Recycling of Pharmaceutical Waste Gelatin for Controlled-Release Applications. I. A 2,4-Dicholorphenoxy Acetic Acid Based System

Sherif Kandil,[1] El-Refaie Kenawy,[2] Azza El-Maghraby[3]

[1]*Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt*
[2]*Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt*
[3]*Mubarak City for Scientific Research and Technological Applications, Alexandria, Egypt*

Received 18 February 2003; accepted 30 July 2003

ABSTRACT: Gelatin is a natural macromolecular protein. It contains a wide variety of amino acids in its polymer structure, and it is colorless to yellowish, water-soluble, and tasteless. It is used as a dispersing agent, sizing medium, and coating for photographic films and in pharmaceutical formulations. In this study, biodegradable mulching, based on waste gelatin from pharmaceutical gelatin scraps (derived from pharmaceutical soft gelatin capsule production), was formulated via the casting of water solutions or suspensions into flexible and consistent films. Gelatin was blended with synthetic materials such as poly(vinyl alcohol) and other natural wastes such as sugar cane bagasse and sawdust. To all formulations, 2,4-dichlorophenoxy acetic acid (2,4-D) was added as a herbicide. The morphology and mechanical properties of the samples were investigated with scanning electron microscopy and tensile testing, respectively. The results showed that the produced films had controlled-release properties. The effects of various additives and crosslinking on the films and the release of the herbicide 2,4-D from the films were also investigated. The introduction of synthetic and natural additives reduced the release rate of 2,4-D. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 91: 2313–2319, 2004

Key words: controlled release; waste gelatin; mulching films; recycling

INTRODUCTION

Gelatin is a purified protein produced by the hydrolysis of the collagenous tissue (e.g., skin and bones) of animals.[1] Gelatin represents a typical renewable material from natural resources of animal origin. It is used as a dispersing agent, sizing medium, coating for photographic films, and stabilizer for food.[2] Gelatin and gelatin derivatives are also used to encapsulate the products of several industries. This includes the encapsulation of medicinal compounds such as drugs and vitamins.[3] However, gelatin scraps generated from these processes may be of concern for the environment.[4] One solution to this problem is to incorporate the gelatin scraps into agroindustrial processes through the formulation of blends of waste gelatin (WG) with poly(vinyl alcohol) (PVA) and composites with sugar cane bagasse (SCB) and sawdust (S) with the addition of agrochemical substances. Gelatin-based films can display a self-fertilizing character as a result of their ultimate bioassimilation by soil microflora because of the presence of protein nitrogen.[5] Agrochemicals are bioactive agents used to improve the production of crops for a plentiful and high-quality food supply for consumers.[6] The contamination of ground water by agrochemicals has become a serious issue.[7] Polymer-supported agricultural chemicals have emerged recently to overcome the serious environmental problems of conventional agrochemicals.[8] The main objective of this work was determining how to use the huge amounts of WG produced by industry for pharmaceutical applications or cosmetics, tannery, and food. This article reports the synthesis of controlled-release formulations based on gelatin scraps of the pharmaceutical industry. The rate of release of the active agents was investigated. The morphology and mechanical properties of blends and composites based on WG, PVA, SCB, and S were determined with scanning electron microscopy (SEM) and tensile testing, respectively.

Correspondence to: E.-R. Kenawy (ekenawy@yahoo.com).

Journal of Applied Polymer Science, Vol. 91, 2313–2319 (2004)

EXPERIMENTAL

Materials

WG was kindly supplied by Rp Scherer Co. (Alexandria, Egypt). These scraps were used as received and contained several additives from the original formulations, such as pigments and glycerol. The chemical microanalysis of WG gave the contents of carbon (42.43%), nitrogen (14.77%), and hydrogen (6.32%).

PVA was a product of Hoechst, Germany, with an average molecular weight of 67 kDa and a hydrolysis degree of 88%. SCB is an agroindustrial byproduct from the juice extraction of sugar cane, and SCB fibers are a waste product of the sugar industry in Egypt. The fibers were dried in an oven at 50°C for 24 h and then pulverized with a blade grinder. The ground SCB was sieved, and the fraction passing through a 300-μm mesh sieve was collected. Glutaraldehyde (GA), a product of Adwic, Egypt, commercialized as a 50% aqueous solution, was used as a crosslinking agent in various weight proportions without any further purification. S was sieved, and the fraction that passed through a 300-μm mesh sieve was collected and used. 2,4-Dichlorophenoxy acetic acid (2,4-D; 99% purity) was a product of Aldrich (USA) and was used as received. Adipic acid (A), a Cambrian product (Canada), was used as received.

SEM

An SEM investigation was carried out with a JSM-820 scanning electron microscope (JEOL, Ltd., USA). We prepared the film samples by critical point drying and sputtering with gold, and we observed them at 15 kV. We prepared the fractured surfaces under liquid nitrogen by breaking the strips, and we examined the specimens with SEM.

Preparation of the buffer solutions

A buffer solution (pH 7) was prepared as follows. To 100 mL of 0.4*M* boric acid (H_3BO_3) were added 100 mL of 0.4*M* acetic acid and 100 mL of 0.4*M* phosphoric acid (H_3PO_4), followed by the addition of a few drops of a sodium hydroxide solution (10%) to reach the required pH. The mixture was increased to 1 L with distilled water.[9]

High-performance liquid chromatography (HPLC)

The release of the bioactive agent 2,4-D was monitored with a Shimadzu HPLC instrument (Japan) with an SPD-6AV UV–vis spectrophotometer, two LC-6AV pumps, an ODS reversed-phase column, and a CTO-6A column oven. The injection parameters were a temperature of 25°C, an injection volume of 10–100 μL, two mobile phases (A, 5-mm KH_2PO_4 and 0.001% CH_3COOH; B, 50% acetonitrile, 50% methanol, and 0.001% CH_3COOH), a gradient (15% B to 50% B at 16 min to 55% B at 20 min to 15% B at 21 min), and a flow rate of 1 mL/min. A Hypersil ODS 5-μm column was used.

Mechanical testing

The break load, maximum load, maximum strain, and break strain for selected films were measured with a Zwicki modular testing system (Germany) for loads up to 2.5 kN. The specimens were cut and then kept in air at 25°C for 10 days before testing. The specimens were prepared according to ASTM D 882-75b. The experiments were run out, and at least five specimens for each sample were tested; then, the average value was recorded.

Release study

The release characteristics of the 2,4-D-containing films were investigated for each formulation with preweighed film samples (500 mg) in flasks containing a 100-mL buffer solution. At regular intervals, the amount of released 2,4-D was monitored by HPLC. After the first few days, the buffer was withdrawn from the flask and was replaced with fresh buffer after each analysis. The release studies were carried out in a neutral medium (pH 7).[10]

Sample preparation

WG (10% dry weight) was suspended in H_2O at 50°C with stirring for 30 min, and 10% of the dry weight of WG of 2,4-D was added. For the preparation of the blends, 20, 35, and 50 wt % of a PVA/water 10% solution were introduced into suspensions, and the resulting mixtures were stirred at 70°C for 20 min. For crosslinked samples, the desired amount of a GA/water or A solution was introduced into a WG/water suspension, and the mixture was stirred for 3 min at room temperature. SCB composites were prepared by the addition of the desired amount of SCB to a WG/water suspension and the bioactive material, and then the mixture was stirred for 5 min at room temperature. The same step was used for the preparation of S composites. Films were obtained by the casting of the water suspensions in Teflon-coated aluminum trays followed by water evaporation at room temperature. The compositions of the prepared blends and composites are reported in Table I.

RESULTS AND DISCUSSION

The gelatin scraps generated in industry represent an environmental problem connected to their disposal. Because gelatin is biodegradable, if it is disposed like other waste, it may create bacterial disasters. Also, if it is dumped into sewage drainage systems, it can shut down the system because of its strong swelling in water, and this consequently requires intensive labor and expensive treatment for corrective management actions. Other proposed solutions are using acids such as mineral acid to degrade gelatin, but this is not an environmentally acceptable solution. In addition to these reasons, it would be a waste of resources to dispose of gelatin without converting it into a useful

TABLE I
Composition (wt %) of the Blends and Composites Based on WG and 2,4-D (D)

Sample	WG	PVA	GA	SCB	S	A
WGD	90	0	0	0	0	0
WGDA	85	0	0	0	0	5
WGDG2	88	0	2	0	0	0
WGDP20	70	20	0	0	0	0
WGDP35	55	35	0	0	0	0
WGDP50	40	50	0	0	0	0
WGDG2P20	68	20	2	0	0	0
WGDG5P20	65	20	5	0	0	0
WGDB10	80	0	0	10	0	0
WGDB20	70	0	0	20	0	0
WGDG2B20	68	0	2	20	0	0
WGDG5B20	65	0	5	20	0	0
WGDS10	80	0	0	0	10	0
WGDS20	70	0	0	0	20	0
WGDS50	40	0	0	0	50	0
WGDG2S10	78	0	2	0	10	0
WGDG5S10	75	0	5	0	10	0

All of the films contained 10 wt % 2,4-D.

material for applications other than its primary applications. One suggested proposal is using gelatin as a mulching film that may be used to replace current polyethylene-based mulching films. Polyethylene mulching films, which are not biodegradable, cause a lot of problems for the next crop. When they are applied, the residues of the polyethylene films interfere with the plant roots of the following crop; therefore, they reduce crop production.

Also, one very interesting feature of WG-based films is their use as a device to control the release of bioactive agents such as the herbicide 2,4-D, which has good biological activity but has many drawbacks because of its excessive use, which consequently causes many environmental problems. Gelatin also has an important feature due to its amino acid content. This enables the crosslinking of gelatin-based blends and composites with low-molecular-weight materials such as GA or A. This crosslinking technique is well documented in the literature. This crosslinking reaction can take place at room temperature.

Because gelatin has a high nitrogen content, its films can enrich the soil with nitrogen as a result of their ultimate bioassimilation by soil microflora.

WG films were cast from solutions containing gelatin and other components by the slow evaporation of water at room temperature and at atmospheric pressure. Smooth films of constant thickness, the size of which depended on the compositions of the films, were obtained. This ranged from 0.3 to 1.0 mm. An elemental analysis of the prepared films is reported in Table II.

SEM

Figure 1 shows SEM photomicrographs of the surface of a characteristic WG/SCB composites film obtained through the solution casting of WG (70%), SCB (20%), and D (10%). The top surfaces of the films were generally smooth [Fig. 1(a)]. However, the bottom layers of the films were less smooth than the top surfaces [Fig. 1(b)].

The composite films obtained from WG/SCB or WG/S revealed coarse surfaces with either SCB or S fibers cemented and covered by gelatin. The freeze-fractured transversal sections of the composite films showed empty tubes in the composite with either SCB or S as the additive. The empty tubes shown in the SEM images of the composite films [Fig. 2(a,b)] revealed that they could be used as spaces for trapping the bioactive ingredient. They could function as another barrier or rate controller for the release of the bioactive agent.

Tensile testing

The tensile data are shown in Table III. The results showed that the tensile strength of the WG films was higher than that of the WG/SCB films. The data revealed that the presence of a crosslinking agent reduced the break load and break strain. The crosslinking of gelatin with GA gave rise to the formation of short aliphatic segments between gelatin chains. When a warm solution of gelatin is cooled, not only is chemical crosslinking present, but physical crosslinking is also present (a recovered collagen triple-helix structure).[11] Consequently, it can be envisaged that the crosslinked

TABLE II
Elemental Analysis of the Prepared Formulations Containing 2,4-D

	Microanalysis		
Formulation code	C (%)	H (%)	N (%)
WGD	42.5	5.3	15.0
WGDA	42.3	5.0	10.2
WGDG2	44.0	6.4	8.6
WGDP20	45.4	5.5	6.5
WGDP35	44.5	6.3	3.0
WGDP50	48.7	6.8	4.6
WGDG2P20	46.3	6.8	6.2
WGDG5P20	46.7	5.8	4.3
WGDB10	41.9	4.9	7.8
WGDB20	42.9	6.9	5.5
WGDG2B20	44.2	7.3	6.8
WGDG5B20	44.5	5.6	4.2
WGDS10	44.6	7.7	8.8
WGDS20	44.6	5.3	6.1
WGDS50	45.1	5.5	7.0
WGDG2S20	45.2	7.1	6.5
WGDG5S20	45.7	7.0	6.0

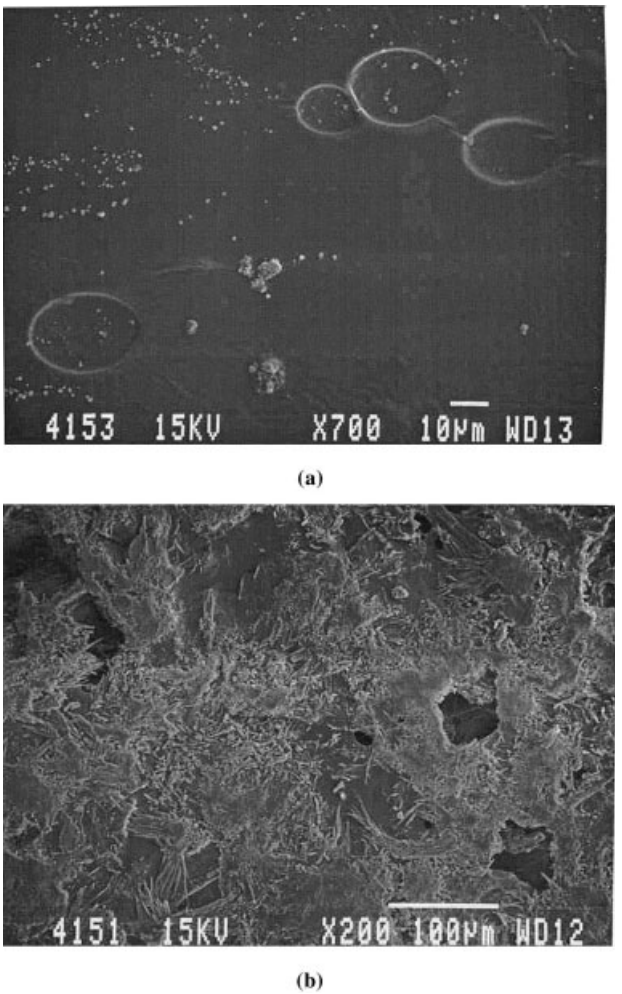

Figure 1 SEM photomicrographs of a WG/SCB/2,4-D composite film containing 20% SCB and 10% 2,4-D: (a) top surface and (b) bottom surface.

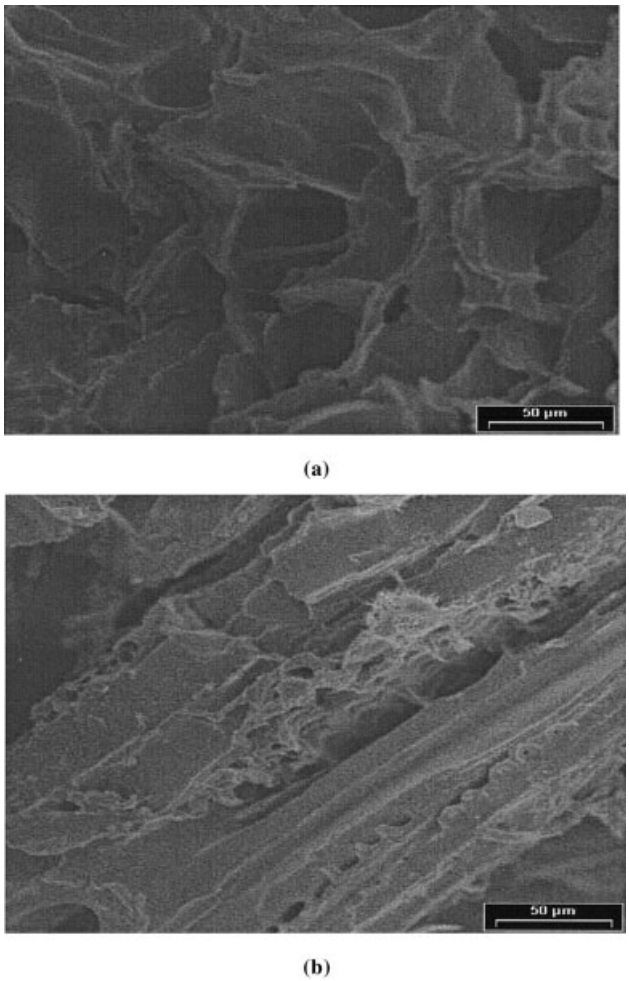

Figure 2 SEM photomicrographs (300×) of freeze-fractured transversal sections of WG-based composite films containing 2,4-D: (a) a film containing 20% SCB and (b) a film containing 20% S (bar = 50 μm).

gelatin at ambient temperature will be the result of these two processes. As the GA concentration increases, the probability of the triple helix recovering will be reduced, and the structure of the crosslinked gelatin will be similar to that of a random coil polymer.[5] The introduction of PVA (20%) reduced the break load but did not significantly affect the strain. When the amount of PVA increased to 35%, it reversed the break load to values similar to those of the samples without PVA. Meanwhile, the elongation (strain break) increased. The presence of 10% SCB reduced the break load as well as the strain break. When the percentage exceeded 10%, the break load decreased too much. Introducing S into WG films sharply reduced the break load, and the elongation was slightly reduced. The presence of 10% S gave much better results than 10% SCB and was equal to the presence of 20% PVA. An increase in S of more than 10% gave a very low break load and bad elongation.

Release studies

To control the release of the bioactive material and the reuse of WG, we prepared different formulations with

TABLE III
Tensile Properties of Bioactive Films

Sample	Break load (MPa)	Maximum load (MPa)	Maximum strain (%)	Break strain (%)
WGD	10.697	11.018	31.56	31.58
WGDG2	3.853	3.854	23.74	23.75
WGDP20	4.619	5.287	28.89	29.82
WGDP35	11.668	11.677	145.11	146.16
WGDG2P20	8.511	8.707	105.10	106.18
WGDG5P20	2.766	3.456	19.79	20.19
WGDB10	1.086	3.623	5.05	10.25
WGDG5B20	0.990	3.365	3.67	4.94
WGDS10	4.198	4.366	21.44	21.8
WGDG2S20	3.800	4.146	2.7	4.19
WGDG5S20	0.366	1.227	3.43	18.05

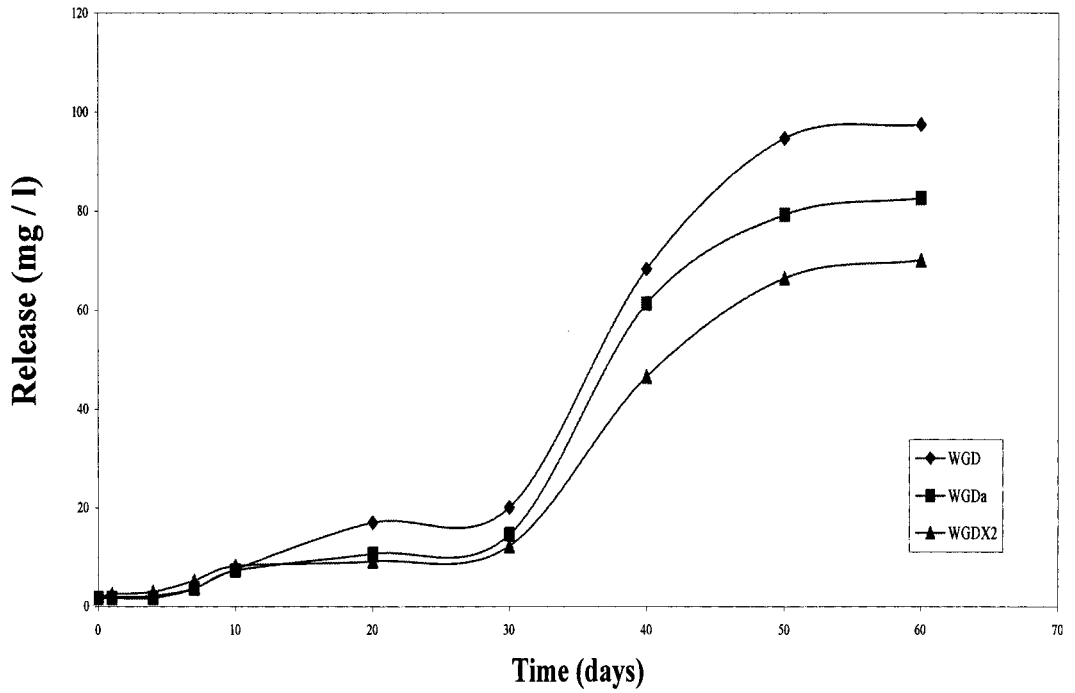

Figure 3 Release profile of 2,4-D from WG and different additives.

different natural and synthetic additives. The release of these formulations was carried out at 25°C in a water solution buffered at pH 7. The amount of herbicide released within the time was monitored by HPLC. The rate of release of the herbicide from WG films likely depended on the compositions of the films. An investigation into the effects of the film compositions was carried out. Release profiles of 2,4-D from the films are shown in Figures 3–6.

In general, the rates of release from these films were slow, and there was no initial fast release in comparison with the previously reported systems based on

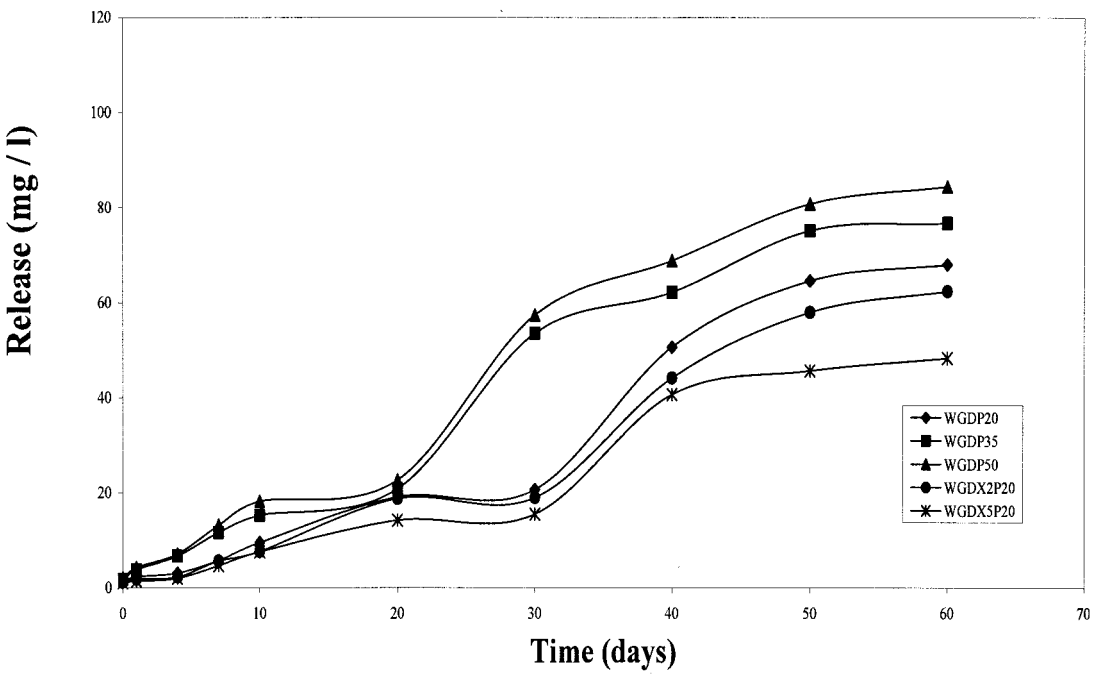

Figure 4 Effect of PVA on the release of 2,4-D from WG.

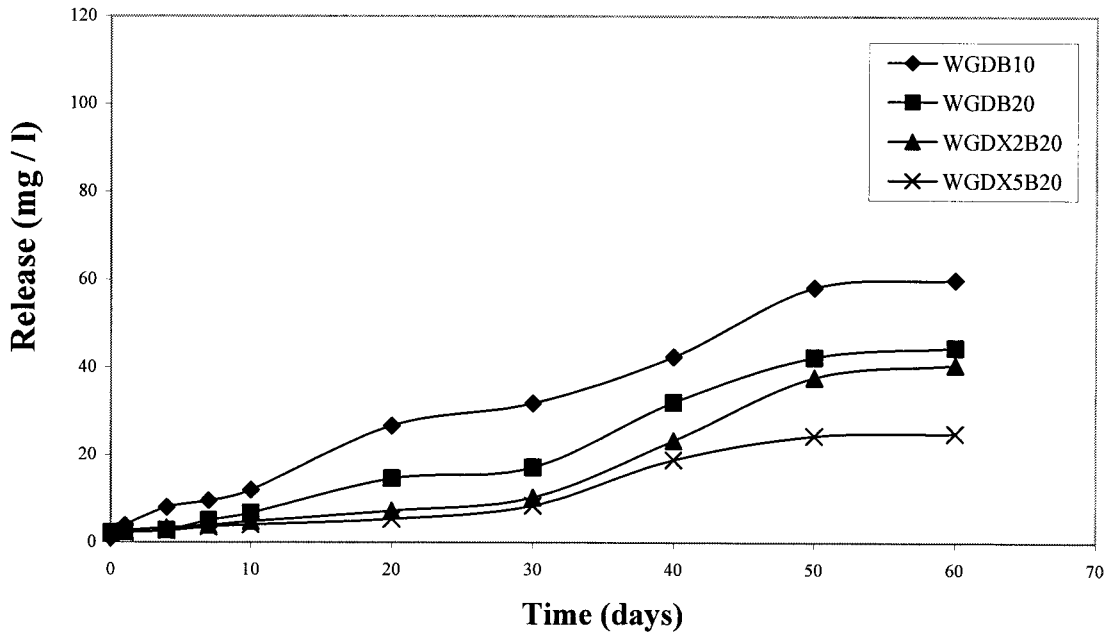

Figure 5 Release profile of 2,4-D from WG in the presence of SCB.

other polymeric materials.[12] The release profiles showed that the presence of 20% PVA in a film reduced the release of 2,4-D from the film from 100 to 70 mg/60 days. This could be due to a possible interaction between the gelatin and PVA, which resulted in a kind of shielding effect or physical crosslinking that protected the bioactive agent and prevented or slowed the release rate. In a previous study, the addition of PVA to gelatin slowed down the degradation of PVA for a reason similar to what we are reporting here.[4,5] However, an increase in the PVA content from 20 to 50% increased the amount released to 84 mg/60 days. This could be attributed to the water solubility of PVA. Therefore, when the amount of PVA exceeded 20%, the excessive amount was dissolved in water during the release process, and this left channels in the

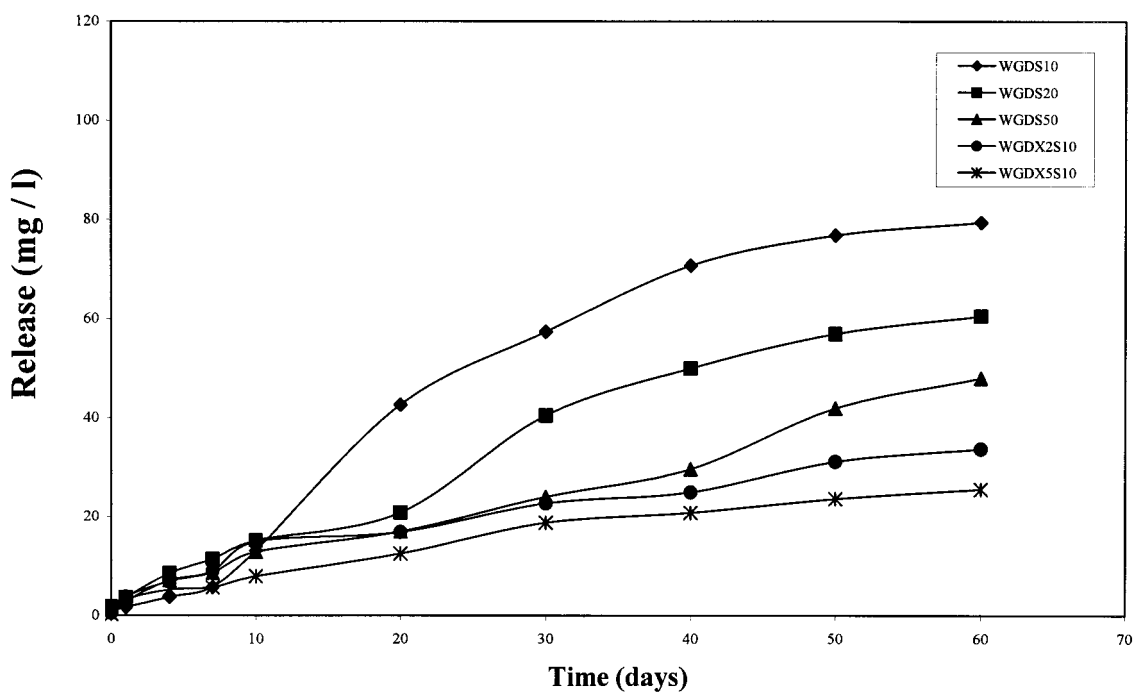

Figure 6 Release profile of 2,4-D from WG in the presence of S.

films, which allowed 2,4-D to be released, or it possibly released 2,4-D while dissolving (Figs. 3 and 4).

The release profiles of 2,4-D from films containing SCB and S showed that an increase in the concentration of SCB or S reduced the release rate. For example, the addition of 10% SCB to a film reduced the amount of released 2,4-D to 60 mg/60 days, and the addition of 10% S reduced the amount of released 2,4-D to 80 mg. An increase in the amount of SCB or S to 50% reduced the released amount of 2,4-D to 50 and 65 mg, respectively.

Because gelatin is soluble in aqueous solutions, gelatin materials for long-term biomedical applications must be submitted to crosslinking, which improves both the thermal and mechanical stability of gelatin as a biopolymer.[13–17]

In this work, we used GA, which is by far the most widely used chemical crosslinker because of its high stabilization efficiency for collagenous materials. The crosslinking process with GA involves the reaction of free amino groups of lysine or hydroxylsine amino acid residues of the polypeptide chain with the aldehyde groups of GA. GA was selected for crosslinking because it is easily available and inexpensive and its aqueous solutions can effectively crosslink collagenous tissues in a relatively short period.[9]

The effect of crosslinking on the release of 2,4-D from the formulations is clear from Figures 3–6. Two crosslinkers were tested, A and GA. GA reduced the rate of release of 2,4-D more than A. For example, the amount released from a film containing 5% A as a crosslinker was 80 mg/60 days, whereas a 2% GA crosslinked film released 70 mg in the same period. Also, the addition of 2% GA to a formulation (WGDG2P20) containing 20% PVA released 65 mg of 2,4-D, whereas a film with the same structure (WGDG5P20) and 5% GA released only 50 mg of 2,4-D. A similar reduction in the release rates was observed for formulations containing S and SCB as fillers (Figs. 3 and 4). These results are logical because the release is diffusion-dependent and crosslinking limits the penetration of water to the site at which the active ingredient is located and increases the hydrophobicity of polymeric formulations.

CONCLUSIONS

Blend and composite films based on pharmaceutical-grade gelatin scraps (WG) were prepared with PVA, SCB, and S. The biologically active agent 2,4-D was incorporated into the films with the aim of these films being using as controlled-release systems for 2,4-D. The WG films were stabilized through crosslinking with solutions with low concentrations of GA or A.

The mechanical properties of the composite films were lower than those of the gelatin blend. The release of 2,4-D from these formulations was found to depend to a large extent on the WG additives. Crosslinked WG displayed a slower rate of release of 2,4-D because of the lower hydrophilicity of the WG formulation and the capacity for the diffusion of water. In all cases, we anticipate that fragments from polymers will enrich soil with nitrogen and will, therefore, have a beneficial fertilizing effect. We also anticipate that every single component will exert a positive effect on soil as a nitrogen source (WG), as a potential humic compound once transferred by soil microflora (SCB), as a soil conditioner (PVA), and as a controlled-release system for herbicides. In addition, such films are expected to be suitable as mulching films and self-fertilizing mulch because they contain, for the most part, waste materials from the pharmaceutical industry or agroindustry.

The authors thank Rp Scherer Co. (Alexandria, Egypt) for the waste gelatin samples and for their cooperation. They also acknowledge Judy Williamson (Anatomy Department, Virginia Commonwealth University) for providing the scanning electron microscopy facility.

References

1. Winfield, A. J.; Richards, R. M. E. Pharmaceutical Practice, 2nd ed.; Churchill Livingstone: New York, 1998; Chapters 12 and 15, p 122, 148.
2. Brady, G. S. Materials Handbook, 10th ed.; McGraw-Hill: New York, 1971; p 358.
3. Schmidt, W. J.; Smith, M. F.; Neal, J. W. U.S. Pat. 5,288,408 (1994).
4. Kenawy, E. R.; Cinelli, P.; Corti, A.; Miertus, S.; Chiellini, E. Macromol Symp 1999, 144, 351.
5. Chiellini, E.; Cinelli, P.; Fernandes, E. G.; Kenawy, E. S.; Lazzeri, A. Biomacromolecules 2001, 2, 806.
6. Kenawy, E. R. J Macromol Sci Rev Macromol Chem Phys 1998, 38, 385.
7. Kenawy, E. R. Indian J Chem Sect B 1997, 36, 886.
8. Akelah, A.; Moet, A. Functionalized Polymers and Their Applications; Chapman & Hall: London, 1990.
9. Kenawy, E. R. Polym-Plast Technol Eng 2001, 40, 437.
10. Kenawy, E. R. React Funct Polym 1998, 36, 31.
11. Rose, P. I. In Encyclopedia of Polymer Science and Engineering; Mark, H. F.; Bikales, N. M.; Overberger, C. G.; Menges, G., Eds.; Wiley: New York, 1987; Vol. 7, p 488.
12. Akelah, A.; Kenawy E. R.; Sherrington, D. C. Eur Polym J 1992, 28, 453.
13. Bigi, A.; Cojazzi, G.; Panzavolta, S.; Rubini, K.; Roveri, N. Biomaterials 2001, 22, 763.
14. Bigi, A.; Bracci, B.; Cojazzi, G.; Panzavolta, S.; Roveri, N. Biomaterials 1998, 19, 2335.
15. Chiellini, E.; Cinelli, P.; Corti, A.; Kenawy, E. Polym Degrad Stab 2001, 73, 549.
16. Van Den Bulcke, A. I.; Bogdanov, B.; De Rooze, N.; Schacht, E. H.; Cornelissen, M.; Berghmans, H. Biomacromolecules 2000, 1, 31.
17. Khor, E. Biomaterials 1997, 18, 95.