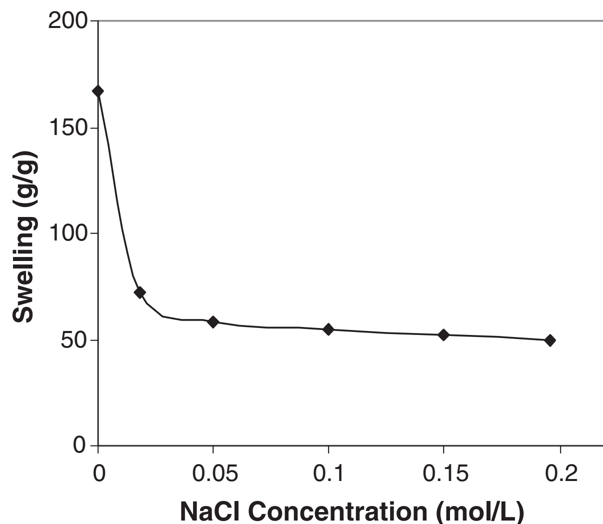

Fig. 8. Effect of NaCl concentration on swelling behavior of the Bio-SAP.

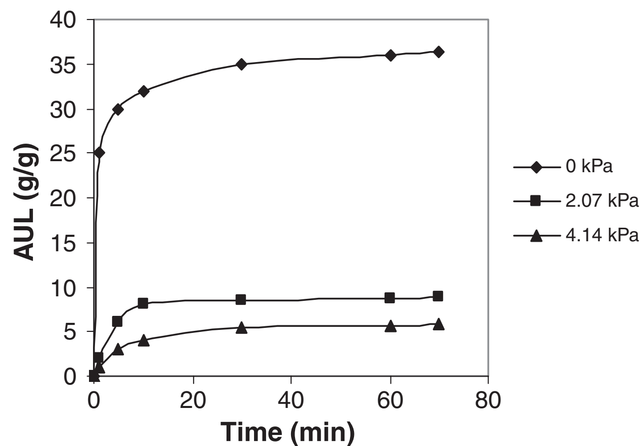

Fig. 9. The absorbency under load (AUL) of the Bio-SAP at different times applied.

Downloaded At: 08:58 24 January 2011

alkaline solutions tested as neutralizing agents of EDTAD-modified ovalbumin before crosslinking (Fig. 2) were diethanolamine (DEA), triethanolamine (TEA), ammonium hydroxide and sodium hydroxide, and the results are summarized in Table 2. The best result obtained for ammonium polyanionic Bio-SAP, was 317 g water per g dry gel after 24 h at 25°C. This swelling capacity is generally related to the characteristics of ammonium salts (NH_4^+) which allow high levels of hydration. In this state, the ammonium cations are surrounded with water due to hydrogen bonds, and result in creating COO^- ions. These hydrated ions move independently in the solution. The solubility of an ionic compound, therefore, depends on the strength of its ionic bonds. It results in lowering the solubility, when the bonds are stronger.

3.7 Absorbency Under Load (AUL)

In general, the rate of swelling under pressure is related to the particle size and particle size distribution, specific surface area and density of the superabsorbent polymer (1). Figure 9 shows absorbency under load (AUL) of the Bio-SAP as the amount of saline uptake under different constant pressures of 2.07 kPa or 4.14 kPa during 70 min, in comparison with no extra load. The results shows drastic reduction of swelling capacity of the Bio-SAP for saline water by increasing the load applied.

Table 2. Effect of the alkali neutralization agent (cf. Figure 2) on the final swelling capacity of the Bio-SAP.

Alkaline neutralizer	*Polyanionic Bio-SAP*	*Equilibrium Swelling ratio (g/g)*
Ammonium hydroxide	Ammonium-salt	306.2 ± 10.6
Sodium hydroxide	Sodium-salt	276.0 ± 20.0
Diethanolamine	DEA-salt	85.6 ± 2.9
Triethanolamine	TEA-salt	40.3 ± 6.2

3.8 Centrifugal Retention Capacity (CRC)

The CRC measurement method is described as the amount of 0.9% saline maintained after the swollen hydrogel has been centrifuged for 5 min at $250 \times g$. The CRC of synthetic SAPs is in the range of 30 to 35 g/g (13). In comparison, the CRC of the Bio-SAP was measured in the range of 18 to 22 g/g at 25 ± 2°C depending on the extent of modification. In fact, the main limiting factor of the saline retention capacity was the amount of carboxyl groups per unit mass of the dry gel.

3.9 Effect of Temperature

The Bio-SAP has a high tendency to absorb water due to hydrophilic groups. This study shows that temperature plays an important role in the swelling capacity of the Bio-SAP at pH 7.0. The temperature dependence of the equilibrium water uptake for Bio-SAP was investigated with temperatures between 15 and 55°C (Fig. 10). Increasing temperature from 15 to 45°C resulted in increasing the swelling capacity from 192 to 310 g/g. However, the Bio-SAP was rapidly shrunk when the temperature increased above a critical temperature of 45°C, which resulted in lower water uptake.

3.10 Effect of Particle Size

The dependence of particle size on the equilibrium swelling ratio of the Bio-SAP was measured by sieve analysis. The results of mesh size from 0.6 to 0.15 mm (25 to 100 mesh) showed that the equilibrium swelling capacity was increased with increasing particle size. This effect might be due to an increase in the volume per unit of the Bio-SAP mass leading

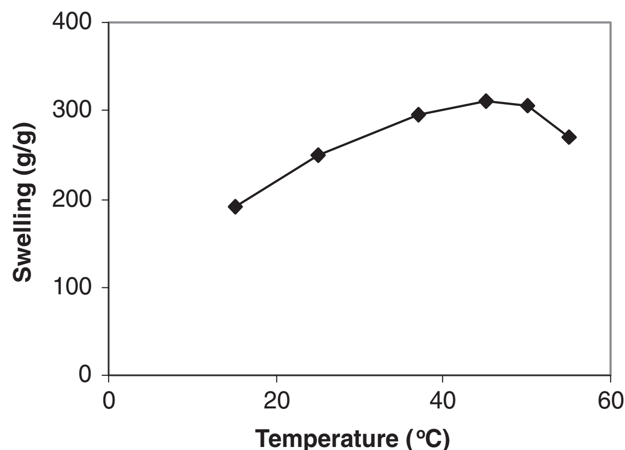

Fig. 10. Effect of temperature on the maximum water uptake of the Bio-SAP.

to rapid water absorption. In this study, the maximum results were obtained by using the average size of 0.5–0.6 mm (25–30 mesh) for determination of swelling behavior of the Bio-SAP.

3.11 FT-IR Spectroscopy Analysis

Figure 11 shows the FT-IR spectra of unmodified ovalbumin protein, EDTAD-modified ovalbumin protein and the Bio-SAP in the frequency region from 700 to 4000 cm^{-1}. In the spectra, the broad band at 3100–3300 cm^{-1} is due to O–H stretching, and an additional peak at 2950–3200 cm^{-1} occurs due to the N–H stretching of amino acids. Amide I ($RCONH_2$) and amide II (RCONHR') bands illustrate two important bands of the protein infrared spectrum, so the stretching band observed at 1640 cm^{-1} can be attributed to amide I and the peak at 1550 $cm^{-1}$is due to amide II in the unmodified ovalbumin protein (Fig. 11(a)). The EDTAD-modified protein carries carboxylate functional groups which are verified by a strong band at 1730 cm^{-1} (Fig. 11(b-c)). Moreover, the medium band detected at 1522 cm^{-1}can be allocated to N–H bending coupled with C–N stretching (14). The Bio-SAP compared with unmodified and EDTAD-modified ovalbumin protein indicates an important peak at 2360 cm^{-1}, which might be due to antisymmetric stretching –NCO (Figure 11(c)), because of the rearrangement mechanism. In this rearrangement, the R– group migrates with its electrons from the acyl carbon to the nitrogen atom in the presence of OH ion. The band at 1450 cm^{-1} represents asymmetric CH_2 bending of end ethyl groups of OVA protein.

4 Conclusions

The results presented in this study show that the protein-based Bio-SAP has outstanding properties as a potential substitute for the synthetic superabsorbent hydrogels. Furthermore, this Bio-SAP might be superior to the current oil-based SAPs, since it is based on renewable materials and also biodegradable. Acylation and crosslinking of the ovalbumin protein resulted in increasing the water binding capacity from its original value of 6 g/g to 317 g/g for deionized water, and to a lesser extent for salt solution and

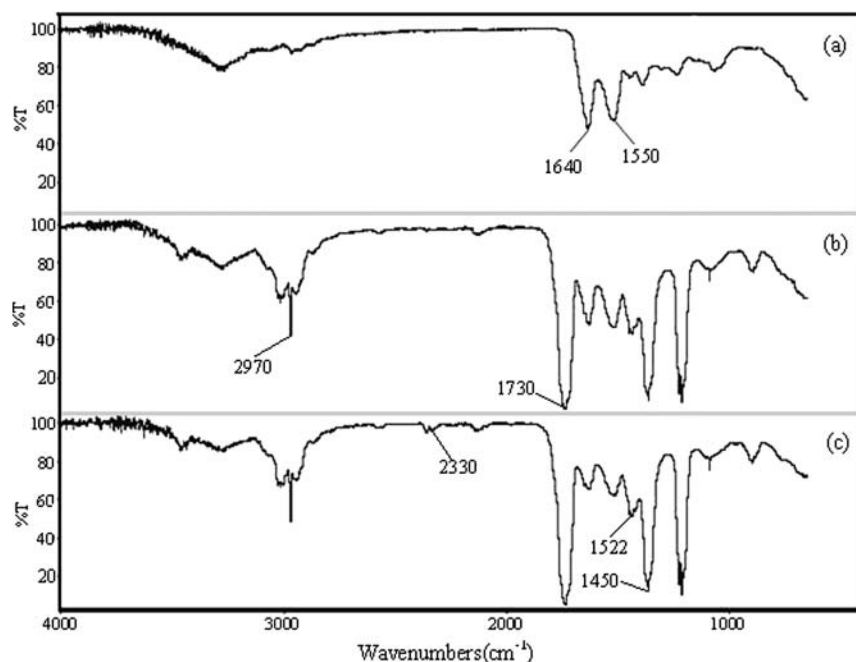

Fig. 11. FT-IR spectra of (a) the unmodified ovalbumin protein, (b) EDTAD-modified ovalbumin protein, and (c) the Bio-SAP.

Downloaded At: 08:58 24 January 2011

synthetic urine. It is in the range of the present commercial SAPs on the market. This biosuperabsorbent may cover many purposes such as immobilized enzymes for chemo-enzymatic reactions, encapsulation of nanoparticles for nano-therapeutics, thermo-responsive drugs, Bio-adhesive and wound dressing, biosensors and artificial cornea.

Acknowledgments

This work was financially supported by the Swedish Knowledge Foundation (KK-Stiftelsen) in collaboration with Medical Equipment Development AB (Sweden) and Rexcell AB (Sweden).

Nomenclature

AUL: Absorbency under load
Bio-SAP: Biosuperabsorbent
CRC: Centrifuge retention capacity
cm^{-1}: Wave number in reciprocal centimeters
DEA: Diethanolamine
EDTAD: Ethylenediaminetetraacetic anhydride
FT-IR: Fourier transform infrared spectroscopy
pI: Isoelectric point
TNBS: 2,4,6-trinitrobenzenesulfonic acid
TEA: Triethanolamine

References

1. Buchholz, F.L. and Graham, A.T., *Modern Superabsorbent Polymer Technology*, (1998) Wiley-VCH: New York, 279.
2. Park, K., Shalaby, W.S.W. and Park, H. *Biodegradable Hydrogels for Drug Delivery*, CRC Press, Pennsylvania, 233, 1993.
3. Shiroya, T., Tamura, N., Yasuri, M., Fujimoto, K. and Kawaguchi, H. (1995) *Coll. and Surf. B: Biointerfaces*, 4(5), 267–274.
4. Yaung, J.F. and Kwei, T.K., (1997) *Appl. Poly. Sci.*, 69(5), 921–930.
5. Karadag, E., Üzum, Ö.B., Kundakci, S. and Saraydin, D. (2007) *J. Appl. Polym. Sci.*, 104(1), 200–204.
6. Hwang, D.C. and Damodaran, S. (1996) *Appl. Poly. Sci.*, 62(8): 1285–1293.
7. Hwang, D.C. and Damodaran, S. (1997) *J. Am. Oil Chem*. Soc., 74.
8. Hall, R.J., Trinder, N. and Givens, D.I. (1973) *Analyst*, 98, 673.
9. Kim, Y.J., Yoon, K.J. and Ko, SW. (2000) *Appl. Poly. Sci.*, 78(10), 1797–1804.
10. Lide, D.R., *Handbook of Chemistry and Physics* 75th ed., CRC Press:Florida, 1913–1995.
11. Hwang, D.C. and Damodaran, S. (1996) *J. Agric. Food Chem.*, 44(3), 751–758.
12. Caillard, R., Remondetto, G.E. and Subirade, M. (2008), *Food Research International,* 42(1), 98–106.
13. Damodaran, S. (2004), *U.S. Pat*., 6821331.
14. Smith, B.C., *Infrared Spectral Interpretation: A Systematic Approach*, CRC Press: Florida, 1998.

Downloaded At: 08:58 24 January 2011