

ORIGINAL

ABSTRACT

The present invention relates to the processes of preparing silkfibroin/polyethylene oxide blended materials, and the resulting materials thereof, which are suitable for biomedical applications such as wound healing. In particular, the electrospun silk fibroin/PEO mats with a silk:PEO blend ratio of 2:1 to 4:1, treated with controlled evaporation, constraint-drying techniques, and/or alcohol treatment, and/or PEO extraction, demonstrate suitable physical and biofunctional properties, such as fiber structure, topography, absorption, water vapor transmission rates, oxygen permeation, and biodegradability, relevant to biomaterial systems with utility for wound dressings.

Refer to figures 1A & 1B

CLAIMS

What is claimed is:

1. A process for producing a silk mat, comprising:
blending a polyethylene oxide (PEO) with an aqueous silk fibroin solution;
electrospinning the blended solution, thereby forming a silk protein/PEO blended mat;
and
constraint-drying the electrospun silk mat.
2. The process of claim 1, further comprising treating the electrospun silk mat with alcohol.
3. The process of claim 1 or 2, further comprising extracting the PEO from the silk mat.
4. The process as in any one of claims 1-3, further comprising embedding at least one active agent in the silk mat.
5. The process of claim 4, wherein the active agent is a therapeutic agent or a biological material, selected from the group consisting of cells, proteins, peptides, nucleic acids, nucleic acid analogs, nucleotides or oligonucleotides, peptide nucleic acids, aptamers, antibodies or fragments or portions thereof, antigens or epitopes, hormones, hormone antagonists, growth factors or recombinant growth factors and fragments and variants thereof, cell attachment mediators, cytokines, enzymes, antibiotics or antimicrobial compounds, viruses, toxins, prodrugs, chemotherapeutic agents, small molecules, drugs, and combinations thereof.
6. The process of claim 5, wherein the active agent is a cell selected from the group consisting of progenitor cells or stem cells, smooth muscle cells, skeletal muscle cells, cardiac muscle cells, epithelial cells, endothelial cells, urothelial cells, fibroblasts, myoblasts, oscular cells, chondrocytes, chondroblasts, osteoblasts, osteoclasts, keratinocytes, kidney tubular cells, kidney basement membrane cells, integumentary cells, bone marrow cells, hepatocytes, bile duct cells, pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic, pituitary, ovarian, testicular, salivary gland cells, adipocytes, precursor cells, and combinations thereof.
7. The process of claim 6, the active agent further comprises a cell growth media.

8. The process of claim 6, wherein the active agent is an antibiotic.
9. A silk material prepared from the process comprising:
blending a polyethylene oxide (PEO) with an aqueous silk fibroin solution;
electrospinning the blended solution, thereby forming a silk protein/PEO blend mat; and
constraint-drying the electrospun silk mat.
10. A silk material encapsulating at least one active agent for dressing a wound to promote wound healing prepared from the process comprising:
blending a polyethylene oxide (PEO) with an aqueous silk fibroin solution comprising at least one active agent;
electrospinning the blended solution, thereby forming a silk protein/PEO blend mat encapsulating the active agent(s); and
constraint-drying the electrospun silk mat encapsulating the active agent(s).
11. The silk material of claim 10, wherein the active agent is a therapeutic agent or a biological material, selected from the group consisting of cells, proteins, peptides, nucleic acids, nucleic acid analogs, nucleotides or oligonucleotides, peptide nucleic acids, aptamers, antibodies or fragments or portions thereof, antigens or epitopes, hormones, hormone antagonists, growth factors or recombinant growth factors and fragments and variants thereof, cell attachment mediators, cytokines, enzymes, antibiotics or antimicrobial compounds, viruses, toxins, prodrugs, chemotherapeutic agents, small molecules, drugs, and combinations thereof.
12. The silk material of claim 11, wherein the active agent is a cell selected from the group consisting of progenitor cells or stem cells, smooth muscle cells, skeletal muscle cells, cardiac muscle cells, epithelial cells, endothelial cells, urothelial cells, fibroblasts, myoblasts, oscular cells, chondrocytes, chondroblasts, osteoblasts, osteoclasts, keratinocytes, kidney tubular cells, kidney basement membrane cells, integumentary cells, bone marrow cells, hepatocytes, bile duct cells, pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic, pituitary, ovarian, testicular, salivary gland cells, adipocytes, precursor cells, and combinations thereof.
13. The silk material of claim 12, the active agent further comprises a cell growth media.

14. The silk material of claim 12, wherein the active agent is an antibiotic.
15. The silk material as in any one of claims 9 to 14, wherein the electrospun silk mat is further treated with alcohol.
16. The silk material as in any one of claims 9 to 15, wherein the PEO is extracted from the electrospun silk mat.
17. An electrospun silk material comprising a silk fibroin protein ranging from about 50 wt % to about 100 wt %, wherein the electrospun silk mat has a thickness of about 20 microns to 80 about microns.
18. The electrospun silk material of claim 17, wherein the content of silk fibroin protein in the electrospun silk mat ranges from about 75 wt% to about 90 wt%.
19. The electrospun silk material of claim 17 or 18, further comprising a blend of a polyethylene oxide (PEO) in the electrospun silk mat, wherein the content of PEO in the electrospun silk mat ranges from about 0 wt% to about 50wt%.
20. The electrospun silk material of claim 19, wherein the content of PEO in the electrospun silk mat ranges from about 10 wt% to about 25 wt%.
21. The silk material as in any one of claims 17 to 20, further comprising at least one active agent.
22. The silk material of claim 21, wherein the active agent is a therapeutic agent or a biological material, selected from the group consisting of cells, proteins, peptides, nucleic acids, nucleic acid analogs, nucleotides or oligonucleotides, peptide nucleic acids, aptamers, antibodies or fragments or portions thereof, antigens or epitopes, hormones, hormone antagonists, growth factors or recombinant growth factors and fragments and variants thereof, cell attachment mediators, cytokines, enzymes, antibiotics or antimicrobial compounds, viruses, toxins, prodrugs, chemotherapeutic agents, small molecules, drugs, and combinations thereof.

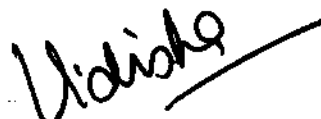
23. The silk material as in any one of claims 17 to 22, wherein the silk mat has a thickness of about 20-30 microns.
24. The silk material as in any one of claims 17 to 23, wherein the silk mats have interconnected pores with the pore throat size surface area averaging from about 0.1 to about 0.3 microns.
25. The silk material as in any of the claims 9 to 24, wherein the resulting silk mat has a water absorption content of more than about 460 %.
26. The silk material as in any of the claims 9 to 25, wherein the resulting silk mat has an equilibrium water content more than about 82%.
27. The silk material as in any of the claims 9 to 26, wherein the resulting silk mat has an oxygen transmission rate of more than about $15460 \text{ cm}^3/\text{m}^2/\text{day}$.
28. The silk material as in any of the claims 9 to 27, wherein the resulting silk mat has a water vapor transmission rate of more than about $1934 \text{ g}/\text{m}^2/\text{day}$.
29. A method of promoting wound healing comprising contacting a wound with at least one electrospun silk mat comprising a silk fibroin protein and, optionally, at least one active agent;
wherein the silk fibroin protein ranges from about 50 wt% to about 90 wt%,
wherein the silk mat has a thickness of about 20 micron to about 80 micron;
wherein the silk mat has a water absorption content of more than about 460 %, or
equilibrium water content more than about 82%; and
wherein the resulting silk mat has an oxygen transmission rate of more than
about $15460 \text{ cm}^3/\text{m}^2/\text{day}$.
30. The method of claim 29, wherein the silk fibroin protein ranges from about 75 wt% to about 90 wt%.
31. A method of promoting wound healing comprising contacting a wound with at least one electrospun silk mat comprising a silk fibroin protein, a polyethylene oxide (PEO) and,
optionally, at least one active agent;
wherein the silk/PEO blend ratio is from about 4:1 to about 2:1;

wherein the silk mat has a thickness of about 20 micron to about 80 micron;
wherein the silk mat has a water absorption content of more than about 460 %, or
equilibrium water content more than about 82%; and
wherein the resulting silk mat has an oxygen transmission rate of more than
about 15460 cm³/m²/day.

32. The method as in any one of claims 29 to 31, wherein said silk mat has a water vapor
transmission rate of more than about 1934 g/m²/day.

33. The method as in any one of claims 29 to 32, wherein the active agent is a therapeutic
agent or a biological material, selected from the group consisting of cells, proteins, peptides,
nucleic acids, nucleic acid analogs, nucleotides or oligonucleotides, peptide nucleic acids,
aptamers, antibodies or fragments or portions thereof, antigens or epitopes, hormones, hormone
antagonists, growth factors or recombinant growth factors and fragments and variants thereof,
cell attachment mediators, cytokines, enzymes, antibiotics or antimicrobial compounds, viruses,
toxins, prodrugs, chemotherapeutic agents, small molecules, drugs, and combinations thereof.

Dated this 16th day of January 2012


OF ANAND AND ANAND ADVOCATES
ATTORNEY FOR THE APPLICANT

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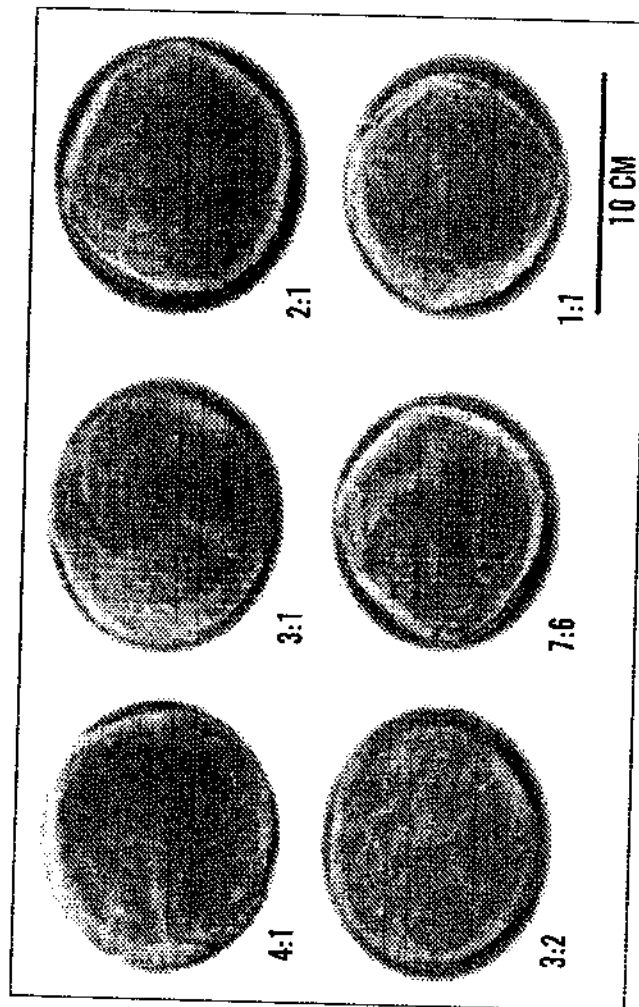


FIG. 1A

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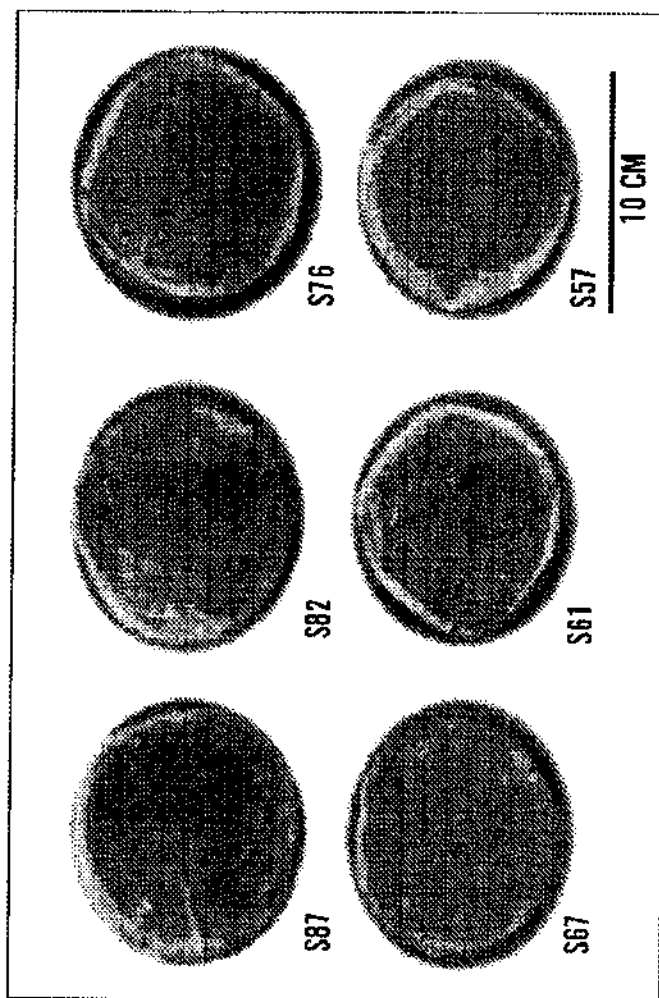


FIG. 1B

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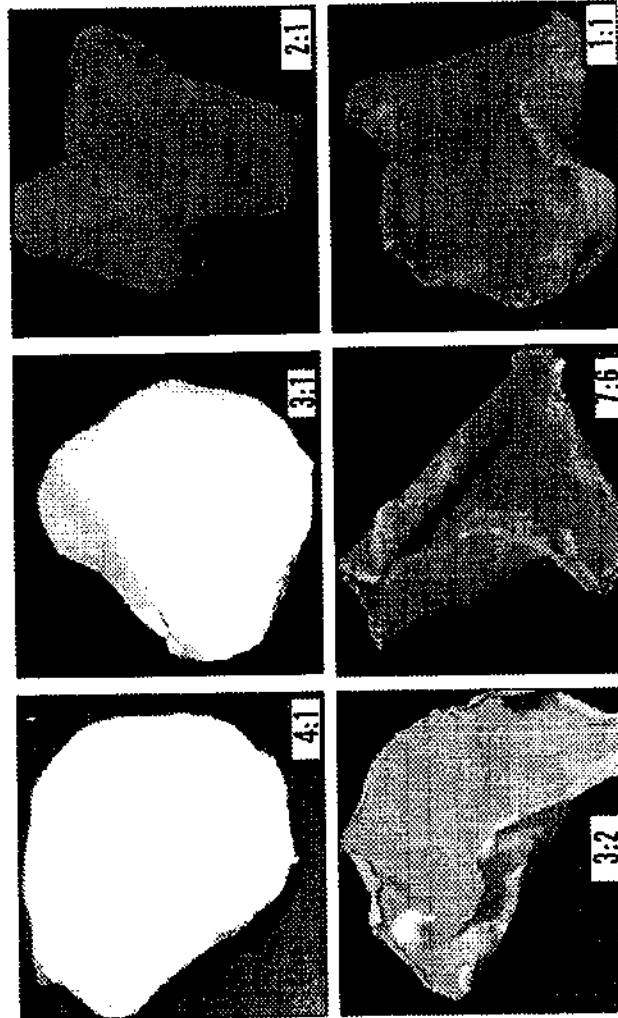


FIG. 2A

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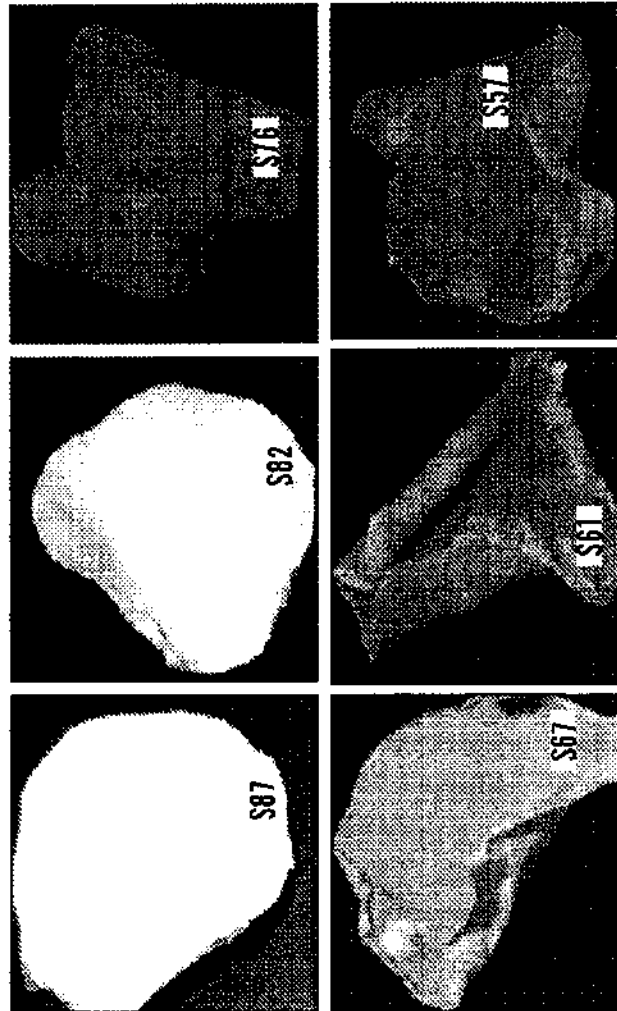


FIG. 2B

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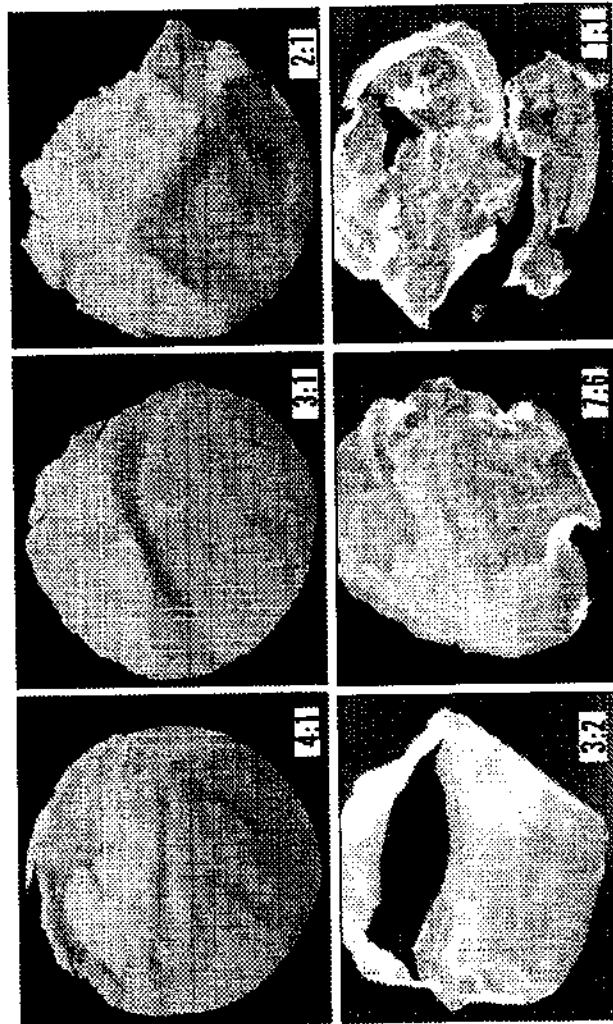


FIG. 3A

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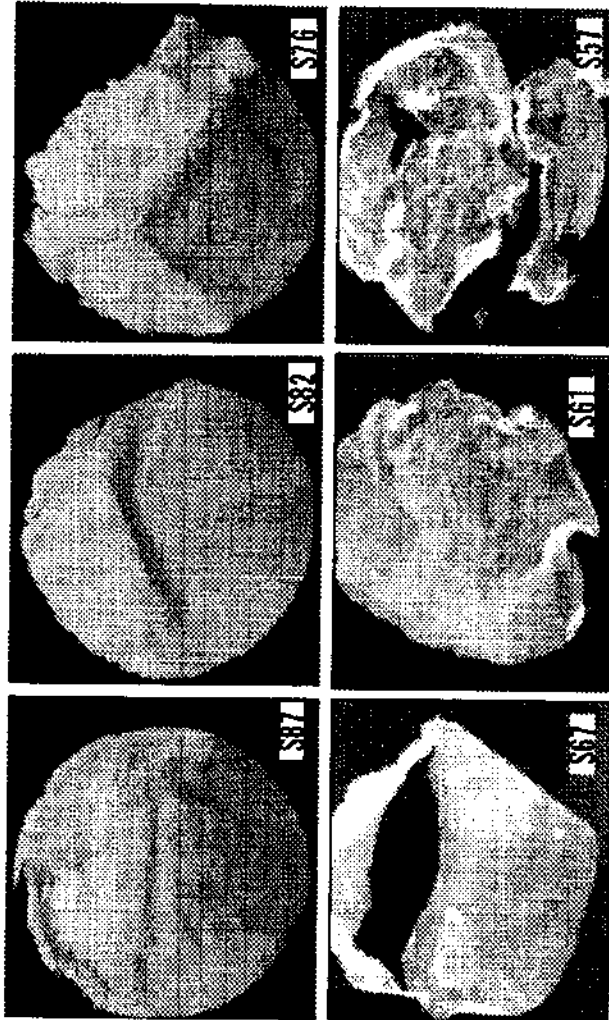


FIG. 3B

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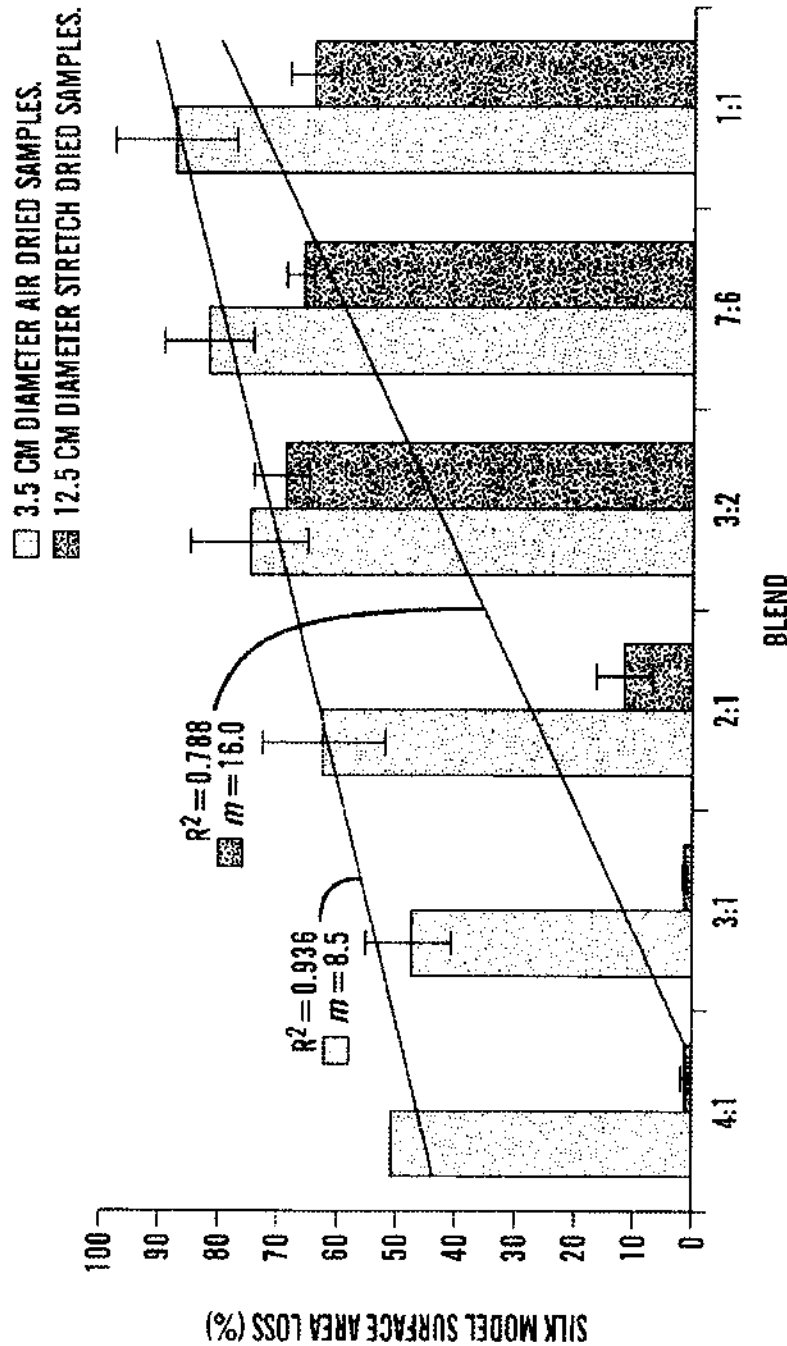


FIG. 4

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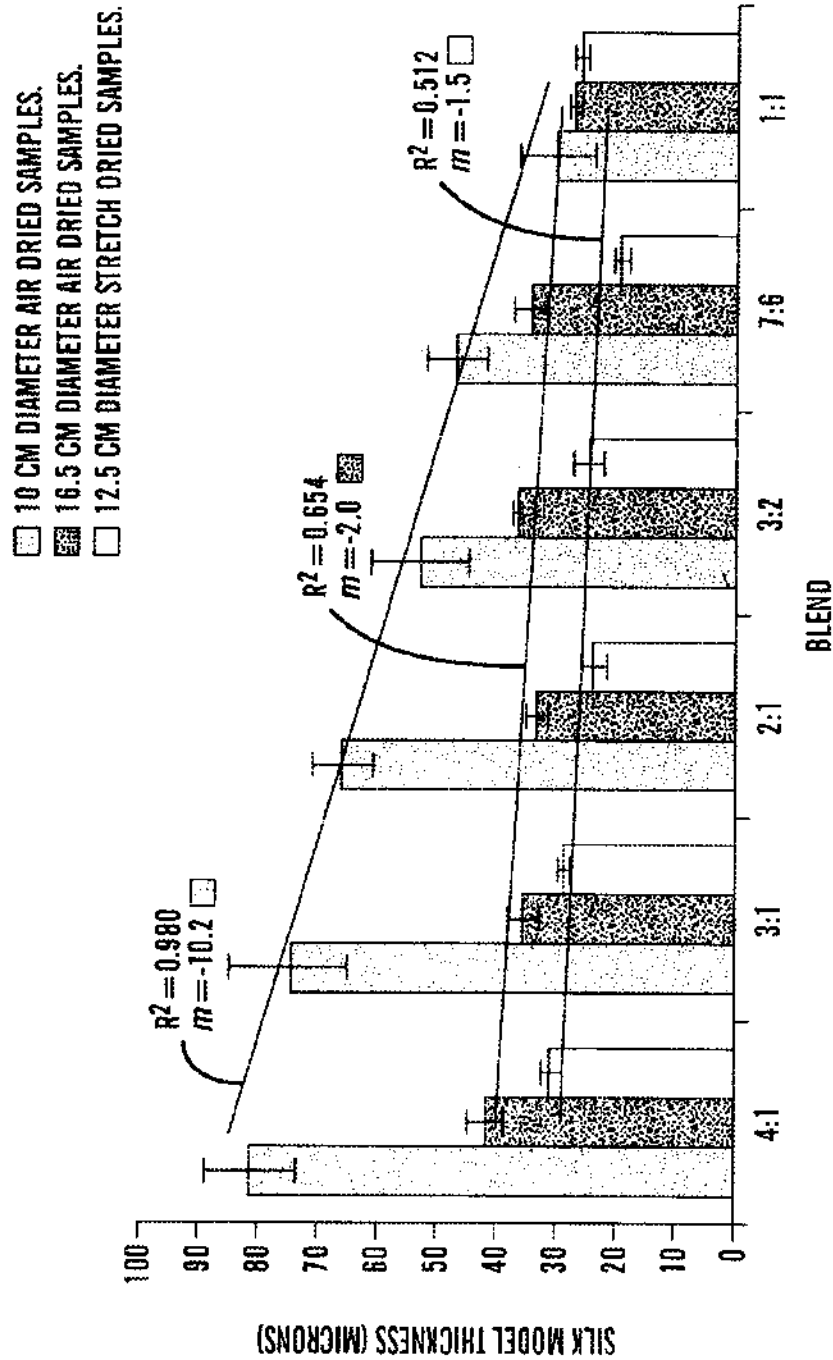


FIG. 5

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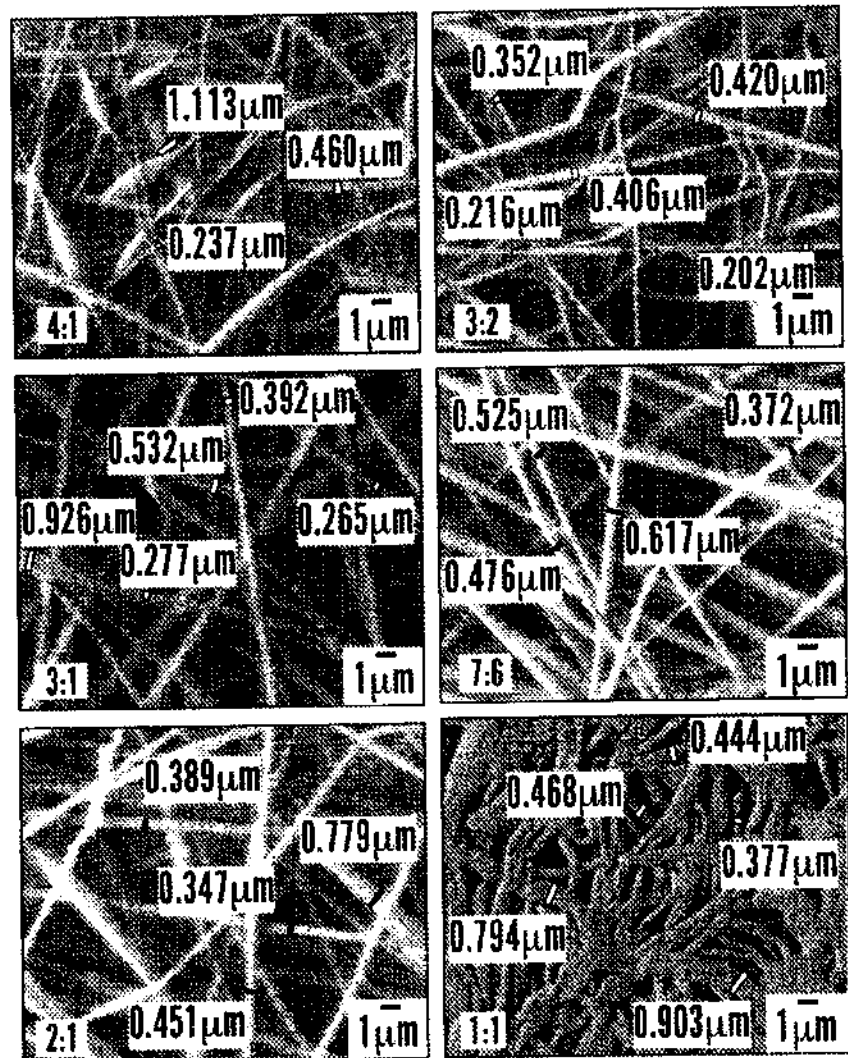


FIG. 6A

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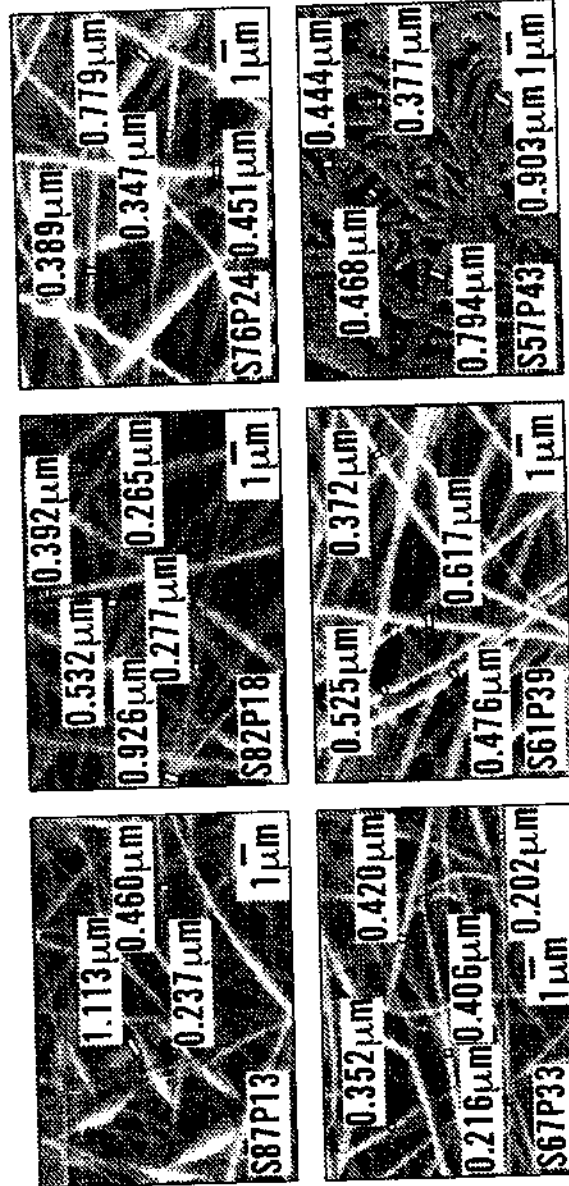


FIG. 6B

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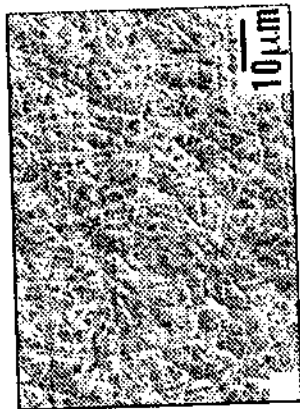
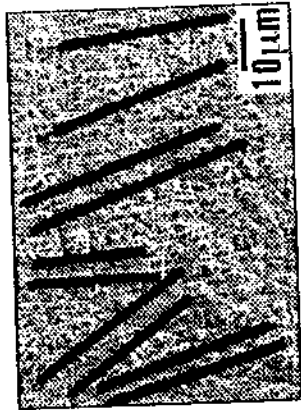


FIG. 7A

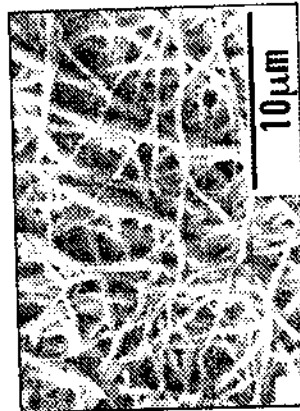


FIG. 7B

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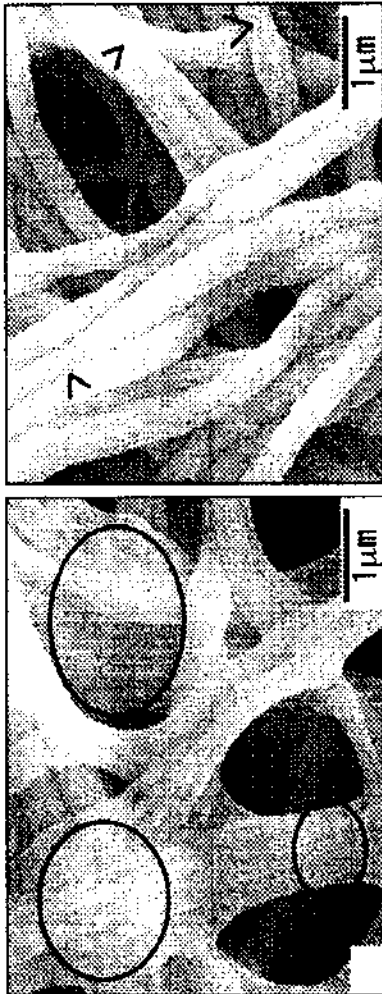


FIG. 7C

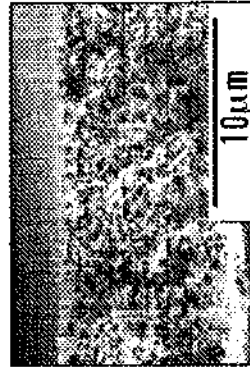


FIG. 7E



FIG. 7D

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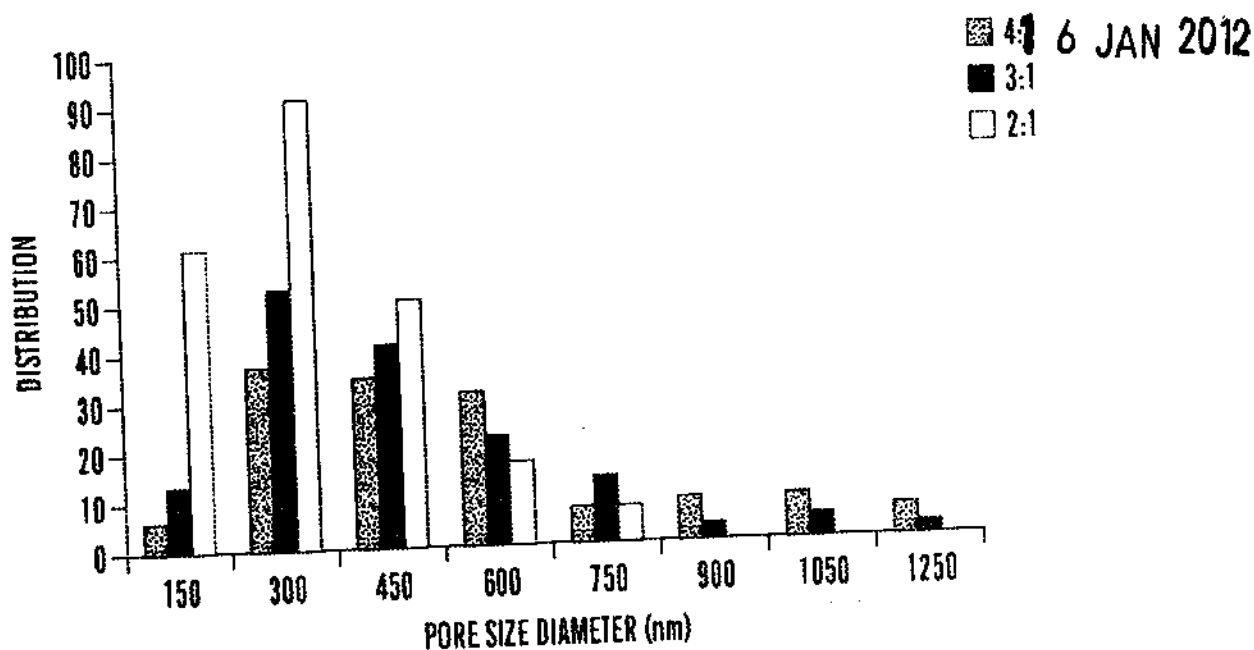


FIG. 8A

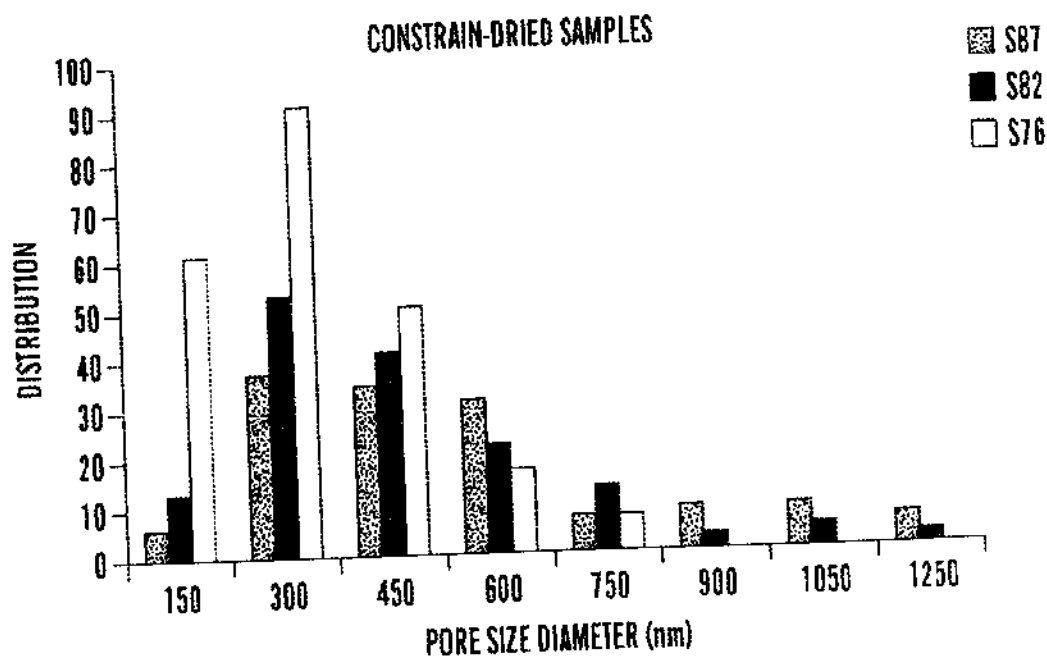


FIG. 8B

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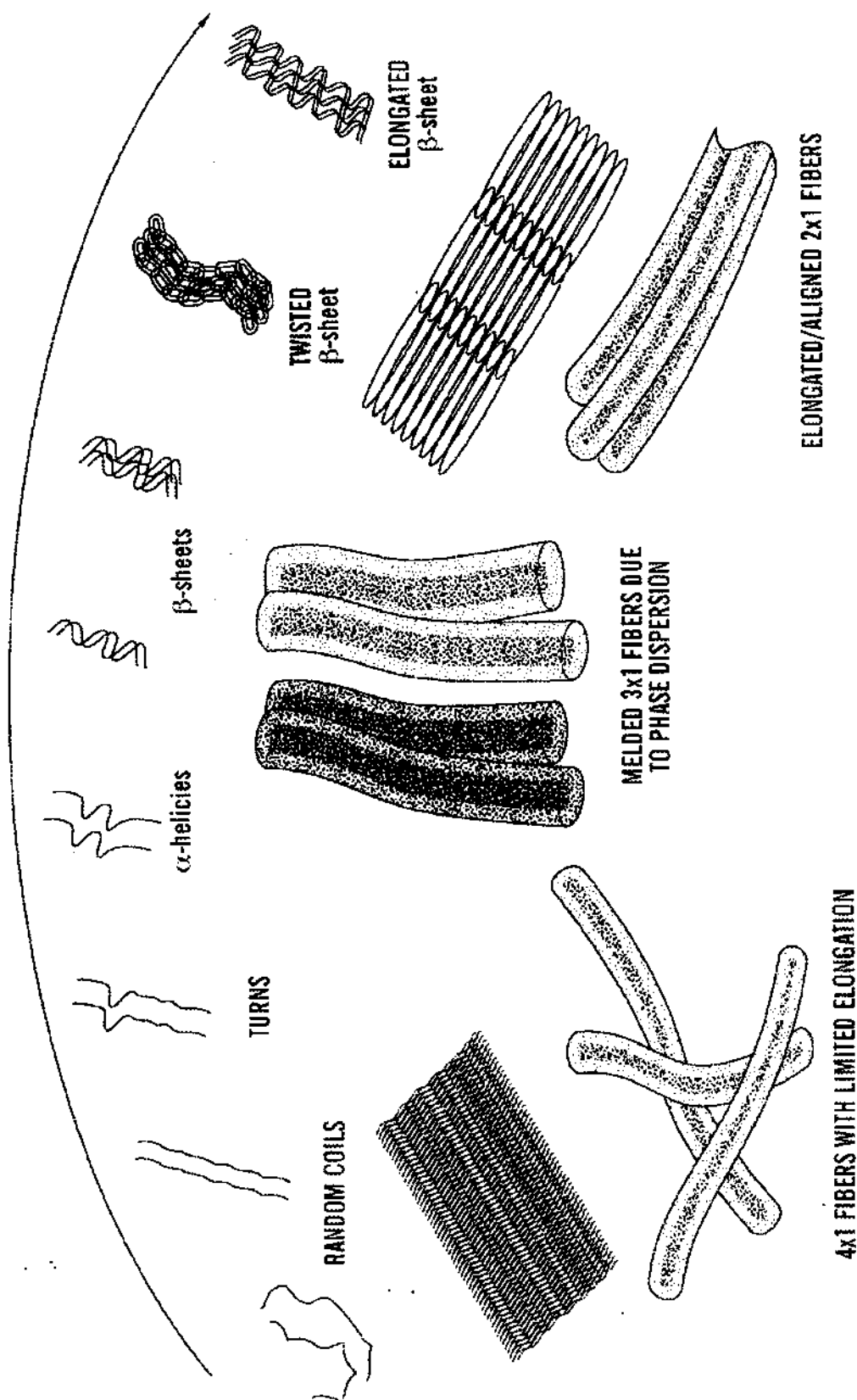


FIG. 9

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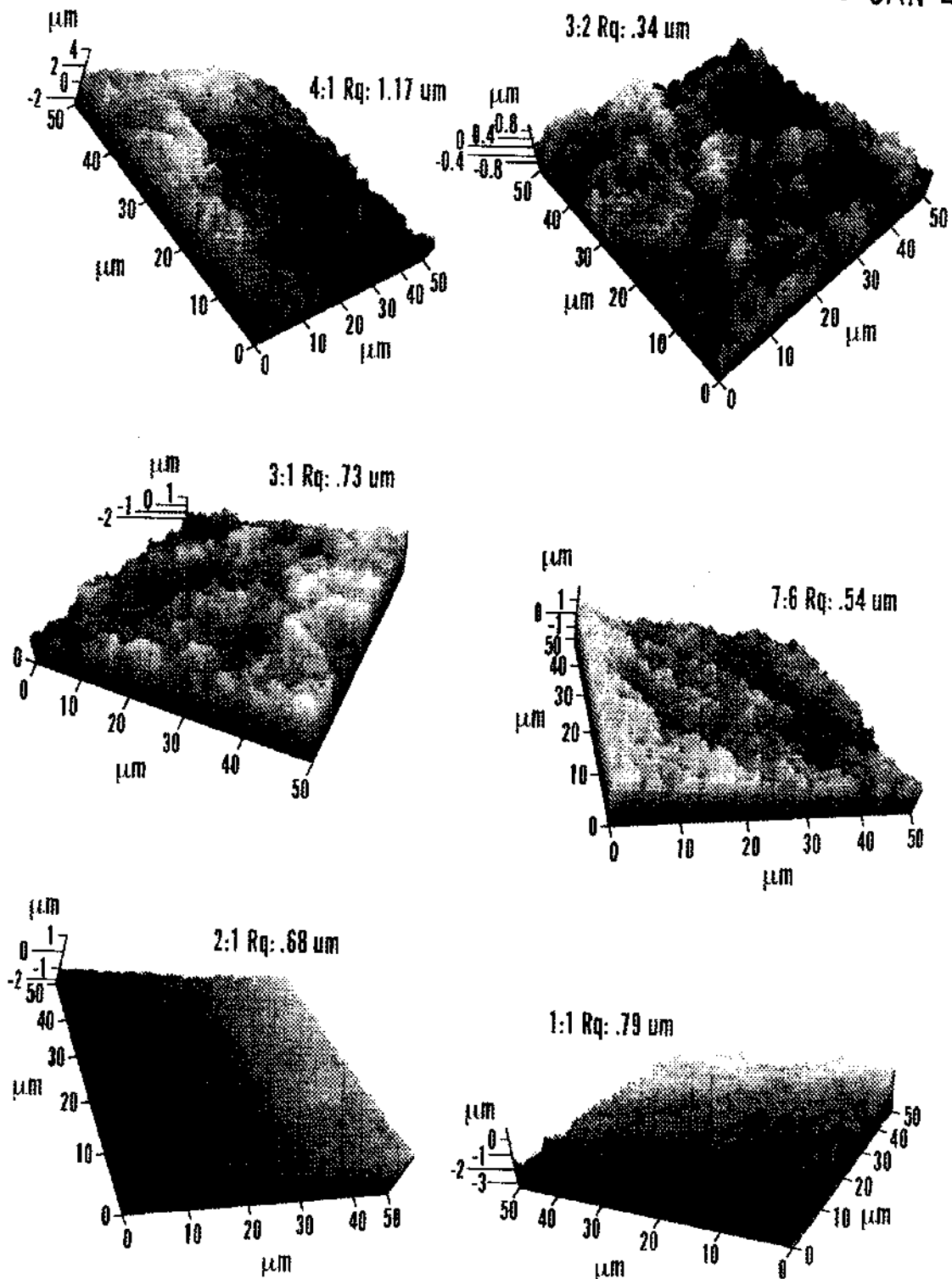


FIG. 10

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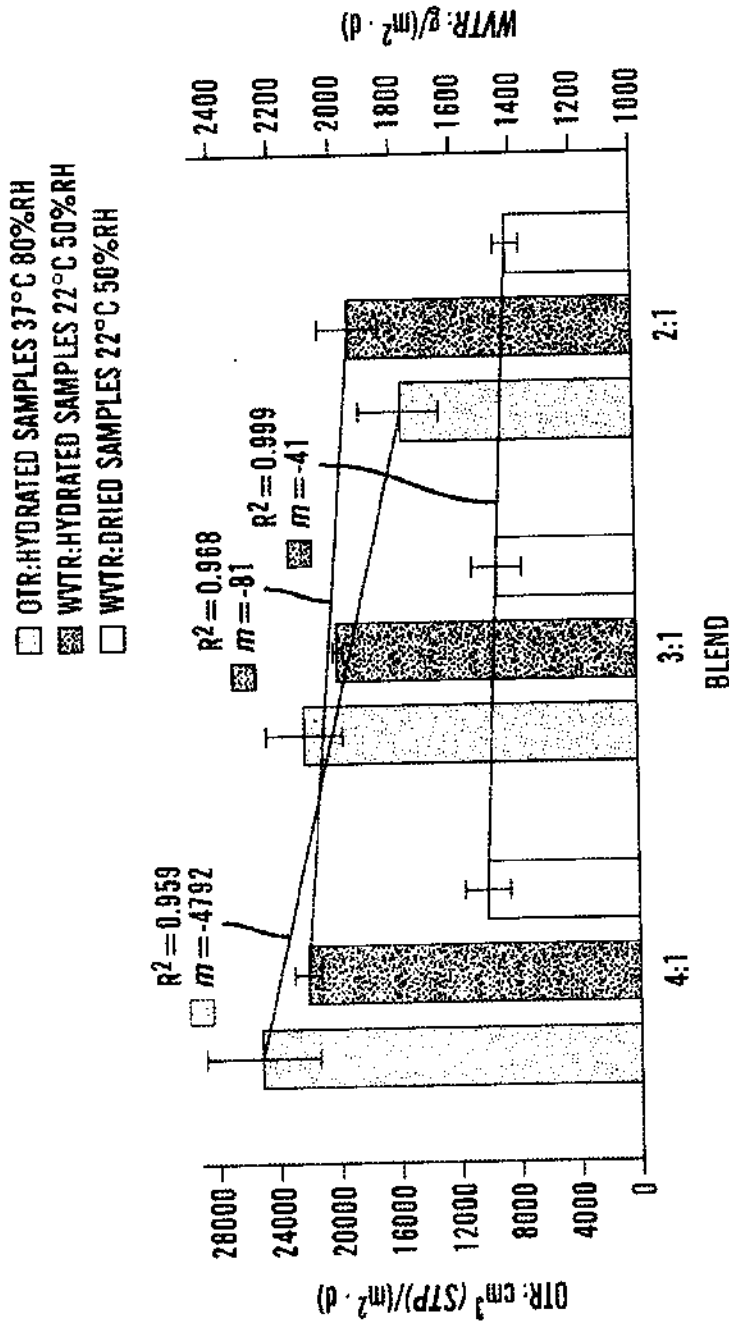


FIG. 11

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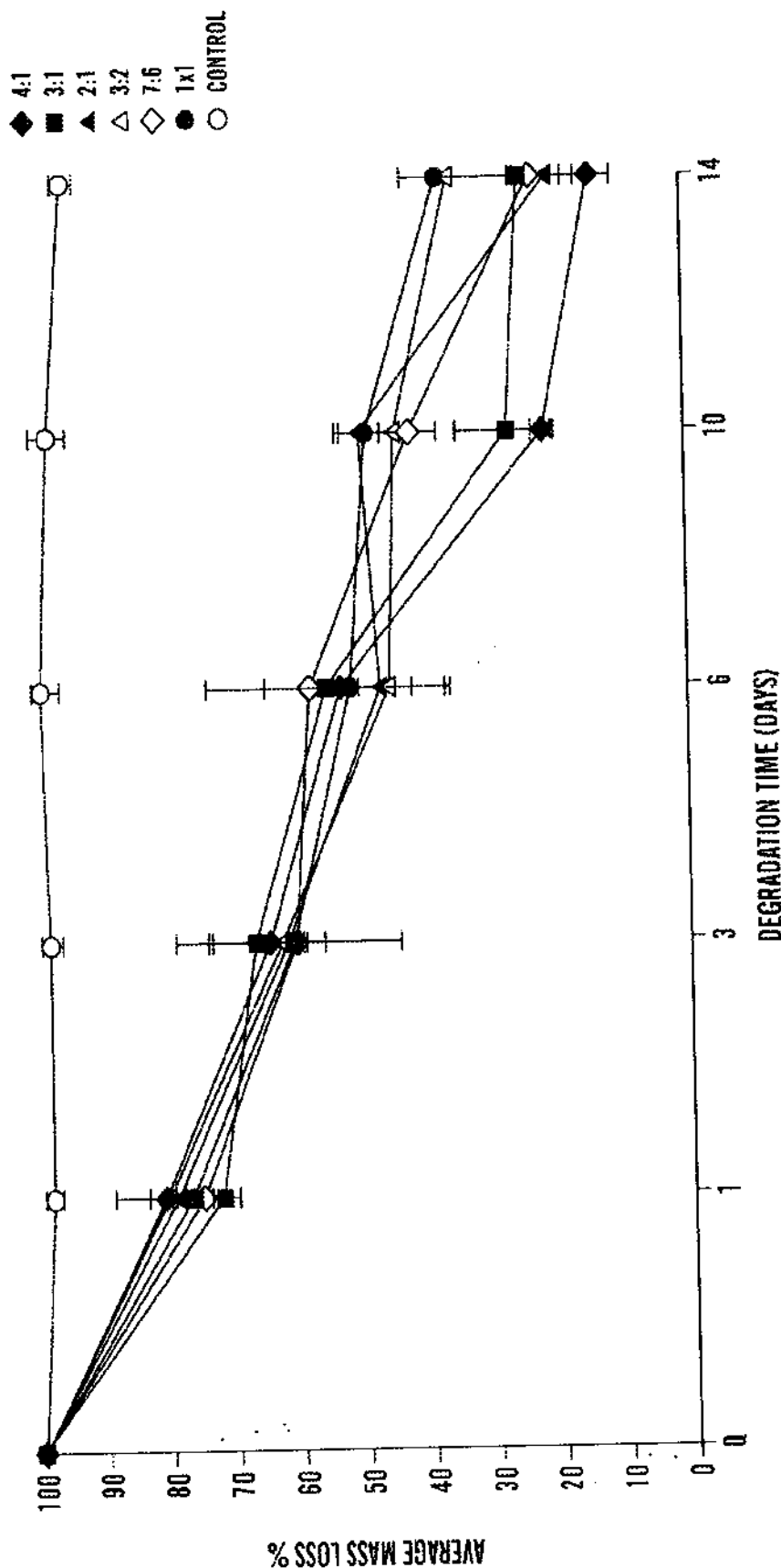


FIG. 12A

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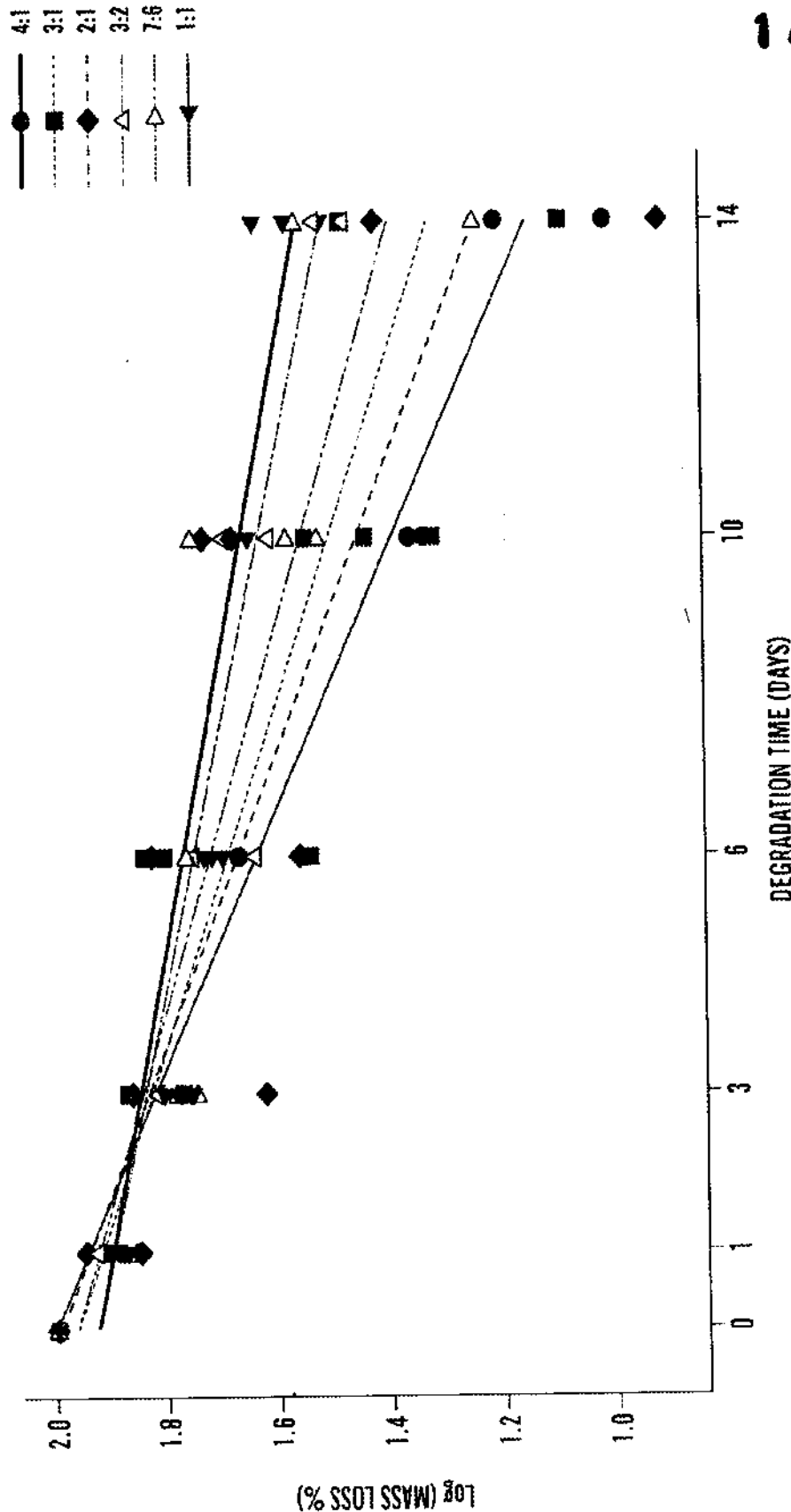


FIG. 12B

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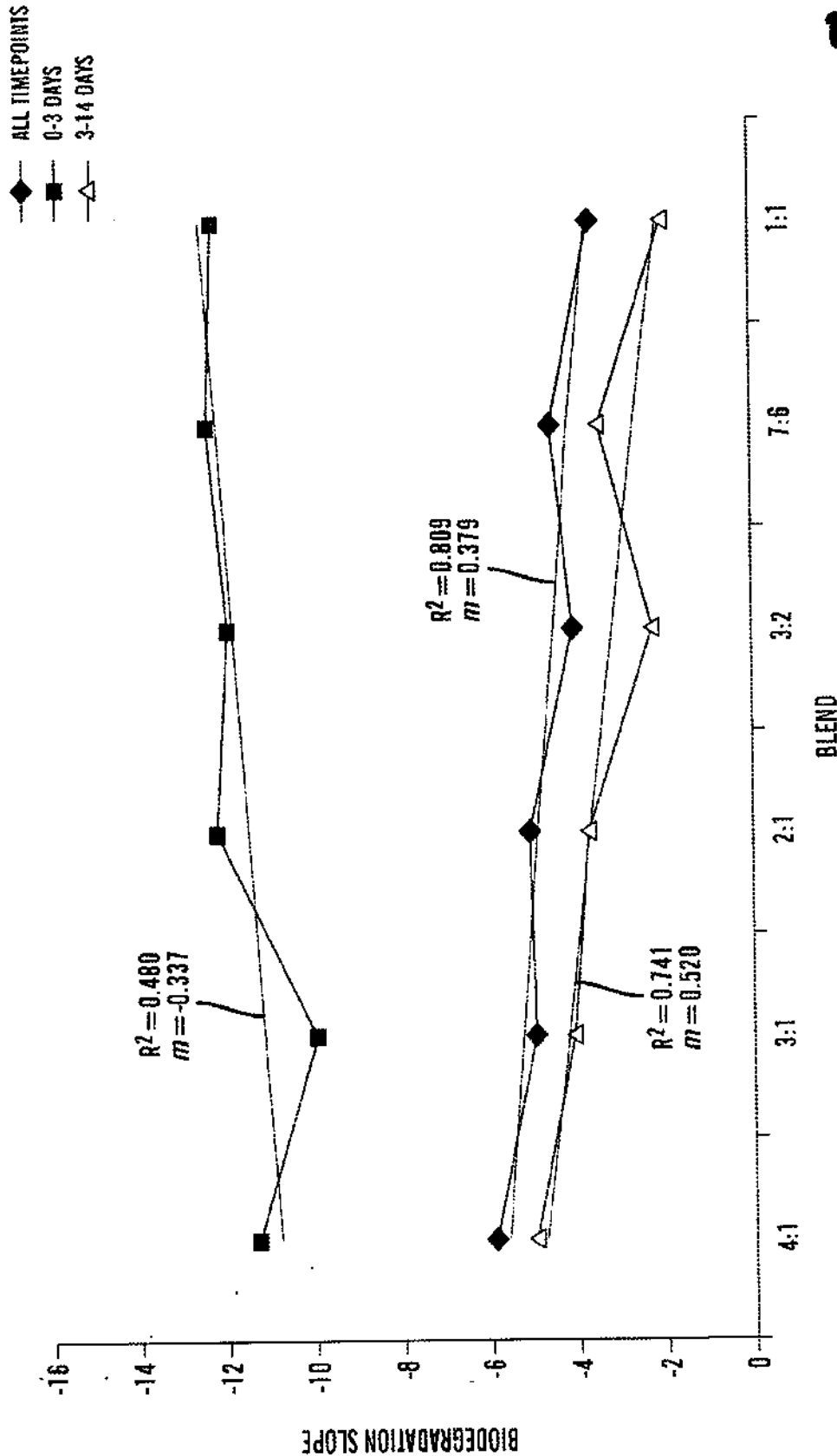


FIG. 12C

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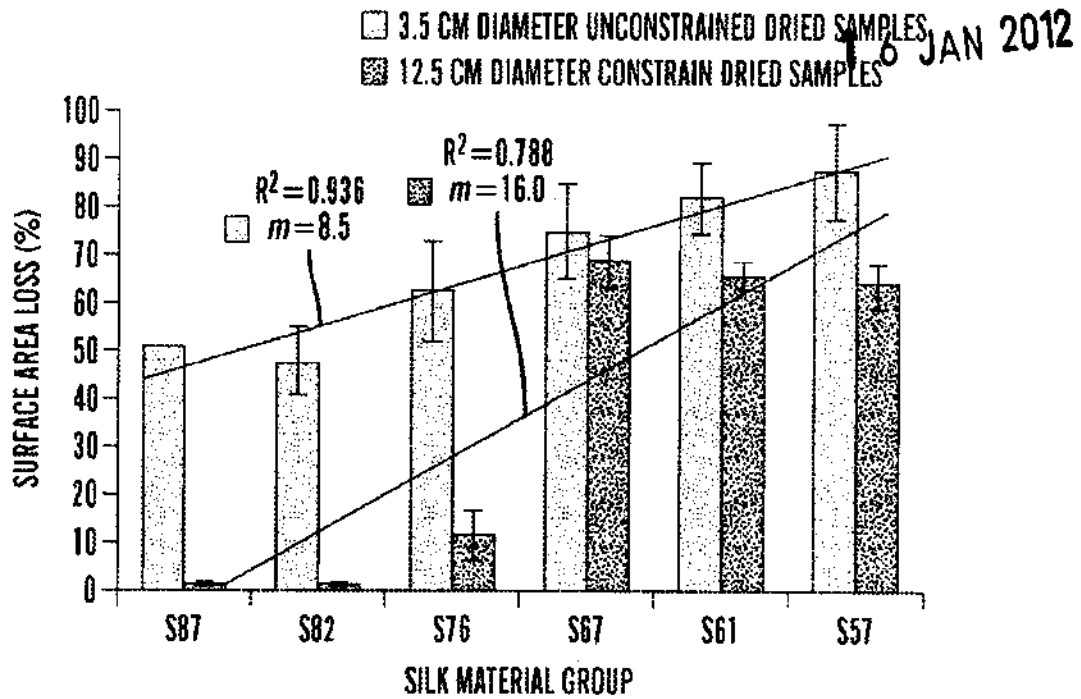


FIG. 13A

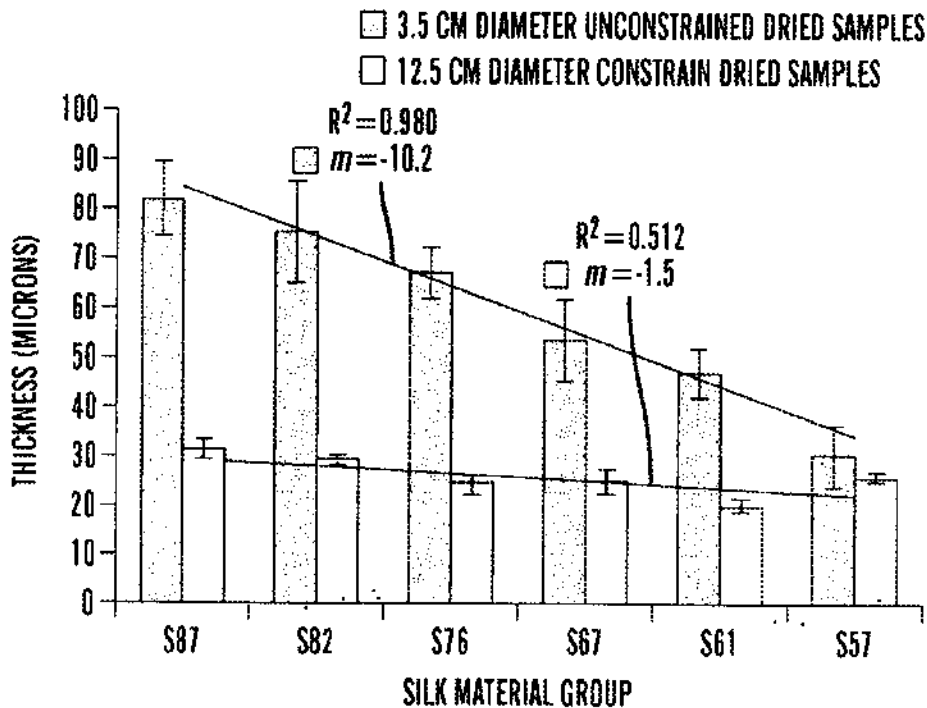


FIG. 13B

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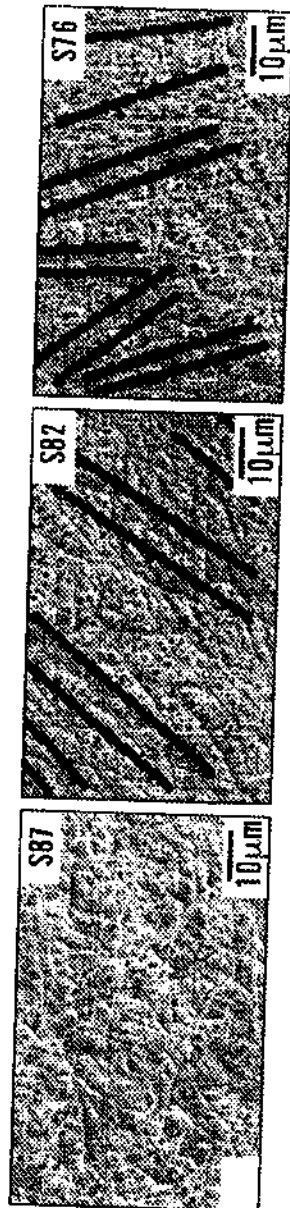


FIG. 14A



FIG. 14B

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FIG. 14D

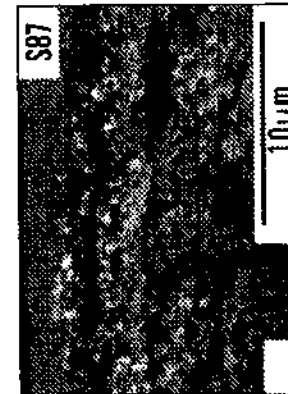
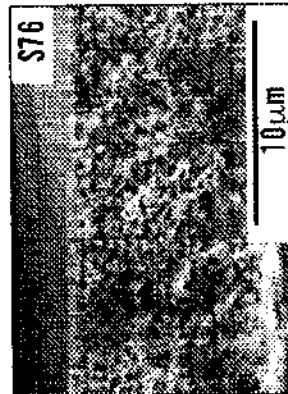


FIG. 14E

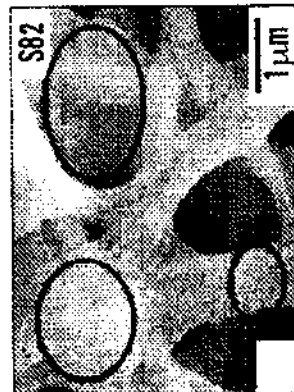
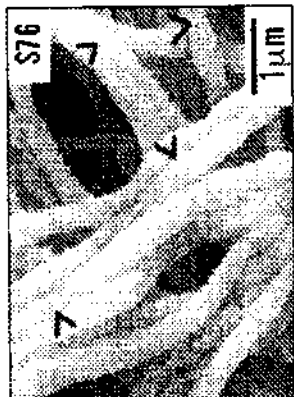


FIG. 14C

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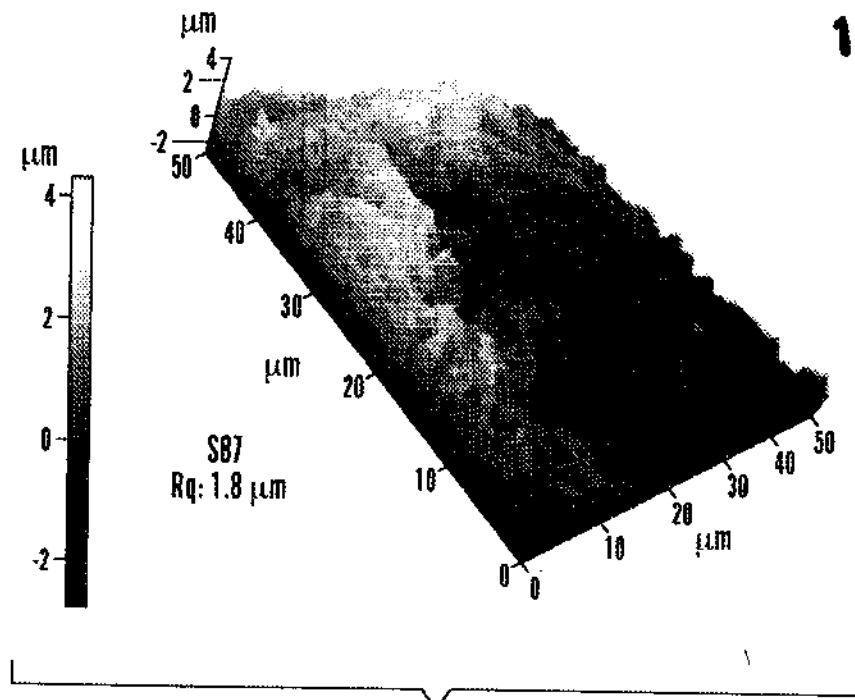


FIG. 15A

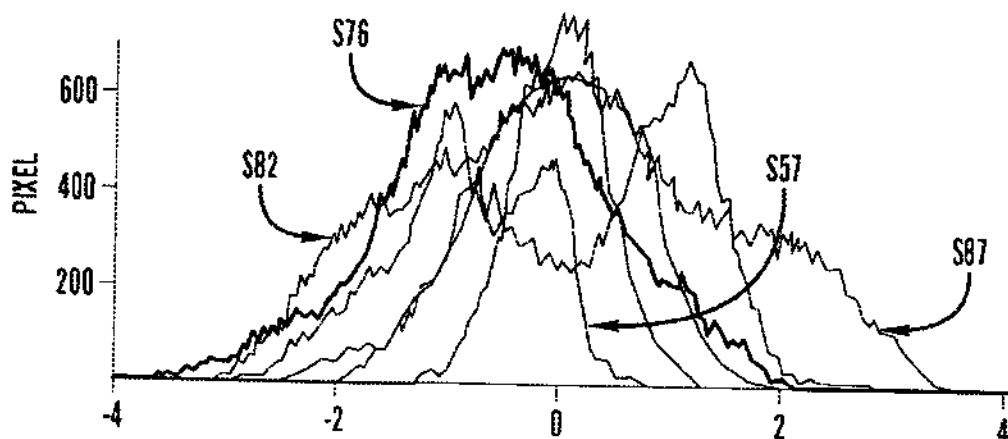


FIG. 15B

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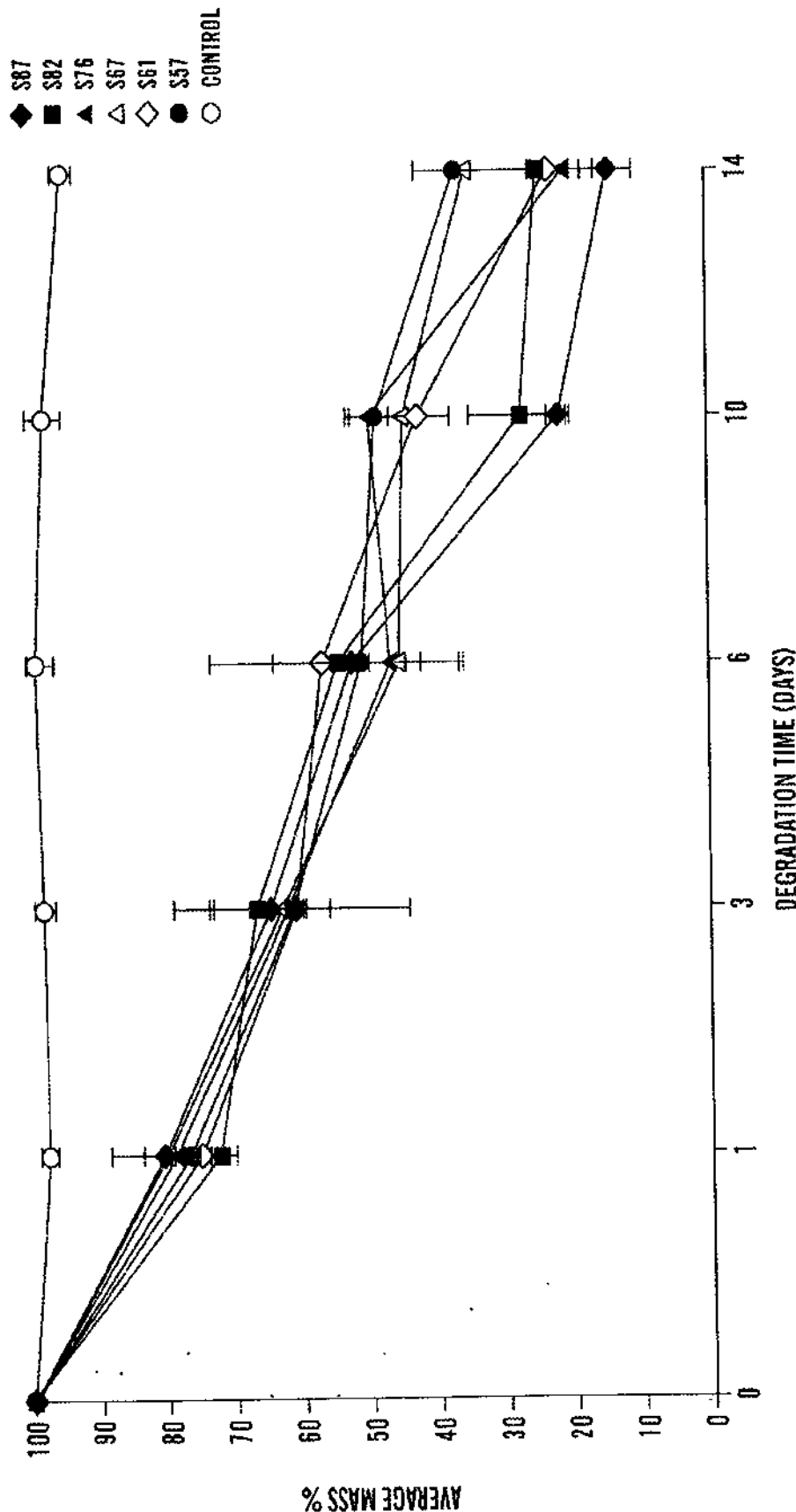


FIG. 16A

S87
S82
S76
S67
S61
S57

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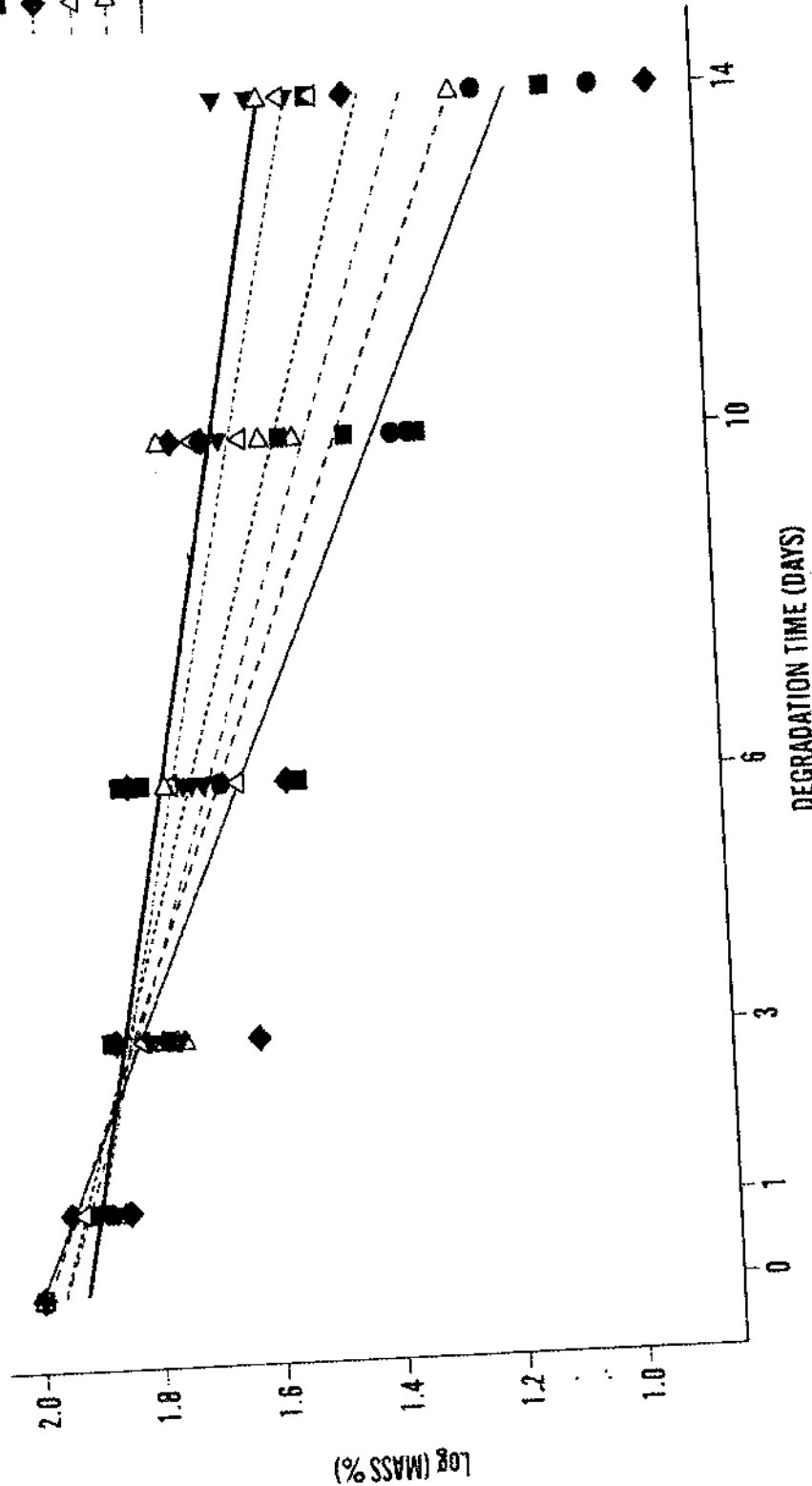


FIG. 16B

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ALL TIMEPOINTS
0-3 DAYS
3-14 DAYS

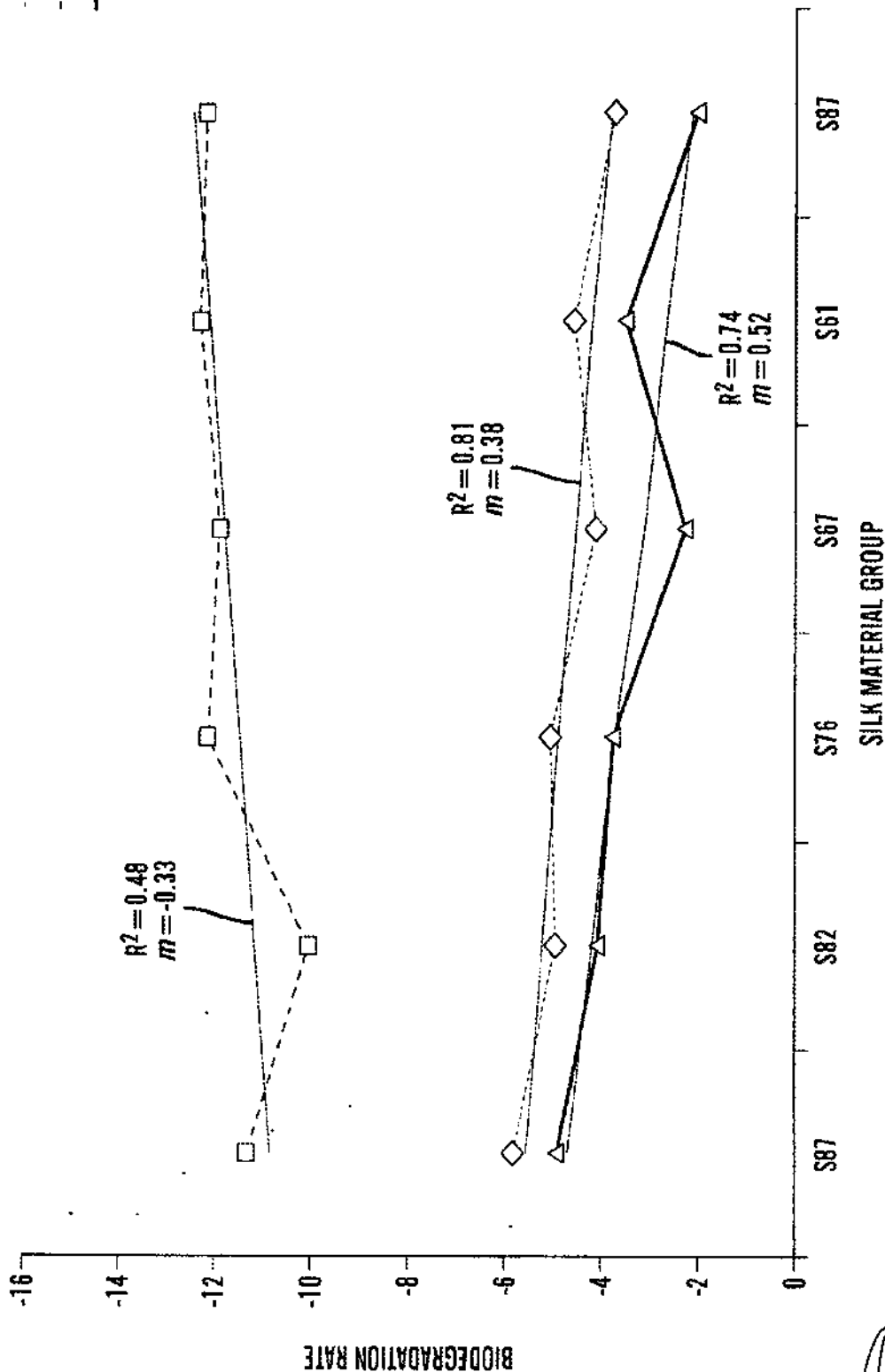


FIG. 16C

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ELECTROSPUN SILK MATERIAL SYSTEMS FOR WOUND HEALING

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 61/225,335 filed July 14, 2009, the content of which is incorporated herein by reference in its entirety.

GOVERNMENT SUPPORT

[0002] This invention was made with funding under grant No. P41 EB002520, awarded by the National Institutes of Health (Tissue Engineering Resource Center). The U.S. government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates to the processes for preparing silk/polyethylene oxide blended materials, and the resulting materials thereof, which are suitable for biomedical applications such as wound healing.

BACKGROUND OF THE INVENTION

[0004] Wound healing, or wound repair, is the body's natural process of regenerating dermal and epidermal tissue. The processes of wound healing are complex and fragile. Among these, the treatment of full-thickness burns continues to be one of the most challenging tasks in medicine. Patients sustaining full thickness injuries over a large percentage body surface area (BSA) often incur complications from eschars, which may lead to systemic bacterial infection, hypovolemia, hypothermia, hypoperfusion, and hemoglobinuria due to rhabdomyolysis and hemolysis. Currently, full thickness burn wounds are generally healed with minimal cicatrization by autologous skin grafting. Autologous skin grafting has limitations, however: Patients incurring full thickness burn wounds over 20% BSA are limited to either temporary stretched meshed allografts from cadavers or artificial dermal regeneration templates such as porcine xenografts and collagen coated semi-permeable synthetic membranes. Along with being immunologically incompatible with the patient, these substitutes induce healing with an acute distribution of wide irregular collagen bands resulting in an uneven grid-like surface and excessive hyperplastic, hypertrophic scarring.

[0005] Various synthetic and natural polymers may be used to develop wound dressing materials, for example, hydrolytically unstable synthetic aliphatic polyesters such as

poly(glycolic acid) (PGA), poly(L-lactic acid) (PLA) or natural-origin polymer such as chitosan. These polymers may suffer from side reactions or reduced performance, however, when subjected to the specific wound environment. For example, the acidity of the hydrolyzed bi-products of PGA or PLA polymers may inhibit full-thickness wound healing cascades; when immersed in an acidic wound environment, chitosan becomes soluble due to amine group protonation which can result in premature loss of mechanical integrity.

[0006] Hence, there is a need for new types of biomaterials that not only have improved biodegradability, biocompatibility and possess the wound healing properties of natural skin, but also have improved physical and mechanical properties, and satisfactory flexibility suitable for an effective wound dressing.

SUMMARY OF THE INVENTION

[0007] The present invention provides a process for production of silk blend mats. The process comprises the steps of blending a polyethylene oxide (PEO) with an aqueous silk fibroin solution; electrospinning the blended solution, thereby forming a silk protein/PEO blended mat; and constraint-drying the electrospun silk mat. A crystallization dish technique may be employed in the constraint-drying step. The process may further comprise the step of treating the electrospun silk mat in alcohol and/or water solution prior to or after the drying step. The alcohol may be methanol, ethanol, isopropyl alcohol (2-propanol) or n-butanol. The process may further comprise the step of extracting the PEO from the silk mat. PEO may be extracted from the silk mat by leaching in water. Additionally, the process may further comprise the step of embedding at least one active agent in the silk mat, such as a therapeutic agent or a biological material.

[0008] The present invention also provides for a silk material prepared by the process comprising the steps of blending a polyethylene oxide (PEO) with an aqueous silk fibroin solution; electrospinning the blended solution, thereby forming a silk protein/PEO blended mat; and constraint-drying the electrospun silk mat.

[0009] Some embodiments of the invention relate to a silk material embedding or encapsulating at least one active agent for dressing a wound to promote wound healing prepared by the process comprising the steps of blending a polyethylene oxide (PEO) with an aqueous silk fibroin solution comprising at least one active agent; electrospinning the blended solution, thereby forming a silk protein/PEO blended mat encapsulating the active agent(s); and constraint-drying the electrospun silk mat. Alternatively, the active agent(s) may be added to the silk fibroin after blending with PEO or added to the electrospun silk material, for example, the electrospun silk/PEO mats may be coated with the active agent(s).

[0010] The present invention also relates to an electrospun silk mat comprising at least a silk fibroin protein, where the content of the silk fibroin protein in the silk mat ranges from about 50 wt% to about 90 wt%, and the silk mat has a thickness of about 20 to 80 microns.

[0011] The present invention also relates to an electrospun silk mat comprising a silk fibroin protein and a polyethylene oxide (PEO). The electrospun silk mat has a silk fibroin protein/PEO blend ratio from 2:1 to 4:1, or silk percentage is about 75% w/w to 90% (w/w); and the silk mat has a thickness of about 20 to 80 about microns.

[0012] In one embodiment, the electrospun silk mat is as thin as about 20 to 30 microns.

[0013] In one embodiment, the electrospun silk mat has interconnected pores with the pore throat size surface area averaging from about 0.1 to about 1 micron.

[0014] The electrospun silk mats prepared by the processes of the invention exhibit good structural, morphological, biofunctional and biocompatible properties suitable for biomedical application, particularly wound dressing. For example, the resulting silk mats of the invention degrade more than about 86% weight in less than 14 days; the equilibrium water content of the silk mats of the invention is more than about 82%; the oxygen transmission rate of the silk mats is more than about 15460 cm³/m²/day; and water vapor transmission rate of the silk mats is more than about 1934 g/m²/day.

[0015] Some embodiments of the invention also relates to a method of promoting wound healing comprising contacting the wound with at least one constraint-dried electrospun silk mat comprising a silk fibroin protein, and optionally, at least one active agent. The electrospun silk mat has a silk fibroin content ranging from about 50 wt% to about 90wt%; and the silk mat has a thickness of about 20 to about 80 microns.

[0016] Some embodiments of the invention also relates to a method of promoting wound healing comprising contacting the wound with at least one constraint-dried electrospun silk mat comprising a silk fibroin protein, PEO, and optionally, at least one active agent. The electrospun silk mat has a silk fibroin/PEO blend ratio from about 2:1 to about 4:1 (or the silk fibroin percentage in the electrospun silk mat is about 75% w/w to 90% w/w, or the PEO percentage in the electrospun silk mat is about 10% w/w to about 25% w/w); and the silk mat has a thickness of about 20 to about 80 microns.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1A shows six electrospun silk mats with silk/PEO ratios of 4:1, 3:1, 2:1, 3:2, 7:6, and 1:1, corresponding to 86.5%, 82.8%, 76%, 70.6%, 65.1% and 61.5% w/w silk fibroin protein percentage for each material group, respectively. The 10 cm diameter silk mats

