The Complete Patents of Ananda M. Chakrabarty

By

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Ananda M. Chakrabarty was born in India on 4 April 1938. He attended Sainthia High School, Belur Bidyamandir and St. Xavier's College, Calcutta. Prof. Chakrabarty received his Ph.D. from the University of Calcutta in Kolkata, West Bengal in 1965.

Ananda M. Chakrabarty -Ph.D. is a microbiologist, scientist, and researcher, known for his most notable work in directed evolution and in developing a genetically engineered organism using plasmid transfer while working at GE.

Chakrabarty genetically engineered a new species of *Pseudomonas* bacteria ("the oil-eating bacteria") in 1971 while working for the Research & Development Centre at General Electric Company in Schenectady, New York. Till this time, four known species of oil-metabolizing bacteria were known to exist.

Chakrabarty discovered a method for genetic cross-linking that fixed all four plasmid genes in place and produced a new, stable, bacteria species (now called *Pseudomonas putida*) capable of consuming oil one or two orders of magnitude faster than the previous four strains of oil-eating microbes. The new microbe, which Chakrabarty called "**multi-plasmid hydrocarbon-degrading *Pseudomonas***," could digest about two-thirds of the hydrocarbons that would be found in a typical oil spill.

The bacteria drew international attention when he applied for a patent—the first-ever patent for living organism. He was initially denied the patent by the Patent Office because it was thought that the patent code precluded patents on living organisms.

Chakrabarty's landmark research has since paved the way for many patents on genetically modified micro-organisms and other life forms and catapulted him into the international spotlight.

He has isolated a bacterial protein, azurin, with potential antineoplastic properties.

In 2001, Prof. Chakrabarty founded a company, CDG Therapeutics, (incorporated in Delaware) which holds proprietary information related to five patents generated by his work at the University of Illinois at Chicago.

In 2008, Prof. Chakrabarty co-founded a second bio-pharmaceutical discovery company, Amrita Therapeutics Ltd., registered in Ahmedabad, Gujarat, to develop therapies, vaccines and diagnostics effective against cancers and/or other major public health threats derived from bacterial products found in the human body.

Chakrabarty is a Distinguished University Professor in the Department of Microbiology and Immunology in the University of Illinois at Chicago College of Medicine. Apart from being an eminent scientist, Ananda Chakrabarty has been an advisor to judges, governments, and the UN. As one of the founding members of a UNIDO Committee that proposed the establishment of the International Centre for Genetic Engineering & Biotechnology (ICGEB), he has been a member of its Council of Scientific Advisors ever since. He has also served the Stockholm Environment Institute of Sweden. He has been on the Scientific Advisory Board of many academic institutions such as the Michigan Biotechnology Institute, the Montana State University Centre for Biofilm Engineering, the Centre for Microbial Ecology at the Michigan State University, and the Canadian Bacterial Diseases Network based in Calgary, Canada. Chakrabarty has also served as a member of NIAG, the NATO Industrial Advisory Group based in Brussels, Belgium. He is a member of the Board of Directors of Einstein Institute for Science, Health and the Courts, where he participates in judicial education. More recently, he has been involved in international judicial work, serving as a Scientific Advisor for meetings in Hawaii and Ottawa, Canada,

organized by the Supreme Court of Canada. For his work in genetic engineering technology, he was awarded the **Padmashri** by the Government of India in 2007.

- **Cytotoxic factors for modulating cell death**
  Patent number: 10421801
  Abstract: Cytotoxic factors having use in modulating cell death, and their use in methods of treating necrosis or apoptosis-related conditions are disclosed. The invention also relates to methods for identifying active agents useful in treating conditions related to cell death or uncontrolled growth. The present inventors have found that different microorganisms produce different cytotoxic factor(s) having anticancer activity. The substantially pure cytotoxic factors can be used in a method of treating an infectious disease or a cancer.
  Type: Grant
  Filed: November 8, 2013
  Date of Patent: September 24, 2019
  Inventors: Ananda M Chakrabarty, Tapas K Das Gupta, Vasu Punj, Olga Zaborina, Yoshinori Hiraoka, Tohru Yamada
- **Cytotoxic factors for modulating cell death**
  Patent number: 10266573
  Abstract: Cytotoxic factors having use in modulating cell death, and their use in methods of treating necrosis or apoptosis-related conditions are disclosed. The invention also relates to methods for identifying active agents useful in treating conditions related to cell death. The present inventors have found that different pathogens produce different cytotoxic factor(s) having anticancer activity. The substantially pure cytotoxic factors can be used in a method of treating an infectious disease or a cancer.
  Type: Grant
  Filed: May 19, 2008
  Date of Patent: April 23, 2019
  Assignee: THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS
  Inventors: Ananda M. Chakrabarty, Tapas K. Das Gupta, Vasu Punji, Olga Zaborina
- **Transport agents for crossing the blood-brain barrier and into brain cancer cells, and methods of use thereof**
  Patent number: 10072049
  Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP. The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particular brain cancer.
  Type: Grant
  Filed: April 11, 2016
  Date of Patent: September 11, 2018
  Assignee: THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS
  Inventors: Chang Soo Hong, Tohru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **TRANSPORT AGENTS FOR CROSSING THE BLOOD-BRAIN BARRIER AND INTO BRAIN CANCER CELLS, AND METHODS OF USE THEREOF**
  Publication number: 20160289276

Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP. The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particular brain cancer.
Type: Application
Filed: April 11, 2016
Publication date: October 6, 2016
Inventors: Chang Soo Hong, Tohru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty

- **Transport agents for crossing the blood-brain barrier and into brain cancer cells, and methods of use thereof**
  Patent number: 9309292
  Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP. The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particular brain cancer.
  Type: Grant
  Filed: September 30, 2013
  Date of Patent: April 12, 2016
  Assignee: The Board of Trustees of the University of Illinois
  Inventors: Chang Soo Hong, Tohru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **Compositions and methods to control angiogenesis with cupredoxins**
  Patent number: 9107881
  Abstract: The present invention relates to compositions comprising cupredoxins, and their use to inhibit angiogenesis in mammalian cells, tissues, and animals, and particularly the angiogenesis that accompanies tumor development and particularly in humans. Specifically, the present invention relates to compositions comprising the cupredoxin(s), and or peptides that are variants, derivatives or structural equivalents of cupredoxins, which retain the ability to inhibit angiogenesis in mammalian cells, tissues or animals. These compositions may be peptides or pharmaceutical compositions, among others. The compositions of the invention may be used to treat any pathological condition that has as a symptom or cause, inappropriate angiogenesis, and particularly inappropriate angiogenesis related to tumor development.
  Type: Grant
  Filed: February 11, 2013
  Date of Patent: August 18, 2015
  Assignee: The Board of Trustees of the University of Illinois
  Inventors: Rajeshwari R Mehta, Brad N Taylor, Tohru Yamada, Craig W Beattie, Tapas K Das Gupta, Ananda M Chakrabarty
- **Cytotoxic Factors For Modulating Cell Death**

Publication number: 20140179617
Abstract: Cytotoxic factors having use in modulating cell death, and their use in methods of treating necrosis or apoptosis-related conditions are disclosed. The invention also relates to methods for identifying active agents useful in treating conditions related to cell death or uncontrolled growth. The present inventors have found that different microorganisms produce different cytotoxic factor(s) having anticancer activity. The substantially pure cytotoxic factors can be used in a method of treating an infectious disease or a cancer.
Type: Application
Filed: November 8, 2013
Publication date: June 26, 2014
Applicant: THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS
Inventors: ANANDA M. CHAKRABARTY, TAPAS K. DAS GUPTA, VASU PUNJ, OLGA ZABORINA, YOSHINORI HIRAOKA, TOHRU YAMADA

- **Transport agents for crossing the blood-brain barrier and into brain cancer cells and methods of use thereof**
  Patent number: 8545812
  Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP. The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particular brain cancer.
  Type: Grant
  Filed: May 25, 2012
  Date of Patent: October 1, 2013
  Assignee: The Board of Trustees of the University of Illinois
  Inventors: Chang Soo Hong, Tohru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **Compositions and methods to control angiogenesis with cupredoxins**
  Patent number: 8372962
  Abstract: The present invention relates to compositions comprising cupredoxins, and their use to inhibit angiogenesis in mammalian cells, tissues, and animals, and particularly the angiogenesis that accompanies tumor development and particularly in humans. Specifically, the present invention relates to compositions comprising the cupredoxin(s), and or peptides that are variants, derivatives or structural equivalents of cupredoxins, which retain the ability to inhibit angiogenesis in mammalian cells, tissues or animals. These compositions may be peptides or pharmaceutical compositions, among others. The compositions of the invention may be used to treat any pathological condition that has as a symptom or cause, inappropriate angiogenesis, and particularly inappropriate angiogenesis related to tumor development.
  Type: Grant
  Filed: February 27, 2012
  Date of Patent: February 12, 2013
  Assignee: The Board of Trustees of the University of Illinois
  Inventors: Rajeshwari R. Mehta, Brad N. Taylor, Tohru Yamada, Craig W. Beattie, Tapas K. Das Gupta, Ananda M. Chakrabarty

- **TRANSPORT AGENTS FOR CROSSING THE BLOOD-BRAIN BARRIER AND INTO BRAIN CANCER CELLS AND METHODS OF USE THEREOF**
  Publication number: 20130004431
  Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP. The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particular brain cancer.
  Type: Application
  Filed: May 25, 2012
  Publication date: January 3, 2013
  Applicant: The Board of Trustees of the University of Illinois
  Inventors: Chang Soo Hong, Tohru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **COMPOSITIONS AND METHODS TO CONTROL ANGIOGENESIS WITH CUPREDOXINS**
  Publication number: 20120196805
  Abstract: The present invention relates to compositions comprising cupredoxins, and their use to inhibit angiogenesis in mammalian cells, tissues, and animals, and particularly the angiogenesis that accompanies tumor development and particularly in humans. Specifically, the present invention relates to compositions comprising the cupredoxin(s), and or peptides that are variants, derivatives or structural equivalents of cupredoxins, which retain the ability to inhibit angiogenesis in mammalian cells, tissues or animals. These compositions may be peptides or pharmaceutical compositions, among others. The compositions of the invention may be used to treat any pathological condition that has as a symptom or cause, inappropriate angiogenesis, and particularly inappropriate angiogenesis related to tumor development.
  Type: Application
  Filed: February 27, 2012
  Publication date: August 2, 2012
  Inventors: Rajeshwari R. Mehta, Brad N. Taylor, Tohru Yamada, Craig W. Beattie, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **Transport agents for crossing the blood-brain barrier and into brain cancer cells, and methods of use thereof**
  Patent number: 8188251
  Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP (SEQ ID NO: 25). The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particularly brain cancer.
  Type: Grant
  Filed: October 4, 2010
  Date of Patent: May 29, 2012
  Assignee: The Board of Trustees of the University of Illinois

Inventors: Chang Soo Hong, Tohru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty

- **Compositions and methods to control angiogenesis with cupredoxins**
  Patent number: 8124055
  Abstract: The present invention relates to compositions comprising cupredoxins, and their use to inhibit angiogenesis in mammalian cells, tissues, and animals, and particularly the angiogenesis that accompanies tumor development and particularly in humans. Specifically, the present invention relates to compositions comprising the cupredoxin(s), and or peptides that are variants, derivatives or structural equivalents of cupredoxins, which retain the ability to inhibit angiogenesis in mammalian cells, tissues or animals. These compositions may be peptides or pharmaceutical compositions, among others. The compositions of the invention may be used to treat any pathological condition that has as a symptom or cause, inappropriate angiogenesis, and particularly inappropriate angiogenesis related to tumor development.
  Type: Grant
  Filed: June 3, 2009
  Date of Patent: February 28, 2012
  Assignee: The Board of Trustees of the University of Illinois
  Inventors: Rajeshwari R. Mehta, Brad N. Taylor, Tohru Yamada, Craig W. Beattie, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **TRANSPORT AGENTS FOR CROSSING THE BLOOD-BRAIN BARRIER AND INTO BRAIN CANCER CELLS, AND METHODS OF USE THEREOF**
  Publication number: 20110077387
  Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP (SEQ ID NO: 25). The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particularly brain cancer.
  Type: Application
  Filed: October 4, 2010
  Publication date: March 31, 2011
  Inventors: Chang Soo Hong, Tobru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **Cytotoxic factors for modulating cell death**
  Patent number: 7888468
  Abstract: Cytotoxic factors having use in modulating cell death, and their use in methods of treating necrosis or apoptosis-related conditions are disclosed. The invention also relates to methods for identifying active agents useful in treating conditions related to cell death or uncontrolled growth. The present inventors have found that different microorganisms produce different cytotoxic factor(s) having anticancer activity. The substantially pure cytotoxic factors can be used in a method of treating an infectious disease or a cancer.
  Type: Grant
  Filed: August 23, 2006
  Date of Patent: February 15, 2011
  Assignee: The Board of Trustees of the University of Illinois

Inventors: Ananda M. Chakrabarty, Tapas K. Das Gupta, Vasu Punj, Olga Zaborina, Yoshinori Hiraoka, Tohru Yamada

- **Transport agents for crossing the blood-brain barrier and into brain cancer cells, and methods of use thereof**
  Patent number: 7807183
  Abstract: The present invention discloses methods and materials for delivering a cargo compound into a brain cancer cell and/or across the blood-brain barrier. Delivery of the cargo compound is accomplished by the use of protein transport peptides derived from Neisseria outer membrane proteins, such as Laz. The invention also provides synthetic transit peptides comprised of the pentapeptide AAEAP (SEQ ID NO: 25). The invention further discloses methods for treating cancer, and specifically brain cancer, as well as other brain-related conditions. Further, the invention provides methods of imaging and diagnosing cancer, particularly brain cancer.
  Type: Grant
  Filed: July 19, 2006
  Date of Patent: October 5, 2010
  Assignee: The Board of Trustees of the University of Illinois
  Inventors: Chang Soo Hong, Tohru Yamada, Arsenio M. Fialho, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **COMPOSITIONS AND METHODS TO CONTROL ANGIOGENESIS WITH CUPREDOXINS**
  Publication number: 20100008919
  Abstract: The present invention relates to compositions comprising cupredoxins, and their use to inhibit angiogenesis in mammalian cells, tissues, and animals, and particularly the angiogenesis that accompanies tumor development and particularly in humans. Specifically, the present invention relates to compositions comprising the cupredoxin(s), and or peptides that are variants, derivatives or structural equivalents of cupredoxins, which retain the ability to inhibit angiogenesis in mammalian cells, tissues or animals. These compositions may be peptides or pharmaceutical compositions, among others. The compositions of the invention may be used to treat any pathological condition that has as a symptom or cause, inappropriate angiogenesis, and particularly inappropriate angiogenesis related to tumor development.
  Type: Application
  Filed: June 3, 2009
  Publication date: January 14, 2010
  Inventors: Rajeshwari R. Mehta, Brad N. Taylor, Tohru Yamada, Craig W. Beattie, Tapas K. Das Gupta, Ananda M. Chakrabarty
- **Compositions and methods to control angiogenesis with cupredoxins**
  Patent number: 7556810
  Abstract: The present invention relates to compositions comprising cupredoxins, and their use to inhibit angiogenesis in mammalian cells, tissues, and animals, and particularly the angiogenesis that accompanies tumor development and particularly in humans. Specifically, the present invention relates to compositions comprising the cupredoxin(s), and or peptides that are variants, derivatives or structural equivalents of cupredoxins, which retain the ability to inhibit angiogenesis in mammalian cells, tissues or animals. These compositions may be peptides or pharmaceutical compositions, among others. The compositions of the invention may be used to treat any pathological condition that has as a symptom or cause, inappropriate angiogenesis, and particularly inappropriate angiogenesis related to tumor development.
  Type: Grant

Filed: July 19, 2006
Date of Patent: July 7, 2009
Assignee: The Board of Trustees of the University of Ilinois
Inventors: Rajeshwari R. Mehta, Brad N. Taylor, Tohru Yamada, Craig W. Beattie, Tapas K. Das Gupta, Ananda M. Chakrabarty

- **CYTOTOXIC FACTORS FOR MODULATING CELL DEATH**
  Publication number: 20090137468
  Abstract: Cytotoxic factors having use in modulating cell death, and their use in methods of treating necrosis or apoptosis-related conditions are disclosed. The invention also relates to methods for identifying active agents useful in treating conditions related to cell death. The present inventors have found that different pathogens produce different cytotoxic factor(s) having anticancer activity. The substantially pure cytotoxic factors can be used in a method of treating an infectious disease or a cancer.
  Type: Application
  Filed: May 19, 2008
  Publication date: May 28, 2009
  Inventors: Ananda M. Chakrabarty, Tapas K. Das Gupta, Vas Punj, Olga Zaborina
- **Cytotoxic factors for modulating cell death**
  Patent number: 7491394
  Abstract: Cytotoxic factors having use in modulating cell death, and their use in methods of treating necrosis or apoptosis-related conditions are disclosed. The invention also relates to methods for identifying active agents useful in treating conditions related to cell death or uncontrolled growth. The present inventors have found that different microorganisms produce different cytotoxic factor(s) having anticancer activity. The substantially pure cytotoxic factors can be used in a method of treating an infectious disease or a cancer.
  Type: Grant
  Filed: November 24, 2003
  Date of Patent: February 17, 2009
  Assignee: The Board of Trustees of the University of Illinois
  Inventors: Ananda M. Chakrabarty, Tapas K. Das Gupta, Vasu Punj, Olga Zaborina, Yoshinori Hiraoka, Tohru Yamada

United States Patent [illegible]

[illegible]

[illegible] 4,299,434

[illegible] Mar. 31, 1981

[illegible]

[illegible] [illegible]

[illegible] General Electric Company, Schenectady, NY

[illegible] [illegible]

[illegible] [illegible]

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FIG. 1

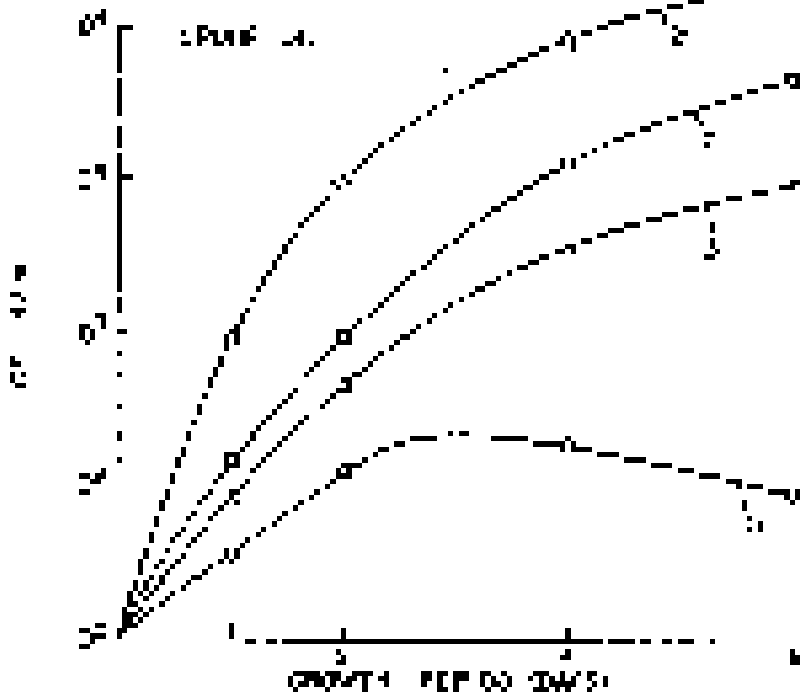
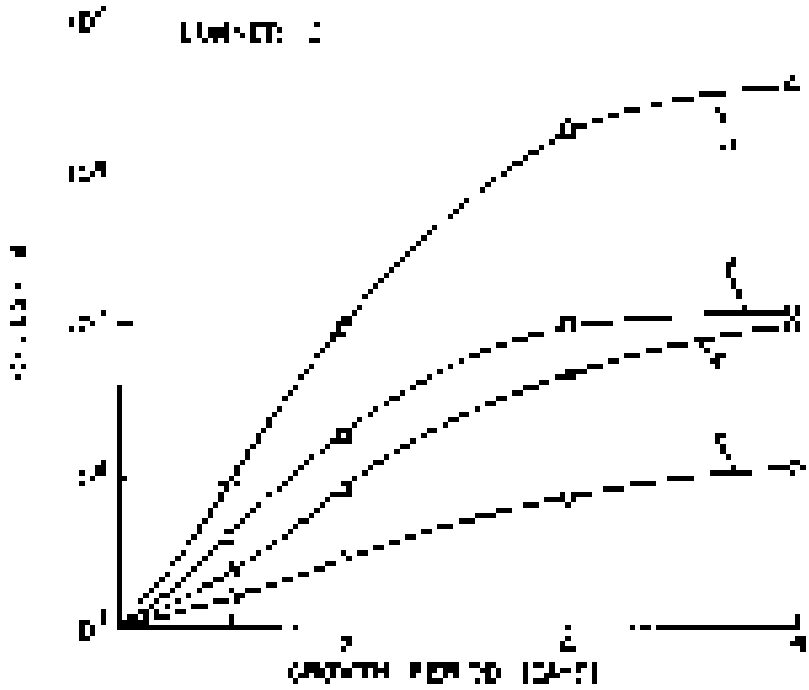


FIG. 2



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BACKGROUND OF THE INVENTION

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TABLE 1

TABLE I-continued

[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]
		[illegible]	[illegible]
[illegible]	[illegible]		
	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]
		[illegible]	[illegible]

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TABLE II

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]		

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[illegible]

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TABLE 13

Plant	Variety	Seeds per Pod	Seed Weight (mg)	Yield (kg/ha)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]

TABLE 13

Plant	Variety	Seeds per Pod	Seed Weight (mg)	Yield (kg/ha)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
			[illegible]	[illegible]
			[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
			[illegible]	[illegible]
			[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
			[illegible]	[illegible]
			[illegible]	[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

TABLE V

[illegible]

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TABLE VI

[illegible]

[illegible]

[illegible]

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[illegible]

[illegible]

TABLE VII

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

[illegible]

United States Patent [illegible]

[illegible]

[illegible] 3,923,[illegible]

[illegible] Dec. 2, 1975

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OTHER PUBLICATIONS

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ABSTRACT

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TABLE 1

Strain Designation	Phenotype	Plasmids	Reference
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]		[illegible]
[illegible]	[illegible]		[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]		
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

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[illegible]

TABLE [illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

TABLE [illegible]—Continued

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

TABLE [illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE [illegible]

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

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BEST MODE CONTEMPLATED

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(54) Process for the production of a bacterial preparation suitable for use as a feed additive for animals, and a feed additive comprising the bacterial preparation.

(73) Proprietor: [illegible]

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(72) Inventor: [illegible]

(74) Representative: [illegible]

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EP 0 161 511 B1

Description

[illegible]

Background of the Invention

[illegible]

Summary of the Invention

[illegible]

Brief Description of the Drawings

[illegible]

Detailed Description

[illegible]

[illegible]

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[illegible] Type Culture Collection [illegible]

[illegible]

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[illegible]

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[illegible]

[illegible]

[illegible]

TABLE 1

BIODEGRADATION OF ORGANIC SOLVENTS THEN WITH
BY SOIL 1 THE STRAIN

Solvent	Absorbance at 540 nm at different times after biodegradation				
	30 min.	2 hrs.	4 hrs.	8 hrs.	22 hrs.
Hexane	0.35	0.07	0.34	0.03	0.045
Octane	0.17	0.07	0.34	0.03	0.015
Decane	0.135	0.215	0.[illegible]3	0.075	0.355
Undecane	0.15	0.[illegible]	0.[illegible]	0.11	0.355
Pentadecane	0.295	0.295	0.[illegible]	0.045	0.358
Heptadecane	0.50	0.535	0.[illegible]	0.12	0.065
Benzene	1.5	1.45	1.45	1.1	1.26
Toluene	1.5	1.5	1.47	1.15	1.17
Xylene	2.0	1.65	1.17	1.12	1.37
Chlorobenzene	1.36	1.24	1.12	1.17	1.2
Dichloromethane	0.12	1.08	—	—	—

[illegible]

[illegible]

Table 2

Reduction of Surface Tension of Water
by Emulsifier Produced by Growth of SD-1 and SB-30
On Different Substrates

Strain	Nutrient Source	Amount of Emulsifier (g/l)	Surface Tension of Water (dynes/cm)
—	—	—	74
NH-1	Hexadecane	20	46
NH-1	Glucose	10	5[illegible]
NH-32	Glucose	1	[illegible]
NH-32	Hexadecane	1	[illegible]
SB-33	Corn oil (LB)	5	46
SD-33	Dodecane	[illegible]	46 [illegible]
SB-30	Butanol	5	37
SB-30	Tetradecane	5	31
SB-30	Tetradecane	5	27
SB-30	DA + Hexadecane	5	32

[illegible]

Claim

[illegible]

References cited

[illegible]

Patentansprüche

[illegible]

FIG. 1

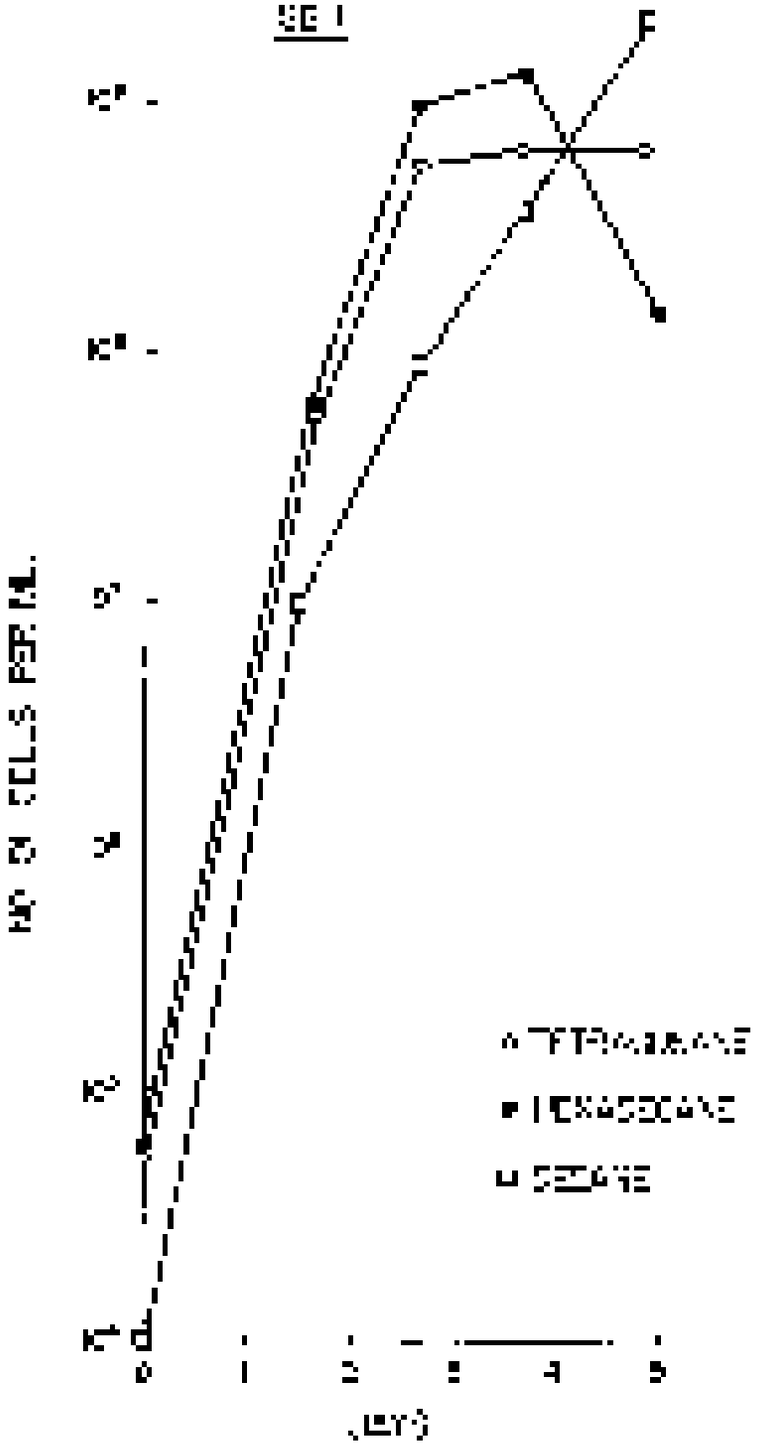


FIG. 2

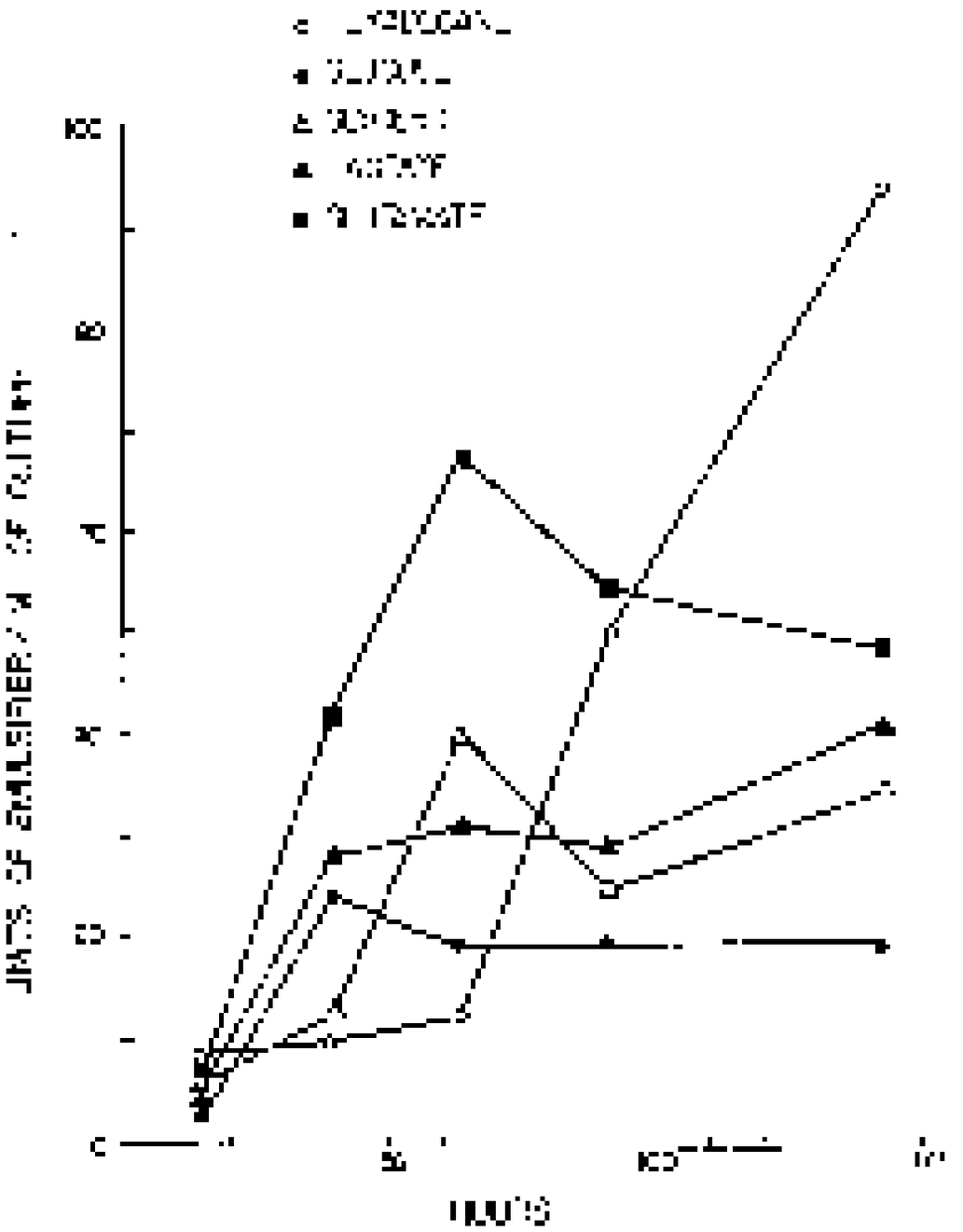


FIG. 3

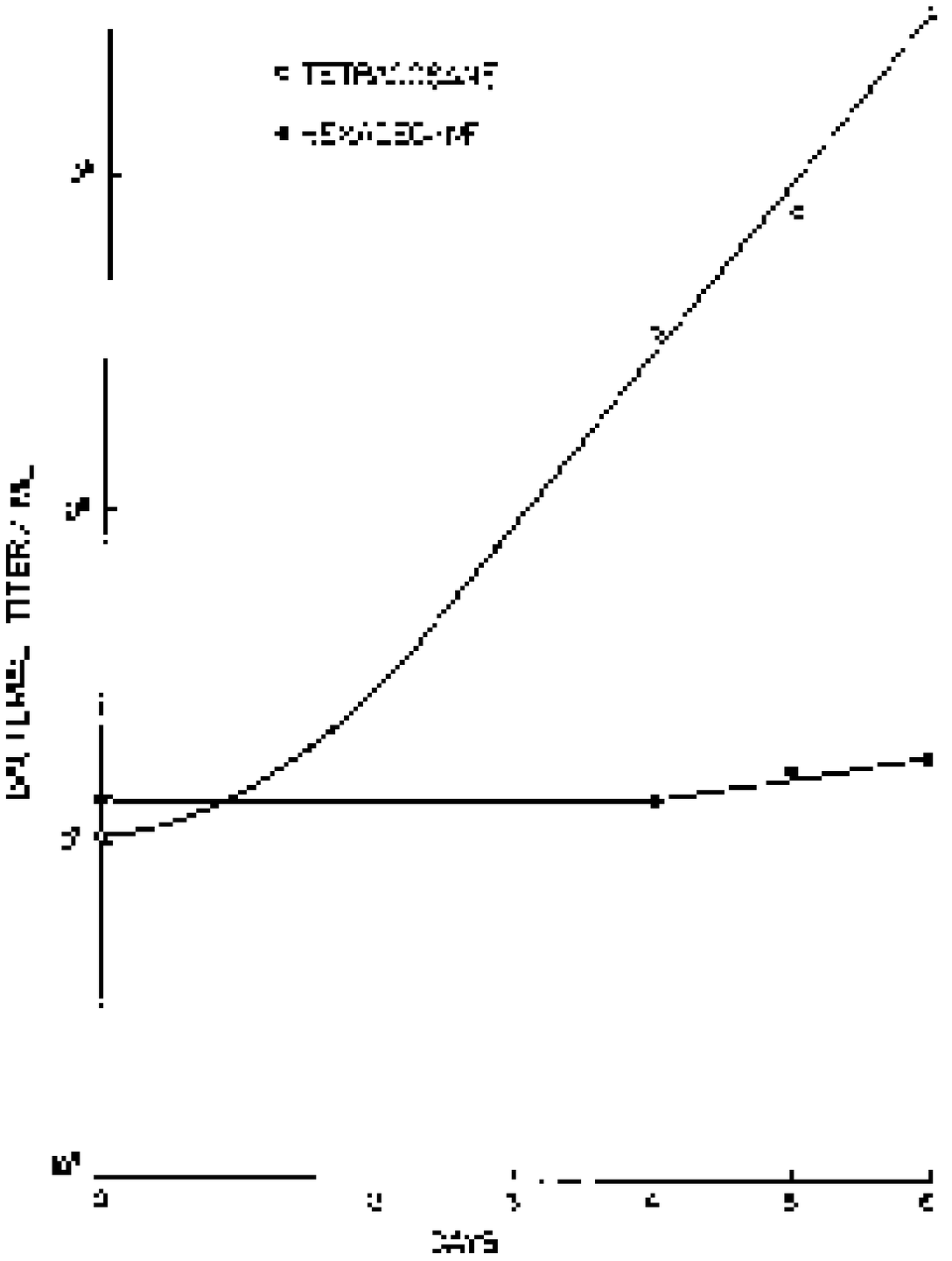
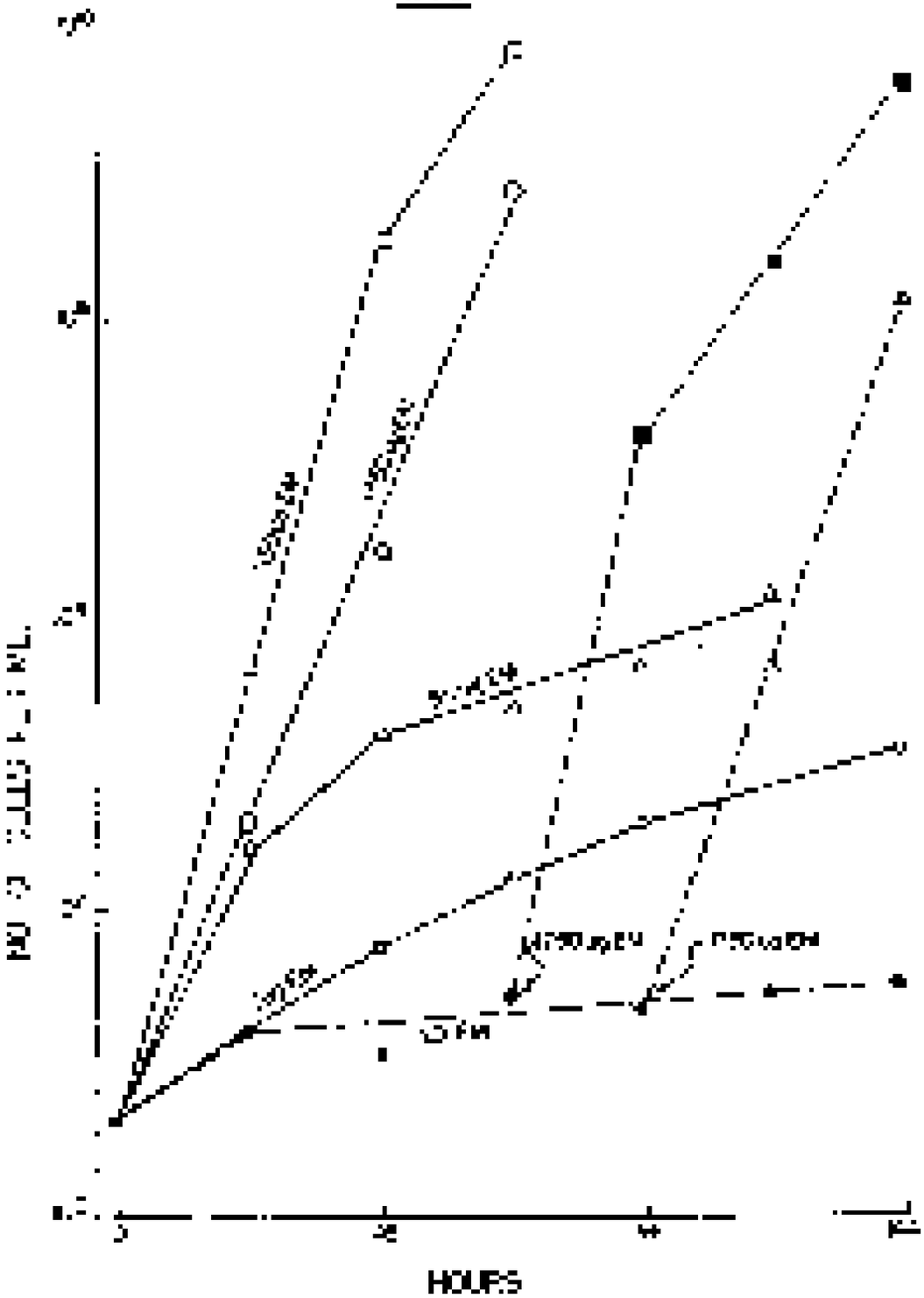
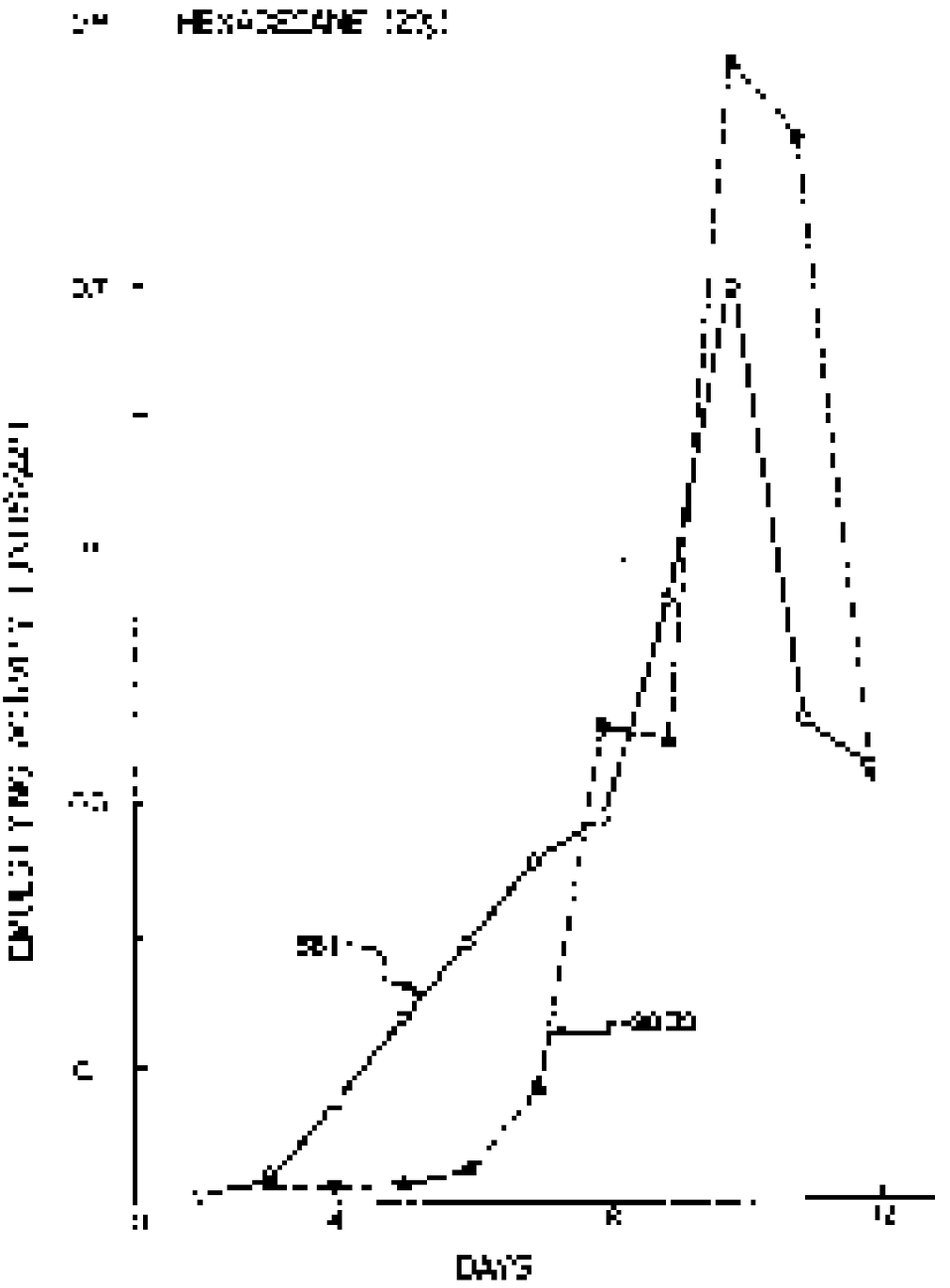
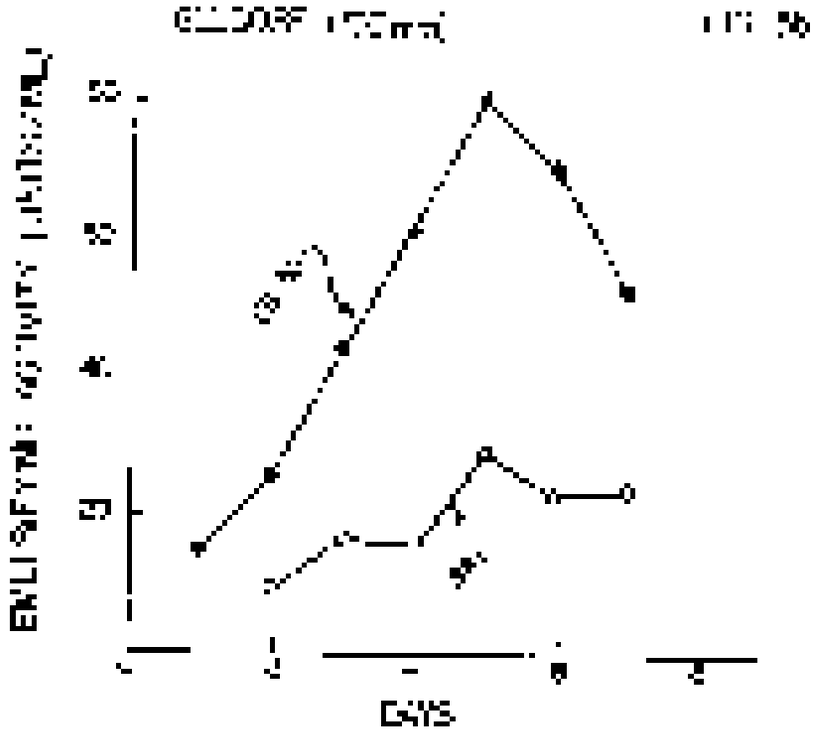


FIG. 4




HEXADECANE (2%)
DAYS


DAYS

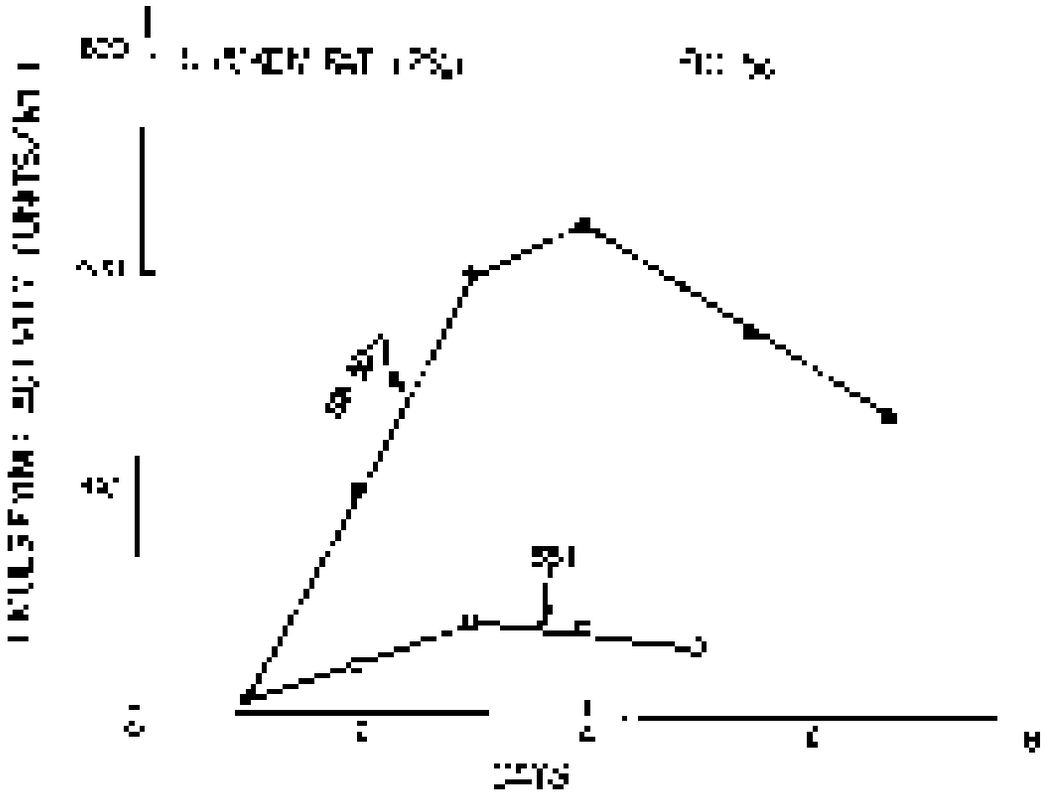
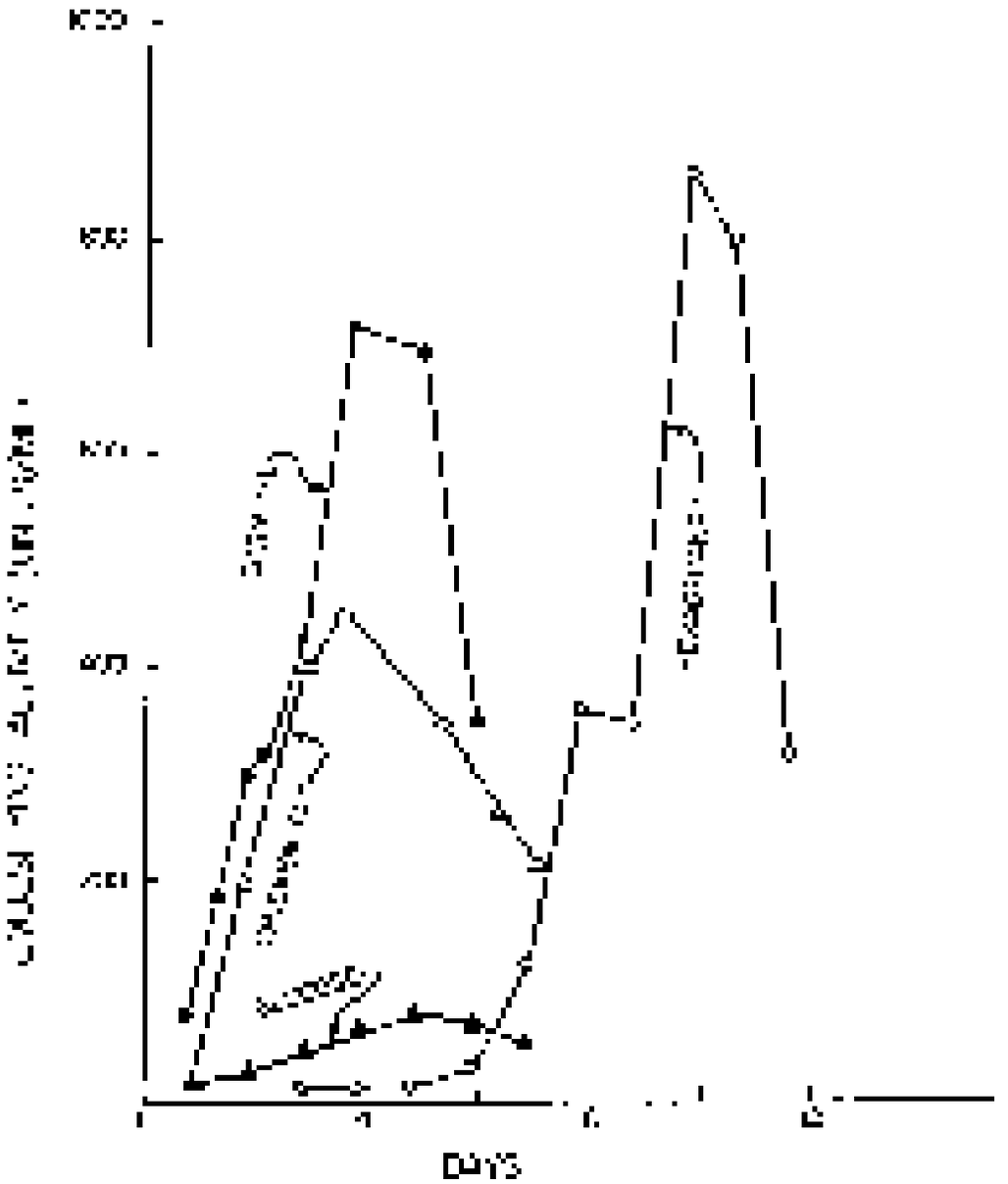

DAYS

FIG. 6



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— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: A NOVEL BIOMEDICAL DEVICE FOR CANCER THERAPY

(57) Abstract: The present invention provides a device comprising stent or catheter containing biofilms or suspension, of microorganisms selected from *Pseudomonas aeruginosa, Streptococcus, Staphylococcus, Salmonella, Clostridia, Mycobacterium bovis* BCG useful in cancer therapy. The microorganisms may be attenuated in their virulence factors and with cloned genes encoding specific proteins with anticancer activity. The microorganism-containing devices may be encased in membranes that allow diffusion of proteins with molecular masses of 25, 50, 75 or 100 kDa but not live organisms *per se.*

BIOMEDICAL DEVICE FOR CANCER THERAPY.

FIELD OF THE INVENTION:

The present invention is in the field of medical devices. Particularly, the invention relates to in-dwelling therapeutic device. The therapeutic device is basically a drug eluting device and may be a stent or a catheter. The invention further relates to a therapeutic device having microorganisms harboured therein, useful in cancer therapy. The microorganisms may be attenuated in their virulence factors and with cloned genes encoding specific proteins with anticancer activity. The microorganisms in **suspension** or biofilm form in the devices may be encased in membranes that allow only diffusion of proteins with molecular masses of 25, 50, 75 or 100 kDa but not live biofilm organisms *per se*. The device of the present invention is intended to deliver drugs of microbial origin directly to the tumor site without reaching the blood stream.

BACKGROUND OF THE INVENTION:

Live bacteria that have been used for treating cancers, primarily in animal models, are strictly anaerobic such as *Clostridia* and *Bifidobacteria* or facultative anaerobic such as *Listeria monocytogenes* or *Salmonella typhimurium* (1, 2). Al-R strain of *S. typhimurium* had significant tumor regressing effect, such as that of orthotopic human pancreatic tumors, in nude mice. In addition, Al-R strain was also shown to significantly reduce the metastasis of lung and high grade osteosarcoma in nude mice as well as spinal cord gliomas (3, 4). Genetically improved avirulent mutants had also been shown to retain the tumor targeting properties (4) and will be excellent candidates for further testing in humans. However, so far, earlier phase I human clinical trials have not shown significant effects in colonization or cancer regression in metastatic melanoma, due to certain toxicity associated with injections of live bacteria in humans (5). Such injections trigger both innate and adaptive immune responses for clearance of the foreign bacteria, thereby enhancing side effects in already debilitated patients.

The reasons bacteria such as *Salmonella, Listeria, Clostridia* and others allow cancer regression are believed to be due to the activation of the immune system elicited by the bacteria as well as active growth of the bacteria in the hypoxic regions of the core of the tumors. Usually, virulence deletion mutants are rapidly cleared from the host compared to the wild type. So lack of toxicity in the blood stream has a negative effect on the retention and proliferation of live bacteria in the host tumors. For example, mutants attenuated in actA and plcB genes in *Listeria monocytogenes* have been shown to have a reasonable safety profile in adult volunteers (5), although no tumor regression effects were studied.

Phase I human clinical trials of a live, attenuated *L. monocytogenes* strain in 15 patients with recurrent metastatic squamous cell carcinoma of the cervix at doses of $1X10^9$, $3.3X10^9$ or $1X10^{10}$ cfu showed toxicity with flu-like syndrome such as fever, chills, nausea and/or vomiting/headache (6). Such symptoms are typical of administration of IL-2, indicating the involvement of innate immune response to the i.v. infusions of live bacteria.

The use of *Clostridia* in cancer therapy has shown efficacy in tumor regression, but with considerable toxicity. Consequently, *Clostridial* strains have been used more as diagnostic agents for detection of tumors because of their high specificity for targeting tumor cells (7).

A very promising *Clostridial* strain, *C. novyi-NT* (8) was used as spores in phase I human clinical trials in 2006 (NCT 00358397, www.clinicaltrials. gov), but the study was suspended for safety issues, again demonstrating the problem of using live bacteria through the i.v. or i.m. route.

From the above discussion, it is interesting to note that most of the efforts, lasting for more than 60 years, for cancer therapy basically involve anaerobic bacteria. Yet, the most successful practical use of bacteria in cancer therapy involves the use of an aerobe, *Mycobacterium bovis* BCG, the vaccine strain for tuberculosis,

to treat superficial urothelial carcinoma of the bladder. Live BCG cells are administered through a urethral catheter directly into an emptied bladder. The catheter is then removed, leaving the live bacteria in the bladder, which then induce an immune reaction in the bladder, leading to tumoricidal activity (9, 10). However, BCG therapy for bladder cancer is associated with a variety of complications, ranging from minor cystitis to life-threatening BCG sepsis, occurring in up to 90% of patients (11, 12).

As of today, there are no live bacteria, other than *M. bovis* BCG which has a very limited application only in superficial bladder cancer therapy, that have gone past phase III human clinical trials successfully and none is on the horizon, given the toxicity associated with intravenous injections of live bacteria in the blood stream evoking strong immune reaction. Attenuated bacteria, with or without cloned genes, get cleared by the immune system quickly, thereby reducing their effectiveness and staying power in the tumor vicinity.

A major problem in the application of live bacteria in cancer therapy (1, 2) is a lack of understanding of how bacteria actually cause tumor regression. Spectacular antineoplastic effect, when *C.* [illegible]-NT was used along with mitomycin C and dolastatin-10, instead of just mitomycin C and dolastatin-10, certainly points out to the active involvement of bacteria in tumor regression and lysis (13). Similarly, the exquisite targeting of tumor and growth in the core of the tumors is known to be a hallmark of *Salmonella*'s ability to cause tumor shrinkage (2, 4, 14). However, such therapies are often known as immunotherapy because the tumor regression is believed to be due to bacteria's preferential growth in the hypoxic core of the tumor, thereby depriving the tumor cells of nutrients, and eliciting immune action that causes tumor regression.

Do certain bacteria actually fight tumors by elaborating anticancer agents, rather than simply targeting tumors or invoking a strong immune response? indeed, an enzyme arginine deiminase (ADI) produced by *Mycoplasma arginini* has been

shown as early as in 1990 to have anti-tumor activity (15). Since then, many studies including phase I human clinical studies, have been performed with ADI and a polyethylene glycol (PEG)-conjugated ADI, termed ADI-PEG20, in patients with hepatocellular carcinoma and malignant melanoma (16). In general, such results have shown modest anticancer effect of ADI-PEG20 in such patients with tolerable side effects and the clinical trials are continuing with additional patient recruitment (16). ADI action is believed to be due to depletion of arginine in such cancer cells as hepatocellular carcinoma, melanoma or renal cell carcinoma which do not express arginino-succinate synthetase in vivo,

The successful use of a bacterial protein, ADI, rather than live bacteria in human clinical trials raises an interesting question: do other bacteria produce similar proteins or small molecule compounds to fight cancer? Arginine deiminase is not unique to *Mycoplasma,* including M *arginini.* The ADI from *Pseudomonas aeruginosa,* an aerobic opportunistic pathogen, has also been shown to have anticancer activity against a range of cancers such as fibrosarcoma, breast and ovarian cancers (17). Most interestingly, a 17 kDa truncated N-terminal part of ADI, called Pa-CARD because it harbors a caspase recruitment domain (CARD), has even higher anticancer activity than ADI in such cancers and in liquid-borne cancers such as chronic myeloid leukemia (CML) or acute myeloid leukemia (AML) cell lines (17; 18).

Pseudomonas aeruginosa, not previously known or tried in any live cell anticancer drug development, not only produces ADI, but another protein with significant anticancer activity called azurin (19). Azurin has not only anticancer activity against a range of cancers, but also strong growth suppressing activity against viruses such as the HIV/AIDS virus HTV-1 or parasites such as the malarial parasite *Plasmodium falciparum* or the toxoplasmosis parasite *Toxoplasma gondii* (19). Most interestingly, azurin, which is an intracellular periplasmic protein, is secreted by *P. aeruginosa* when *P. aeruginosa* cells are exposed to cancer cells, suggesting that azurin is a weapon that *P. aeruginosa* uses

to keep cancer cells in check. ***Pseudomonas aeruginosa*** is a biofilm-forming extracellular pathogen that prefers to establish long term residence in human tissues without causing much harm to the host normal cells but becomes very protective of the host as its habitat and has developed promiscuous protein weapons to target other invaders of the human body such as cancers, viruses and parasites (19). A similar azurin-like protein, termed Laz, is produced by ***gonococci/meningococci*** which also retains such activity against cancers, HIV-1, ***P.falciparum*** and ***T. gondii*** (19). A 28 amino acid peptide derived from azurin, azurin 50-77 termed p28, has no toxicity or immunogenicity in animals, including non-human primates (20). In phase I human clinical trials in Chicago, p28 (NSC 745104, www.clinicaltrials.gov) has shown no side effects in 10 advanced cancer patients where no drug is working and who have an average life expectancy of 8 weeks. p28 has shown significant beneficial effect in such patients in a dose dependent manner without demonstrating any side effects.

The therapeutic efficacy of azurin in mouse xenograft models, and the lack of side effects of the p28 peptide with anticancer activity in both animal and phase I human clinical trials raises an important question: can live cells of P. ***aeruginosa*** fight cancer similar to the anaerobic or facultative anaerobic bacteria such as ***Clostridia, Salmonella,*** etc, as mentioned earlier? As a known pathogen, ***P. aeruginosa,*** even attenuated strains lacking its major virulence factors such as Exotoxin A, elastase, and other toxins such as ExoS, ExoT and ExoU, that are injected in host cells by a type III secretion mechanism, will evoke strong immune response resulting in considerable toxicity when given intravenously. A unique feature of many pathogenic bacteria such as ***P. aeruginosa, Streptococcus*** and many others is their ability to form biofilms which allows surface adhesion of such bacteria on biotic or abiotic surfaces and acquisition of enough nutrients to allow a slow mode of growth. Indeed, biofilm formation of ***P. aeruginosa*** in both the lungs of cystic fibrosis patients and in-dwelling medical devices is well known for its pathogenesis, allowing the biofilm bacteria to resist both immune attack and antibiotic treatment. It is also conceivable that ***P. aeruginosa*** biofilms in an

in-dwelling medical device such as a catheter or stent inserted near a tumor can keep the tumor growth in check through elaboration of weapons such as azurin. This approach can contribute to effective cancer therapy, alone or in combination with common anticancer drugs.

Since azurin is a small 14 kDa protein secreted in presence of cancer cells, other pathogenic bacteria with long term residence in human bodies that can secrete small (less than 25 to 30 kDa) protein with anticancer activity may proe to be a potential component for cancer treatment. Having access to such bacterial protein weapons, with inhibitory activity against a range of cancers, viruses, parasites and pathogenic bacteria, perhaps even multiply drug resistant ones, will not only provide us with promiscuous, multi-disease-targeting drugs, but will provide a general principle of method development for isolation of such potential drugs. Indeed, we have recently described the isolation of a 17 kDa protein, MPT63, produced and secreted by *Mycobacterium tuberculosis* and *M. bovis* BCG, and a 30 amino acid peptide derived from MPT63, termed MB30, that, similar to azurin-p28, have strong anticancer activity against a range of cancers such as bladder, colon, etc.. Similar to azurin, MPT63 demonstrates promiscuity by strongly inhibiting the growth of the HIV/AIDS virus HIV-1. The production of such a protein weapon by *M. bovis* BCG, widely used in the treatment of superficial bladder cancer as mentioned earlier (9, 10), clearly suggests that bacterial regression of cancer is not just due to growth inside tumors or induction of an immune response, as is widely believed (24), but is due to active participation by the microorganisms in secreting or exposing on the surface various protein or other weapons to suppress invasion and growth of a variety of invaders of the human body including cancers, viruses, parasites and pathogenic bacteria/fungi. It is thus quite possible that anaerobic/facultative anaerobic bacteria such as *Clostridia, Salmonella,* etc, mentioned earlier, may produce and secrete similar weapons to fight cancers and other invaders. This kind of an approach will allow them to be used as biofihns on catheters/stents or other in-dwelling devices and inserted in the vicinity of the tumor, singly or in combination with other in-

dwelling medical devices harboring other types of bacteria as biofilms. The protein weapons can also be isolated and used as diagnostic agents and/or therapeutic compounds. The in-dwelling devices containing the biofilms of ***P. aeruginosa, M. tuberculosis/M. bovis, Clostridia, Salmonella,*** etc, either wild type or attenuated by mutations/deletions in various genes contributing to their virulence, can be used as is, or preferably encased by membrane filters with pore sizes that allow diffusion of only molecules with a mass of 25 kDa or 40 kDa. For example, a catheter with attenuated ***P. aeruginosa*** harboring deletions in exotoxin A, elastase (lasA/lasB) and type III secretion systems (but with or without hyperexpression of the azurin gene), and encased by a membrane filter with a cut-off molecular mass of 25 kDa will allow secretion and diffusion of azurin to reach the tumor but not any residual toxins with molecular mass higher than 25 kDa. In none of the cases, the biofilm bacteria can escape from the catheter to reach the blood stream but remains very close to the tumor to sense its presence. Such an approach greatly reduces any toxicity associated with an immune response, as is normally observed with live bacteria given orally, intravenously or by other means. Multiple catheters with multiple different bacterial biofilms will produce a synergistic effect without concomitant side effects, in absence or in presence of treatments with other drugs.

OBJECTIVES OF THE INVENTION:

The main objective of the present invention is to provide a biomedical device for cancer therapy.

Specifically the objective of the invention is to provide an in-dwelling or inserted / catheterized therapeutic device with regulated delivery of drug of microbial origin. Further, neither the microbes nor the drug enter the blood stream. Thus, the invention provides a device with novel drug delivery system.

The other objective is to provide a therapeutic device that may be a stent or a catheter.

Yet another objective is to provide a therapeutic device with microorganisms harboured therein in the form of **suspension** or biofilms useful in cancer therapy.

The device may be made of any physiologically acceptable material comprising plastic, ceramic, wood, metal, polymer (natural or synthetic) or hydrogel.

Still another objective is to provide a therapeutic device containing live *Pseudomonas aeruginosa biofilms. Pseudomonas aeruginosa* may be wild type, or virulence compromised or mutated to reduce toxin and/or cloned with the azurin gene under a strong promoter to hyper express the desired protein or derivative thereof..

The mutations may be in genes encoding type III secretion and Exotoxin A and the elastase genes las A/las B that are important virulence factors for *P. aeruginosa.* For other biofilms comprising of other bacteria such as *Salmonella, Clostridia, M. bovis* BCG, etc, in the in-dwelling device, appropriate attenuated strains may be used.

Yet another objective is to insert a therapeutic device at the tumor site wherein the biofilm microorganisms be encased in a membrane surrounding the device. The membrane may be provided with pore sizes capable of preventing diffusion of macromolecules with masses greater than 25 KDa to 40 kDa. Such membranes with defined pore sizes will prevent the release of any bacteria from the biofilm to the blood stream. It is to be noted that while biofilm bacteria are resistant to antibiotics or immune attack, in the absence of membrane encasing, any released bacteria from the device will be susceptible to antibiotics or immune attack, facilitating their clearance.

SUMMARY OF THE INVENTION:

Accordingly the present invention provides a biomedical device for cancer therapy comprises indwelling medical device encased in a filter with cut off molecular mass of 25 kDa, the said device harbor live microorganisms selected from *Pseudomonas aeruginosa, Streptococcus, Staphylococcus, Salmonella, Clostridia Mycobacterium bovis* BCG.

In one of the embodiment, the device may be a stent or catheter or any other similar means that can be implanted at tumor site.

In other embodiment, the device could be made of any physiologically acceptable biocompatible material that will not hamper the growth of the microorganism and selected from plastic, wood, polymer (natural or synthetic) or hydrogel.

In yet other embodiment, the therapeutic device may harbor suspension or biofilms of microorganism either wild type or attenuated or modified, wherein the organisms preferably be *Pseudomonas aeruginosa. Pseudomonas aeruginosa* may be virulence compromised or mutated to eliminate toxin production, and cloned with azuringene under a strong promoter to hyperproduce azurin protein.

The mutations may be in genes encoding type III secretion and Exotoxin A and the elastase genes las A/las B.

The biofilms may be composed of different sets of bacteria such as *Salmonella, Clostridia, M. bovis* BCG, etc. Such medical devices can be used in conjunction with other traditional anticancer drug treatment.

DETAILED DESCRIPTION:

There are emerging reports on the use of live and virulence attenuated bacteria for the treatment of cancers. Few bacterial species such as *Salmonella, Clostridia, Mycobacterium bovis,* when injected intravenously, intramuscularly or other

means, have the ability to enter into the human tumors and allow tumor regression. *Mycobacterium bovis* BCG is used in the treatment of superficial bladder cancer and is thought to induce cancer regression through activation of the immune system.

However, there is no report on the use of live wild type or attenuated *Pseudomonas aeruginosa* for the treatment of cancers.

In prior art, the organisms being administered parenterally via injections or orally pose problem in reaching and targeting the poorly vascularized tumors, which have no or low level oxygen. Additionally, eradication of the disseminated/migrated organisms to other parts of the body is another problem. Further, these pathogenic organisms, even when attenuated, evoke strong immune response, leading to toxicity problems.

The biomedical device of the present invention having confinement of microorganisms encased in the membrane of the device eliminates the problem of dissemination in other parts of the body or blood stream and toxicity development. Further the right choice of microorganism, attenuated *Pseudomonas aeruginosa* with mutations/deletions in various toxin genes helps in solving certain problems of accumulation of toxins.

The novel device of the present invention is a novel drug delivery system (NDDS) that provides the benefits of protein / peptide therapy via a combination medical device. The device is a drug eluting oncology stent a first-ever combination medical device designed to deliver in situ microbial therapy directly to the tumor site without reaching the blood stream. This revolutionary approach to cancer therapy will provide an important alternative or adjunct treatment to surgery, radiation or chemotherapy, particularly for inoperable or hard to reach cancer tumors.

The device preferably encases a biofilm in a membrane filter, enabling secretion and diffusion of bacterial proteins/peptides to reach the tumor and blocking release of any residual toxins with molecular mass higher than 25 kDa. The

concentration of anticancer molecule released from bacteria to the tumor site allow tumor regression effectively, while the device will prevent the bacterium as well as the toxic molecules having size more than 25 kDa to be released thereby minimizing risk of potential side effects of using live bacterium in cancer therapy. A range of cancers may be treatable by this method, either as an alternative or adjunct to surgery, radiation or chemotherapy.

The device was tried in preclinical trials wherein the experiments were conducted on nude mice. The two experimental nude mice (Figures 1 A, B) were subcutaneously injected with one million cells of human breast tumor (cancer cell line MCF-7) to instate infection. After 5 days, when both the mice showed small visible tumors, a device of the present invention (drug eluting stent) harboured with *Pseudomonas aeruginosa* was inserted in the experimental mouse, while the other (control) mouse was devoid of such stent. The sizes of tumors in both mice were measured twice a week by an electronic caliper over a period of five weeks. Such measurements demonstrated that the size of the tumor in the experimental mouse (with catheter) was about half the size of the tumor in the control mouse up to about three and a half weeks. Most surprisingly, the tumor in the experimental mouse went necrotic and burst after about three and a half weeks (Figure IB, experimental mouse) while the tumor in the control (without any catheter) mouse kept growing (Figure 1A, control mouse). At about 5 weeks, while the tumor in the control mouse kept growing and the mouse was extremely lethargic and sick, the necrotic tumor and the wound surrounding it of the experimental mouse gradually healed (Figure 1C, experimental mouse). This mouse exhibited good mobility and activity like any mouse without tumor.

The device was also tested on human subjects with high grade prostate cancer. Insertion of a catheter containing pseudomonas proved to be promising in regression of tumor

The applicant had recently described the production of anti-cancer proteins, or peptides derived from microorganisms,, such as **azurin,** arginine deiminase (ADI), Pa-CARD and azurin-p28 that allow tumor regression both *in vitro* and *in vivo* in mice (1, 17, 18, 19). Although, as described earlier, live bacteria such as *Clostridia, Salmonella,* etc., have been shown to allow tumor regression, there is no report on the use of live or attenuated *Pseudomonas aeruginosa* for the treatment of cancers. *Pseudomonas aeruginosa* is known to form biofilms on the abiotic or biotic surfaces and can grow under microaerophilic conditions. The use of a stent, catheter or other similar therapeutic device that contains biofilm bacteria of the wild type or virulence compromised *P. aeruginosa* strains may provide potential and viable treatment of cancers in humans. The stent, catheter, hydrogel or other similar therapeutic device containing *P. aeruginosa* is administered in the human body at the site of the tumors or near to the tumors where the anticancer proteins or small molecules secreted by the bacterium allow regression of solid tumors. The use of wild type or virulence compromised *P. aeruginosa* is an excellent way to treat a variety of human cancers. For example, a patient with prostate cancer may have a catheter containing *P. aeruginosa* biofilms in the urovesicular region near the tumor. While the *P. aeruginosa* persists in the catheter, the patient's condition will be relatively stable with the tumor either stabilized or undergoing shrinkage. Additional therapy with anticancer drugs will help in the complete elimination of the tumor.

Further, it is clear that such a stent, catheter, or similar therapeutic device with *P. aeruginosa* also may be encased in membranes with pore sizes that allow diffusion of only 25 Kda to 40 kDa macromolecules. Because the *P. aeruginosa* toxins such as Exotoxin A or ExoS/T are larger than 40 Kda, the live cells of *P. aeruginosa* or their released toxins cannot come out of the catheter to enter the tumors or surrounding tissues, leaving the release of low molecular weight anticancer proteins such as azurin to fight cancer.

Here, we focus on the use of plastic, ceramic, wooden or metal stent, catheter, or similar therapeutic device containing a biofilm of the live bacterium *Pseudomonas*

aeruginosa that is either wild type or genetically modified to eliminate its pathogenicity and to be inserted at the site of the tumors to allow tumor regression in cancer patients.

The strain of the *P. aeruginosa* in the biofilm could be laboratory or clinical isolates of wild-type bacteria or laboratory-derived mutants of such bacteria defective in the production of toxins such as Exotoxin A, Elastase or toxins elaborated through the type III secretion system (ExoS, ExoT, ExoU, etc). *P. aeruginosa* may have cloned azurin gene under a strong promoter to hyperexpress azurin gene with its signal sequence to allow secretion of the azurin.

Other biofilm-forming bacteria such as *Streptococcus, Staphylococcus, Mycobacterium bovis* BCG, etc, also may be used in place of P. *aeruginosa.*

The cancers to be treated by the live cells of *P. aeruginosa* or other biofilm-forming bacteria as part of catheters or stent surfaces are prostate, melanoma, sarcoma, breast, lung, ovarian, kidney, cervical, liver, bladder, colon, pancreas or other tumor types. The use of catheter/stent or similar therapeutic device containing *P. aeruginosa* or other bacterial biofilms inserted at the site of the tumor(s) is a treatment modality that can be given singly or in conjunction with other treatments such as radiation, chemotherapy or the emerging treatments with bacterial proteins or peptides given via intravenous/intra muscular routes.

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WE CLAIM:

1. A biomedical device for cancer therapy comprises indwelling medical device encased in a filter with cut off molecular mass of 25 kDa, the said device harbor live microorganisms selected from *Pseudomonas aeruginosa, Streptococcus, Staphylococcus, Salmonella, Clostridia, Mycobacterium bovis* BCG.

2. The device as claimed in claim 1 wherein the microorganisms are either in the form of biofilm or suspension.

3. The device as claimed in claim 1 wherein the indwelling medical device is a stent or a catheter or any other similar means that can be implanted at tumor site.

4. The device as claimed in claim 3 wherein the indwelling medical device is made of any physiologically acceptable biocompatible material selected from plastic, ceramic, wood, metal, polymer (natural or synthetic) or hydrogel.

5. The device as claimed in claim 1 wherein the live microorganisms harbored are either wild type or attenuated or modified

6. The device as claimed in preceding claims wherein the organism is *Pseudomonas aeruginosa.*

7. The device as claimed in claim 6 wherein *Pseudomonas aeruginosa is* virulence compromised, mutated to eliminate toxin production, or cloned with azurin gene under a strong promoter to hyperproduce *azurin* protein.

8 The device as claimed in claim 7 wherein the mutations are in genes encoding type III secretion and Exotoxin A and the elastase genes las A/las B.

The device as claimed in claim 1 for use to treating cancers of prostate, melanoma, sarcoma, breast, lung, ovarian, kidney, cervical, liver, bladder, colon, pancreas or other tumor types and HIV/AIDS without entering in blood stream.

The device as claimed in claim 9 for use in conjunction with other traditional anticancer drug to treating cancers of prostate, melanoma, sarcoma, breast, lung, ovarian, kidney, cervical; liver, bladder, colon, pancreas or other tumor types and HTV/AIDS.

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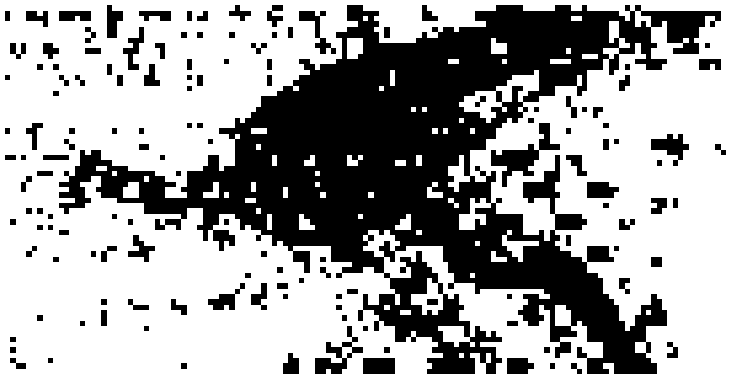

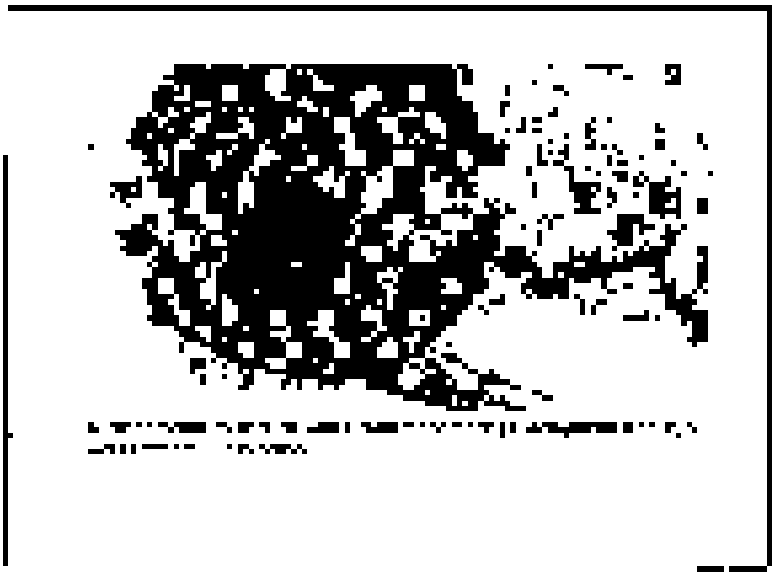

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(54) Title: ANTICANCER AGENT

(57) Abstract: The present invention thus provides microbial products as anticancer agents and pharmaceutical compositions comprising isolated and purified proteins or synthetic peptides, and methods of using them for the treatment of cancer. It is very important to develop new anticancer bioactive peptides having high activity and low toxicity; given that most currently available anticancer therapies either have significant toxicity, and/or are prone to development of resistance.

ANTICANCER AGENT

FIELD OF THE INVENTION:

The present invention particularly relates to a broad spectrum anticancer agent that is of microbial origin. More particularly, the invention relates to proteins either secreted by or surface associated in microorganisms including but not limiting to bacteria both pathogenic and nonpathogenic, viruses, and/or parasites. The anticancer agents of the present invention are purified proteins isolated from bacteria and parasites. Specifically, the agent of the present invention comprising at least proteins isolated from *Mycobacterium bovis* BCG, and *Toxoplasma gondii.* It also encompasses peptides derived from such proteins, synthetically prepared peptides, and proteins or peptides modified by PEGylation, acetylation, phosphorylation, etc. The proteins or various truncated derivatives thereof possess enhanced efficacy and reduced toxicity. The modifications made to the active purified proteins and peptides extend half life of the active ingredient or reduce immunogenecity in the patient blood stream.

The present invention also discloses the compositions, pharmaceutical compositions and the manner of its applications as therapeutic agent to treat mammalian cancer. The pharmaceutical composition comprises an active ingredient i.e. proteins, peptides, including PEGylated, acetylated, phosphorylated form thereof in isolation or in combination and physiologically and pharmaceutically accepted adjuvants or excipients.

BACKGROUND OF THE INVENTION:

Bladder cancer is one of the deadliest forms of cancer, considered the sixth most common cause of cancer-related death in the United States (Jemal et al., 2008). One of the major weapons in the arsenal of cancer fighting drugs is the use of the bacterium *Mycobacterium bovis* (Bacillus Calmette-Guerin, BCG) since 1976 to fight superficial urothelial carcinoma of the bladder (Herr and Morales, 2008; Kresowik and Griffith, 2010). For its use as an anticancer therapy, **live BCG cells** are taken from lyophilized powders and introduced into emptied bladders through a urethral catheter. After a residence varying from a few minutes to a few hours,

the BCG cells are eliminated by the patients through emptying of the bladder. The patients are subsequently monitored by cystoscopy, conventional cytology and FISH analysis. The BCG effect is believed to be mediated through induction of an immune reaction in the bladder such as release of cytokines IL 8 and TRAIL that leads to tumoricidal activity (Herr and Morales, 2008; Kresowik and Griffith, 2010). This immune response is greatly amplified with repeated instillations of BCG, demonstrating the importance of elevated cytokine levels including IL2, IL6, IL8, TNF, and IFNs (Shintani et al., 2007; Bisiaux et al., 2009) and the subsequent infiltrations of neutrophils, lymphocytes, and monocytes/macrophages. Indeed, BCG-stimulated neutrophils have been shown to kill bladder cancer cells in vitro in a TRAIL-dependent manner (Ludwig et al., 2004).

Unfortunately, therapeutic use of live BCG cells for bladder cancer treatment is associated with many debilitating and/or serious side effects, ranging from cystitis and gross hematuria to life-threatening BCG sepsis. Such major side effects during intravesical BCG therapy can affect treatments in 30% of patients while mild cystitis, malaise, low grade fever and other side effects are common in about 90% of patients (Bohle et al., 2003; Sylvester et al., 2003).

Further, some recent observations endorses that certain single nucleotide polymorphisms (SNPs) in humans can promote disease progression in spite of BCG therapy (Basturk et al., 2006; Decobert et al., 2006), making use of the immune response-invoking live BCG bacteria less effective in such patients.

Thus toxicity and efficacy issues have been a major deterrent in the use of live BCG cells in bladder cancer immunotherapy.

The ability of *M. bovis* BCG to attack cancer cells and suppress their growth is not unique to this organism. Indeed, many other bacteria such as *Salmonella, Clostridia, Listeria,* etc, are known to allow cancer regression both by inducing an immune response as well as actively growing in the core of the tumor (Mahfouz et al., 2007; Fialho and Chakrabarty, 2010a). Even many viruses have been designed for cancer therapy (Fialho and Chakrabarty, 2010a).

Recently pathogenic bacteria such as *Pseudomonas aeruginosa or gonococci/ meningococci* such as *Neisseria meningitides* reported to produce proteins such as azurin or Laz that demonstrate strong anticancer activity both in vivo and in vitro (Chakrabarty, 2010; Fialho and Chakrabarty 2010 a,b). Not only the full-length proteins, but peptides derived from them such as the 28 amino acid peptide P28 or the 26 amino acid peptide P26, derived from different parts of azurin, show entry specificity in cancer cells (Taylor et al., 2009) and high cytotoxicity in cancers such as breast, melanoma, prostate, brain, etc (Taylor et al., 2009, Chaudhari et al., 2007). Proteins such as azurin, considered a bacterial weapon against cancer (Chakrabarty, 2010; Fialho and Chakrabarty, 2010b), are secreted in response to the presence of cancer cells (Mahfouz et al., 2007), while the *Neisserial* protein weapon Laz is surface-exposed (Hong et al., 2006). The phase I human clinical trials of p28 were performed as per US-FDA guidelines. The p28 peptide has shown lack of toxicity and partial tumor regression in 2 patients and complete regression in 2 other patients out of 15 advanced stage (stage IV) cancer patients where no drugs were working and where the patients had less than 6 months life span. When given in 5 escalating doses 3 times a week for 4 weeks, followed by a break of 2 weeks, several patients showed stunted tumor growth but 2 patients showed partial regression and 2 patients showed complete regression of their tumors (where no drugs were working any longer). The 2 patients where the tumors completely regressed (making them disease free) as well as another patient (altogether 3) are alive today (middle of August 201 1) beyond one and a half year (Richards et al., 201 1), showing that such bacterial peptides as p28, derived from azurin, have unique modes of action so that they work against drug-resistant cancers. We hope that the newly identified peptide MB30 will behave similarly.

To the best of the knowledge of the applicant and/or inventors, no efforts have been made to look for protein/peptide weapons produced by such bacteria. However, considering the ability of microbial products being used as potential anticancer agent, the applicant/inventors carried out pains taking research to arrive at other protein weapons, either secreted or surface-associated in microorganisms including pathogens, or that could be designed as synthetic peptides, that would

demonstrate anticancer activity for use as anticancer therapies instead of the live bacteria.

OBJECT OF THE INVENTION:

The main object is to provide an anticancer agent pharmaceutical compositions and application thereof eliminating the limitations of prior art.

The other object is to provide an anticancer agent, particularly broad spectrum anticancer agent of microbial origin, more particularly, proteins either secreted by or surface associated in microorganisms including but not limiting to bacteria both pathogenic and nonpathogenic, viruses, and/or parasites.

Yet another object is to provide purified proteins isolated from bacteria and parasites specifically, proteins isolated from *M. bovis* BCG, and *Toxoplasma gondii* useful as an anticancer agent.

Still other object is to provide peptides derived from such proteins, synthetically prepared peptides, and proteins or peptides modified by PEGylation, acetylation, phosphorylation, etc. useful as an anticancer agent.

Still another object is to also provide anticancer agent comprising the proteins or various truncated derivatives thereof that possess enhanced efficacy and reduced toxicity.

Yet other object is to provide purified proteins and peptides, as an anticancer agent, with extended half life and reduced immunogenicity in the patient blood stream.

The other object of the present invention also discloses the compositions, pharmaceutical compositions and the manner of its applications as therapeutic agent to treat mammalian cancer.

The pharmaceutical composition comprises an active ingredient i.e. proteins, peptides, including PEGylated, acetylated, phosphorylated form thereof in isolation or in combination and physiologically and pharmaceutically accepted adjuvants or excipients.

DESCRIPTION OF THE DRAWINGS:

Figure 1: depicts the cytotoxicity effect of varying concentrations of peptides on HTB-9 bladder cancer cell line, which was comparable with the controls cisplatin and azurin. 1 x 10^4 cancer cells were seeded and after 24h, media were changed with varying concentrations of anticancer peptides (TG20, TG23, and MB30), azurin and cisplatin as positive controls. The viability of the cells was estimated by using MTT assay on the basis of formazan formed, which was detected spectrophotometrically by optical density at 570 nm at 24h, 48h and 72h [Bar- mean $\pm$ S.E.]

Figure 2: Depicts the cytotoxicity effect of varying concentrations of peptides on UM-UC-3 bladder cancer cell line, which was comparable with the controls cisplatin and azurin. 1 x 10^4 cancer cells were seeded and after 24h, media were changed with varying concentrations of anticancer peptides (TG20, TG23, and MB30), azurin and cisplatin as positive controls. The viability of the cells was estimated by using MTT assay on the basis of formazan formed, which was detected spectrophotometrically by optical density at 570 nm at 24h, 48h and 72h [Bar- mean+ S.E.].

Figure 3: Depicts the cytotoxicity effect of varying concentrations of peptides on COLO 205 colorectal cancer cell line, which was comparable with the controls cisplatin and azurin. 1 x 10^4 cancer cells were seeded and after 24h, media were changed with varying concentrations of anticancer peptides (TG20, TG23 and MB30), azurin and cisplatin as positive controls. The viability of the cells was estimated by using MTT assay on the basis of formazan formed, which was

detected spectrophotometrically by optical density at 570 nm at 24h, 48h and 72h [Bar- mean+ S.E.]

Figure 4: Depicts the cytotoxicity effect of varying concentrations of peptides on HCT 116 colorectal cancer cell line, which was comparable with the controls cisplatin and azurin. 1 x 10^4 cancer cells were seeded and after 24h, media were changed with varying concentrations of anticancer peptides (TG20, TG23 and MB30), azurin and cisplatin as positive controls. The viability of the cells was estimated by using MTT assay on the basis of formazan formed, which was detected spectrophotometrically by optical density at 570 nm at 24h, 48h and 72h [Bar- mean + S.E.].

Figure 5: Depicts the cytotoxicity effect of varying concentrations of peptides on SiHa cervix cancer cell line which was comparable with the controls cisplatin and azurin. 1 x 10^4 cancer cells were seeded and after 24h, media were changed with varying concentrations of anticancer peptides (TG20, TG23 and MB30), azurin and cisplatin as positive controls. The viability of the cells was estimated by using MTT assay on the basis of formazan formed, which was detected spectrophotometrically by optical density at 570 nm at 24h, 48h and 72h [Bar- mean ± S.E.].

Figure 6: Depicts the cytotoxicity effect of varying concentrations of peptides on CaSki cervix cancer cell line which was comparable with the controls cisplatin and azurin. 1 x 10^4 cancer cells were seeded and after 24h, media were changed with varying concentrations of anticancer peptides (TG20, TG23 and MB30), azurin and cisplatin as positive controls. The viability of the cells was estimated by using MTT assay on the basis of formazan formed, which was detected spectrophotometrically by optical density at 570 nm at 24h, 48h and 72h [Bar- mean ± S.E.].

Figure 7: Depicts the cytotoxicity effect of MB30 peptide and MPT-63 protein on U87 human glioblastoma, HepG2 liver cancer and MDA-MB-231 breast cancer

cell lines. 1 x 10^4 cancer cells were seeded and after 24 hours it was treated separately with varying concentrations of either MB30 peptide or MPT63 protein or cisplatin as a positive control. The viability of the cells were estimated by using MTT assay on the basis of formazan formed, which was detected spectrophotometrically by measuring optical density at 570 nm at 24h and % cell cytotoxicity was determined.

SUMMARY **OF THE INVENTION:**

The present invention relates to an anticancer agent with enhanced activity and reduced toxicity, which is a protein of microbial origin having SEQ ID NO. 1 and/or SEQ ID NO. 2 and variants thereof.

The anticancer agent of SEQ ID NO. 1 may be a secreted protein of *Mycobacterium tuberculosis,* and/or *Mycobacterium bovis* BCG and termed as MPT 63.

The anticancer agent of SEQ ID NO. 2 may be a surface protein from the parasite *Toxoplasma gondii,* and termed as SAG1.

The variants of SEQ ID NO. 1 are peptides such as MB30 of SEQ ID NO. 3 and other peptides from other regions having 10 to 50% variation in the amino acid sequence without affecting the anticancer activity.

The variants of SEQ ID NO. 2 are peptides such as TG20 of SEQ ID NO. 4 and TG23 of SEQ ID NO. 5 and other peptides from other regions having 10 to 50% variation in the amino acid sequence without affecting the anticancer activity.

The anticancer agent of the present invention exhibits anticancer activity against the group consisting of melanoma, leukemia, breast, ovarian, cervical, lung, pancreatic, colon, bladder, prostate, liver, renal and brain cancers.

The invention also relates to a pharmaceutical composition comprising an anticancer agent as herein before described.

The pharmaceutical composition may further comprises adjuvant(s).

The anticancer agent may be modified by PEGylation, acetylation, phosphorylation or prepared synthetically. The modification thus made extends the half life of the active ingredient and/or reduce immunogenecity in the blood stream of the subject.

The pharmaceutical composition may be useful for intravenous (iv), intramuscular, oral, subcutaneous or topical application, in presence or absence of adjuvants or excipients for the therapeutic and /or prophylactic treatment of melanoma, leukemia, breast, ovarian, cervical, lung, pancreatic, colon, bladder, prostate, liver, renal and brain cancers.

The applicant has found out that the secreted protein MPT63 and of a peptide, termed MB30, derived from it possess anticancer activity and can be used for treating cancers in mammals. Similarly, they also found out the anticancer activity of a surface protein from the parasite *Toxoplasma gondii,* termed SAG1, and two peptides TG20 and TG23 derived from it. Such proteins and peptides proved to be an excellent candidate drugs to treat a variety of human cancers. The present invention thus provides microbial products as anticancer agents and pharmaceutical compositions comprising isolated and purified proteins or synthetic peptides, and methods of using them for the treatment of cancer. It is very important to develop new anticancer bioactive peptides having high activity and low toxicity; given that most currently available anticancer therapies either have significant toxicity, and/or are prone to development of resistance.

The inventors carrying out research over long period could demonstrate that the *Mycobacterium-specific* secreted protein, MPT63, of hitherto unknown function produced by both the human pathogen *M. tuberculosis* and the bovine strain *M. bovis* and a peptide derived from it termed MB30, exhibit its use in bladder and other cancer therapy.

MPT63 is a small (16 kDa) protein which is secreted after 2-3 weeks of culturing and decreases with longer cultivation. This protein has been shown to have

immunogenic property and has been implicated in virulence. It is specific to *mycobacteria* as homologues of MPT63 have only been found in *mycobacterial* species like *M. smegmatis, M. bovis and M. avium.* A pseudogene of MPT63 has been found within the *M. lapre* genome, but is thought not to be translated into protein. The analysis of DNA sequence encoding MPT63 revealed an ORF encoding a protein of 159 amino acids (aa). It consists of a 130 aa mature protein preceded by 29 aa signal peptide. The X-ray crystal structure of MPT63 was determined to 1.5-Angstrom resolution with the hope of yielding functional information about MPT63. The structure of MPT63 is a β-sandwich consisting of two antiparallel β-sheets similar to an immunoglobulin like fold, with an additional small, antiparallel β-sheet. Apart from immunoglobulin structure, MPT63 has some structural homology to cell surface binding proteins such as Homo sapiens β-adaptin, bovine arrestin and *Yersinia pseudotuberculosis* invasin. It has also structural similarity to eukaryotic fibronectin-binding proteins, major histocompatiblity domains and T-cell receptors. The function of MPT63 has hitherto been unknown and could not be predicted by its structural features as it has an extremely common immunoglobulin like fold that occurs in about 24% of the structures in the Protein Data Bank. The β-sandwich fold that MPT63 resembles is at the core of many proteins with diverse functions.

Interestingly, the inventor also found out that not only pathogenic bacteria and viruses have been shown to allow cancer regression, but a few parasites have also been implicated in this process. For example, concurrent infection by enteric helminth, a parasite, and *Helicobacter felis* in mice can attenuate gastric atrophy, a pre-malignant lesion in mice. The helminth pre-infection was shown to reduce helicobacter gastritis but not *H. felis* colonization, presumably due to a shift in the balance of Thl to Th2 response. However, the production of anticancer agent(s) by the helminths, just as secretion of azurin by *P. aeruginosa* or MPT63 by *M. bovis,* cannot be ruled out.

An interesting case of tumor regression by a parasite is the regression of B16 melanoma cells in mice by *Toxoplasma gondii,* an obligate intracellular parasite

that is the causative agent of toxoplasmosis. In absence of *T. gondii* infection, challenge with B16 melanoma cells in mice led to tumor formation. In *T. gondii*-infected mice, however, B16 cells failed to form subcutaneous tumors. To determine if the immune system activation in *T-gondii-mfected* mice was responsible for the failure of the B 16 melanoma cells to form tumors, a variety of mutant mice lacking major immune functions such as iNOS or perforin where cytolytic functions of lymphocytes were severely reduced, were used. These investigators also used scid-beige mice in which cytotoxic T lymphocytes are absent and NK cells are not cytotoxic. Even in scid-beige and perforin knock-out mice where cytolytic functions of lymphocytes are severely impaired and immune functions are greatly reduced, the anticancer activity of *T. gondii* was clearly evident, suggesting the presence of active anticancer agents produced by *T. gondii.* Further experimentations with blood vessel formation demonstrated that hemoglobin levels in Matrigels from *T. gondii-v[illegible]e d* mice were 50 to 100 fold lower than the control mice without *T. gondii* infection with very little histopathologically-observed vascular channels. This suggested that *T. gondii* infection led to systemic suppression of angiogenesis, although the nature or the origin of the anti-angiogenic agent(s) could not be determined. There was some evidence that the circulating anti-angiogenic factors were soluble and not cell-associated but whether such factors were produced by the mice cells or *T. gondii* could not be determined.

The applicant also demonstrated that the anticancer activity of *T. gondii* can be attributed, at least in part, to the presence of the surface antigen called SAG1. SAG! is one of the more prominent surface proteins in *T. gondii* involved in host cell attachment and is considered a key virulence factor for *T. gondii.* SAG1 is a 30 kDa immunodominant surface antigenic glycoprotein, also known as P30, highly conserved in *T. gondii* strains, particularly in tachyzoites. The monomelic SAG1 has two domains, a N-terminal 130 amino acid domain D1 and a C-terminal 120 amino acid domain D2. Remarkably, SAG1 shows structural similarity to azurin and interacts with azurin, but it interacts much more strongly with the azurin-like protein Laz. Because SAG1 is surface-associated similar to

Laz, and shows structural similarity with the anticancer agents azurin and Laz, it was of great interest to the applicant to determine if similar to Laz, SAGl might possess anticancer activity against some of the human cancers. To eliminate the likely toxicity of the protein itself in human patients, two peptides TG20 and TG 23, were designed taking advantage of the structural similarities between azurin (and the azurin-like protein Laz) and SAGl. These two peptides were though to harbor the potential anticancer activity of SAGl, somewhat similar to azurin peptides P28 and P26 with known anticancer activity.

Further, TG20 and TG23, derived from different parts of SAGl, did demonstrate anticancer activity against bladder and colon cancers. For comparison purposes, the applicant has also included azurin and cisplatin in assays.

MATERIALS AND METHODS:

The complete amino acid sequence of MPT63 protein (SEQ ID NO. 1) from *Mycobacterium bovis* is given below. The first 29 amino acids (underlined) in the following MPT63 sequence form secretion signal peptide (leader) sequence and the MB30 peptide (amino acids 44-73 of mature protein) sequence is highlighted in bold.

<u>MKLTTMIKTAVAVVAMAAIATFAEPVALAAYPITGKLGSELTMTDTVGO</u>
WLGWKVSDLKSSTAVIPGYPVAGQVWEATATVNAIRGSVTPAVSQFN
ARTADGINYRVLWQAAGPDTISGATIPQGEQSTGKIYFDVTGPSPTrVAMN
NGMQDLLIWEP

The peptide sequence of 30 amino acids derived from MPT63 protein, called MB30, peptide is given below.

MB30 peptide sequence (SEQ ID NO.3):

GQVWEATATVNAIRGSVTPAVSQFNARTAD

The complete amino acid sequence of SAGl protein (SEQ ID NO. 2) from *Toxoplasma gondii* is given below.

MFPKAVRRAVTAGVFAAPTLMSFLRCGVMASDPPLVANQWTCPDKKST
AAVILTPTENHFTLKCPKTALTEPPTLAYSPNRQICSAGTTSSCTSKAVTLS
SLIPEAEDSWWTGDSASLDTAGIKLTVPIEKFPVTTQTFWGCIKGDDAQS
CMVTVTVQARASSVVNNVARCSYGANSTLGPVKLSAEGPTTMTLVCGK
DGVKVPQDNNQYCSGTTLTGCNEKSFKDILPKLTENPWQGNASSDKGAT
LTIKKEAFPAESKSVIIGCTGGSPEKHHCTVKLEFAGAAGPAKSAAGTASH
VSIFAMVIGLIGSFAACVA

The peptide sequence of 20 amino acids derived from SAG1 protein of ***T. gondii,*** termed TG20, is given below.

TG20 peptide sequence (SEQ ID NO. 4): **NHFTLKCPKTALTEPPTLAY**

The peptide sequence of 23 amino acids derived from SAG1 protein ***T. gondii,*** termed TG23, is given below.

TG23 peptide sequence (SEQ ID NO. 5):

TAGIKLTVPIEKFPVTTQTFWG

For illustrating the invention, experiments detailing the anticancer activity of the bioactive peptides are described below. It should be noted that the following examples are intended to describe such activities and not to limit the invention. The isolation and purification of the proteins have previously been described in the literature while all the peptides were chemically synthesized by commercial concerns. We have cloned and expressed the MPT-63 protein and its anticancer activity was evaluated in various cancer cell lines.

Cloning and expression of MPT63 protein:

The MPT63 gene was amplified by PCR using the following primers with ***Ndel*** restriction site on the forward primer and ***Hindlll*** restriction site on the reverse primer:

Forward primer: 5'-TCGATCCATATGGCCTATCCC ATCACCGGA-3', and

Reverse primer: 5'- TCGATCAAGCTTCTACGGCTCCC AAATCAG-3'.

The ***NdeI*** and ***HindIII*** PCR product is ligated in the pUC18 cloning vector. The MPT63 gene is excised from pUC18 and ligated into prerestricted pET28a expression vector and transformed into ***E. coli*** BL21de3. For MPT63 purification, a single colony of BL21de3 was inoculated into LB broth and inoculated at 37°C until the OD reached to 0.5. The culture was induced with ImM IPTG for overnight at 37°C.

After overnight incubation period, the culture was centrifuged and the cell pellets were incubated on ice for 15 minutes. Cells were re-suspended in 10ml native lysis buffer with benzonase and lysozyme, incubated on ice for 30 minutes, and intermittently mixed so as to ensure homogenous suspension. Cell lysate was centrifuged at 14000g/4°C for 30 minutes to pellet the cellular debris. The cell lysate supernatant was loaded on the Ni-NTA column supplied by Qiagen, and the flow-through fraction was collected. The column was washed twice with wash buffer. The bound 6xHis-tagged protein was eluted with two 1 ml aliquots of native elution buffer. Both the elution fractions were collected in separate tubes. At each step of the process, samples were analyzed by SDS PAGE. This purified protein was used in the further experiments at concentration of 1 μM, 5μM and 10 μM to determine its cytotoxic effect on the various cell lines.

Cell culture:

Bladder cancer cell lines HTB-9 [Grade II Bladder carcinoma], CRL-1749 (UM-UC-3) [High grade bladder carcinoma], and colorectal carcinoma cell lines CCL-222 (COLO 205) [Dukes Type D colorectal adenocarcinoma], CCL-247 (HCT-116), SiHa, CaSki, MDA-MB-231, U87 and HepG2 were procured from American Type Culture Collection (ATCC). All cell lines were grown under standard conditions in humidified 37°C incubator in 5% $C0_2$ as described earlier (Garg et al., 2009; Kanojia et al., 2010).

ATCC: **HTB-9:** Human cell line with epithelial-like characteristics established from a urinary bladder carcinoma (grade II) of a 68 year old male patient. This cell line is tumorigenic in nature. These cells lack a functional retinoblastoma protein, Rb. HTB-9 cells proliferate in response to a variety of growth factors, including IL2, IL3, G-CSF, GM-CSF, and M-CSF. Goselink et al (1996) have shown that the serum-free conditioned medium of HTB-9 cells can replace serum to support the growth of hematopoietic progenitor cells (HPC) in semi-solid cultures.

ATCC: **UM-UC-3:** Human cell line with epithelial-like characteristics established from a high grade invasive urinary bladder carcinoma of a male patient. This is a hypertriploid human cell line and IFN non responsive. This cell line is tumorigenic in nature.

ATCC **COLO 205:** Human cell line derived from colorectal adenocarcinoma Dukes' type D of 70 year old male patient. The cells are CSAP negative. The cells are positive for keratin by immunoperoxidase staining. COLO 205 cells express a 36000 Dalton cell surface glycoprotein related to GA733-2 tumor associated antigen.

ATCC **HCT 116:** Human cell line with epithelial-like characteristics established from colorectal carcinoma from an adult male patient. The cells are positive for keratin by immunoperoxidase staining. HCT 116 cells are positive for transforming growth factor beta 1 (TGF beta 1) and beta 2 (TGF beta 2) expression. This line has a mutation in codon 13 of the ras protooncogene, and can be used as a positive control for PCR assays of mutation in this codon.

ATCC: **SiHa:** Human cell line established from fragments of a primary tissue sample obtained after surgery from a Japanese patient. Electron microscopic observations revealed presence of typical desmosomes at the cell junctions and an abundance of tonofilaments in the cytoplasm. The line is reported to contain an

integrated human papillomavirus type 16 genome (HPV-16, 1 to 2 copies per cell).

ATCC: **CaSki:** Human cell line derived from 40 year-old Caucasian female patient. The line was established from cells from a metastasis in the small bowel mesentery. The cells are reported to contain an integrated human papillomavirus type 16 genome (HPV-16, about 600 copies per cell) as well as sequences related to HPV-18.

ATCC: **MDA-MB-231:** It is a human cell line with epithelial like characteristics established from mammary gland (breast) adenocarcinoma from a 51 year old Caucasian female patient. The cells express the WNT7B oncogene.

ATCC: **U87:** It is a human glioblastoma cell line formally known as U-87 MG. It has epithelial morphology, and was obtained from 44 year old Caucasian female patient.

ATCC: **HepG2:** Human cell line derived from the liver tissue of a 15 year old Caucasian American male with a well differentiated hepatocellular carcinoma. These cells are epithelial in morphology and are not tumorigenic in nude mice. The cells secrete a variety of major plasma proteins; e.g., albumin, transferrin and the acute phase proteins fibrinogen, alpha 2-macroglobulin, alpha 1-antitrypsin, transferrin and plasminogen.

Cytotoxicity Assay:

The cytotoxicity assays performed in this investigation were designed to evaluate the cytotoxic potential of bioactive peptides from Table 1 in cancer cell lines at the given concentrations. MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay was carried out for measurement of the cytotoxicity of different anticancer peptides. The water soluble tetrazolium salt, MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] is metabolized to the water insoluble formazan by intact mitochondrial dehydrogenases. The formazan

is then solubilized by adding 2-propanol + 40 mM HC1 for 4h incubation. 1 x 10^4 cells were seeded and after 24h, media were changed with varying concentrations of anticancer peptides (TG20, TG23 and MB30), azurin (known to have anticancer activity, Chakrabarty, 2010) and cisplatin, also a known anticancer compound (Sedletska et al., 2005), as positive controls [Table 1]. The viability of the cells was estimated on the basis of formazan formed, which was detected spectrophotometrically by optical density at 570 nm at 24h, 48h and 72h. At every time point such as 24h, 48h and 72h, 1 x 10^4 cells were seeded without the peptide in triplicate in each plate for a given treatment of peptide. This was used as control cytotoxicity levels to calculate the percent cell cytotoxicity of every peptide treatment. The cytotoxicity of full length protein MPT-63 was also determined using this assay, where protein was used instead of peptides. All the experiments were carried out in triplicates and repeated three times.

Table 1:

Peptide TG20	1 μM	10 μM
Peptide TG23	1 μM	10 μM
Peptide MB30	1 μM	10 μM
Azurin		10 μM
Cisplatin		10 μM

RESULTS;

Treatment with various peptides in this investigation revealed anticancer properties of the peptides. *In vitro* analysis of cytotoxicity results distinctly revealed reduction in cellular proliferation. All the experiments were carried out in triplicates and repeated three times.

MTT assays demonstrated the cytotoxic activity in HTB-9 bladder cancer cells of all the 3 peptides (TG20, TG23, and MB30, Table 1) when used at concentrations of 1.0 and 10.0 micromolar, along with the other positive controls azurin and cisplatin. All the three peptides demonstrated cytotoxicity upto 40% and 60% at 48 and 72 h respectively, comparable to azurin and cisplatin (Fig. 1). Such results demonstrated that peptide concentrations as low as 1.0 micromolar were enough

and higher concentrations did not add substantially to the cytotoxicity. Also, while low at 24 h, the cytotoxicity levels increased with increasing times of incubation up to 72 h (Fig. 1).

In order to assess the cytotoxicity of all these peptides in different bladder cancer cell lines, UM-UC-3 bladder carcinoma cell line was incubated with the peptides at 1.0 and 10 μM concentrations and cytoxicity was monitored at 24, 48 and 72 hours. At 24 h, TG23 and MB30 peptides demonstrated higher cytotoxicity levels than the other peptide, comparable to azurin but less than cisplatin. With prolonged exposure up to 72h, all the three peptides demonstrated about 45% cytotoxicity in the UM-UC-3 bladder cancer cell line (Fig. 2).

Although the ability of live cells of *M. bovis* BCG to allow regression of cancers such as melanoma, prostate or leukemia has been reported in the past, such effects were not consistent. The consistent efficacy in human patients was only against bladder cancer. To evaluate if the MB30 peptide or the other peptides would demonstrate cytotoxicity against other cancers, the cytotoxicity of all the three peptides was also assessed in a colon cancer cell line, called COLO 205, at various concentrations up to 72 hour. All the three peptides demonstrated varying cytotoxicity, about 10-20%, during 48h incubation (Fig. 3).

The anticancer activity of all the three peptides was also checked in the colon cancer cell line HCT 116 and in cervical cancer cell lines (SiHa and CaSki). The peptide MB30 killed about 40% of the colon and cervical cancer cells at both the concentrations when incubated up to 24 h, higher than azurin but somewhat less than cisplatin. The other peptides TG20 and TG23 showed less cytotoxicity (Fig. 4, 5 and 6). Such data clearly indicate that peptides like MB30, as well as TG20 and TG23, demonstrate significant cytotoxicity in bladder and other cancer cells. The full length MPT-63 protein was purified and its cytotoxicity was compared with MB30 peptide in U87, HepG2 and MDA-MB-231 cell lines. The MPT-63 protein has also shown cytotoxicity in all the three cell lines (brain, liver and breast cancers) tested (Fig. 7). However, the MB30 peptide has demonstrated better cytotoxicity in comparision to the full length protein MPT-63 in all the

three cell lines (brain, liver and breast cancers) at 24 h (Fig. 7). This indicates that the full length protein as well as MB30 peptide shows anticancer activities in a variety of cancer cell lines.

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WHAT IS CLAIMED IS:

1. An anticancer agent with enhanced activity and reduced toxicity is a protein of microbial origin having SEQ ID NO. 1 and/or SEQ ID NO. 2 and variants thereof.

2. The anticancer agent as claimed in claim 1, wherein the protein of SEQ ID NO. 1 is a secreted protein of *Mycobacterium tuberculosis,* and/or *Mycobacterium bovis* BCG and termed as MPT 63.

3. The anticancer agent as claimed in claim 1, wherein the protein of SEQ ID NO. 2 is a surface protein from the parasite *Toxoplasma gondii,* and termed as SAG1.

4. The anticancer agent as claimed in claim 1 wherein the variants of SEQ ID NO. 1 are peptides such as MB30 of SEQ ID NO. 3 and other peptides from other regions having 10 to 50% variation in the amino acid sequence without affecting the anticancer activity.

5. The anticancer agent as claimed in claim 1, wherein the variants of SEQ ID NO. 2 are peptides such as TG20 of SEQ ID NO. 4 and TG23 of SEQ ID NO. 5 and other peptides from other regions having 10 to 50% variation in the amino acid sequence without affecting the anticancer activity.

6. The anticancer agent as claimed in claims 1 exhibits anticancer activity against the group consisting of melanoma, leukemia, breast, ovarian, cervical, lung, pancreatic, colon, bladder, prostate, liver, renal or brain cancers.

7. A pharmaceutical composition comprising an anticancer agent as claimed in claims 1 and 2.

8. The pharmaceutical composition as claimed in claim 7 further comprises adjuvant(s).

The anticancer agent as claimed in claims 1 and 2 is modified by PEGylation, acetylation, phosphorylation or prepared synthetically.

The pharmaceutical composition as claimed in claims 7 and 8 is useful for intravenous (iv), intramuscular, oral, subcutaneous or topical application, in presence or absence of adjuvants or excipients for the therapeutic and /or prophylactic treatment of melanoma, leukemia, breast, ovarian, cervical, lung, pancreatic, colon, bladder, prostate, liver, renal or brain cancers.

An anticancer agent is a protein of microbial origin having SEQ ID NO. 1 and/or SEQ ID NO. 2 variants thereof substantially such as herein described with reference to figures.

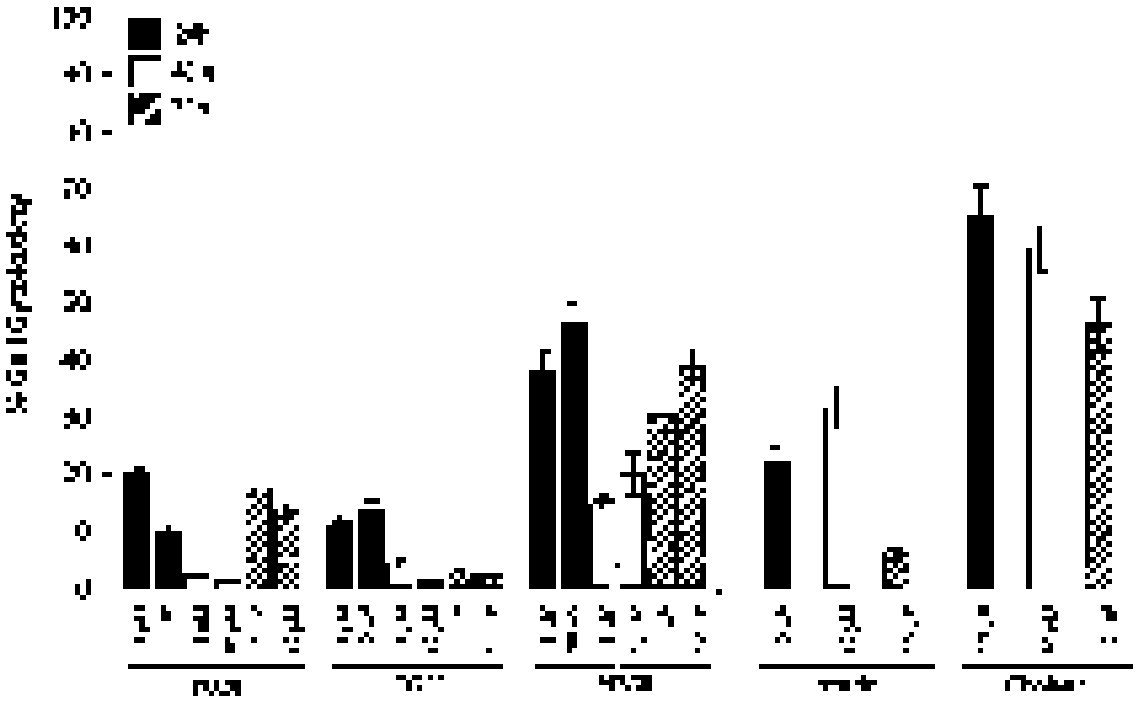
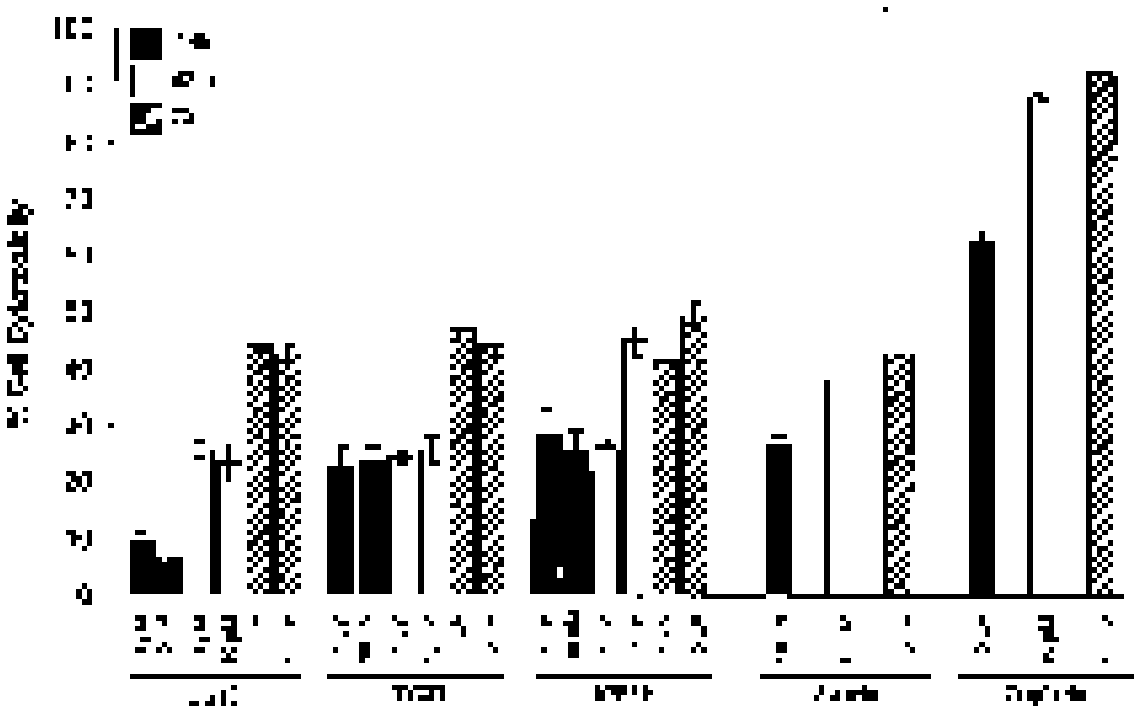


FIG 1



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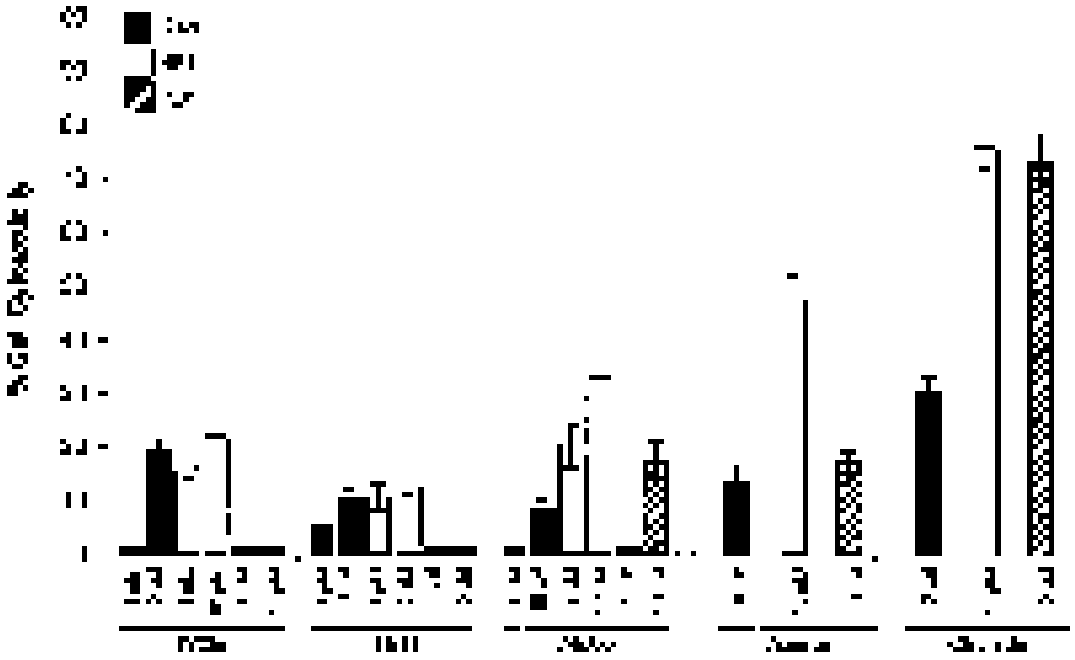

FIG. 5

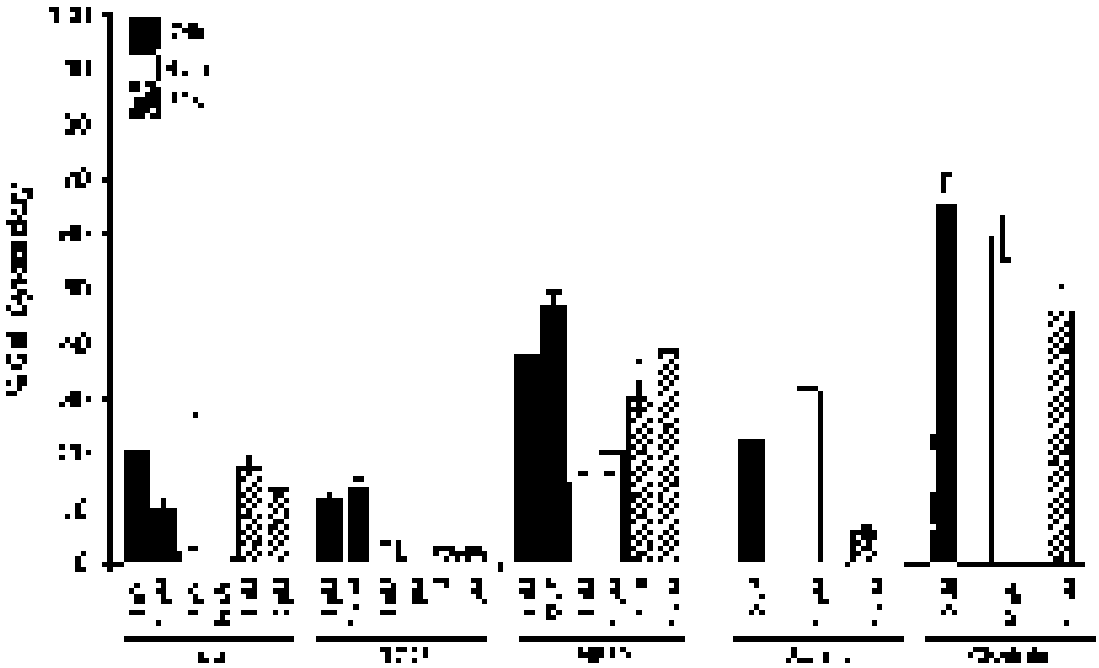

FIG. 4

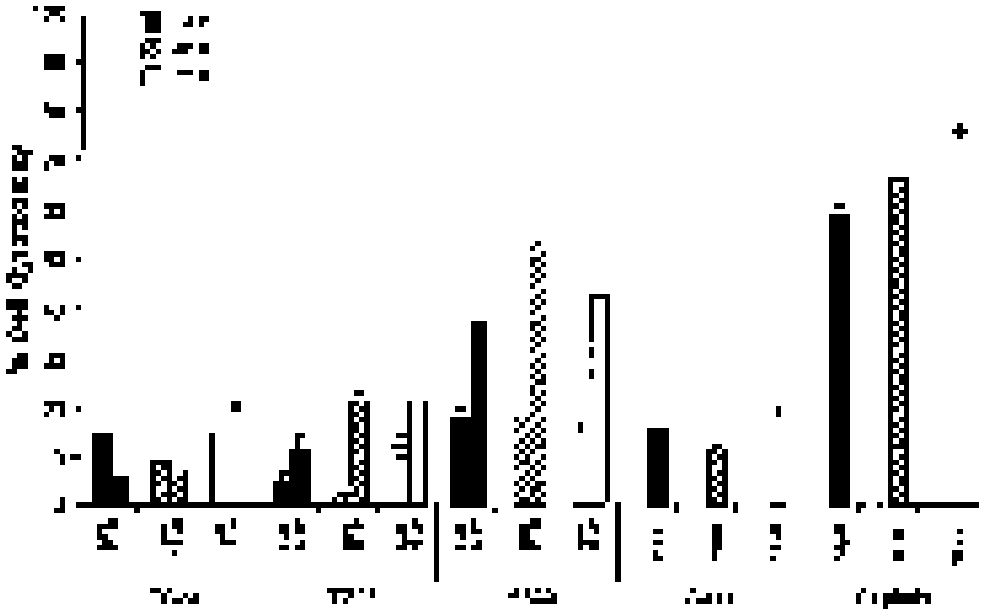

FIG. 3

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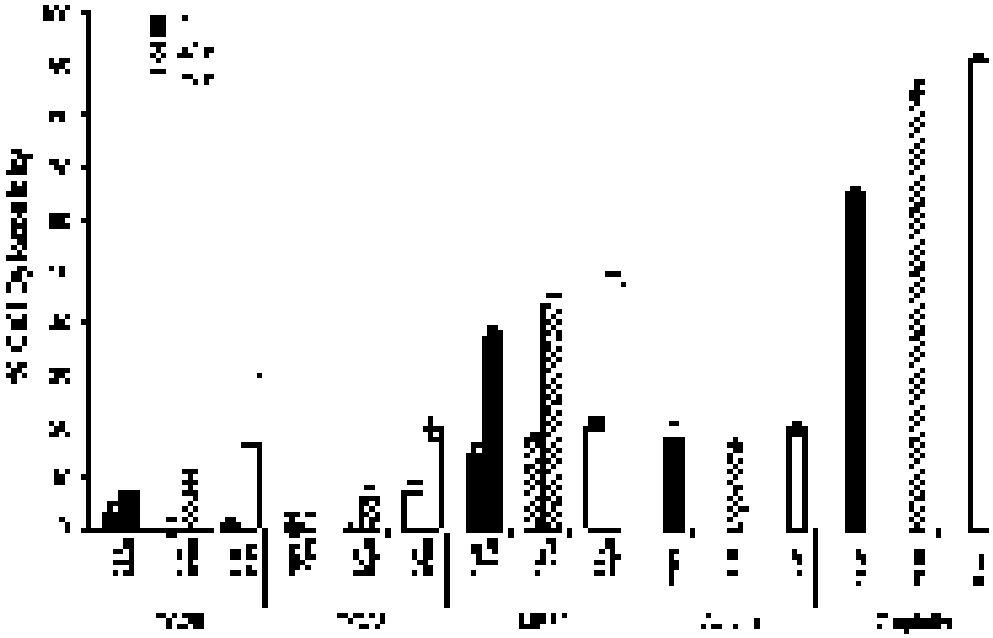

FIG. 8

37

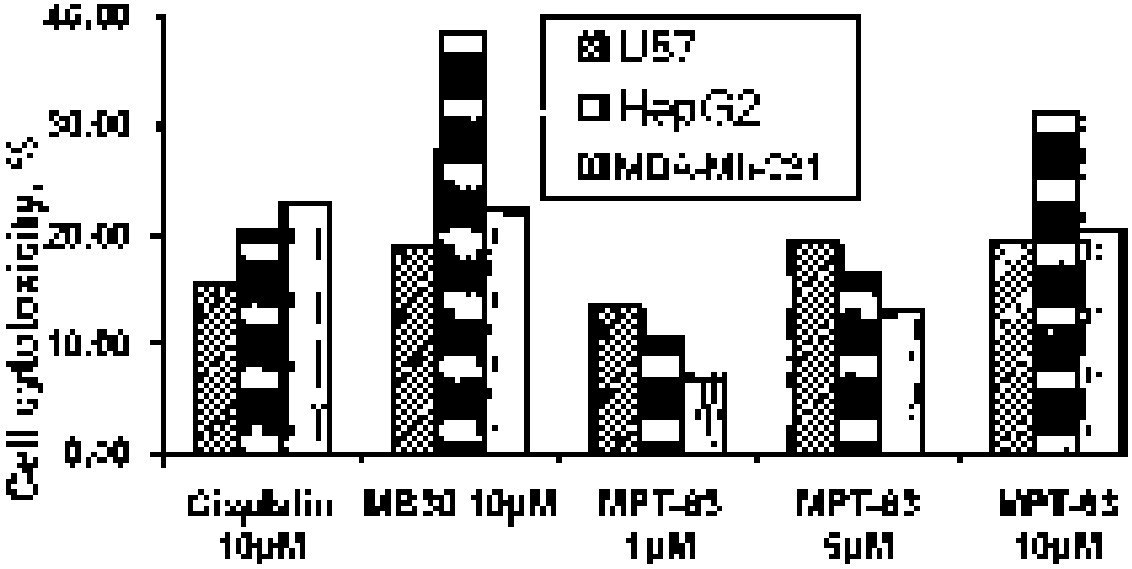
40.00
30.00
20.00
10.00
0.00
Cell cytotoxicity, %
U87
HepG2
MDA-MB-231
Cisplatin 10μM
MB30 10μM
MPT-83 1μM
MPT-83 5μM
MPT-83 10μM

FIG 7

United States Patent [19] [11] Patent Number: [illegible]

[illegible] et al. [45] Date of Patent: [illegible]

[54] [illegible]

[75] Inventors: [illegible]

[73] Assignee: [illegible]

[21] Appl. No.: [illegible]

[22] Filed: [illegible]

[51] Int. Cl. [illegible]

[52] U.S. Cl. [illegible]

[58] Field of Search [illegible]

[56] References Cited

U.S. PATENT DOCUMENTS

[illegible]

OTHER PUBLICATIONS

[illegible]

Primary Examiner—[illegible]

Attorney, Agent, or Firm—[illegible]

[57] ABSTRACT

[illegible]

[illegible] Claims, No Drawings

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DETAILED DESCRIPTION

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[illegible]

[illegible]

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. [illegible]

DATED [illegible]

INVENTOR(S) [illegible]

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column [illegible], line [illegible], change "[illegible]" to "[illegible]".

Column [illegible], line [illegible], change "[illegible]" to "[illegible]".

Column [illegible], line [illegible], change "[illegible]" to "[illegible]".

Column 13, line [illegible], change "[illegible]" to "[illegible]".

Column 14, line [illegible], change "[illegible]" to "[illegible]".

Signed and Sealed this

Eleventh Day of December, 1990

[illegible]

Commissioner of Patents and Trademarks

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. [illegible]

DATED [illegible]

INVENTOR(S): [illegible]

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : [illegible] Page 1 of 1

DATED : [illegible] 1992

INVENTOR(S) : [illegible] and [illegible]

[illegible]

[illegible]

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[illegible]

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Signed and Sealed this

Fifth Day of February, 1991

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

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DATED : [illegible]

INVENTOR(S) : [illegible]

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : [illegible]

DATED : August [illegible]

INVENTOR(S) : [illegible] Ballagg

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

[illegible] Column 53, [illegible] read --[illegible]--

[illegible] Column 53, [illegible] read --[illegible]--

Signed and Sealed this Twenty-[illegible] Day of November, 2004

Signature of Patent Examiner

[illegible]

United States
Patent Application Publication
Chakraborty et al.

Pub No. US 2008/0312413 A1
Pub Date: Dec. 18, 2008

[75] Inventors:

[73] Assignee:

[21] Appl. No.:

[22] Filed:

Related U.S. Application Data

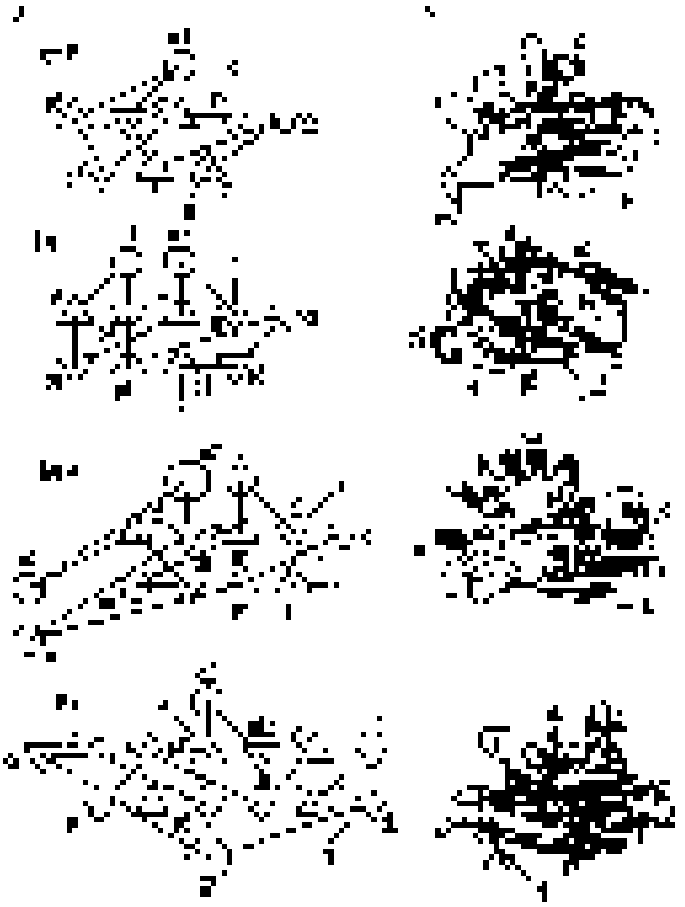
[63]

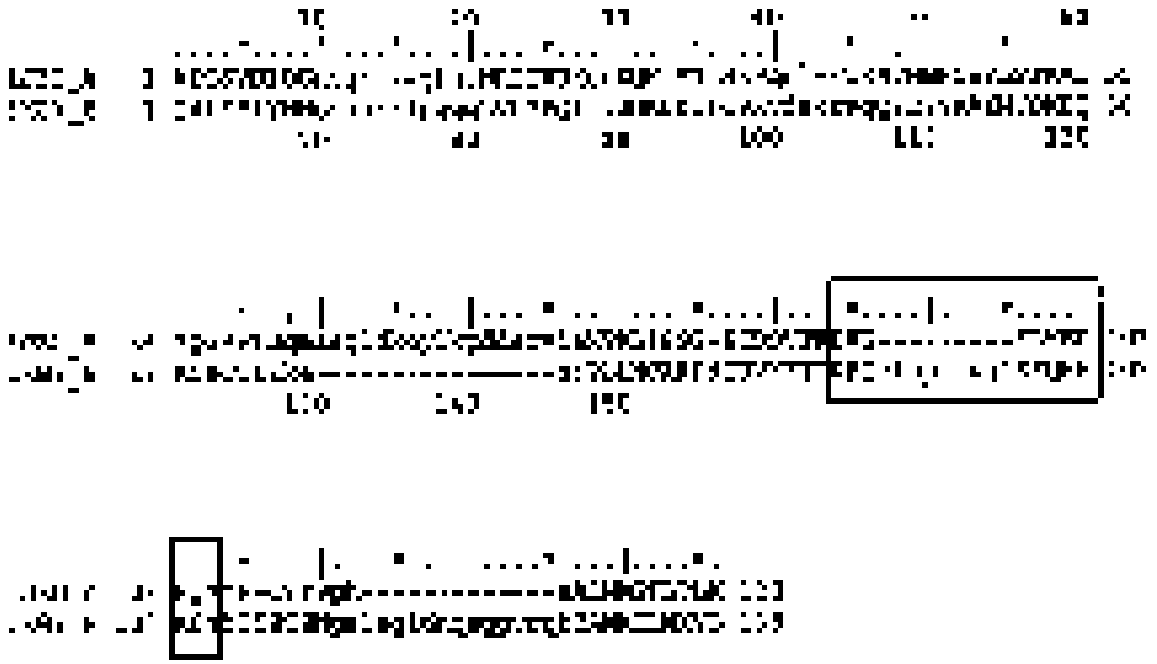
[51] Int. Cl.

[52] U.S. Cl.

[57] ABSTRACT

[illegible]





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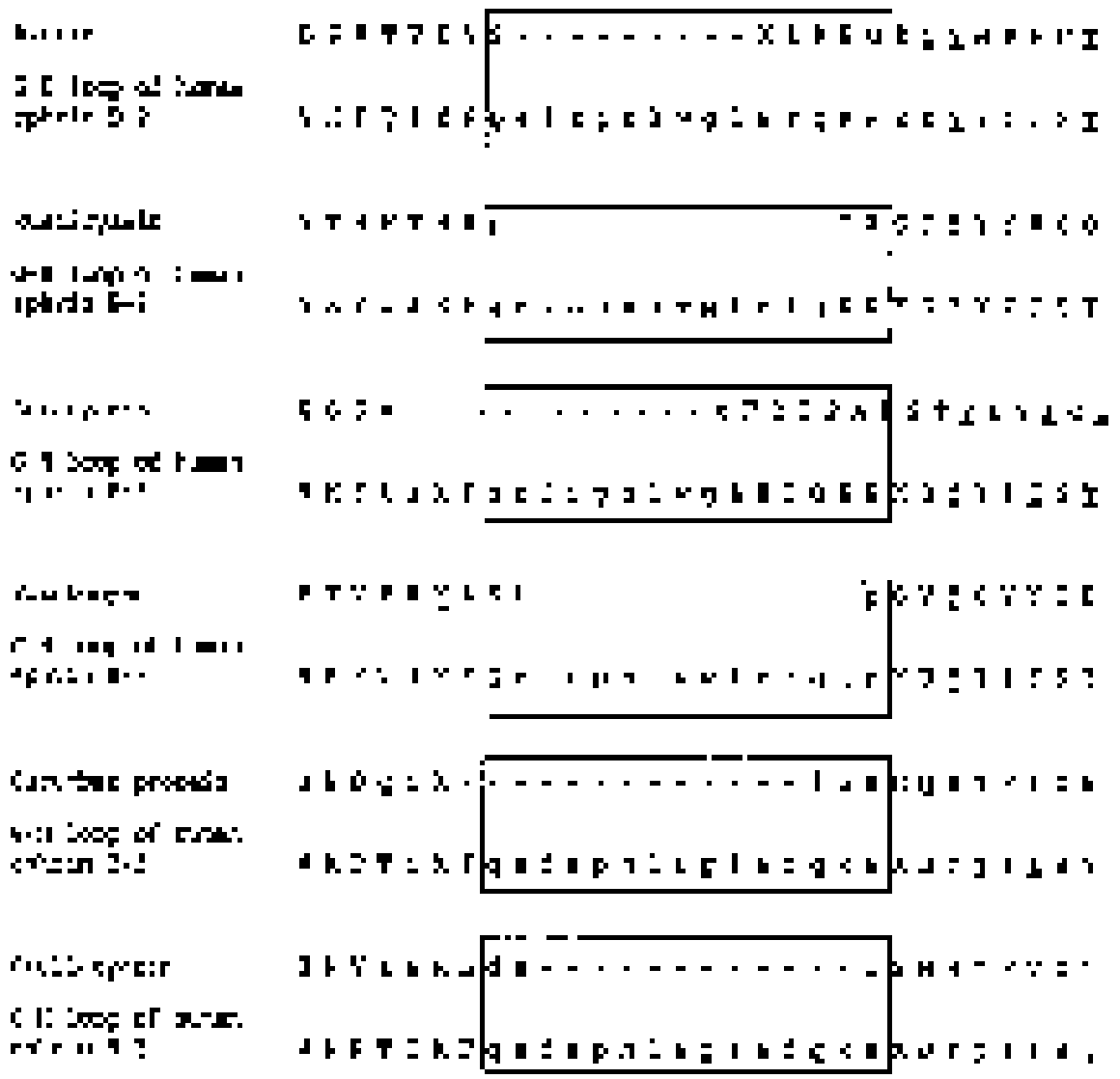
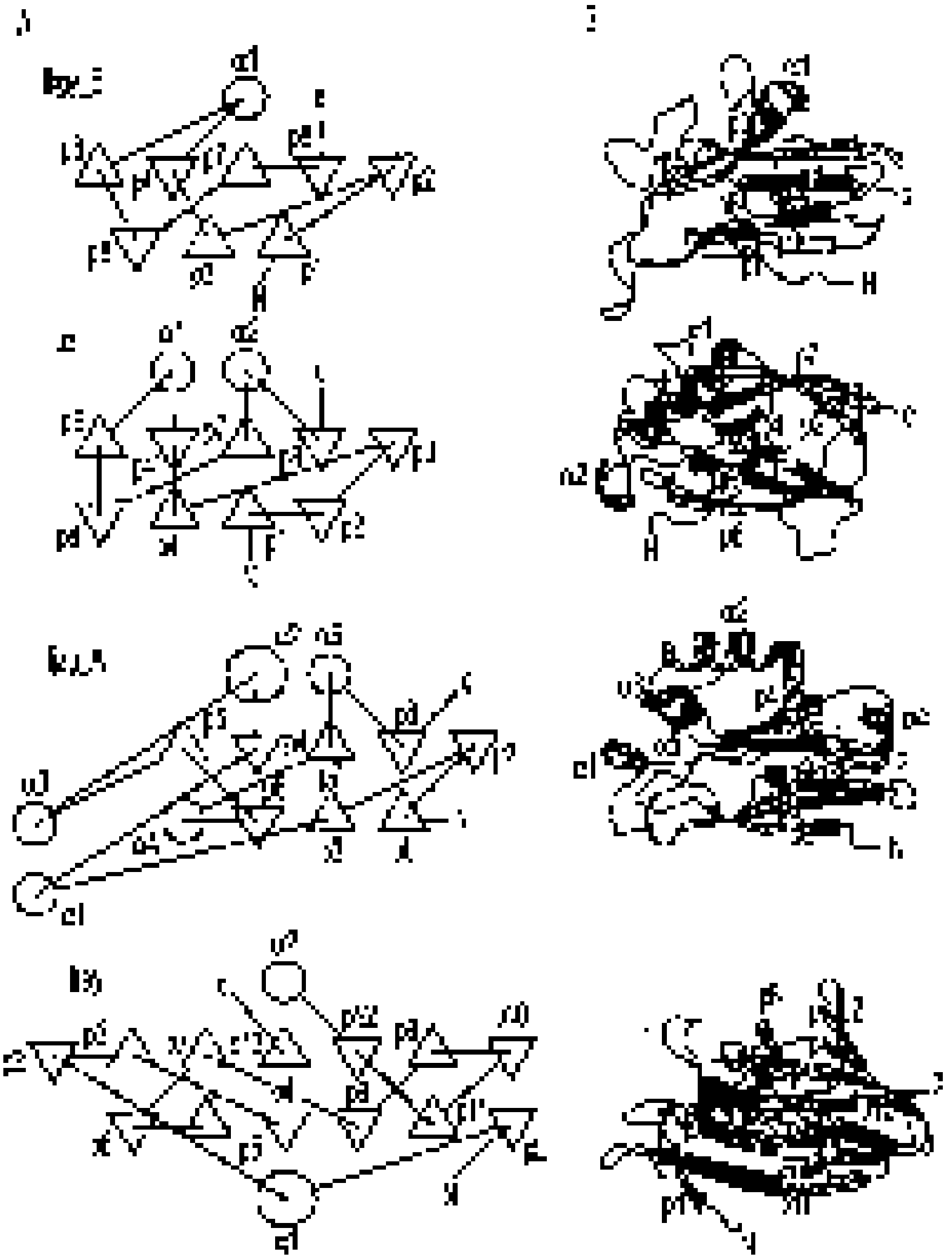


FIG. 2



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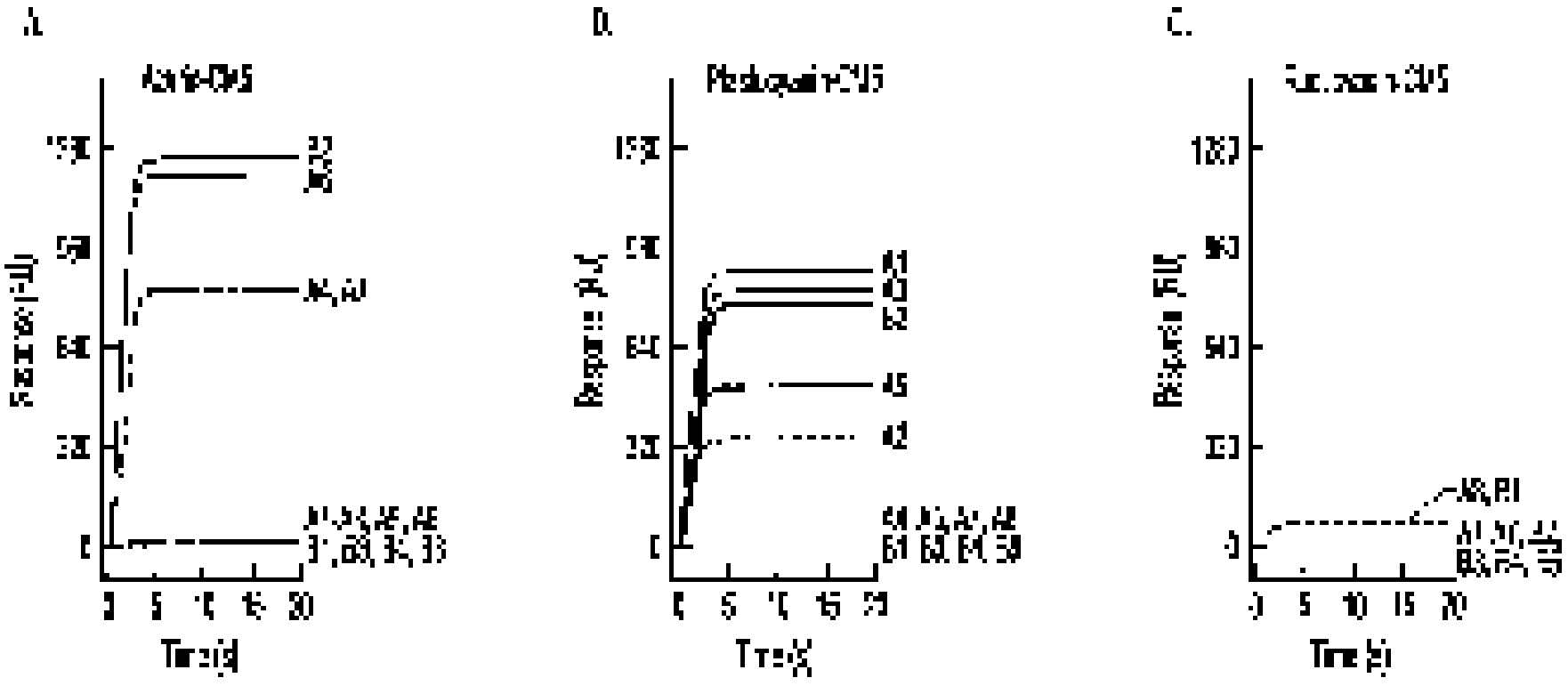

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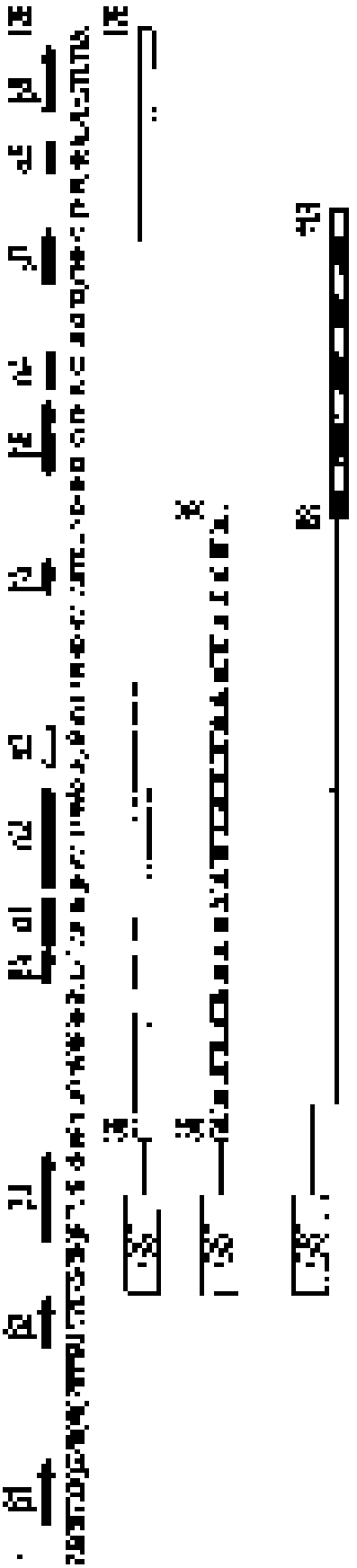

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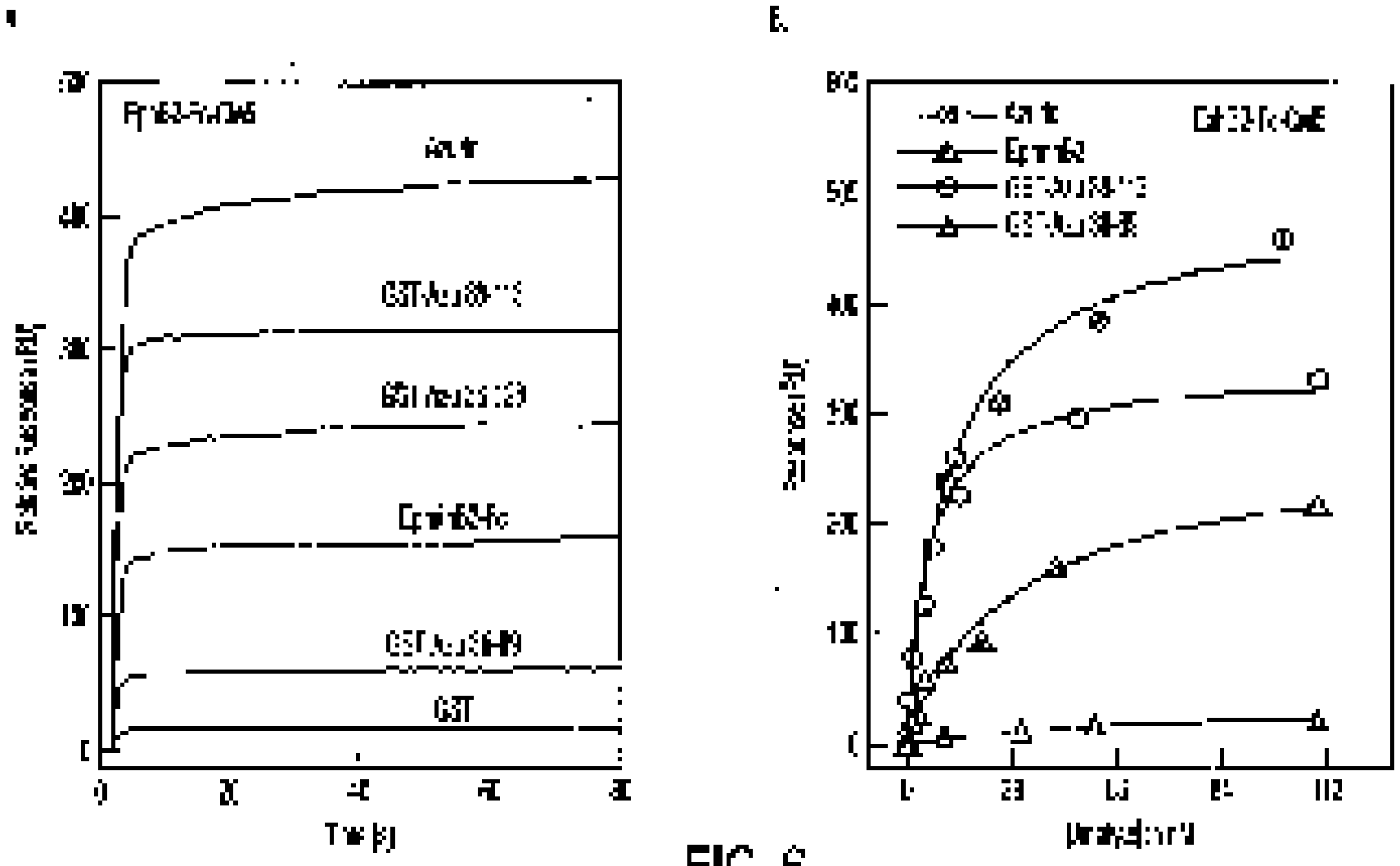
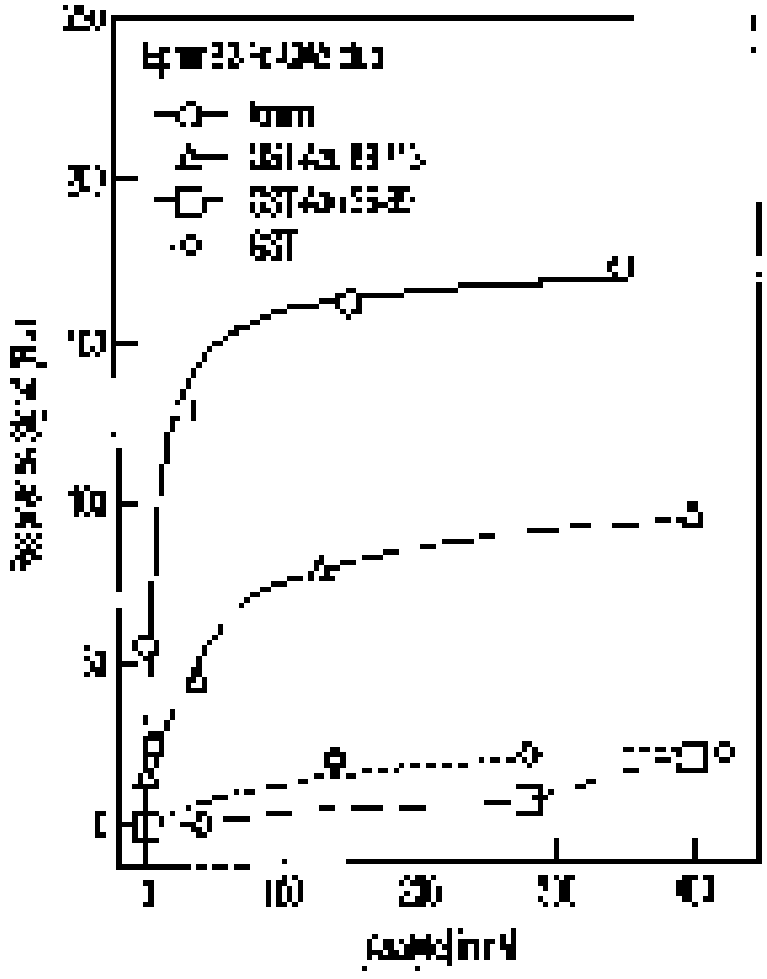
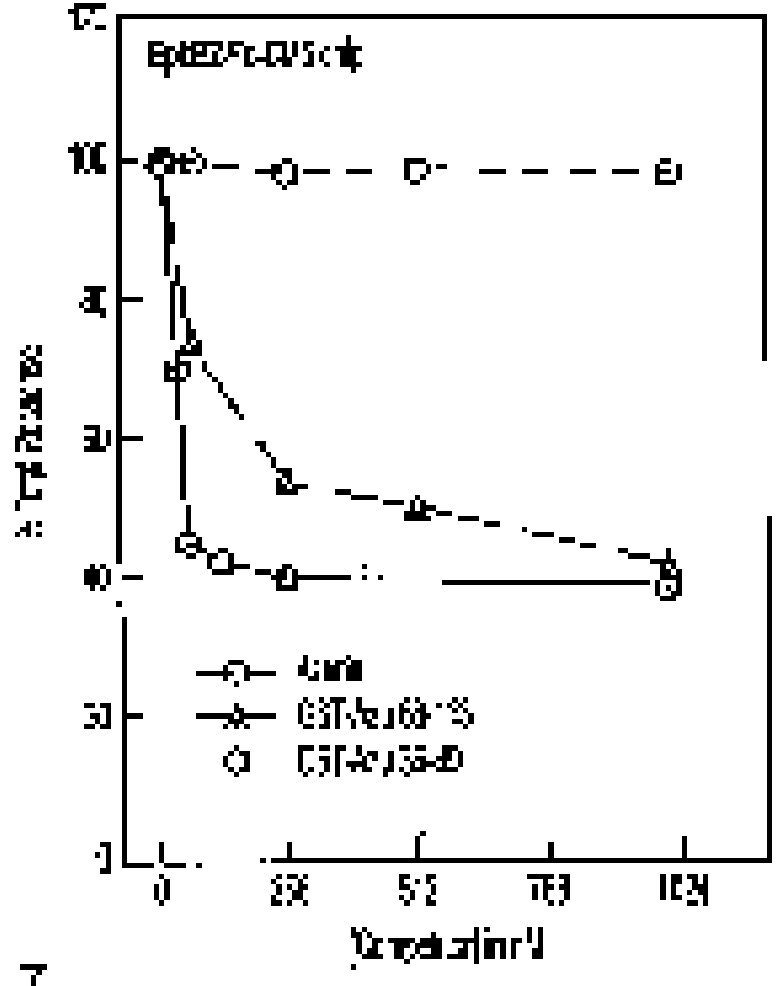


FIG. 6





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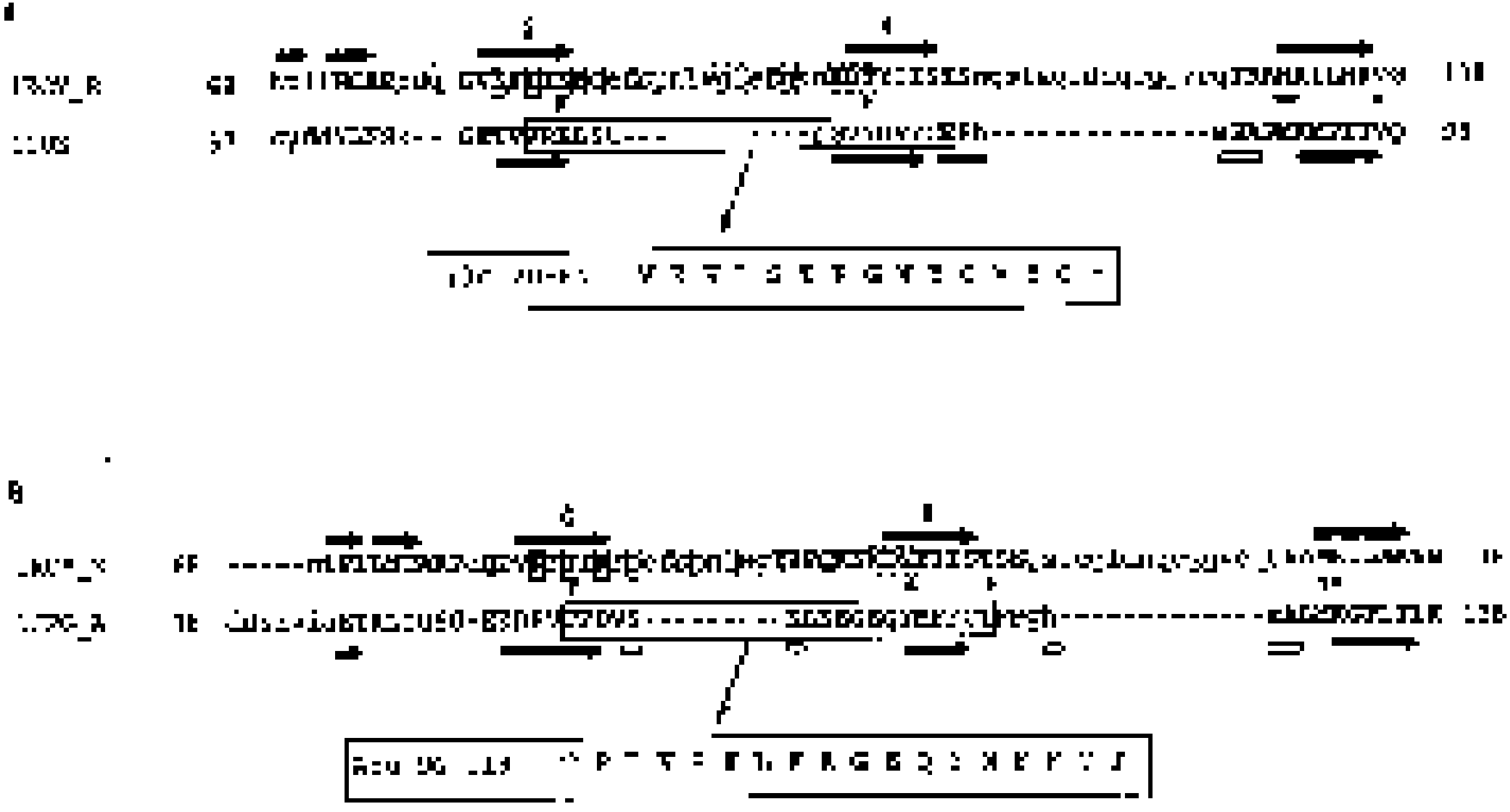

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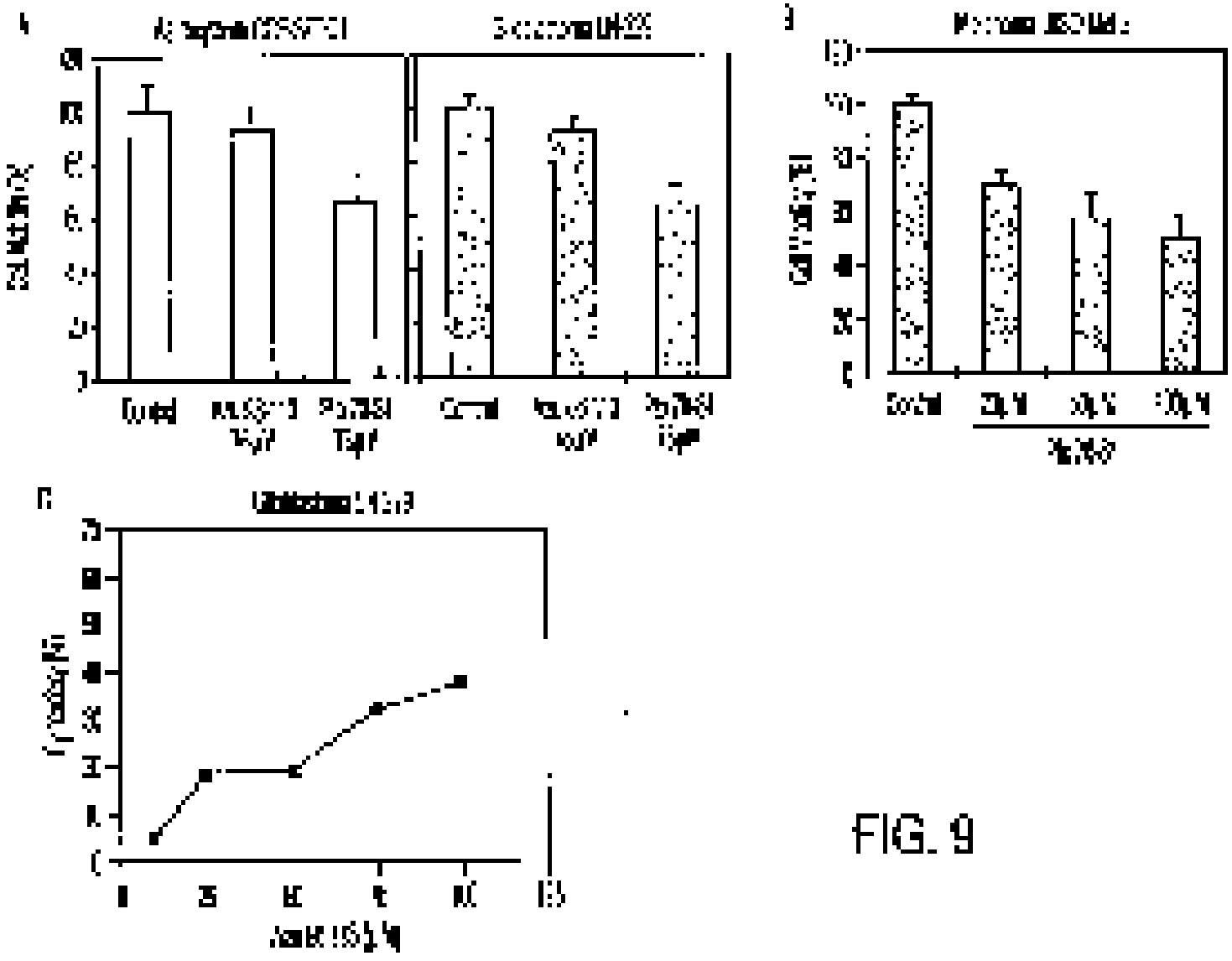

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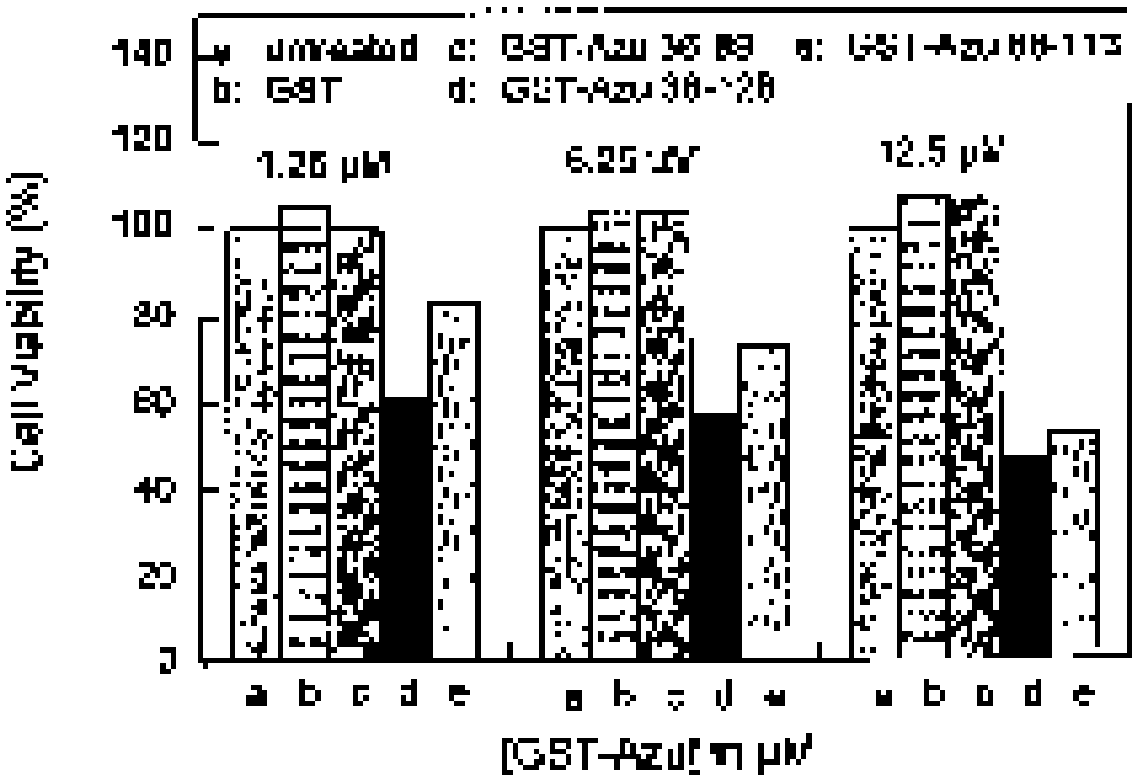
a: untreated
b: GST
c: GST-Azu 36-89
d: GST-Azu 38-128
e: GST-Azu 88-113
1.25 μM
6.25 μM
12.5 μM
Cell viability (%)
140
120
100
80
60
40
20
0
a b c d e
a b c d e
a b c d e
[GST-Azu] in μM

FIG. 10

[illegible]

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Example [illegible]

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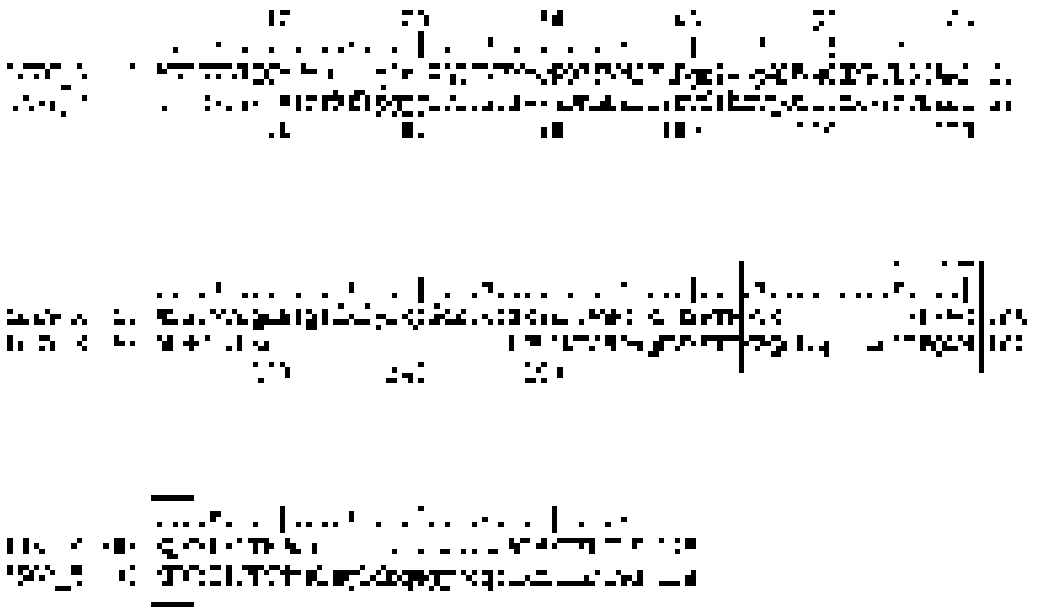

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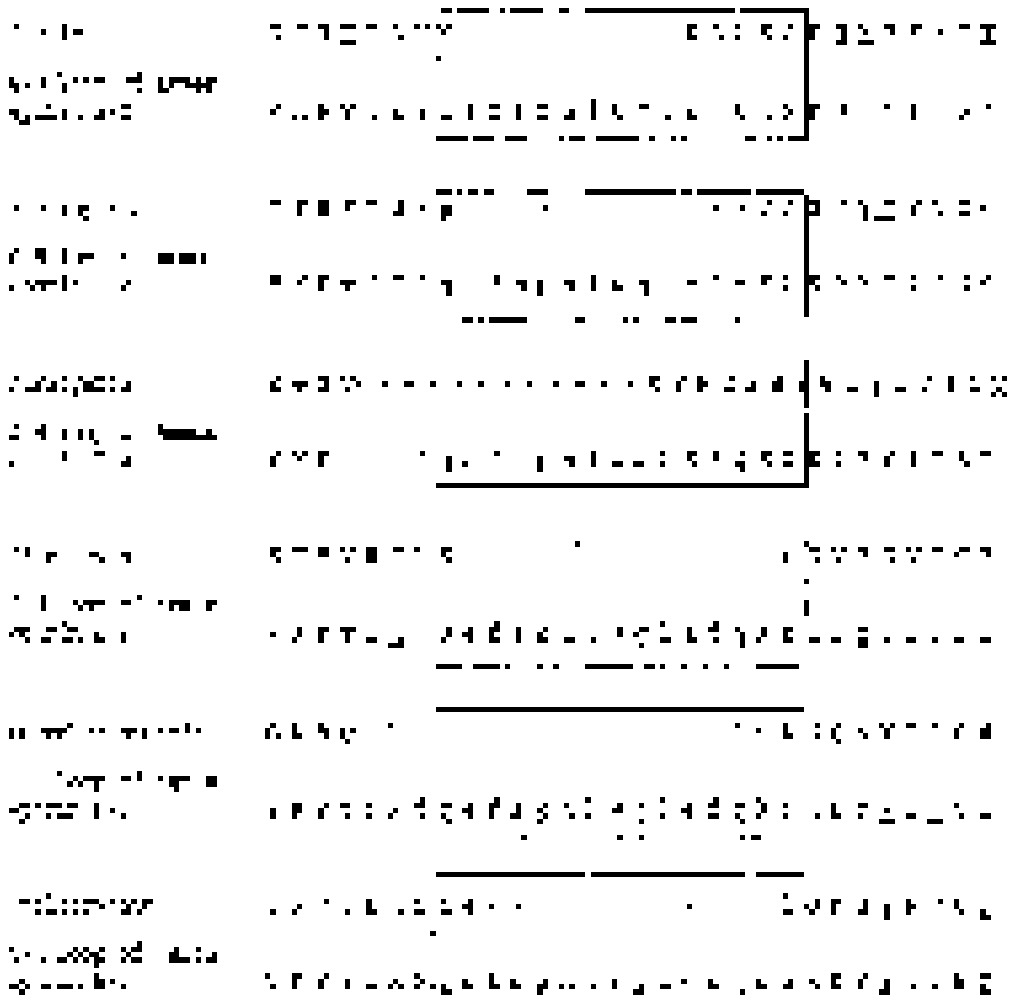

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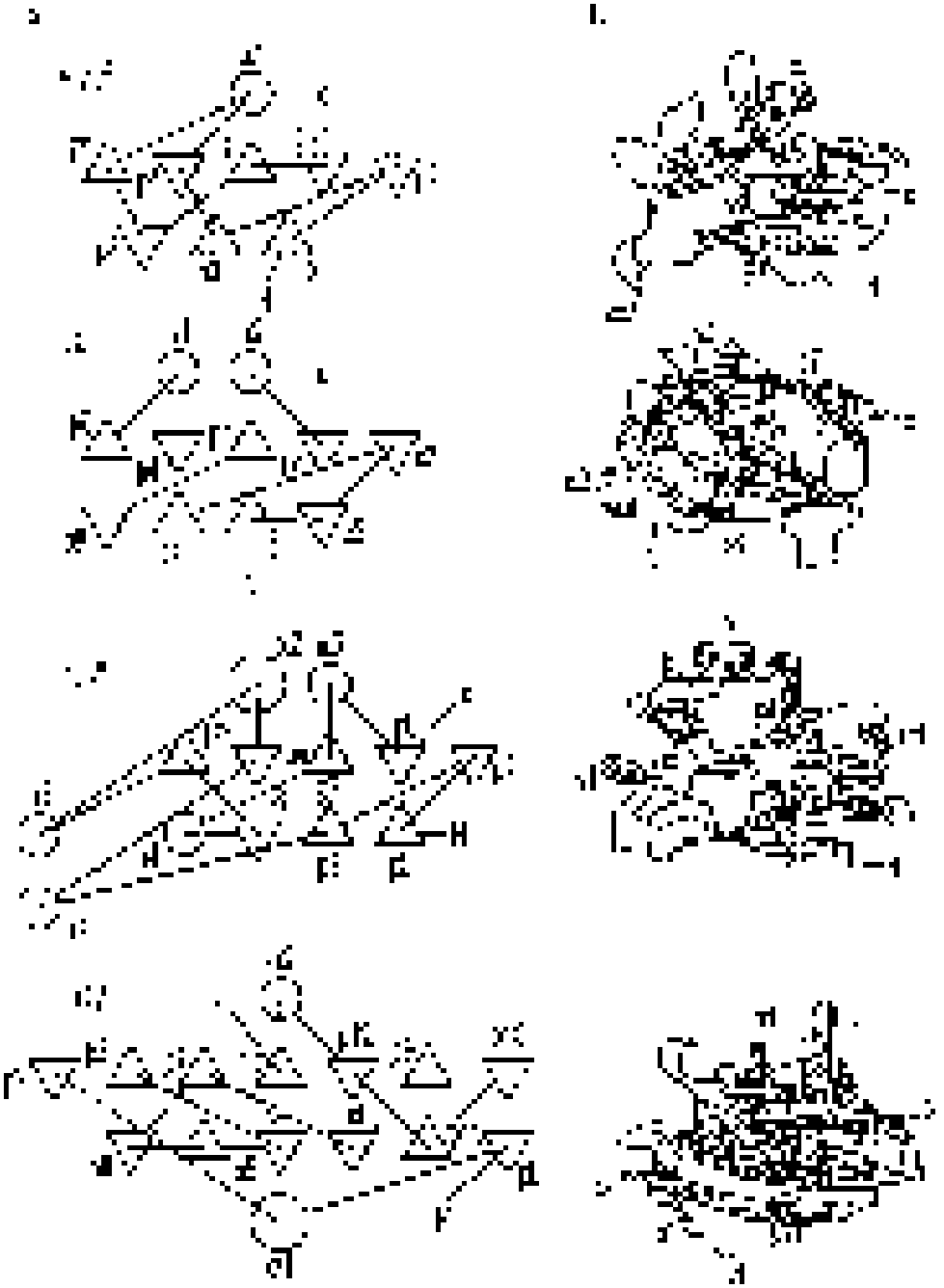

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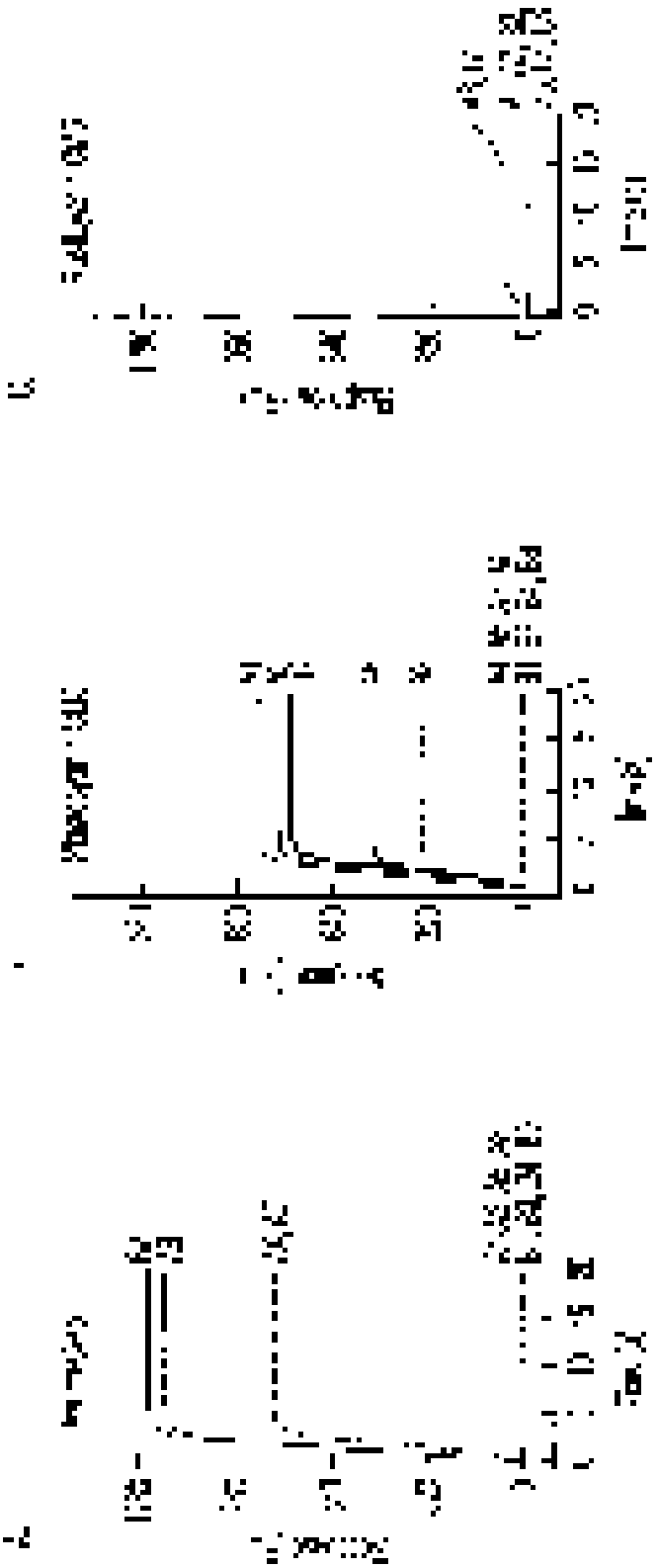
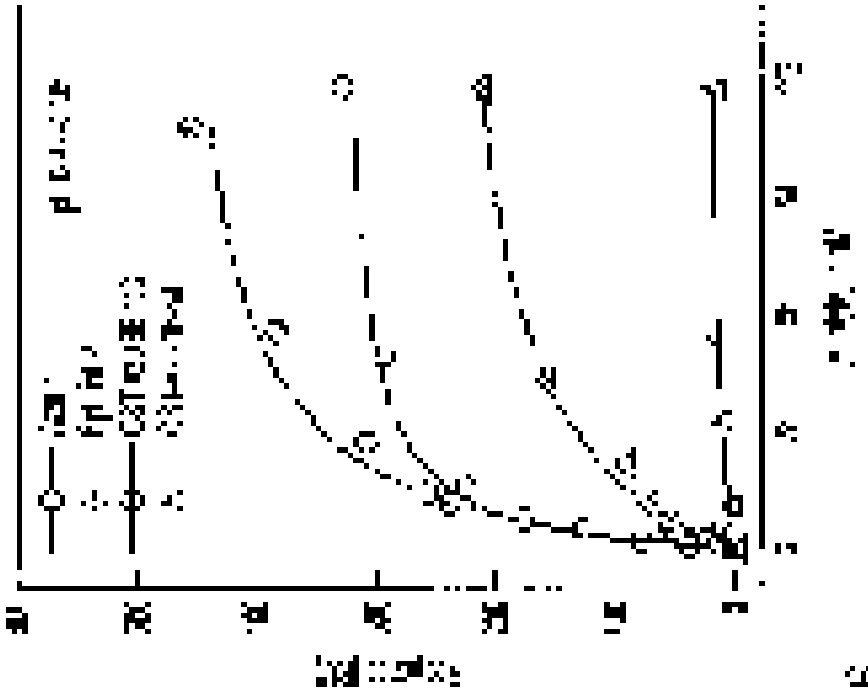


FIG. 4

FIG. 5



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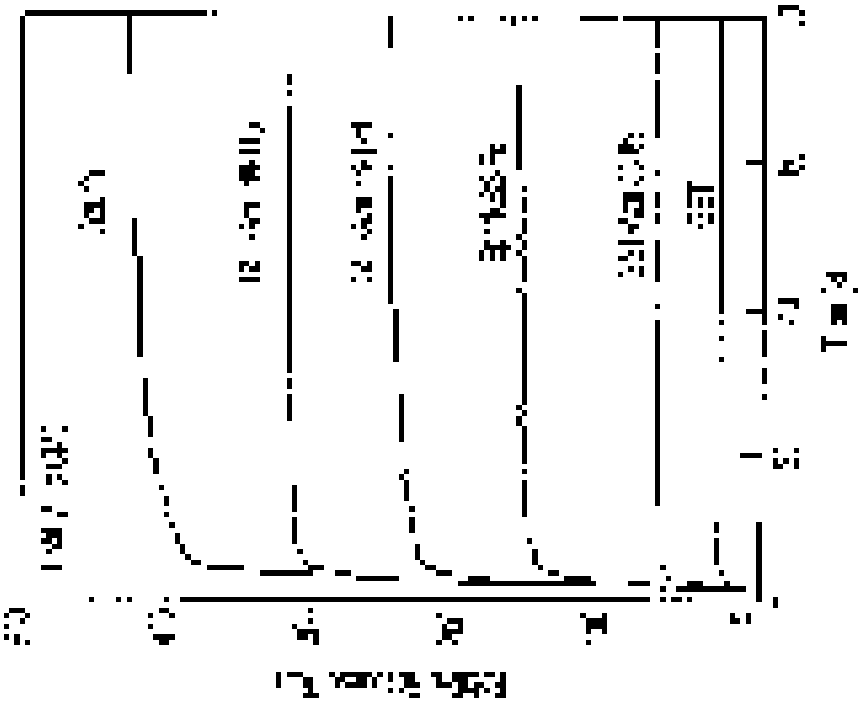

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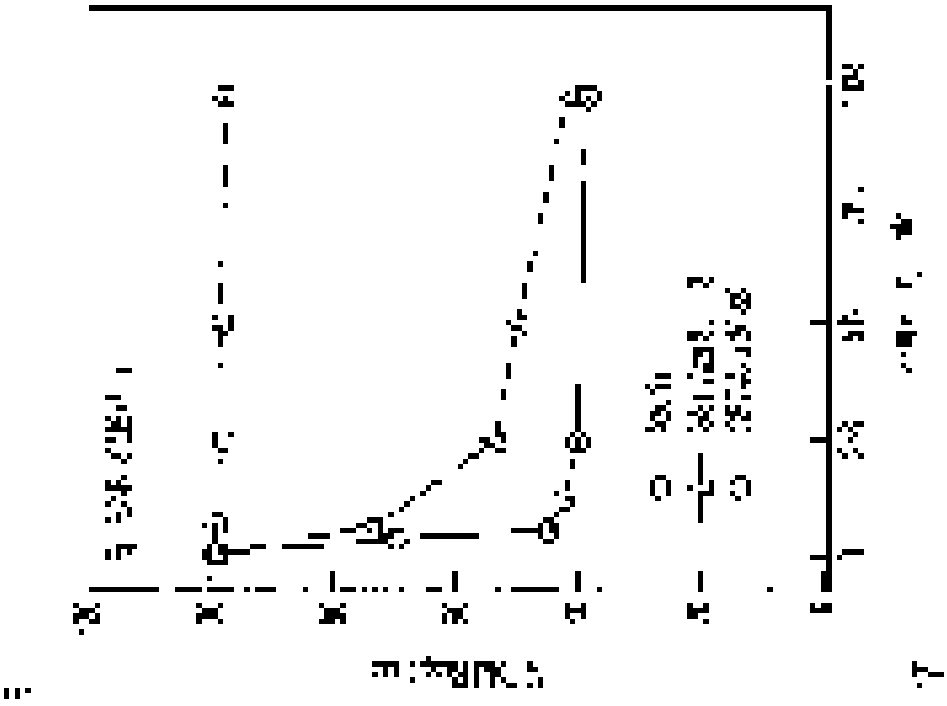

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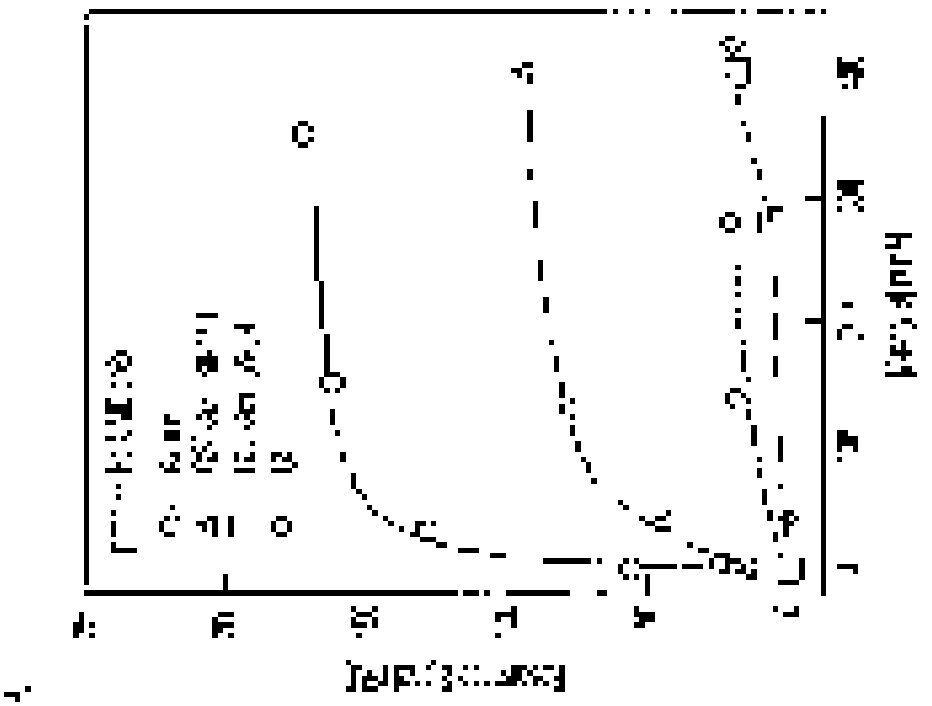


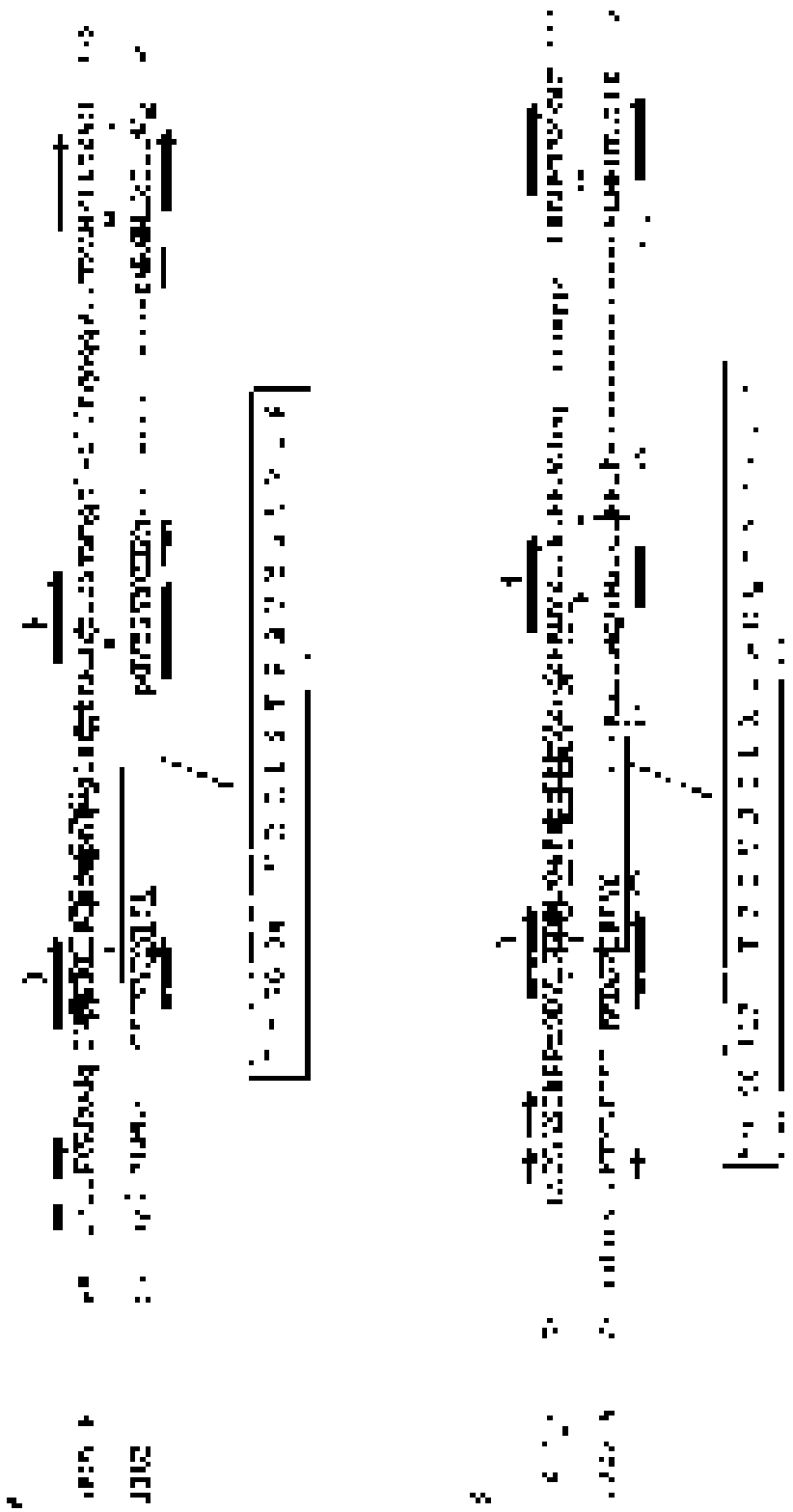


FIG. 8

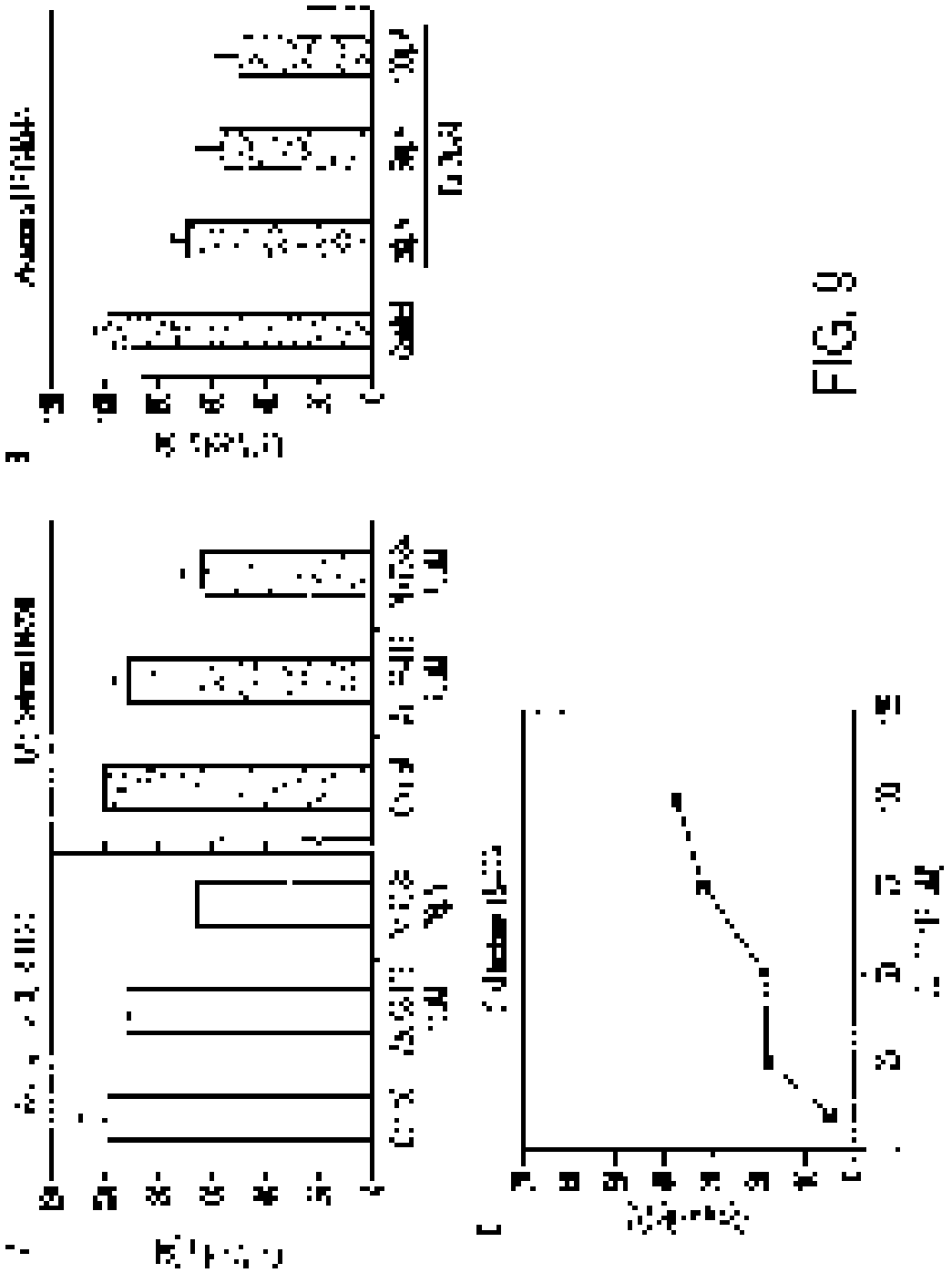
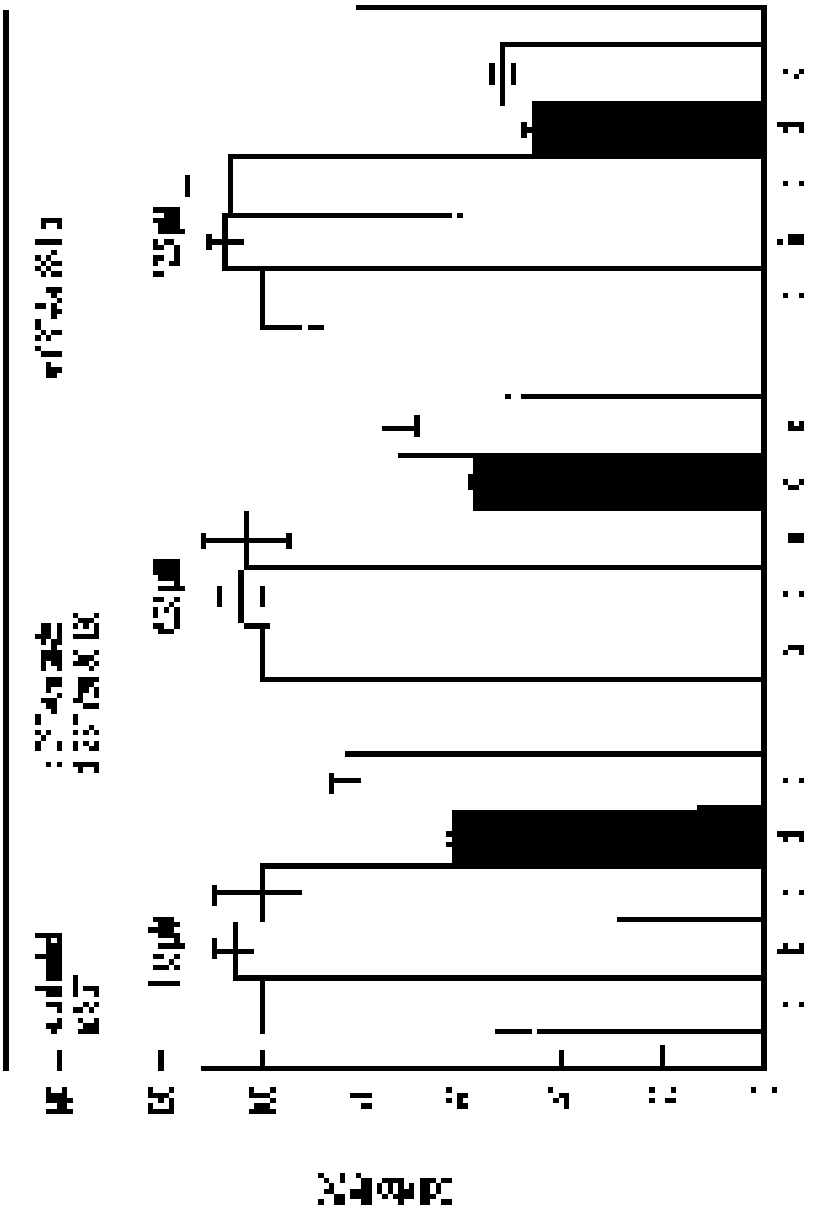


FIG. 9



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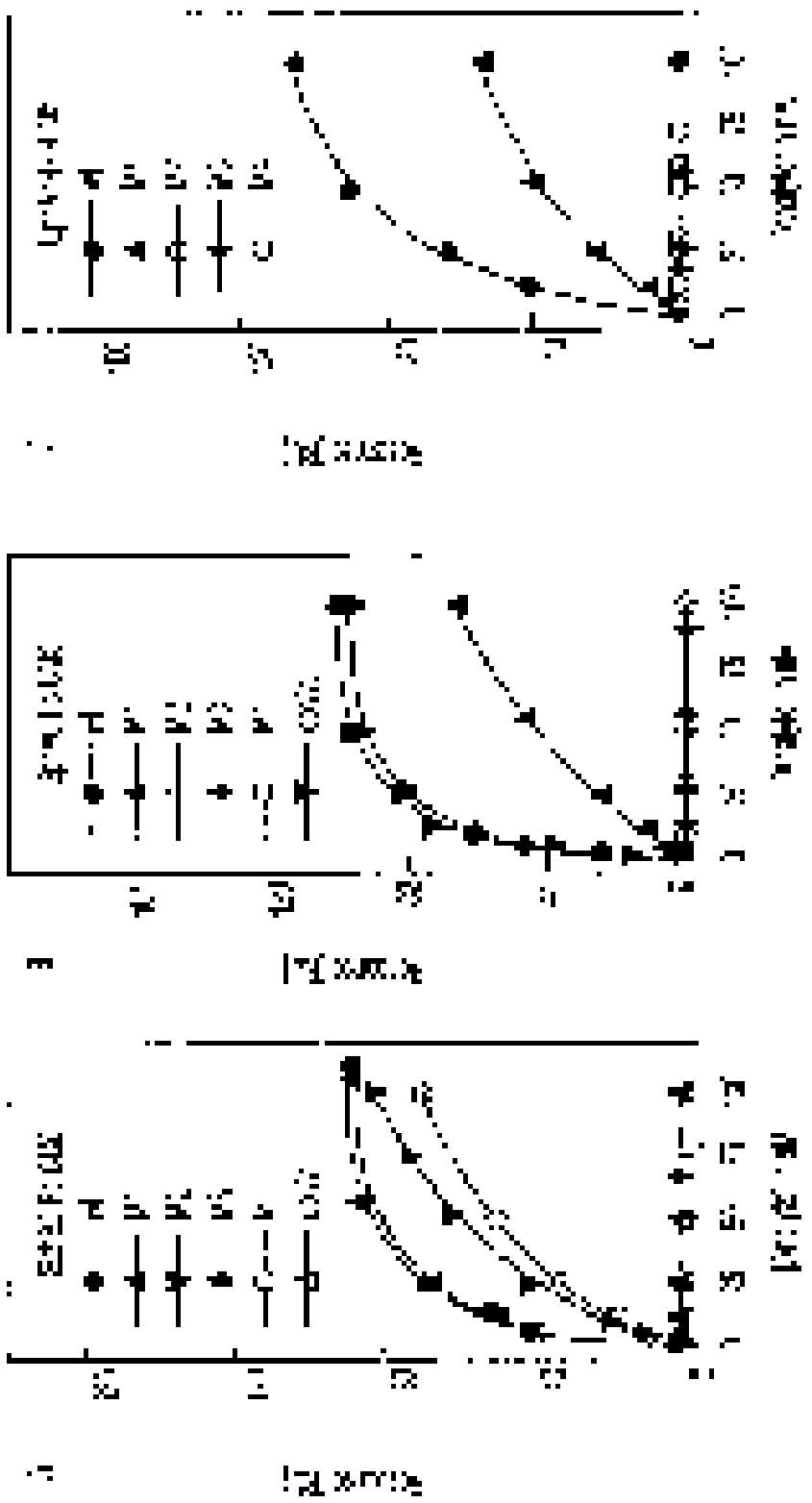

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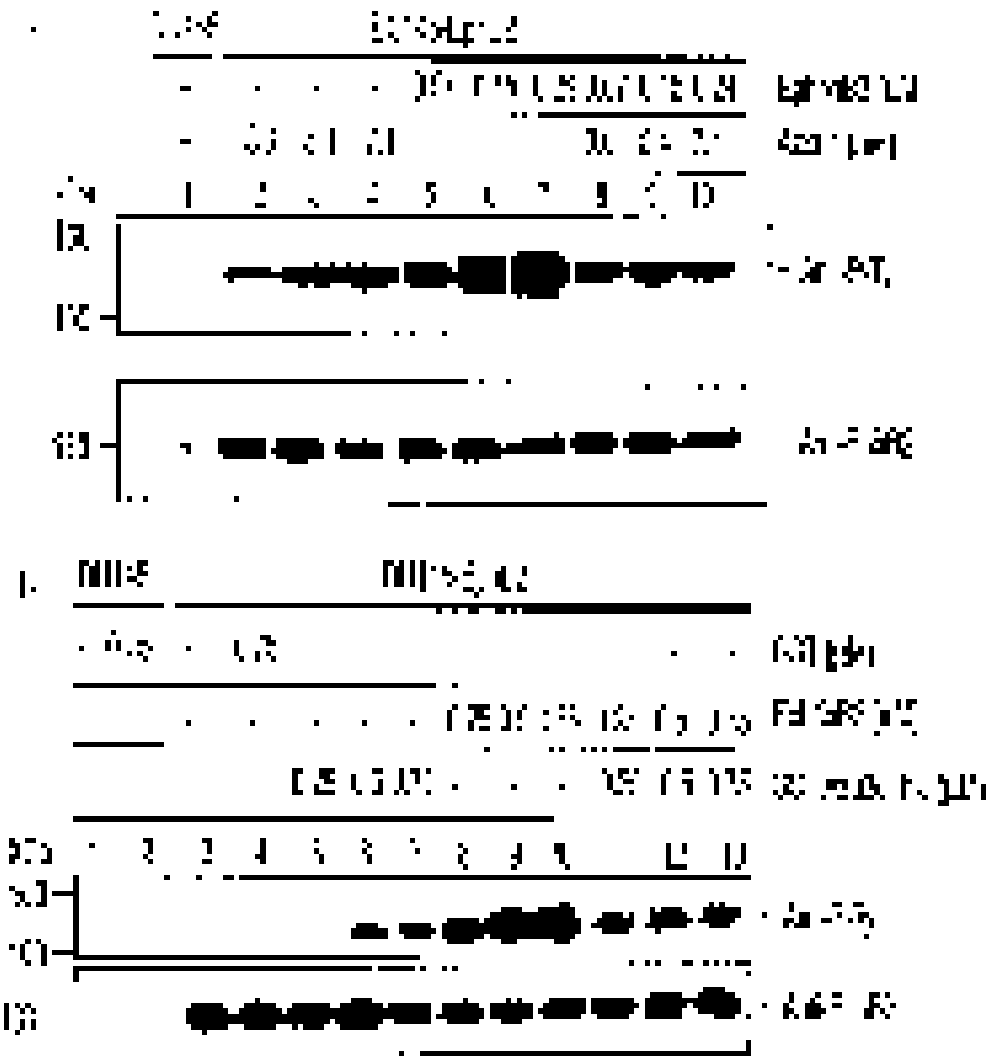

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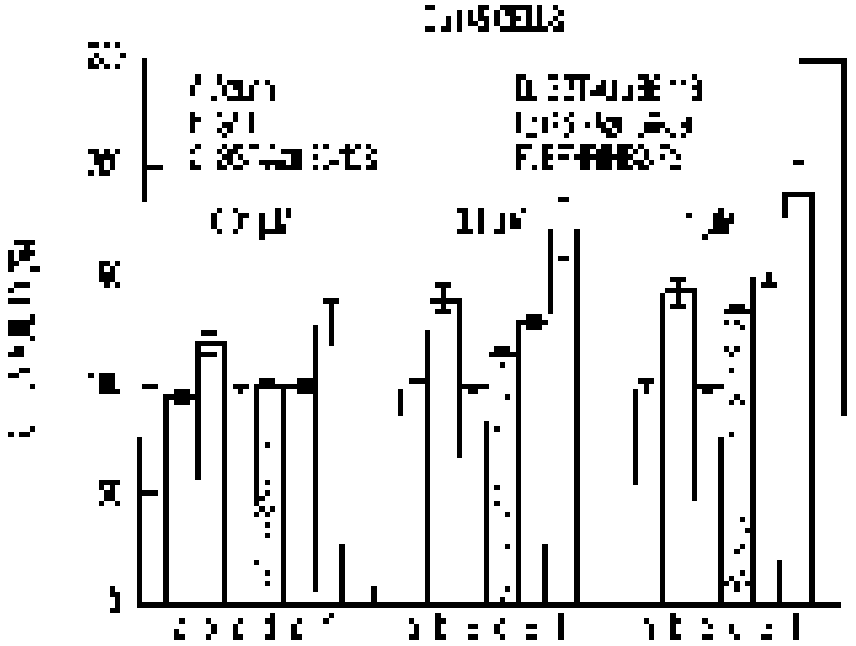

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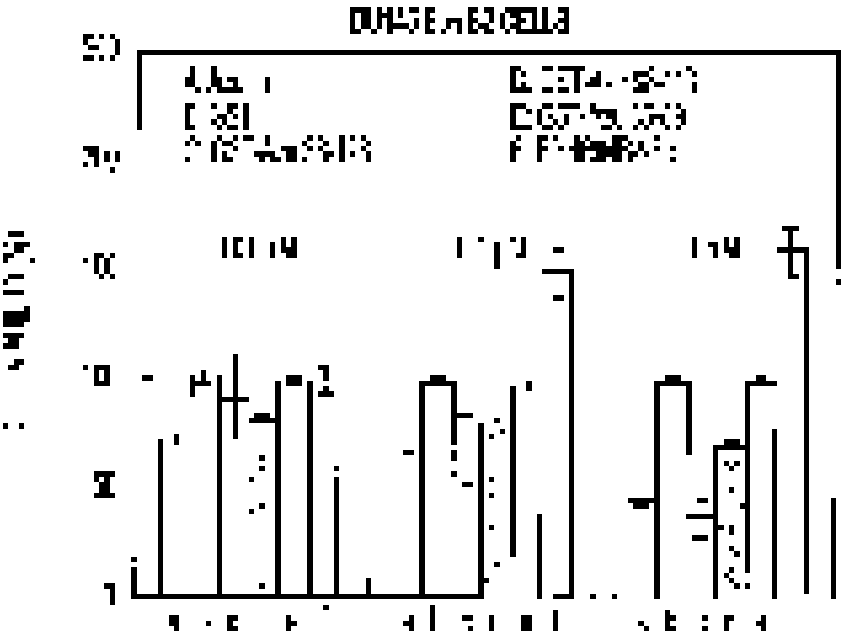

FIG. 13B

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RELATED APPLICATIONS

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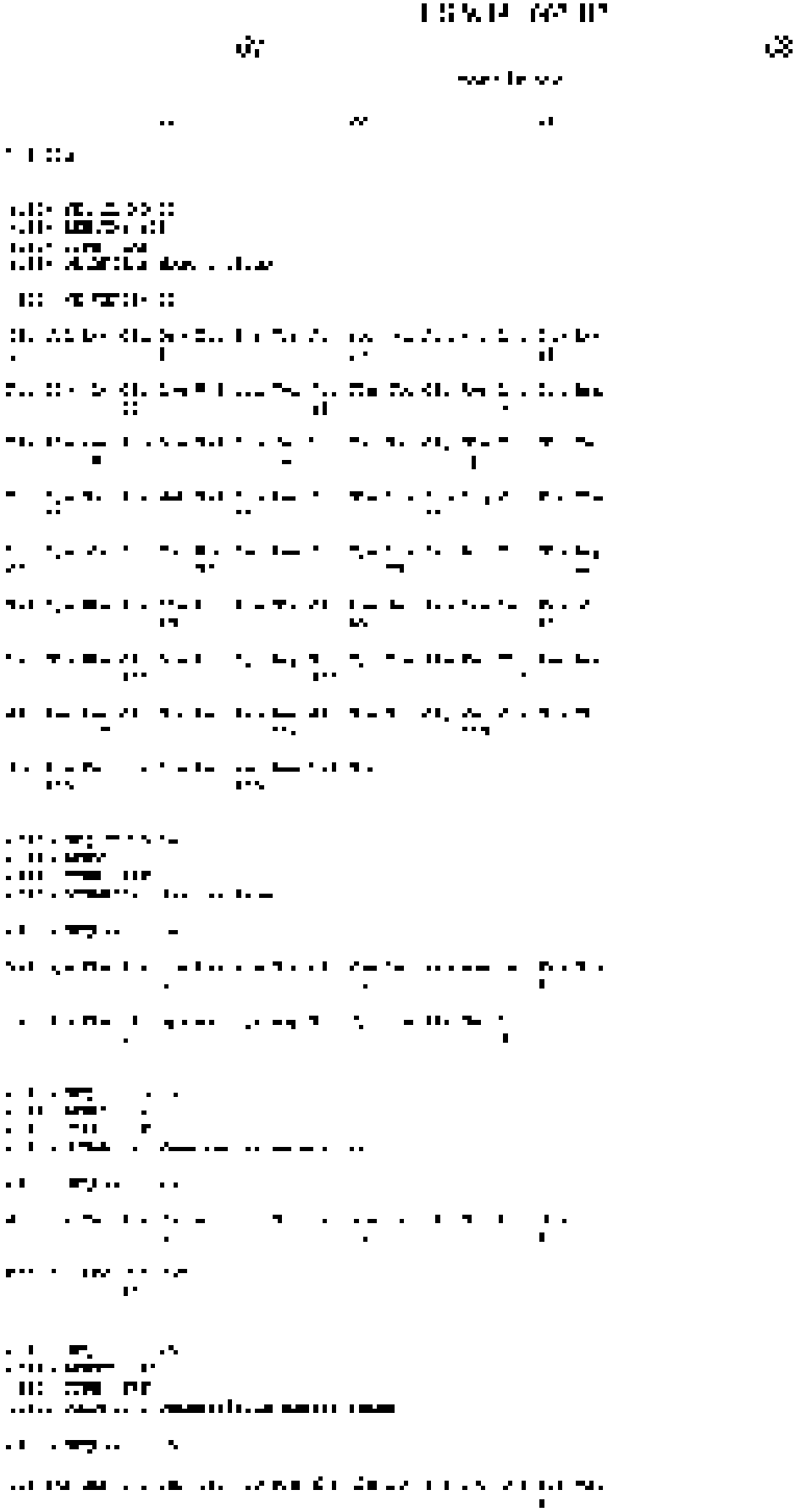

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(19) United States
(12) Patent Application Publication
Chandhari et al.

(10) Pub. No.: US 2016/0151452 A1
(43) Pub. Date: Jun. 2, 2016

(54) [illegible]

(71) Applicant: [illegible]

(72) [illegible]

(21) [illegible]

(22) [illegible]

Related U.S. Application Data

[illegible]

(51) Int. Cl.
[illegible]

(52) U.S. C.
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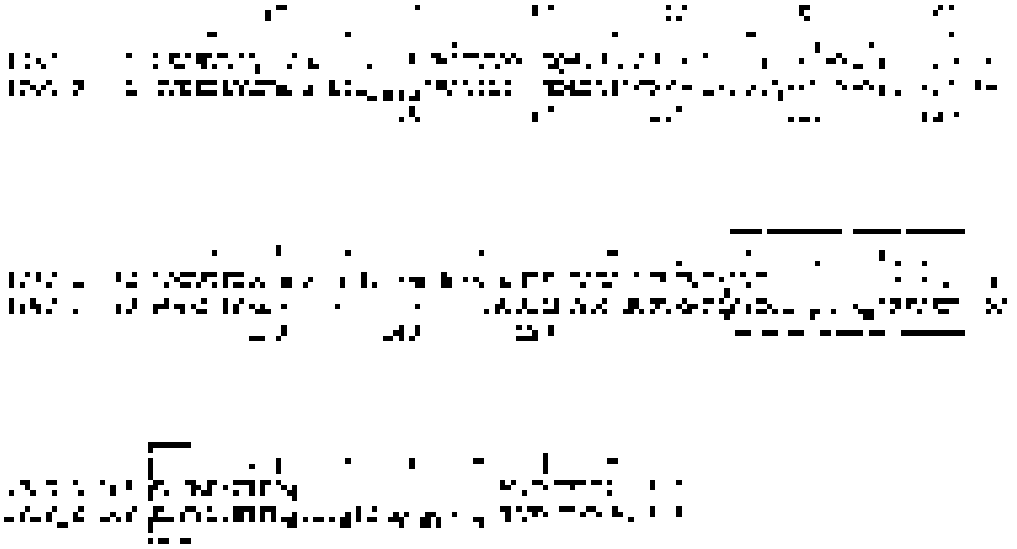
(57) ABSTRACT

[illegible]



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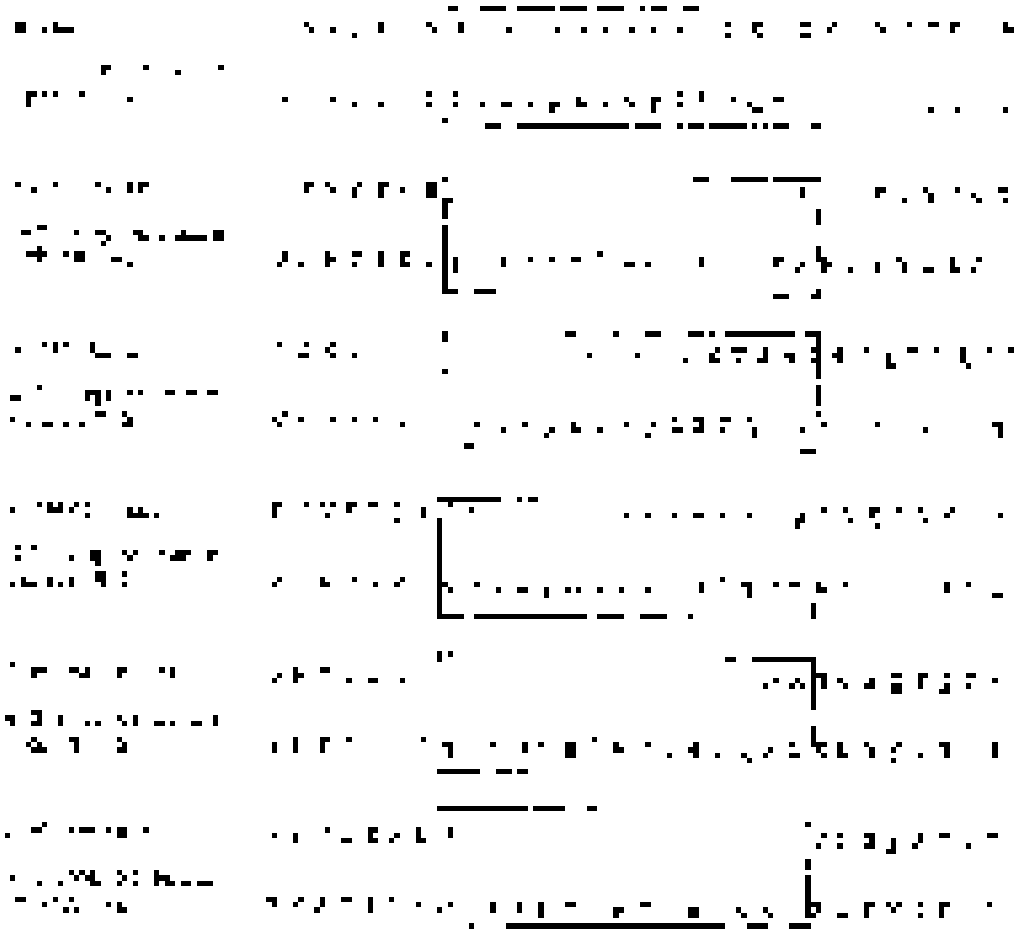


FIG. 2

FIG. 9A

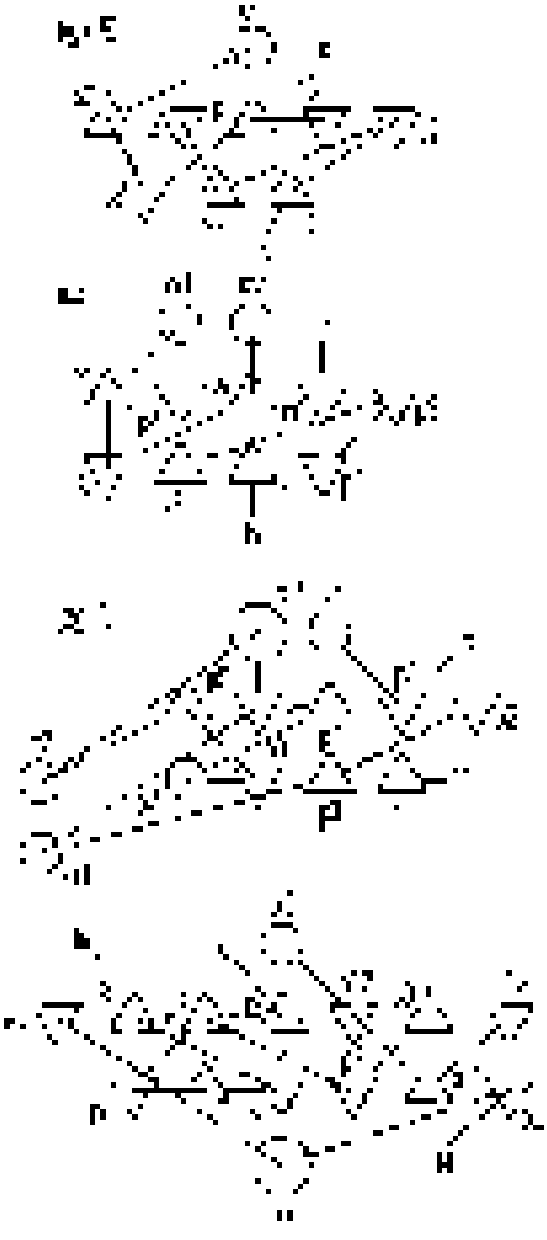


FIG. 9B

FIG. 4C

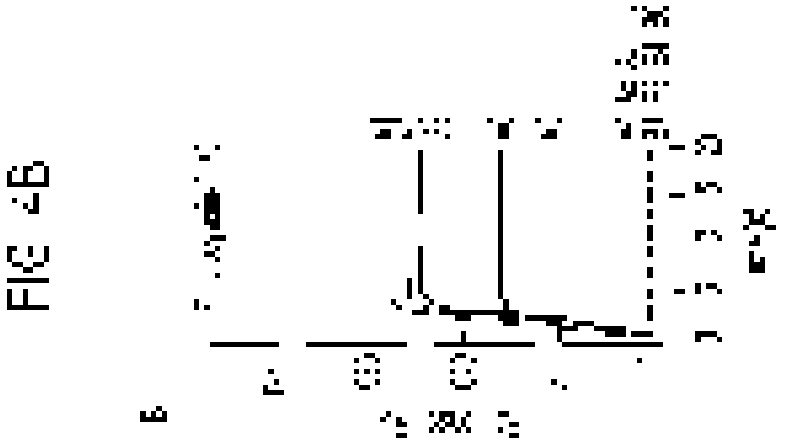


FIG. 4B

FIG. 4A

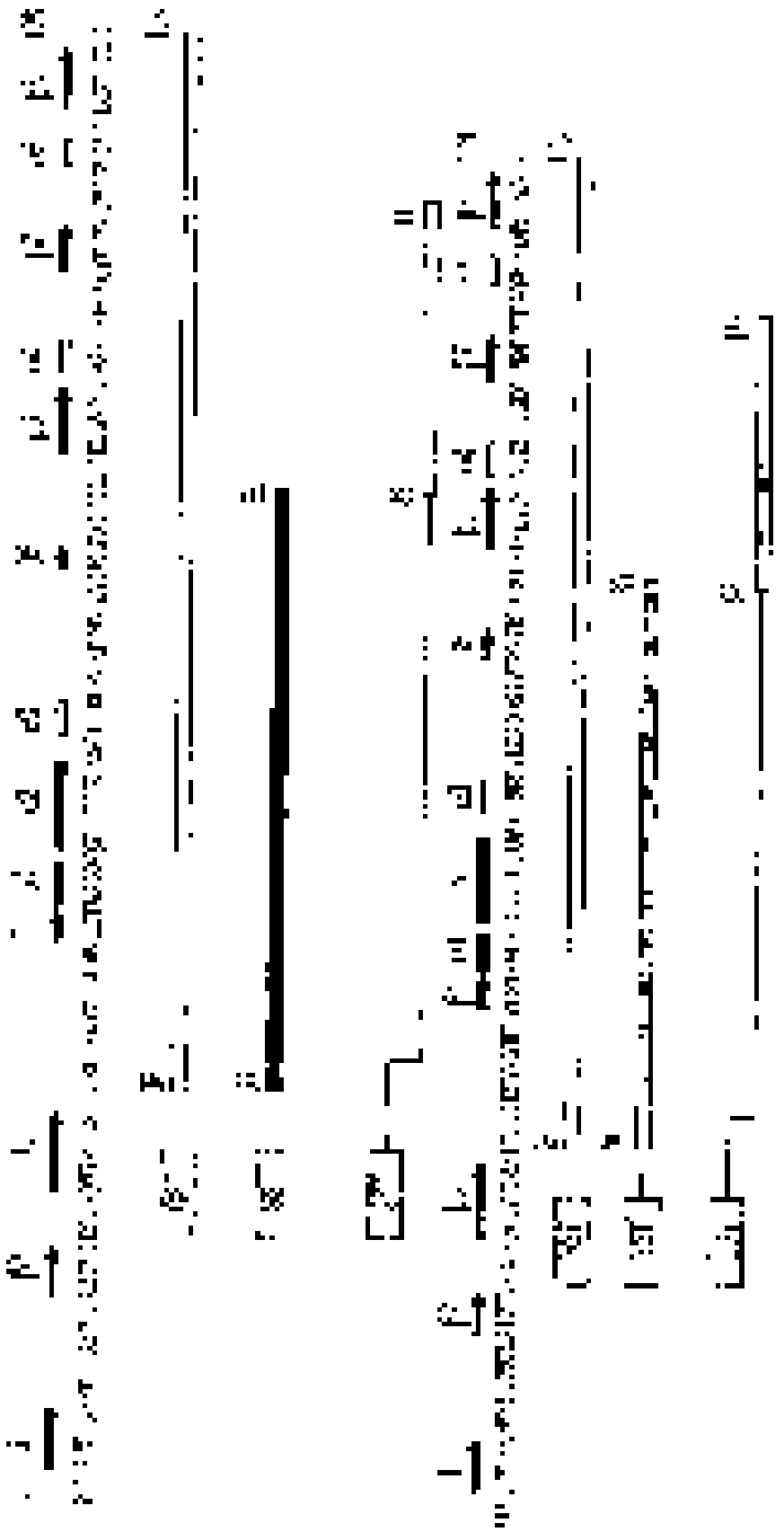
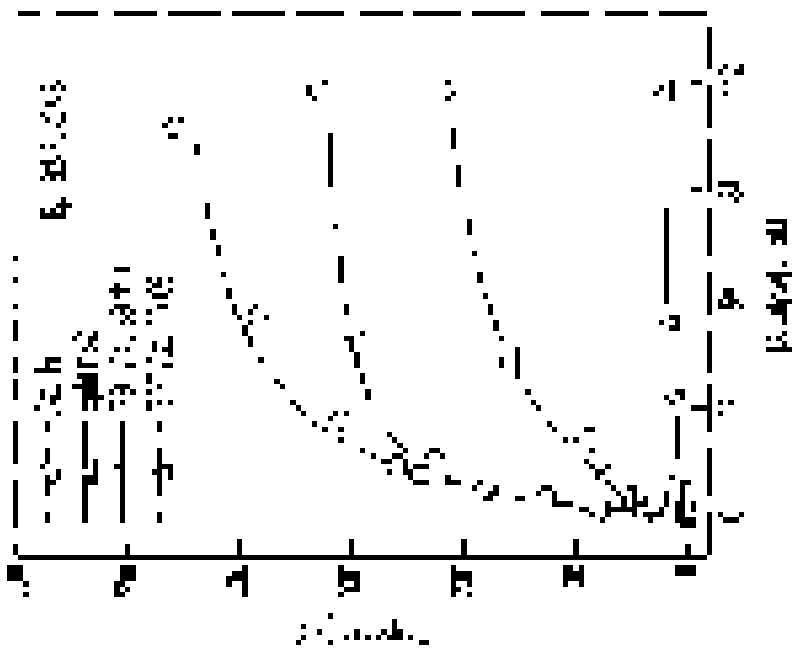


FIG. 5

FIG. 8B



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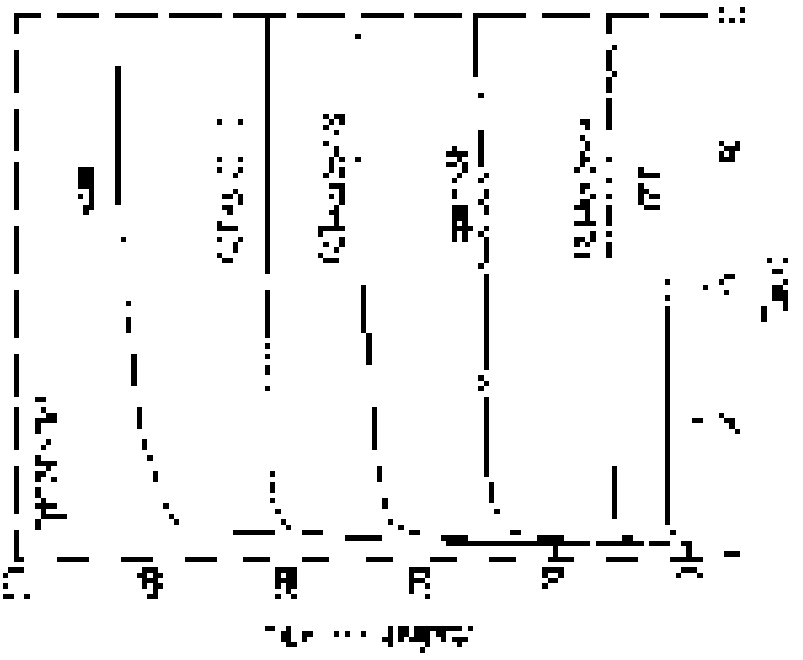


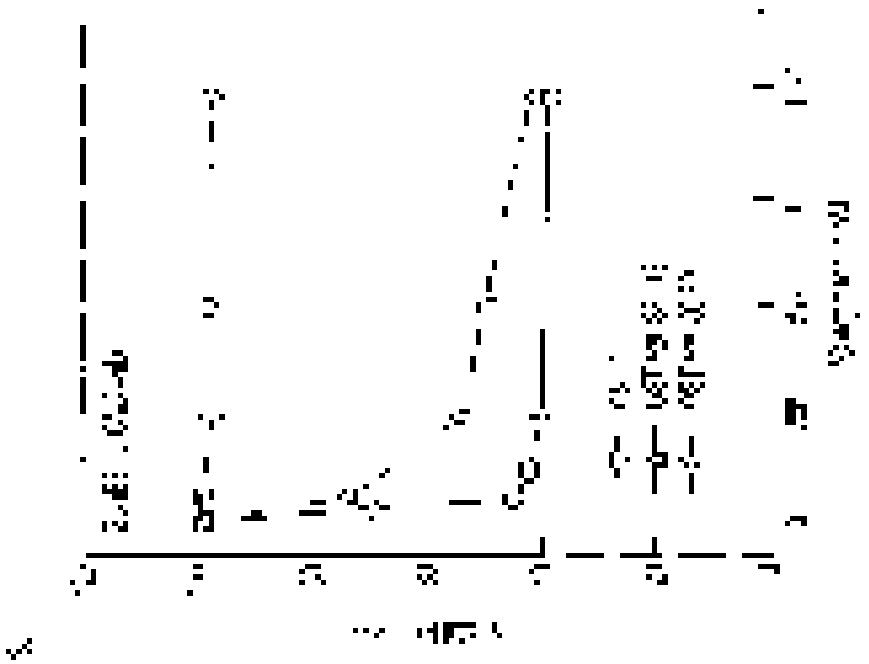


FIG. 7E

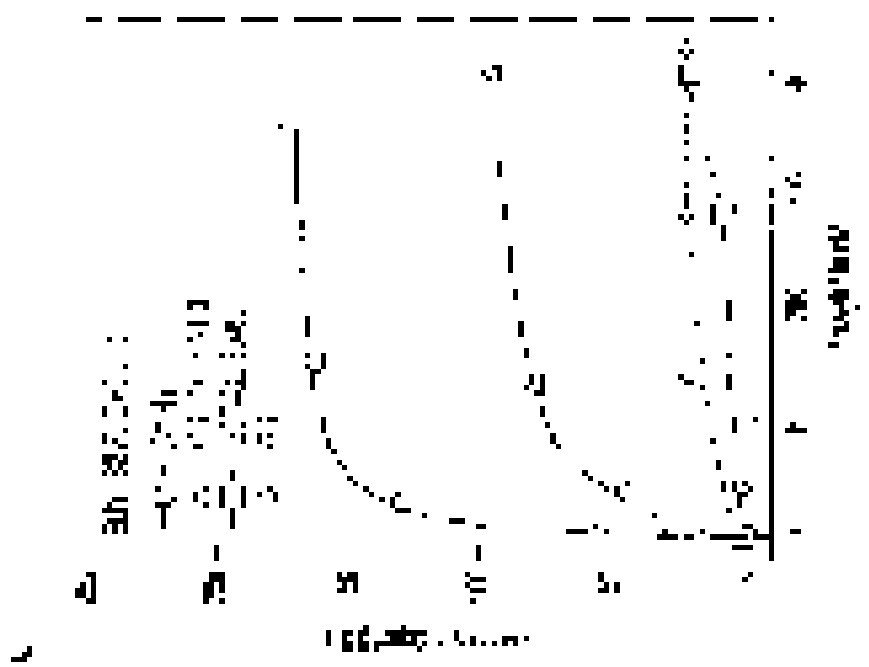
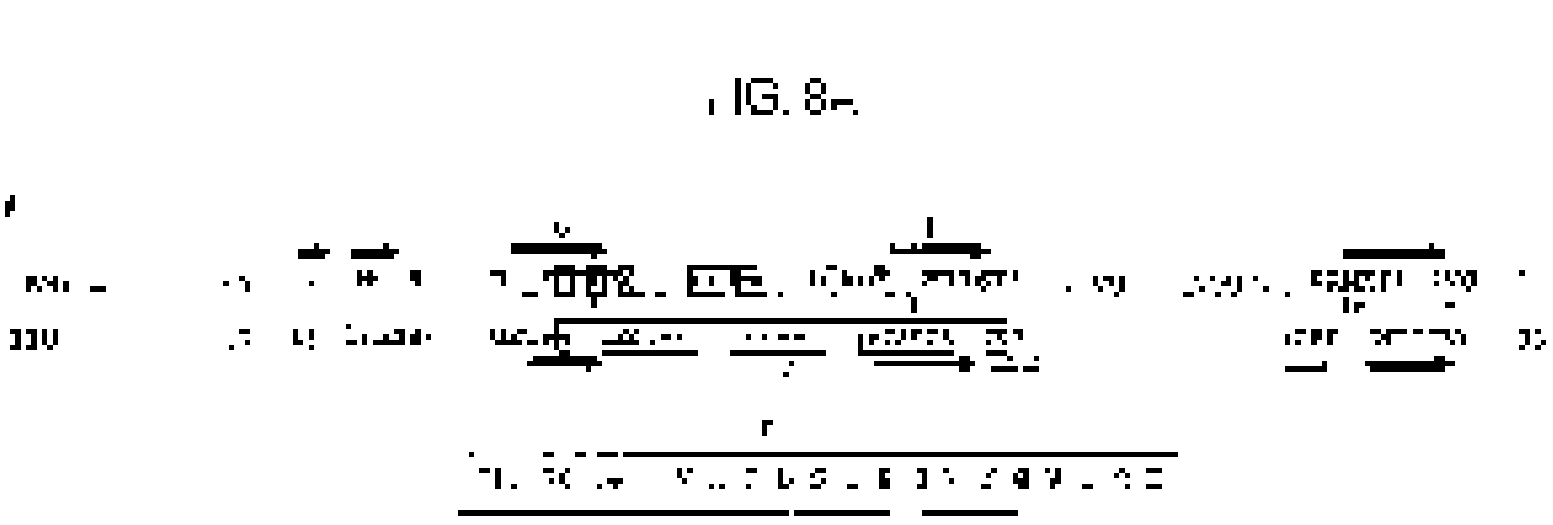


FIG. 7F

FIG. 8A



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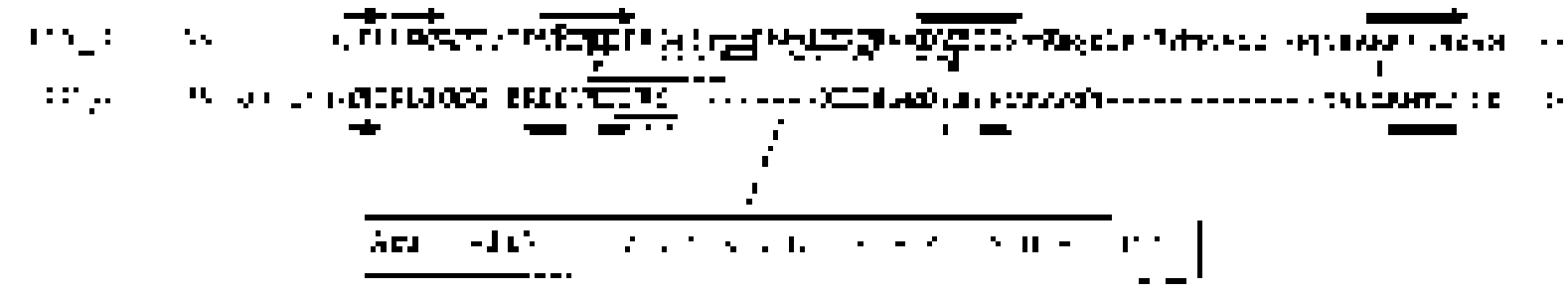

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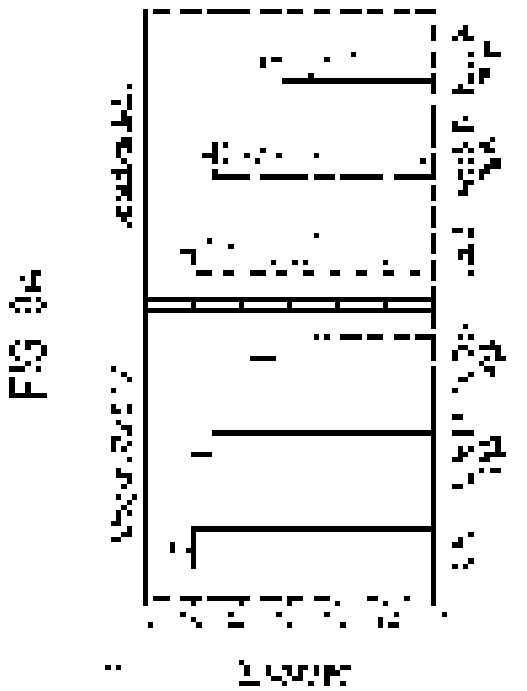

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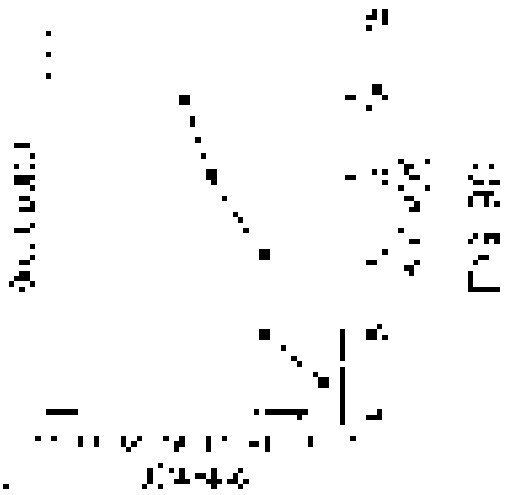


FIG. 8C

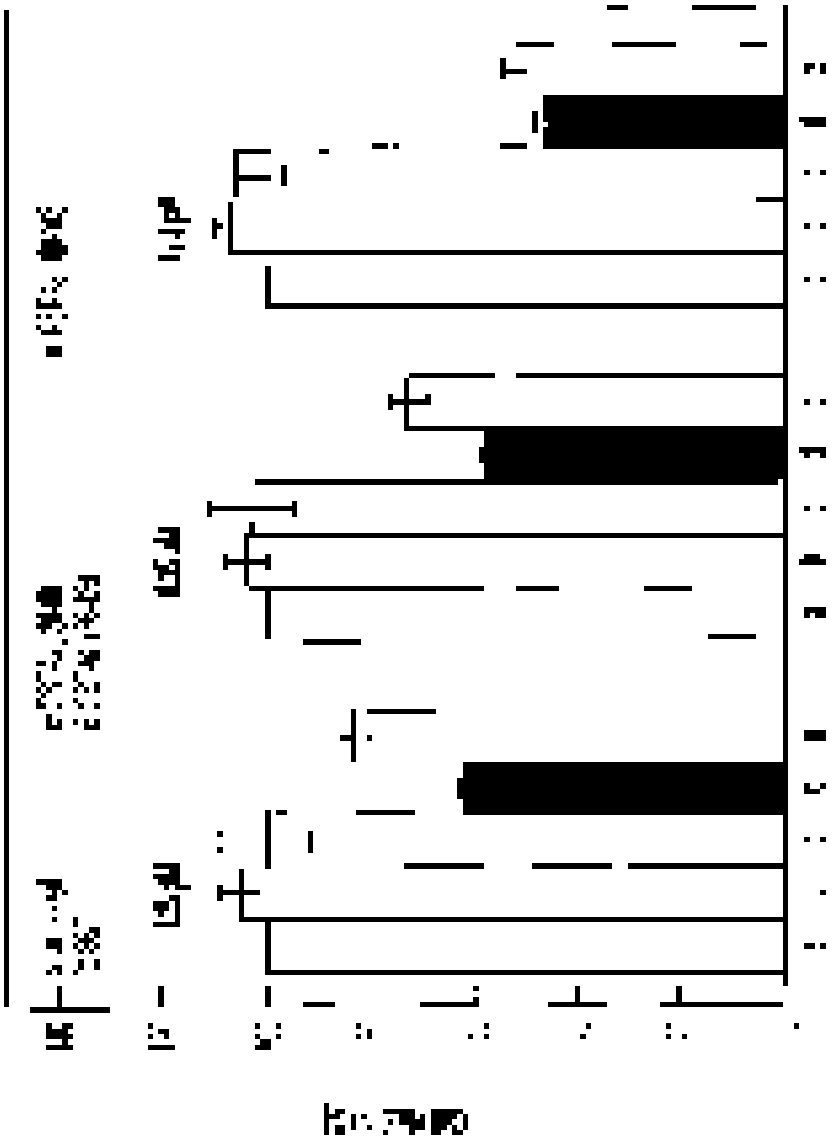


FIG. 10

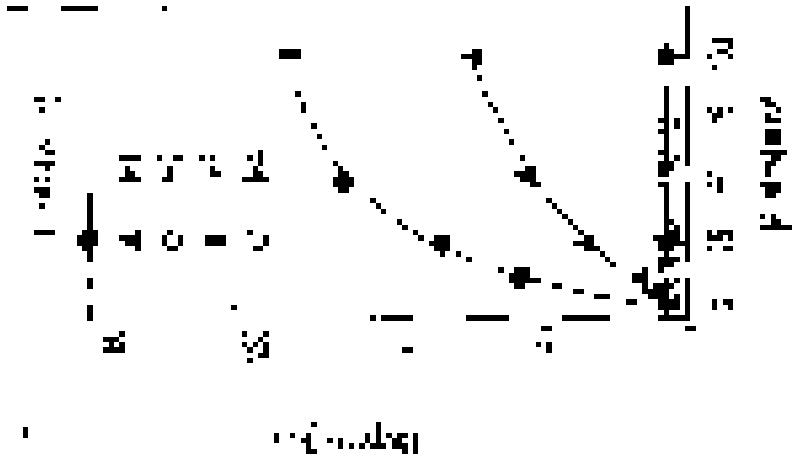


FIG. 11C

FIG. 11B

FIG. 11A

FIG. 12A

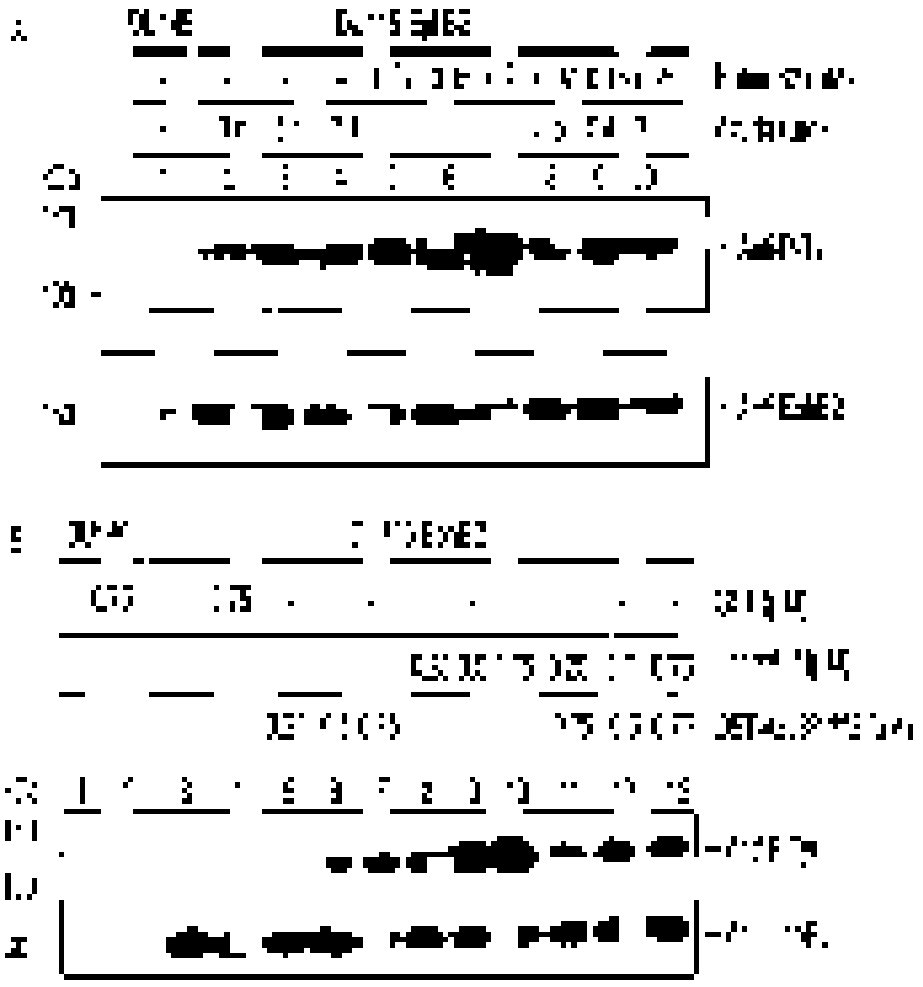
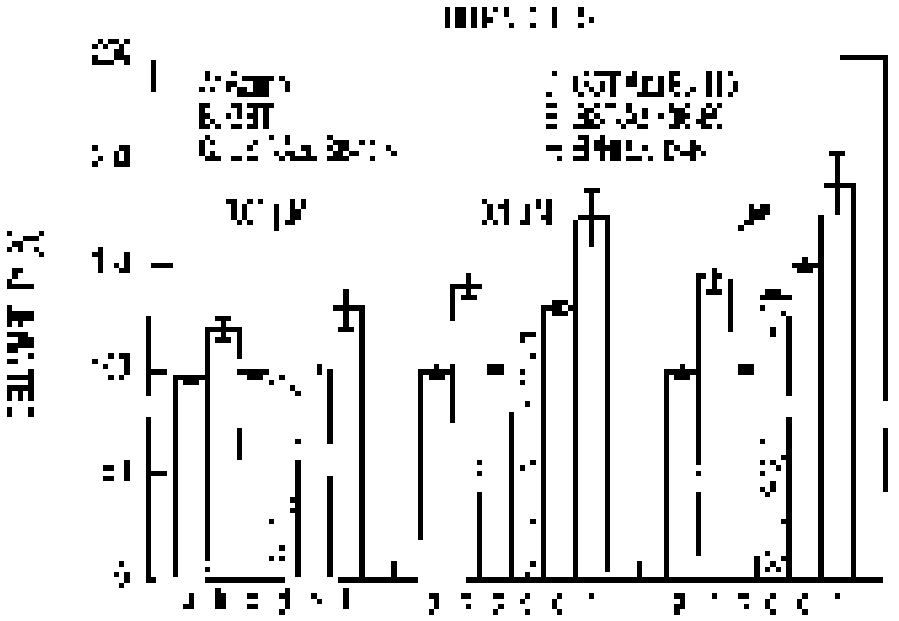


FIG. 12B



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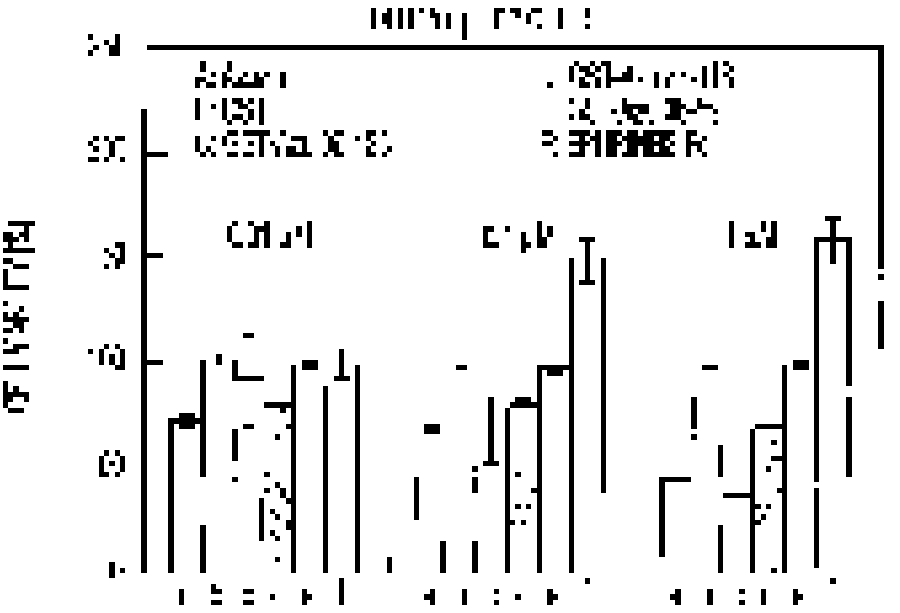

FIG. 13B

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EXAMPLES

Example 1

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Example 2

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Example 3

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Example 10

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Example 11

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(12) **United States Patent**
[illegible] et al.

(10) Patent No.: US 7,795,4[illegible] B2
(45) Date of Patent: Sep. 14, 2010

(54) [illegible]

(75) Inventors: [illegible]

(73) Assignee: [illegible]

(*) Notice: [illegible]

(21) Appl. No.: [illegible]

(22) Filed: [illegible]

(65) **Prior Publication Data**

[illegible]

Related U.S. Application Data

(63) [illegible]

(60) [illegible]

(51) Int. Cl.
[illegible]

(52) U.S. Cl. [illegible]

(58) Field of Classification Search [illegible]

(56) **References Cited**

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FOREIGN PATENT DOCUMENTS

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OTHER PUBLICATIONS

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(57) **ABSTRACT**

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OTHER PUBLICATIONS

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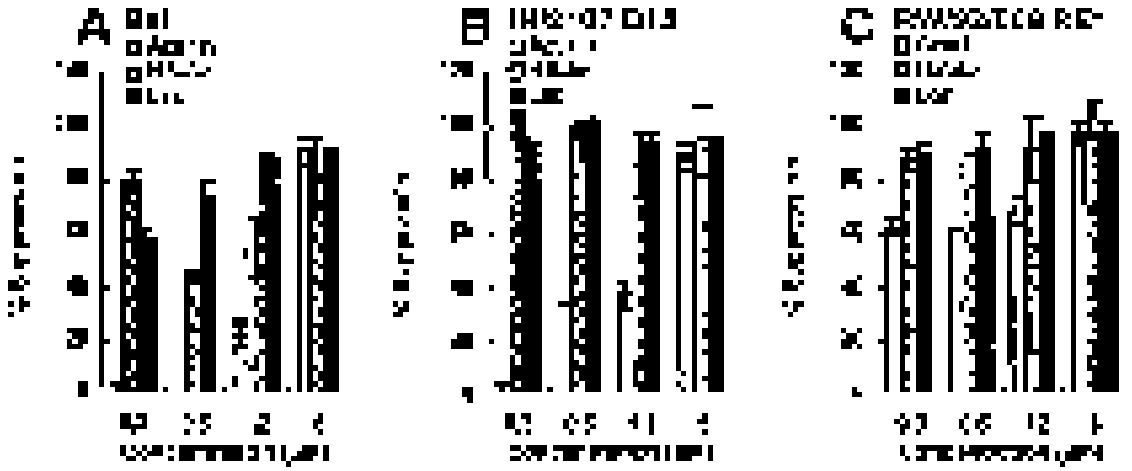

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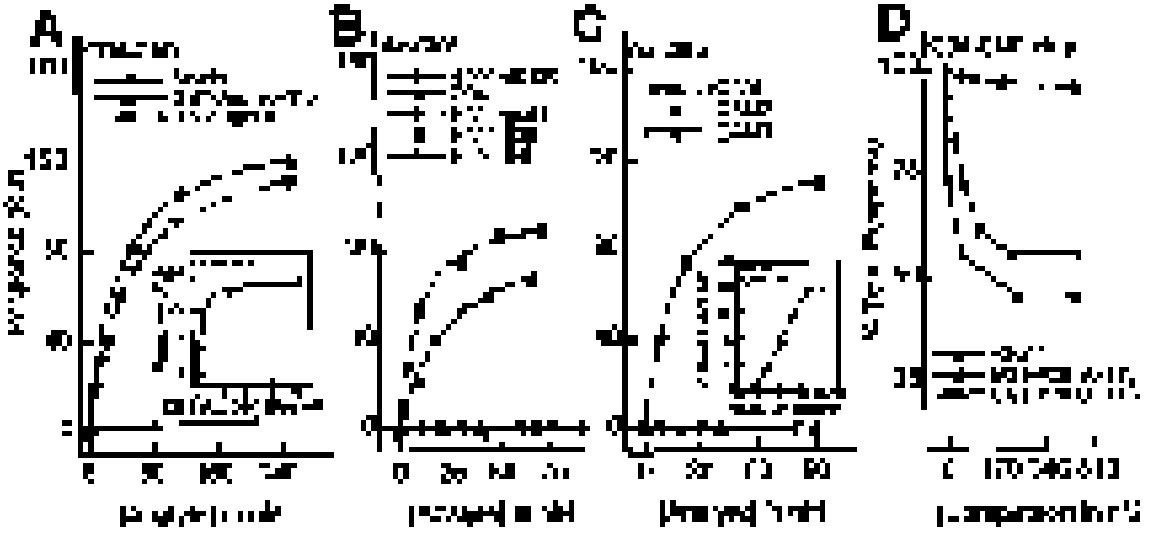

Figure 2

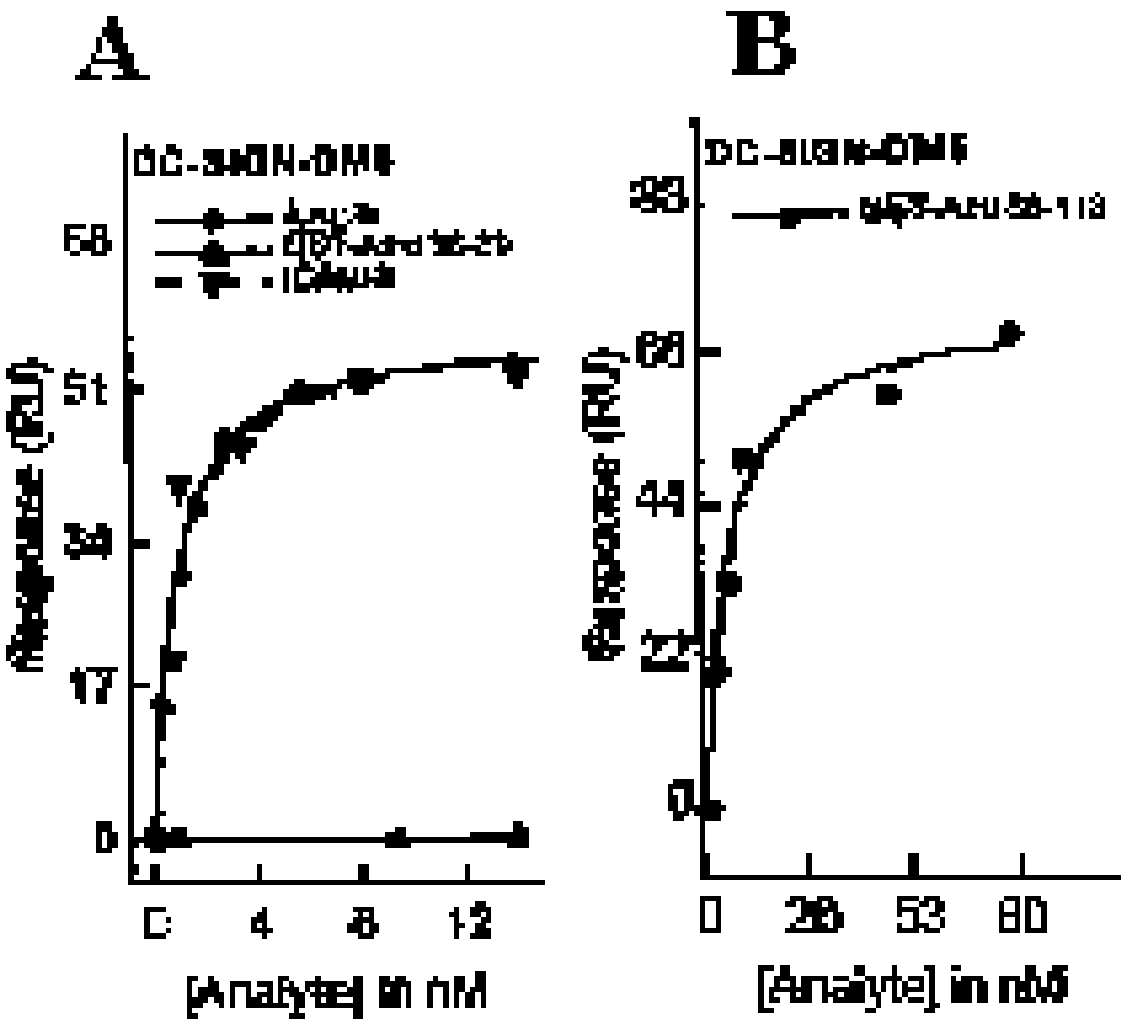

Figure 3

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. [illegible] Page 1 of

APPLICATION NO. [illegible]

ISSUED: [illegible]

INVENTOR(S): [illegible]

[illegible]

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Signed and sealed this

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(12) **United States Patent**
Chakrabarty et al.

(10) Patent No: US 8,623,816 B2
(45) Date of Patent: Jan. 7, 2014

(54) [illegible]

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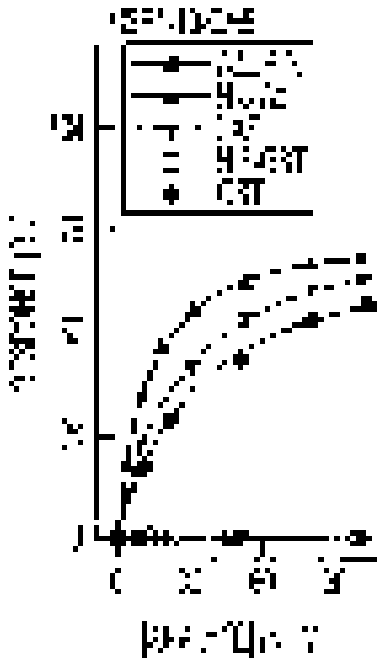
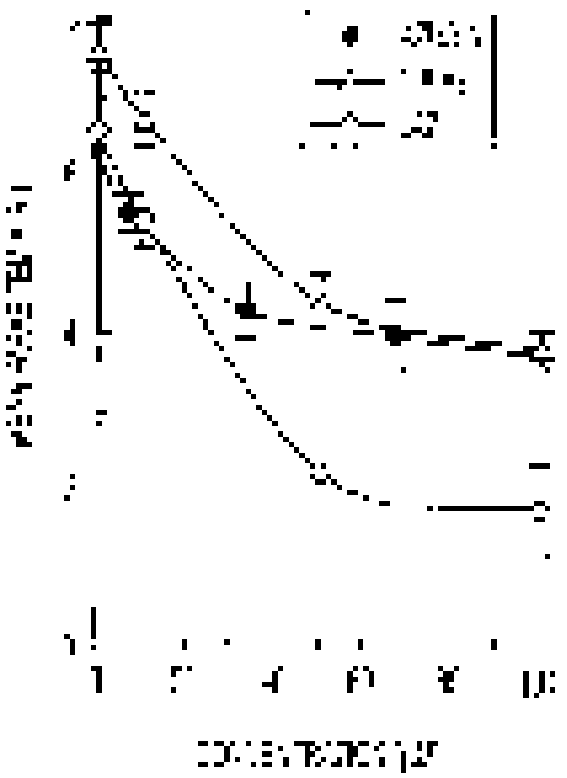


FIG. 1A

FIG. 1B

FIG. 1C



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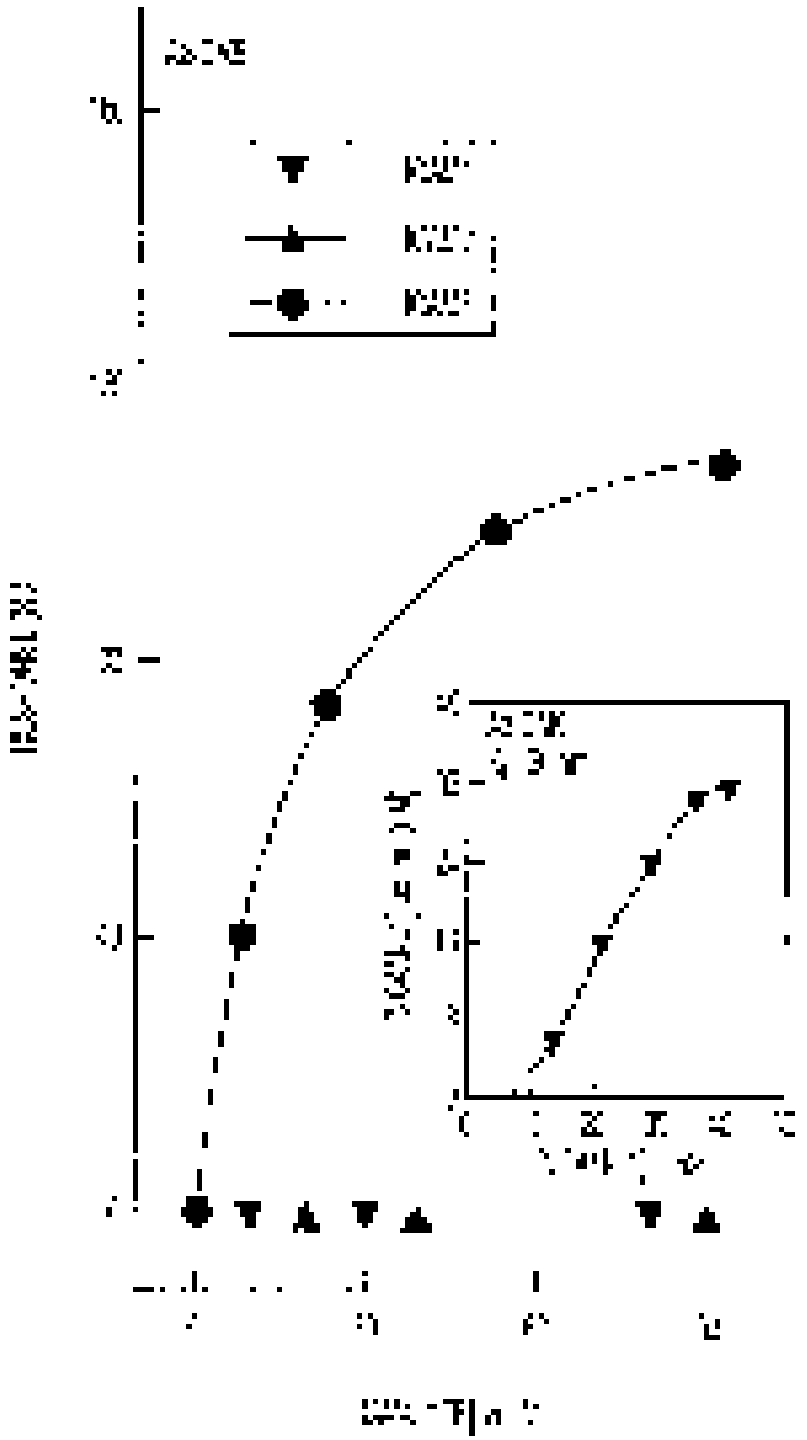

FIG. 3

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United States Patent

[illegible] et al.

Patent No.: [illegible]

Date of Patent: Feb. 28, 2012

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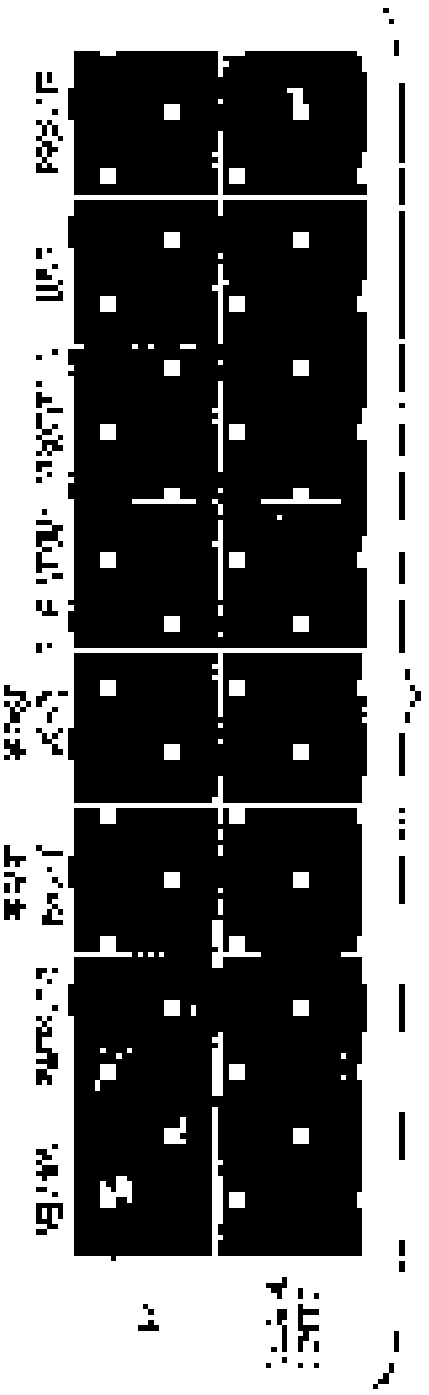
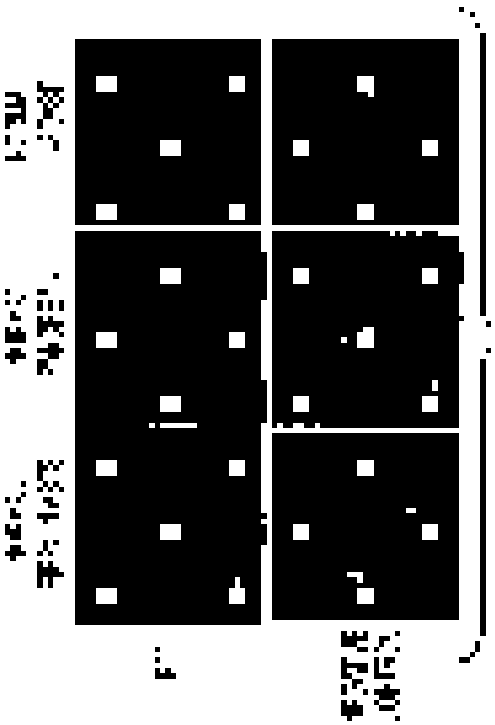


FIG. 1A

FIG. 1C



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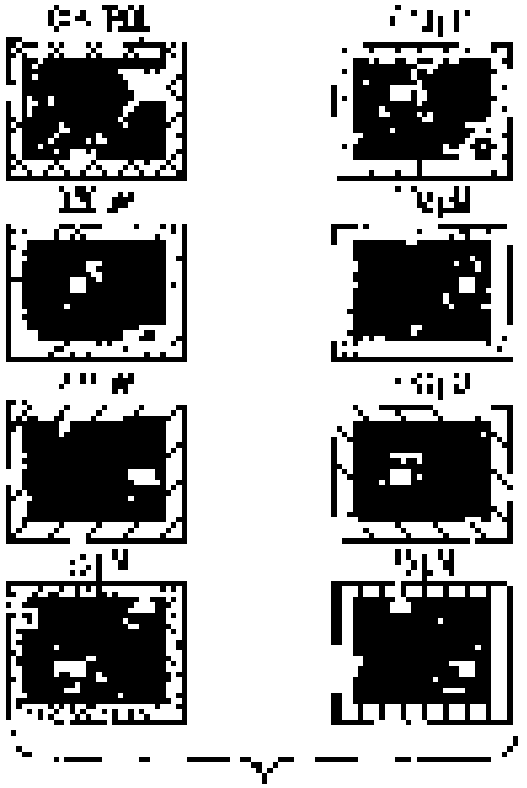

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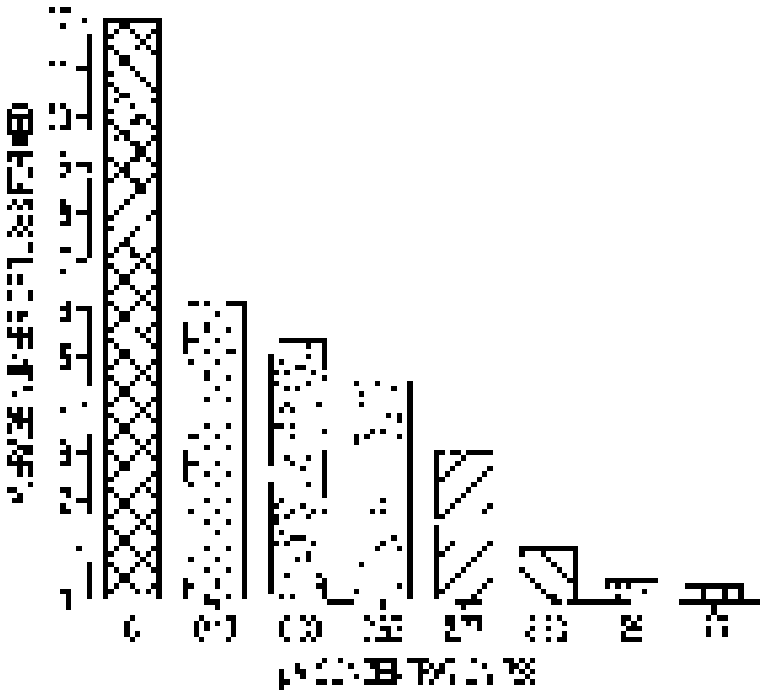


FIG. 2B

FIG. 3A

FIG. 3B

FIG. 3C

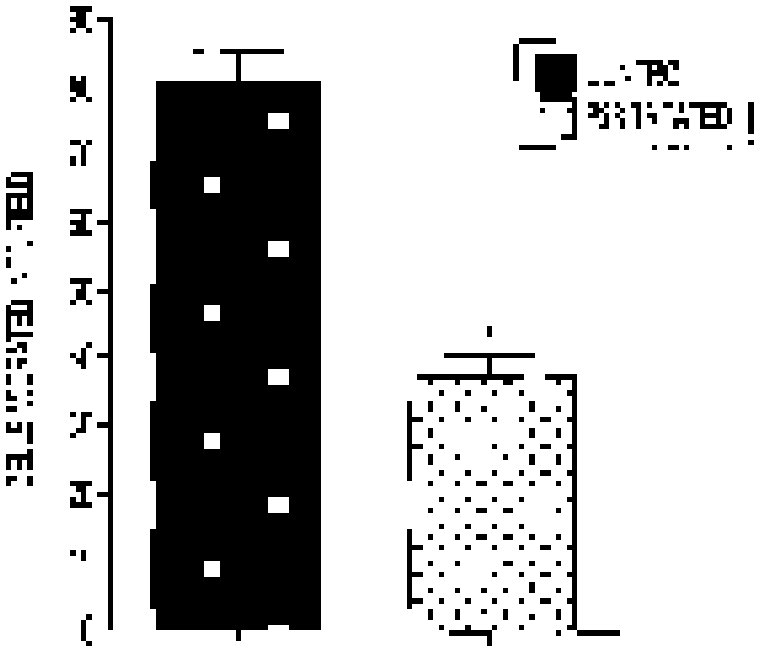
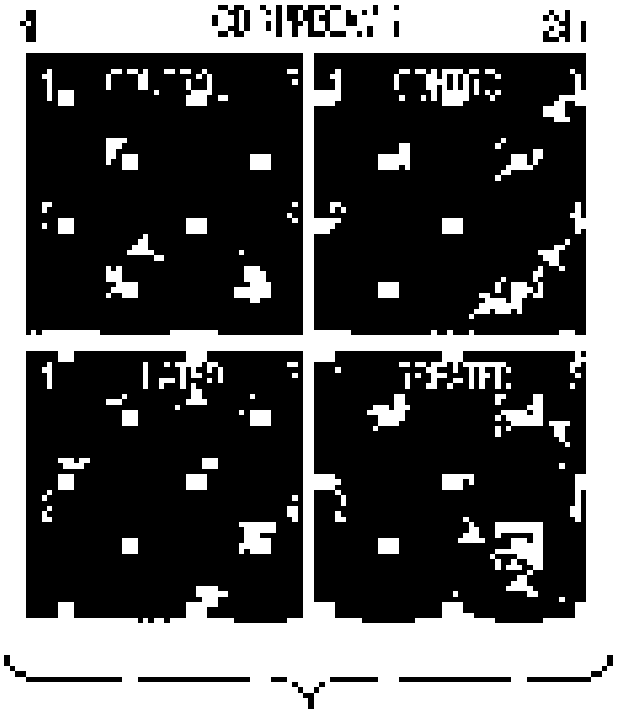


FIG. 3D



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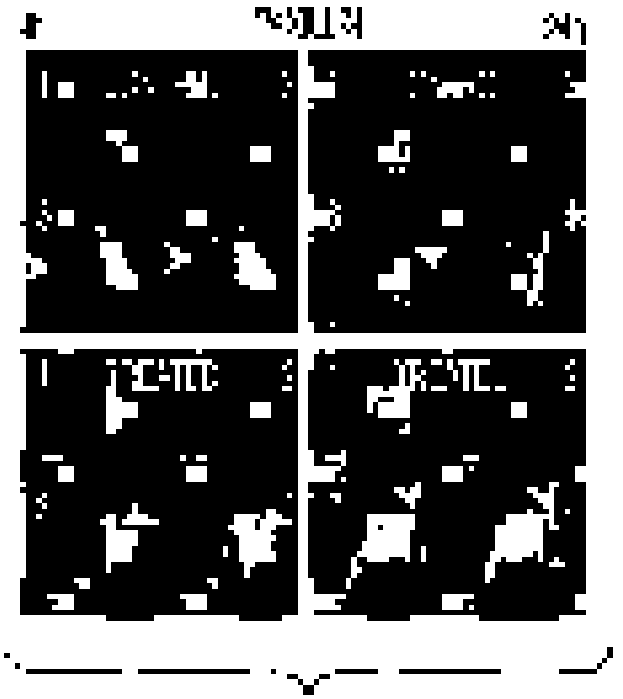

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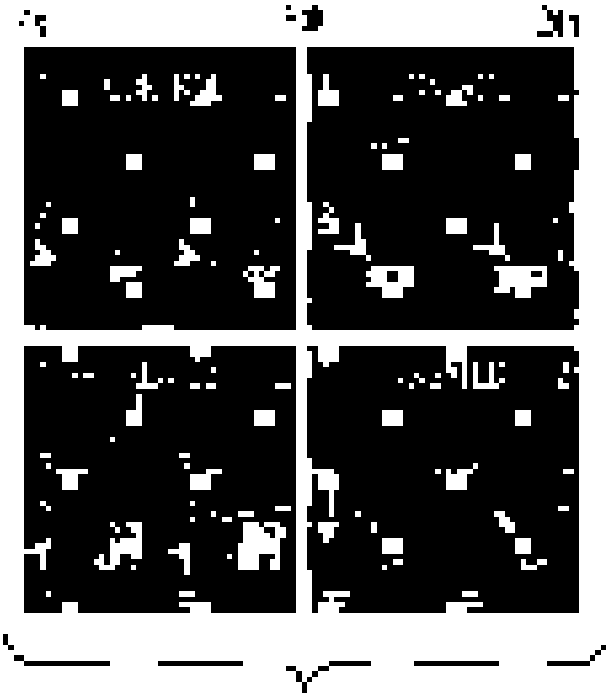

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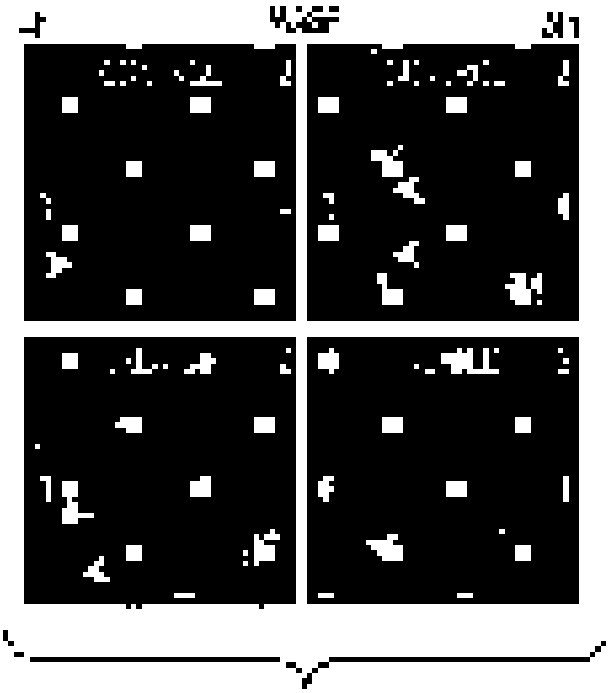

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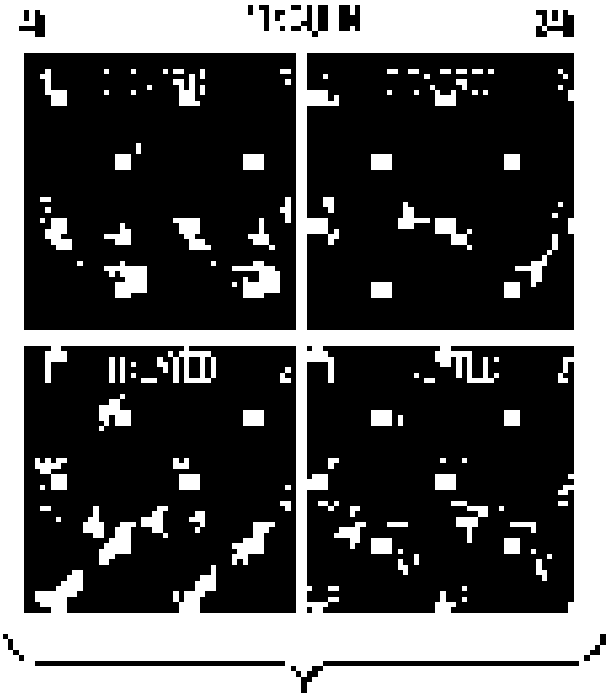

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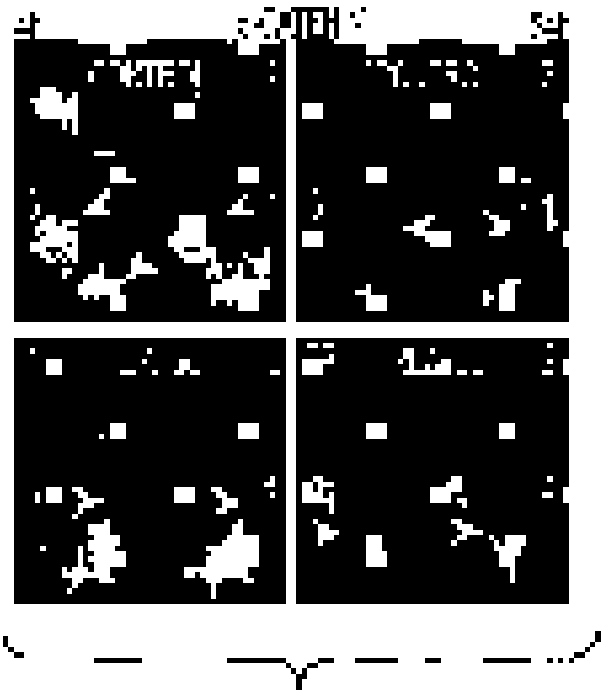

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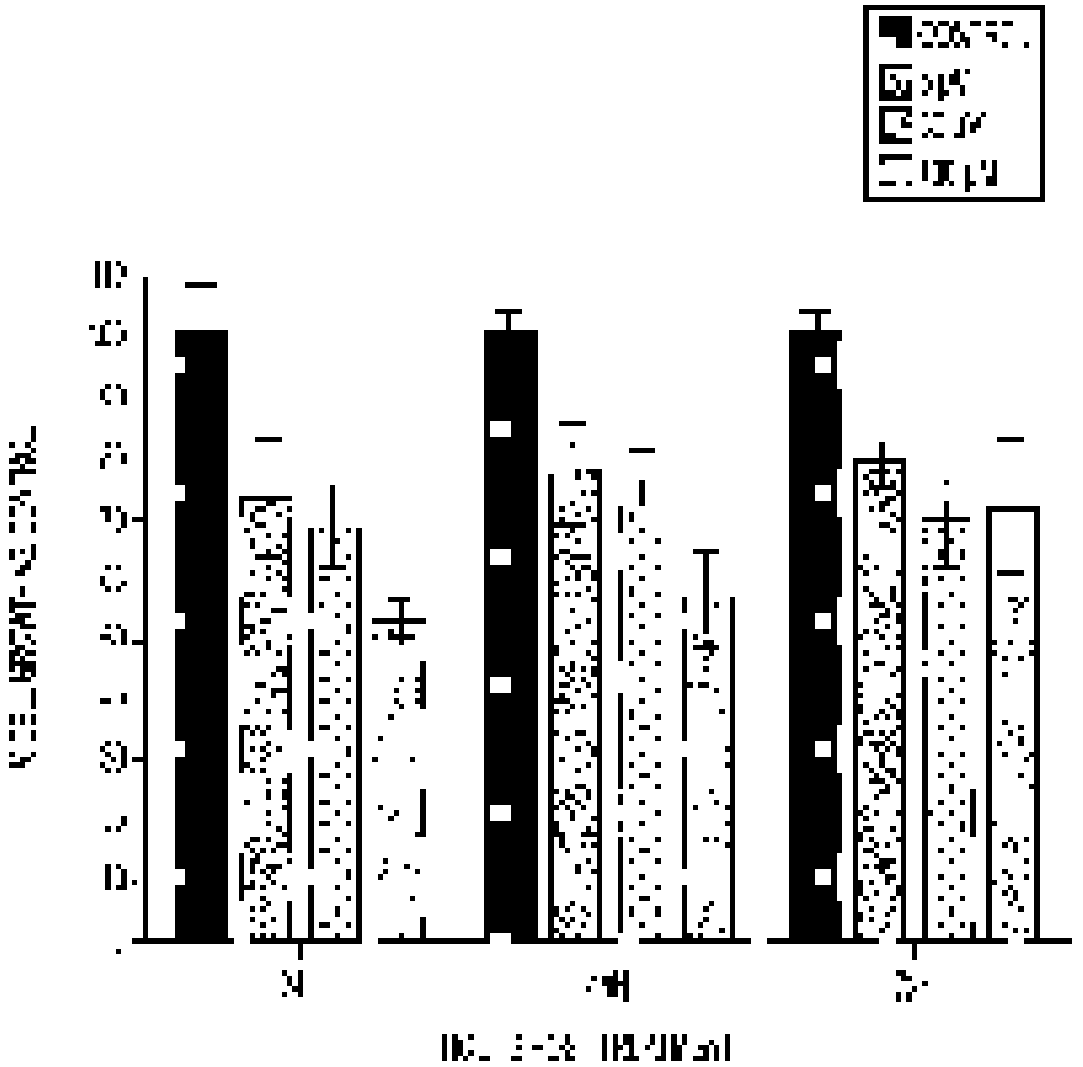

FIG. 5

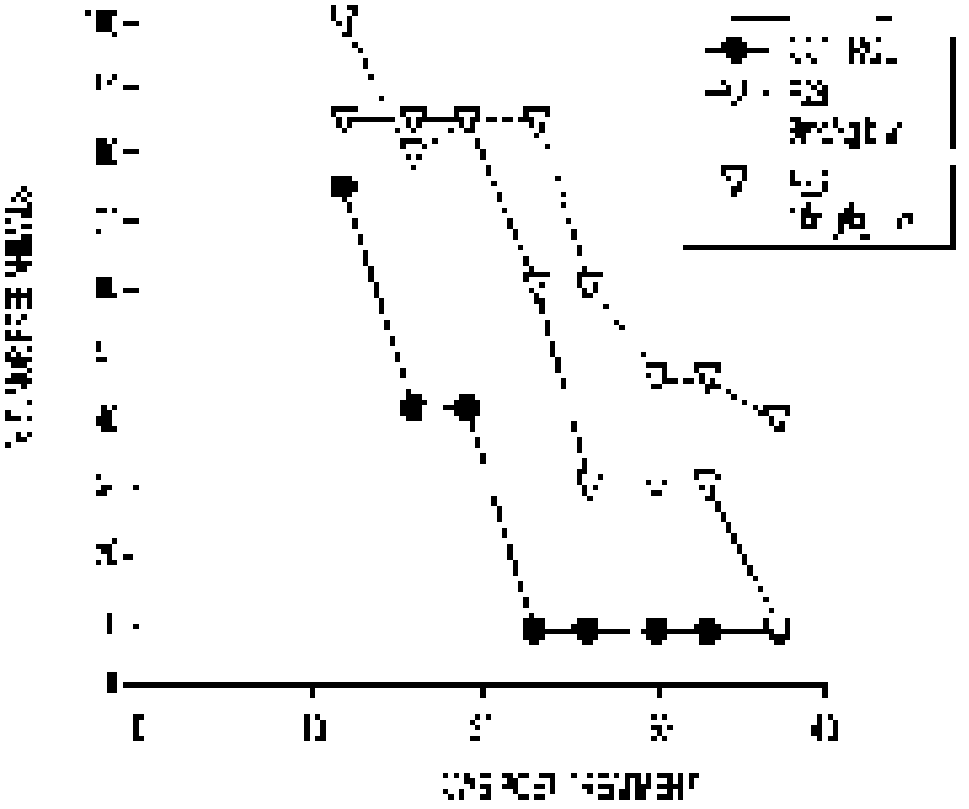

FIG. 6A

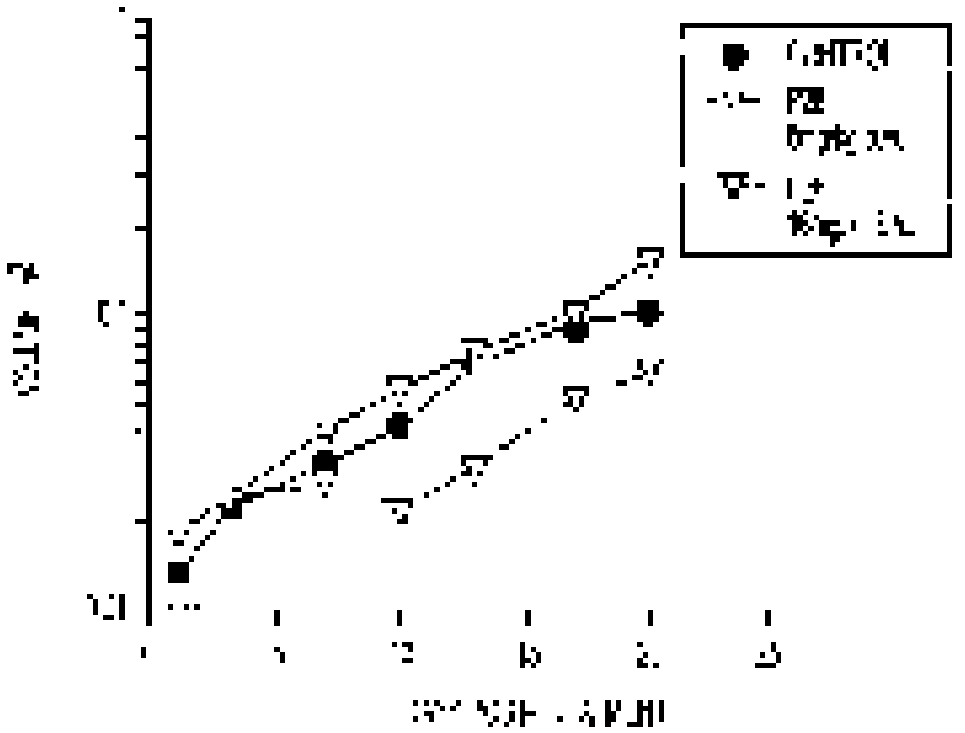

FIG. 6B

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DETAILED DESCRIPTION OF THE INVENTION

Definitions

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Table 1

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TABLE 1

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United States Patent

Mahm et al.

Patent No.: US 8,124,065 B2

Date of Patent: Feb. 28, 2012

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OTHER PUBLICATIONS

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ABSTRACT

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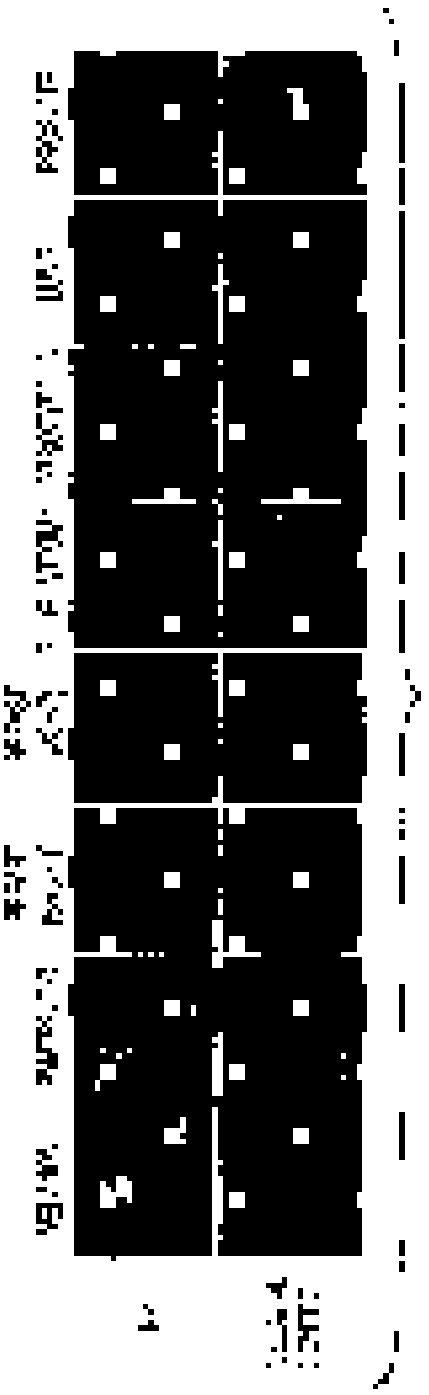

FIG. 1A

FIG. 1C

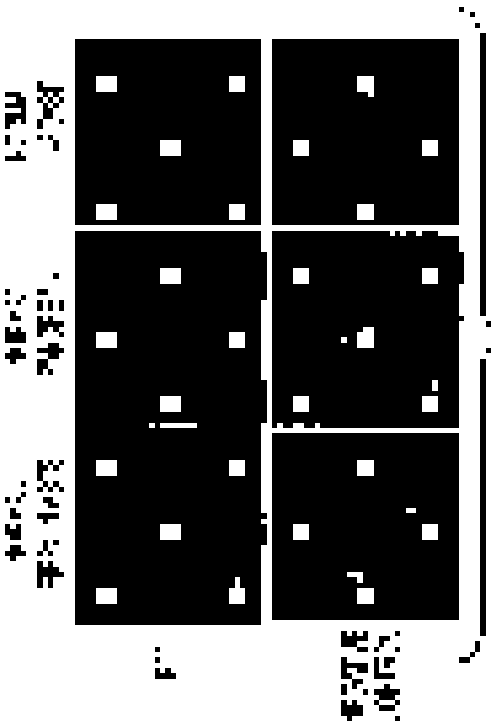

FIG. 1B

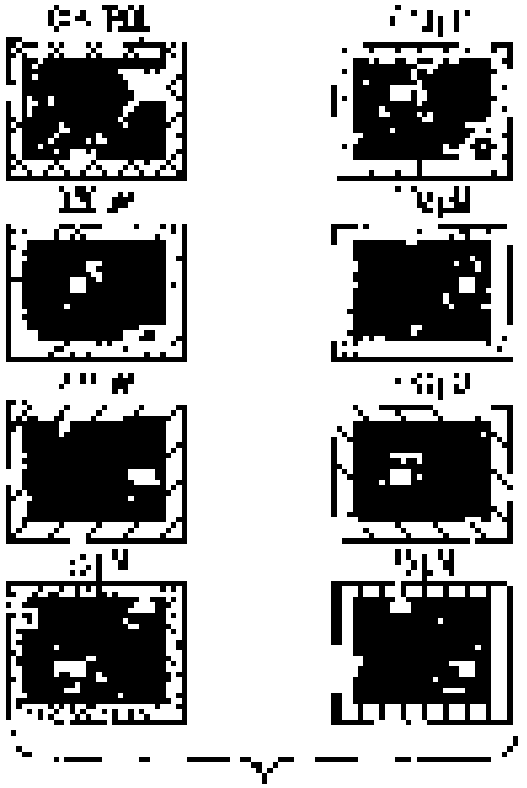

FIG. 2A

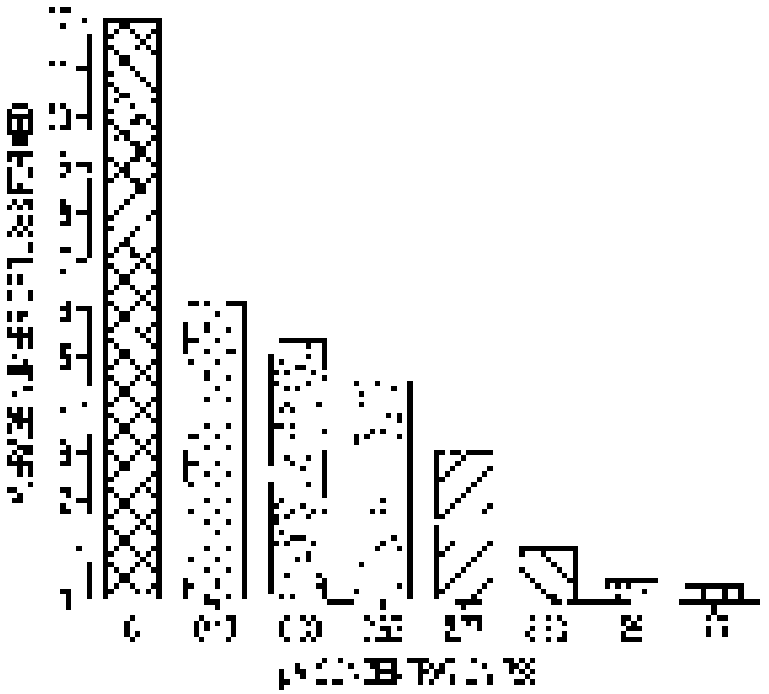

FIG. 2B

FIG. 3A

FIG. 3B

FIG. 3C

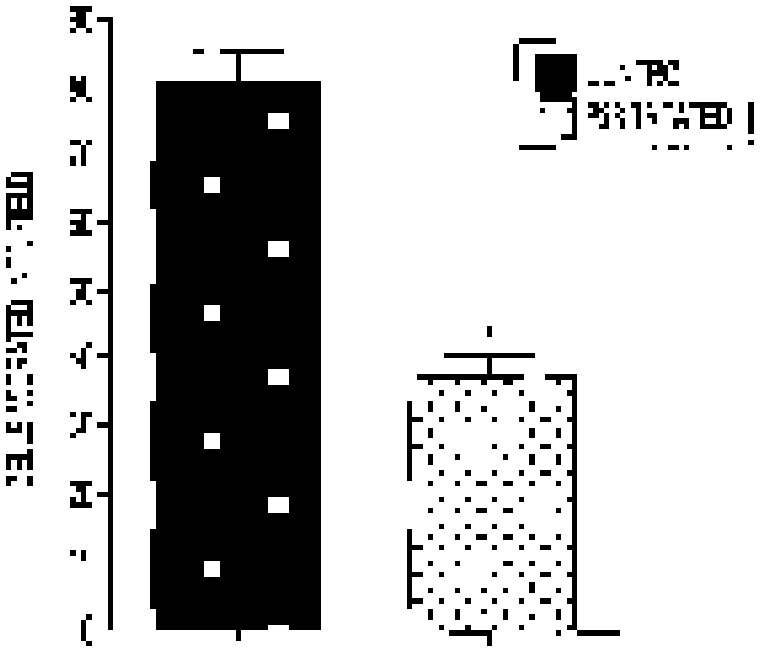

FIG. 3D

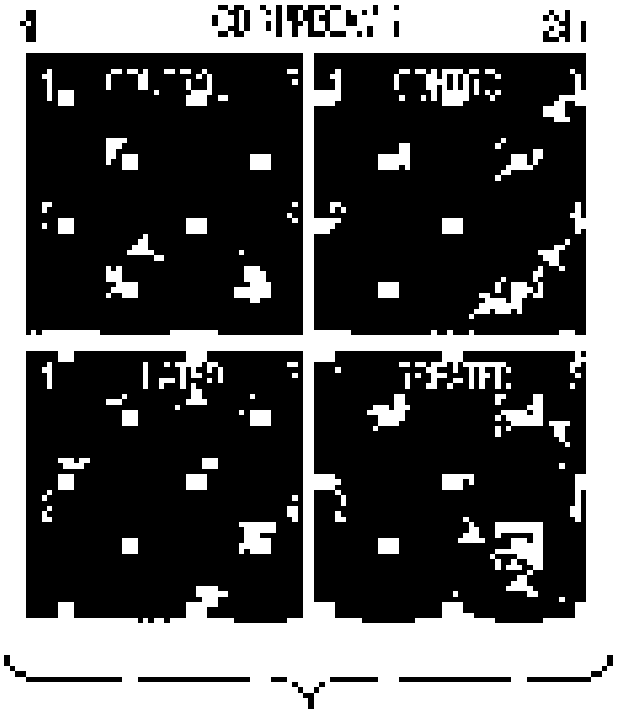

FIG. 4A

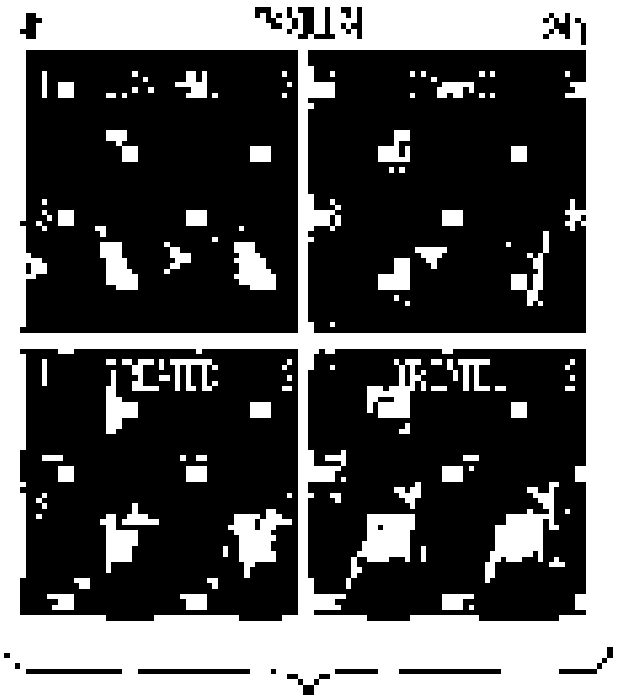

FIG. 4B

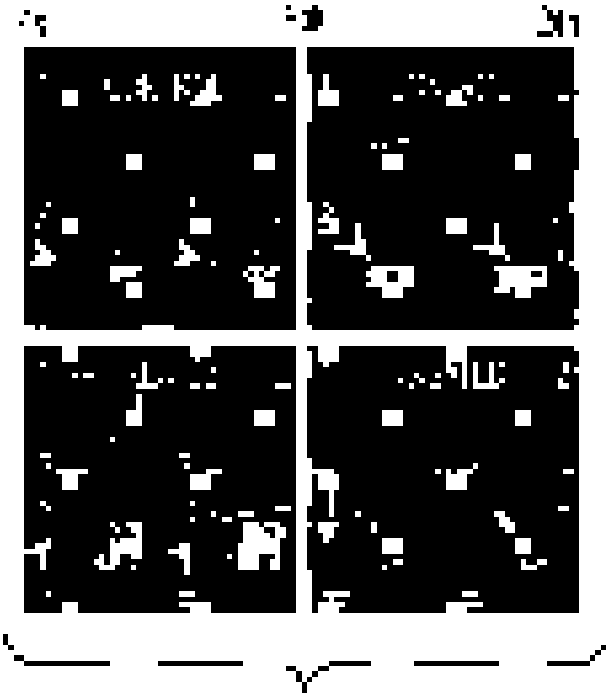

FIG. 4C

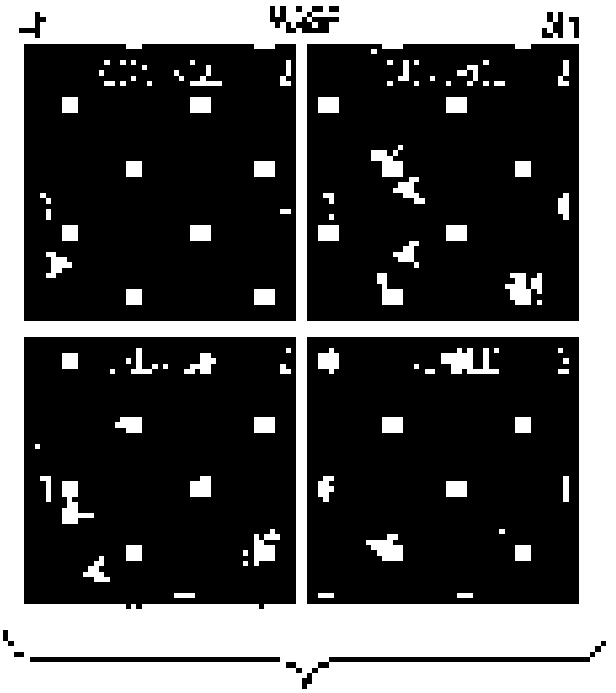

FIG. 4D

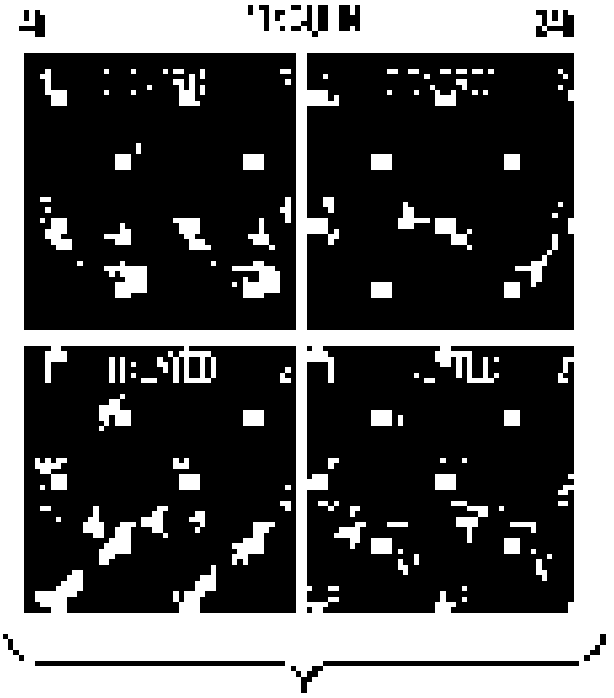

FIG. 4E

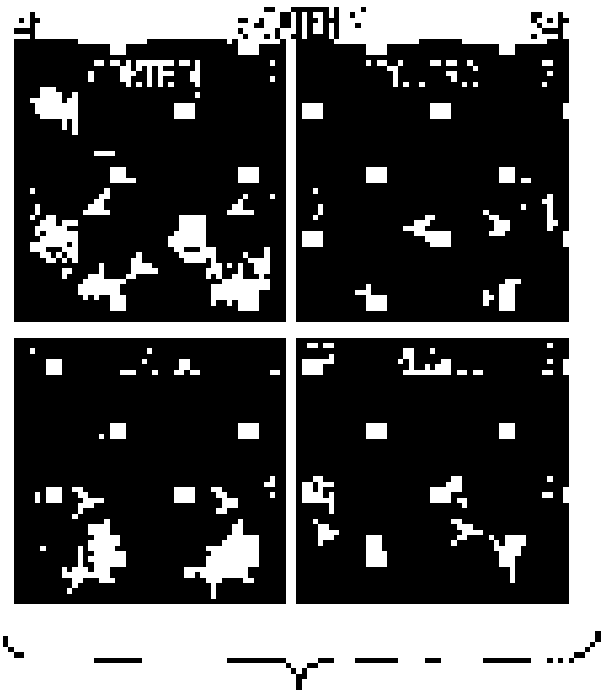

FIG. 4F

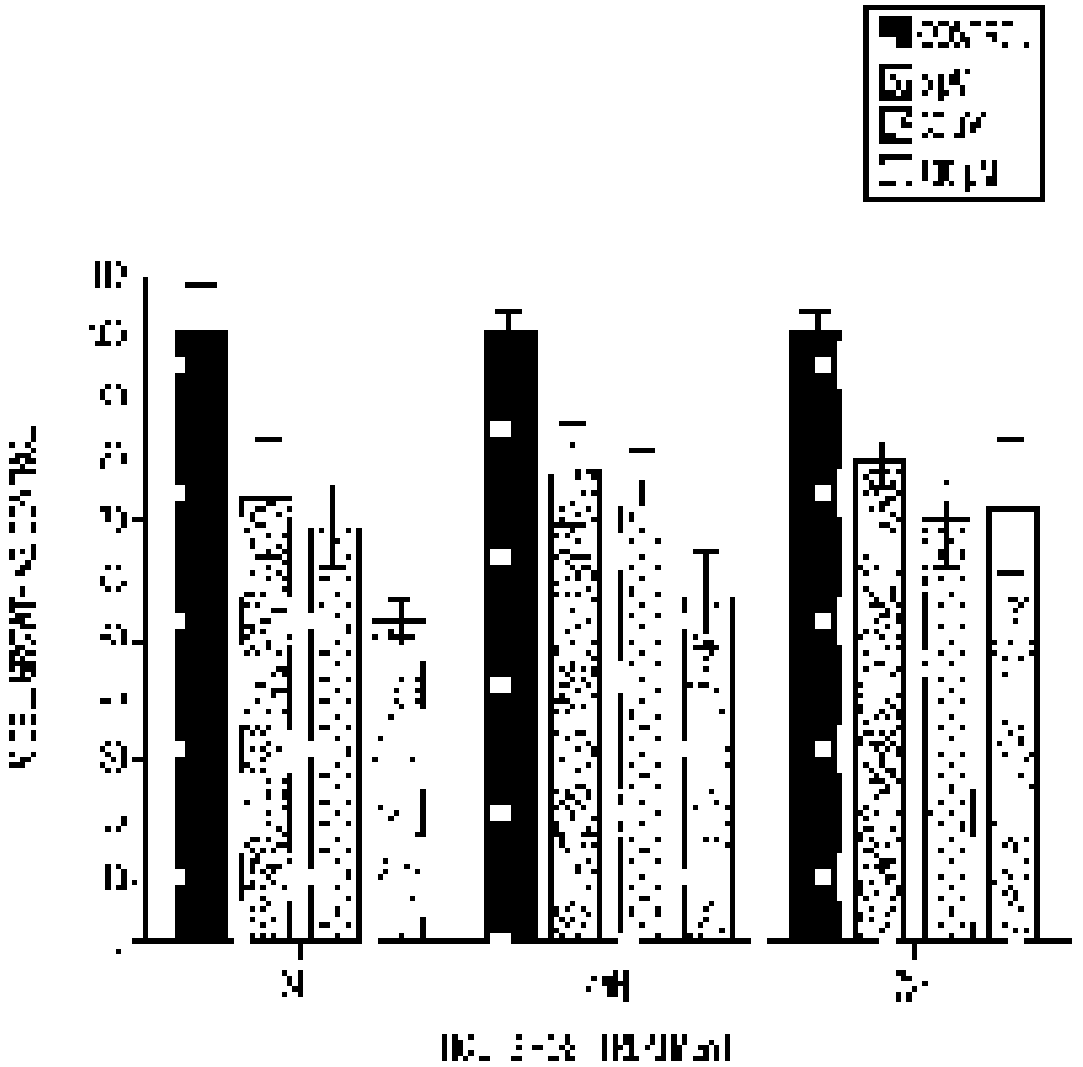

FIG. 5

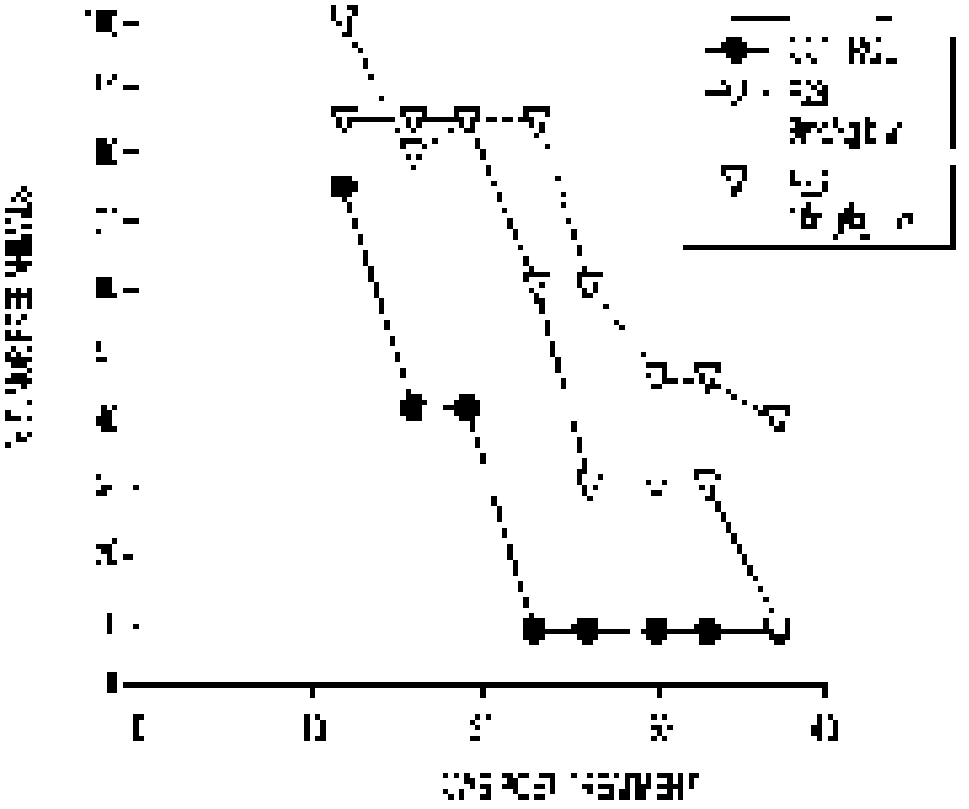

FIG. 6A

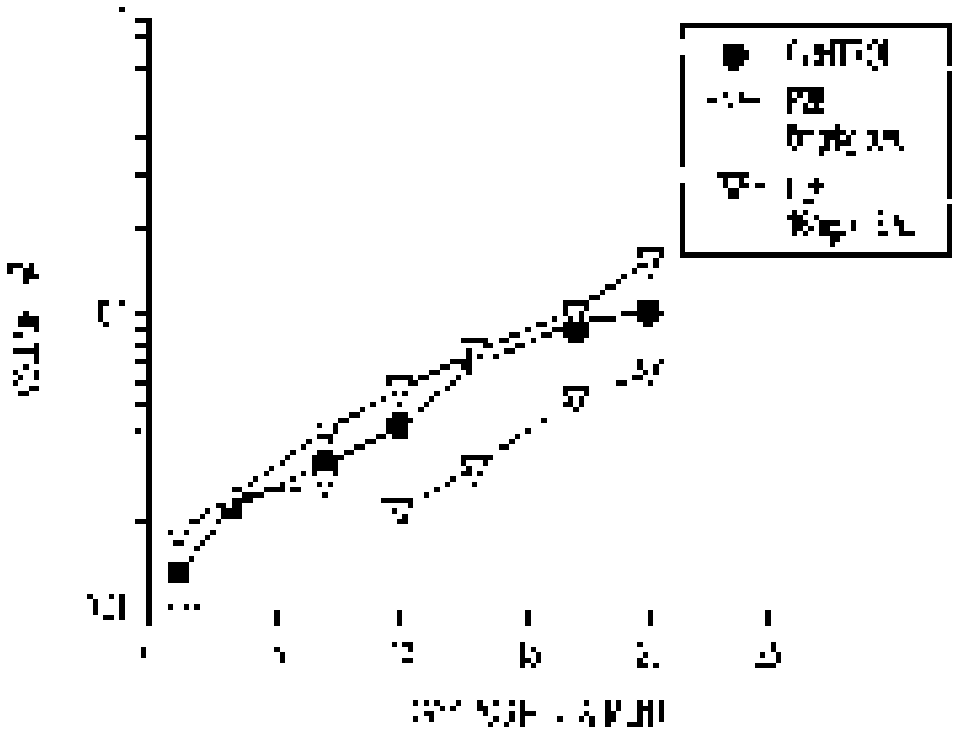

FIG. 6B

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BRIEF DESCRIPTION OF THE DRAWINGS

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DETAILED DESCRIPTION OF THE INVENTION

Definitions

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TABLE 1

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EXAMPLES

Example 1

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Example 2

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Example 3

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US 8,232,244 B2

United States Patent

Patent No: US 8,232,244 B2

Date of Patent: Jul. 31, 2012

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Figure 1A
Comparison of glands with or without lesions in an enlarged picture

Lesions

No Lesions

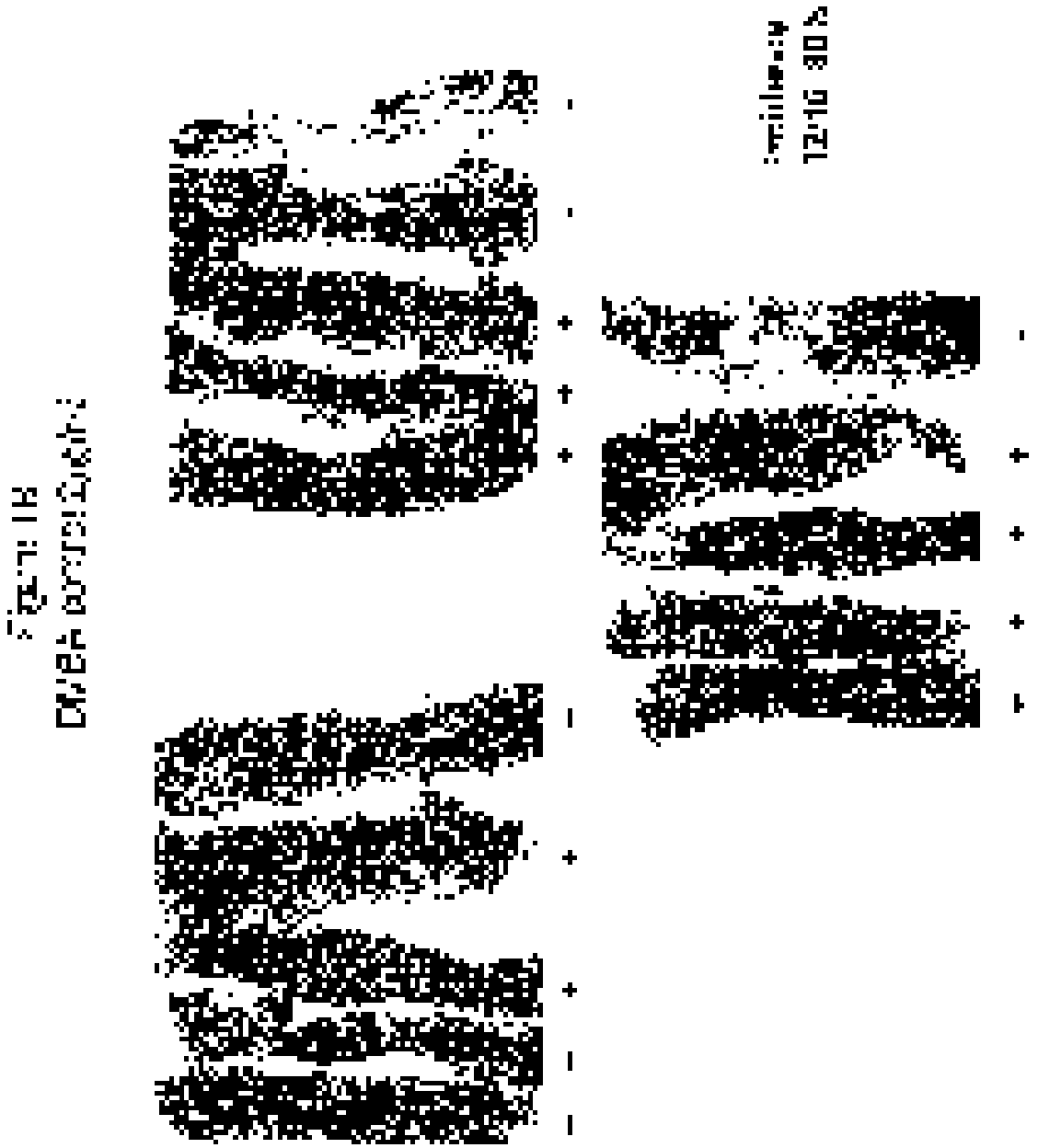

Cytochrome c
Mitochondria
12 16 30 %
Cytochrome c
Mitochondria
12 16 30 %

Figure 1B

Figure 1C
LMBA – P28 (12.5 g/ml)

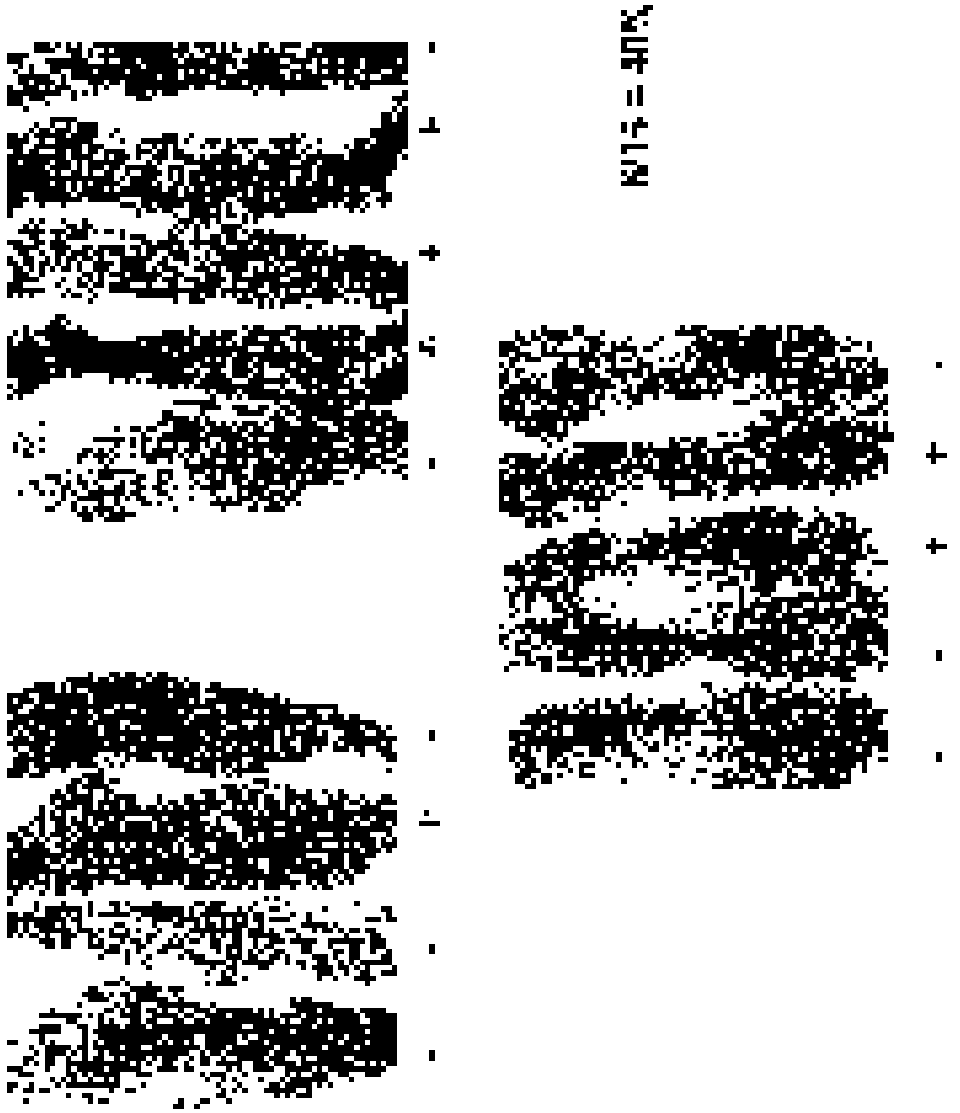
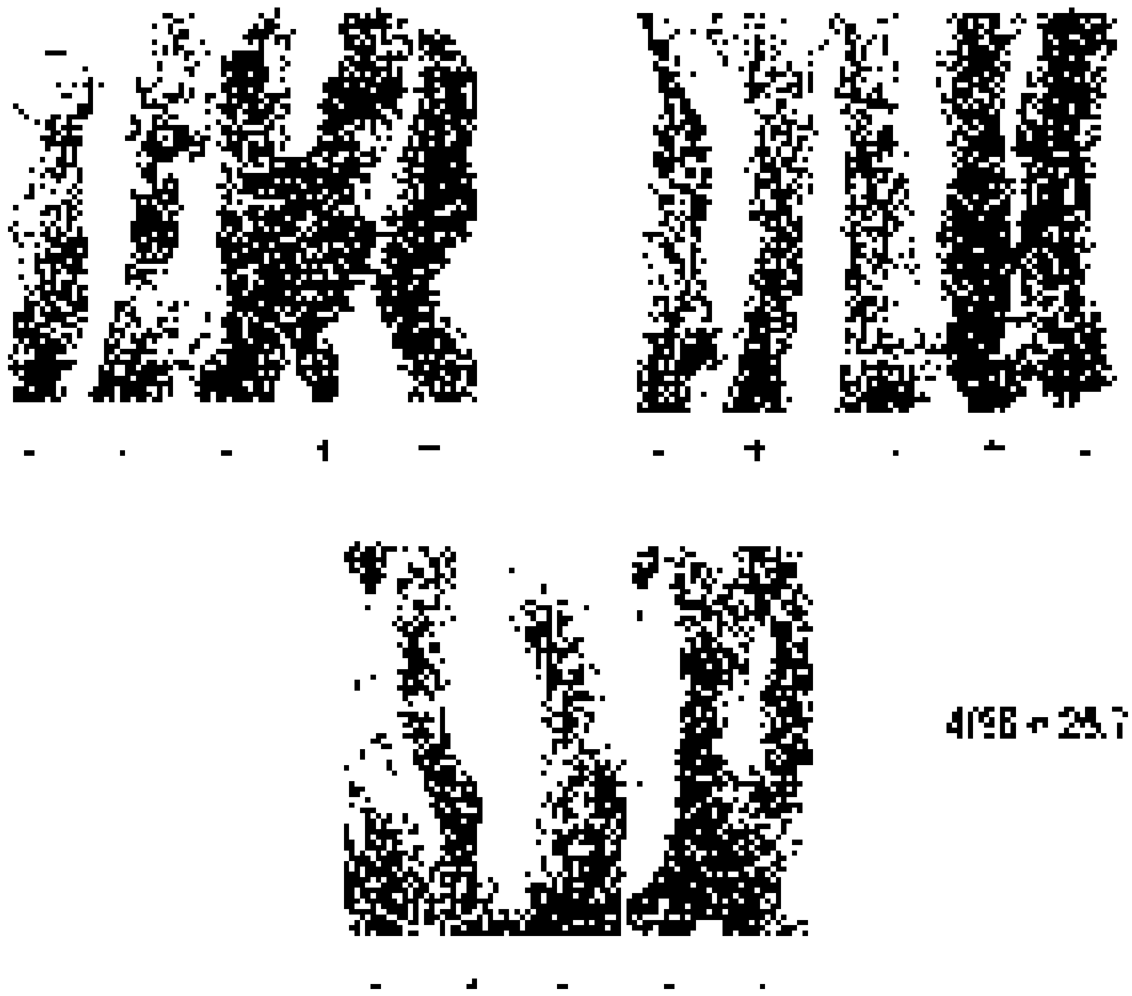


Figure 1C

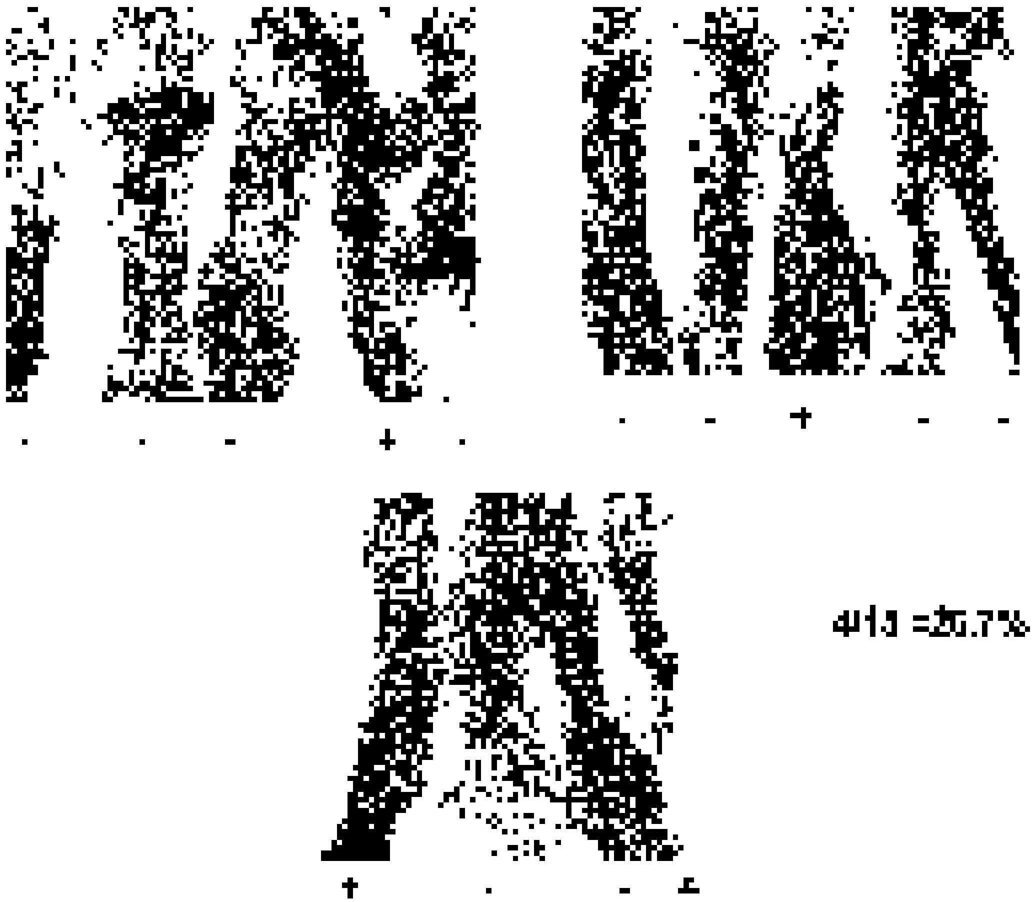
DMBA + P28 (25µg/ml)



9/35 = 25.7% Incidence

Figure 5E

DMBA + P28 (5mg/kg)



4/15 =26.7% Incidence

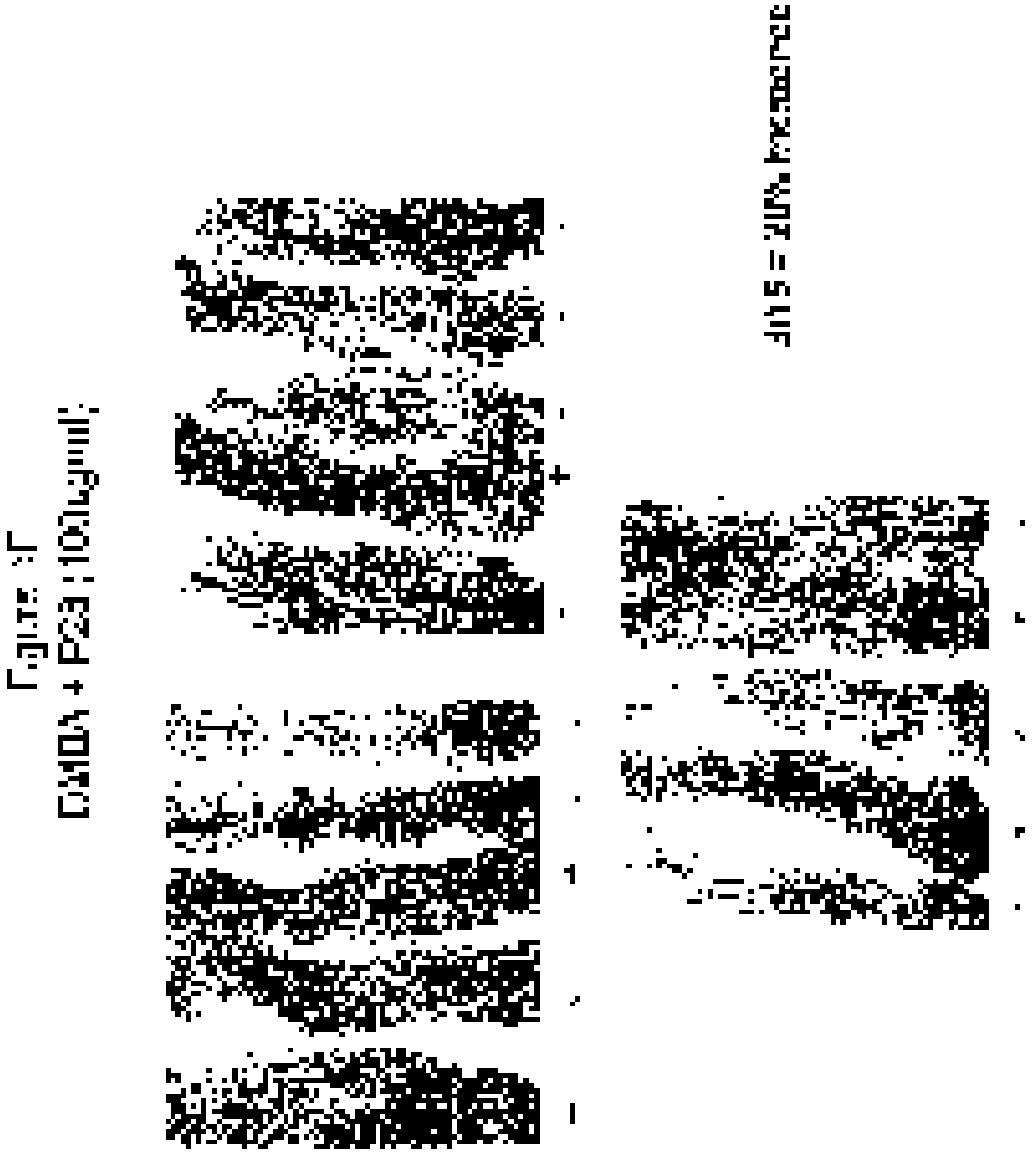


Figure 7

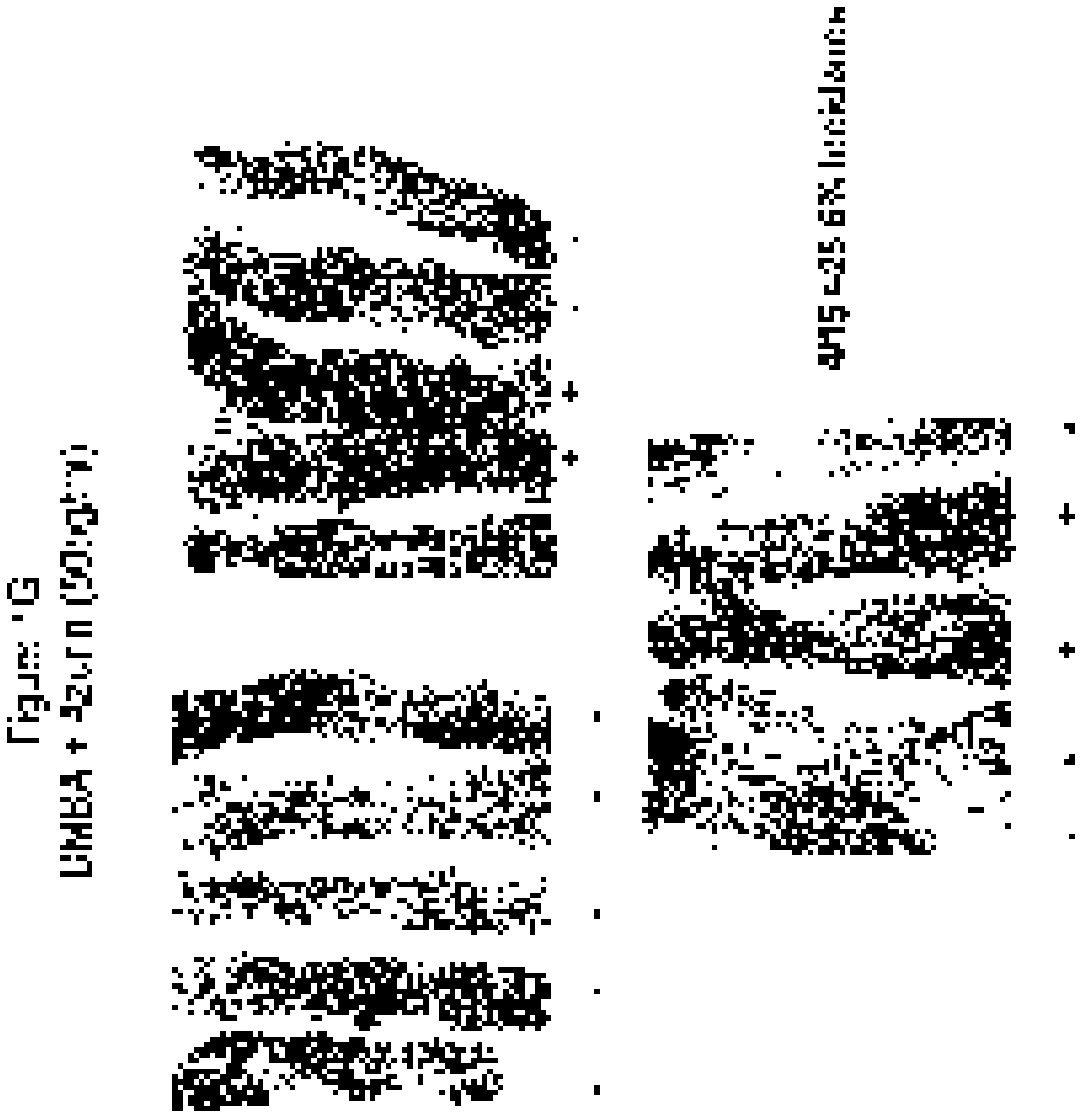


Figure 1G

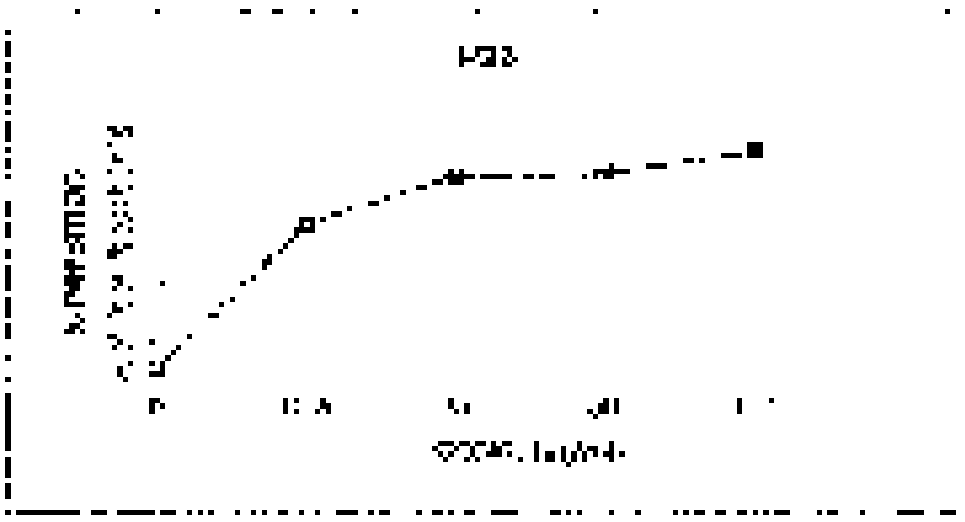
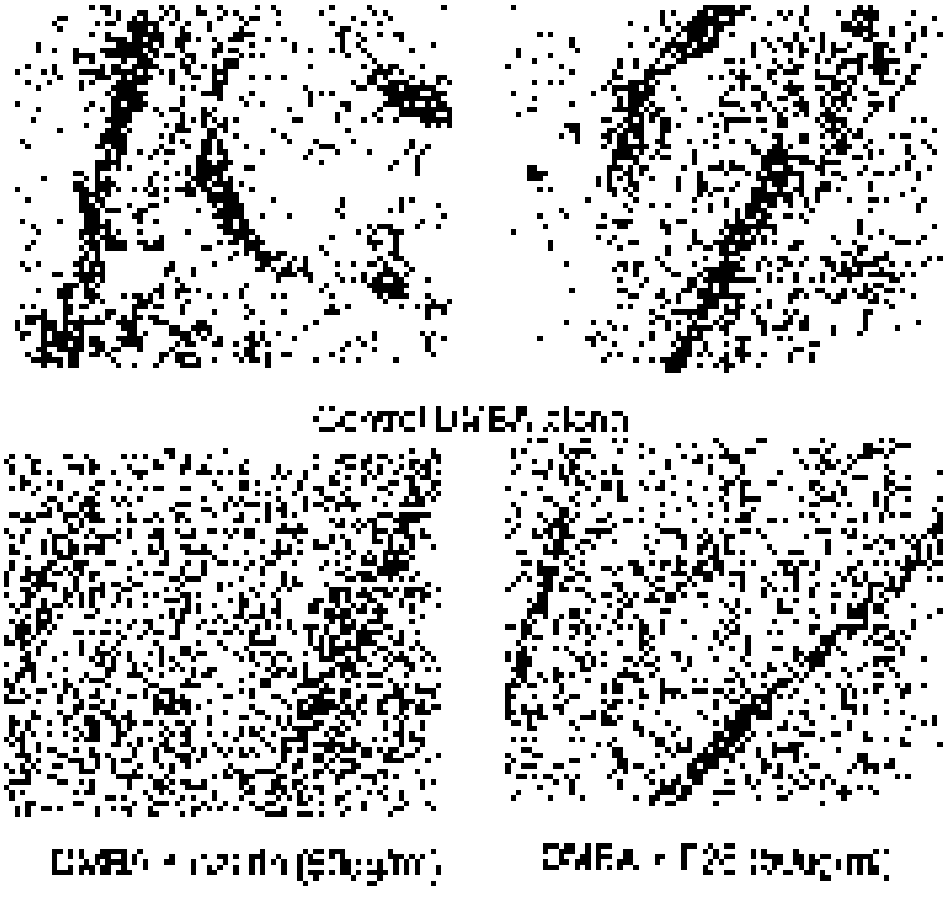


Figure 2

Figure 3

Examples of DMBA-induced mammary ductal lesions



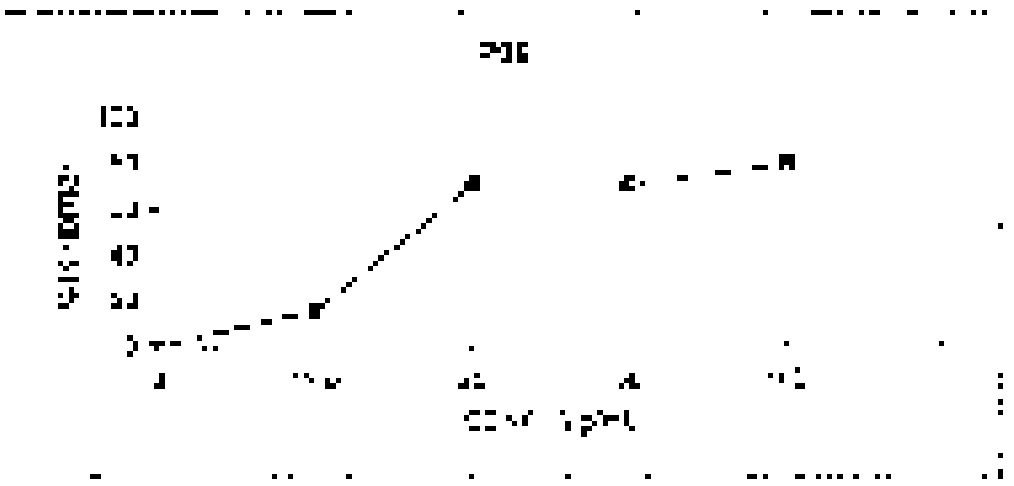


Figure 4

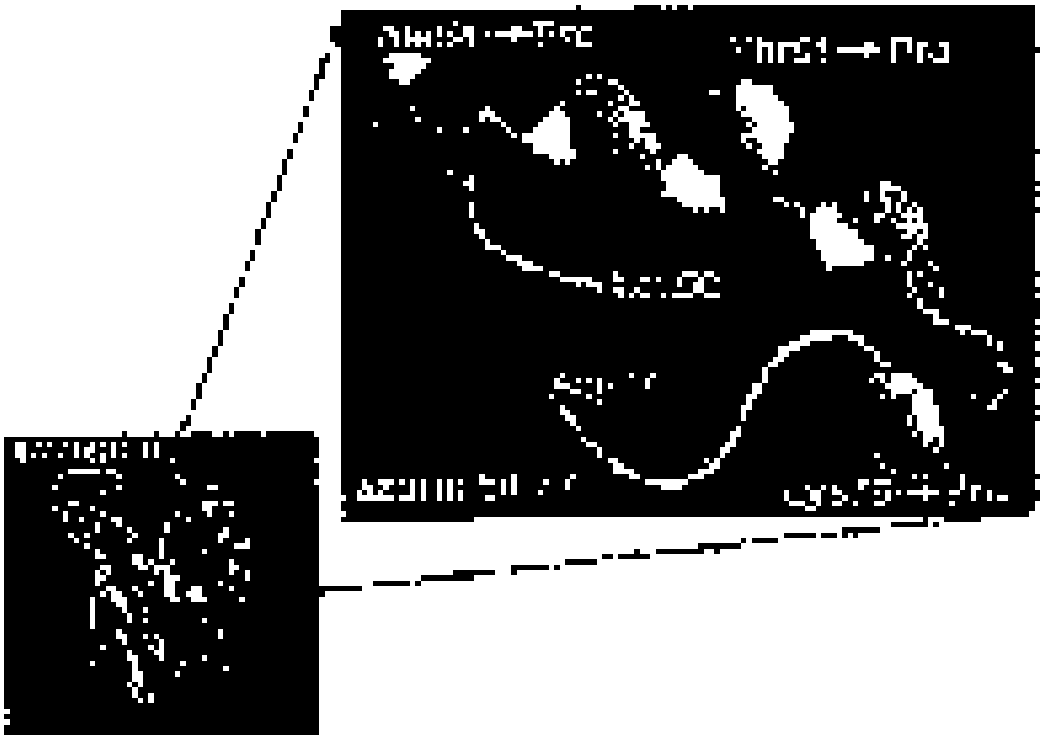


FIG. 5

FIG. 8A

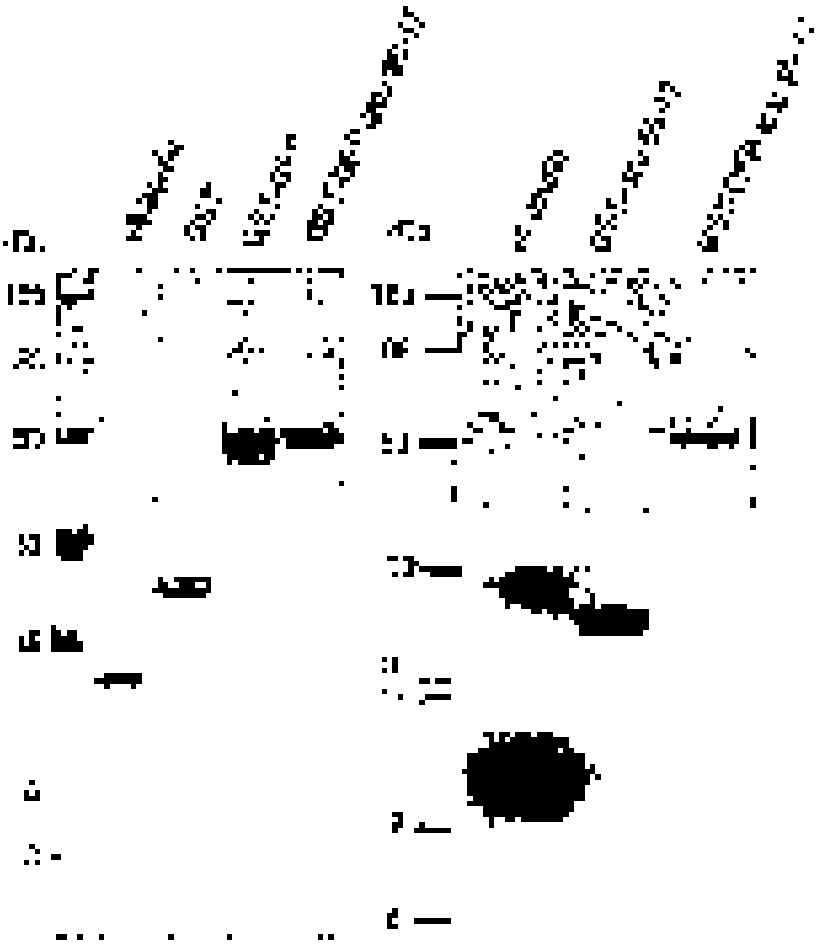


FIG. 8B FIG. 8C

FIG. 7A

FIG. 7B

FIG. 7C

FIG. 8A

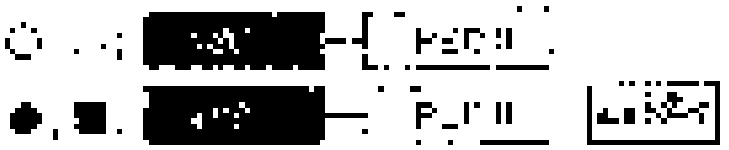


FIG. 8B

FIG. 8C

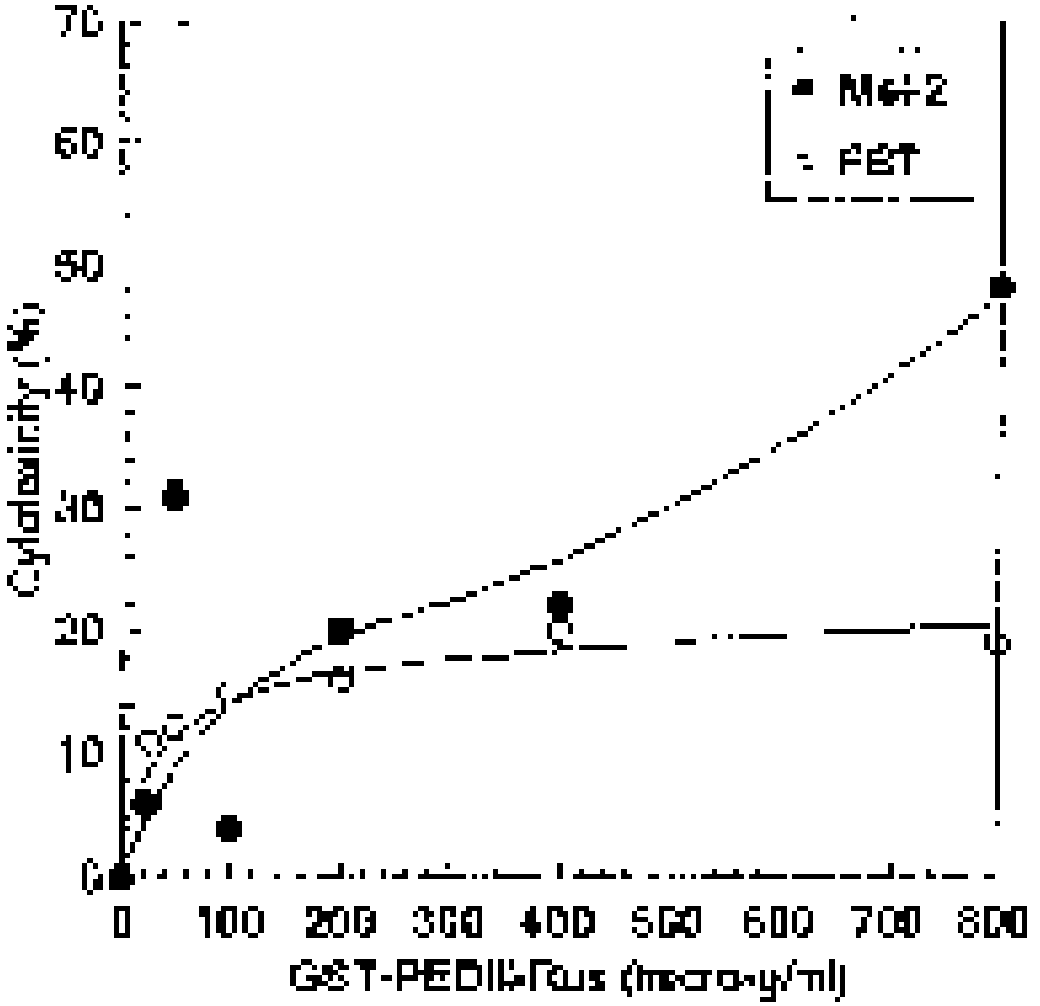

70
60
50
40
30
20
10
0
Cytotoxicity (%)
0
100
200
300
400
500
600
700
800
GST-PEDII-Fous (micro-g/ml)
Mel-2
FBT

FIG. 9

[illegible] DIRECT CONDUCTIVE CONTACTS

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TABLE 2

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Example 1

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Example 2

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(19) United States Patent
[illegible]

(10) Patent No: US 8,817,749 B2
(45) Date of Patent: Sep 15 2011

(54) [illegible]

(75) Inventors: [illegible]

(73) Assignee: [illegible]

(1) [illegible]

(2) [illegible]

(3) [illegible]

(4) [illegible]

Related U.S. Application Data

(6) [illegible]

(5) [illegible]

(51) [illegible]

(52) [illegible]

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FIG. 1A-1

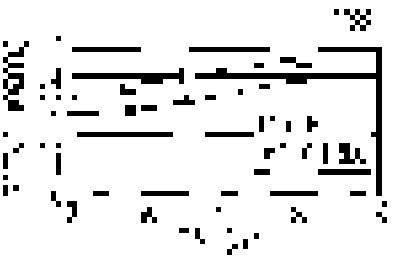

FIG. 1A-2

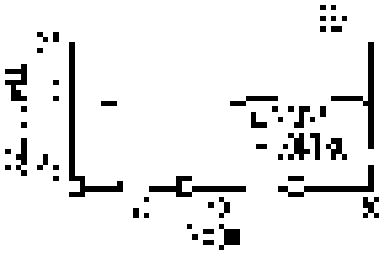

FIG. 1A-3

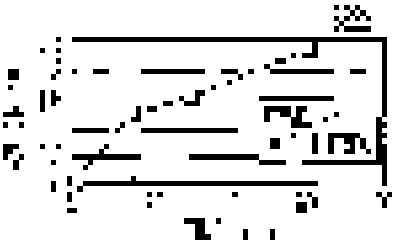

FIG. 1A-1

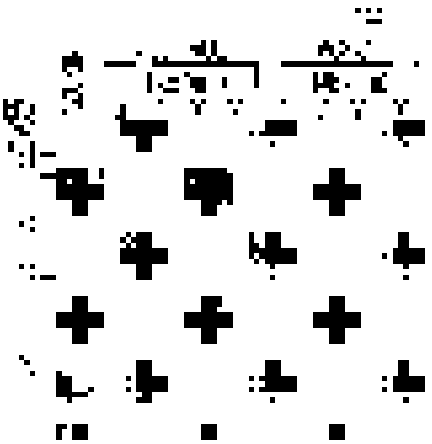

FIG. 1A-5

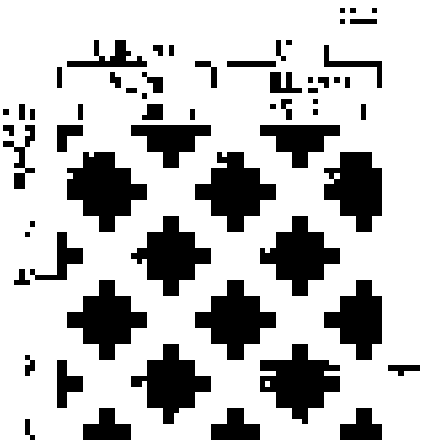

FIG. 1A-6

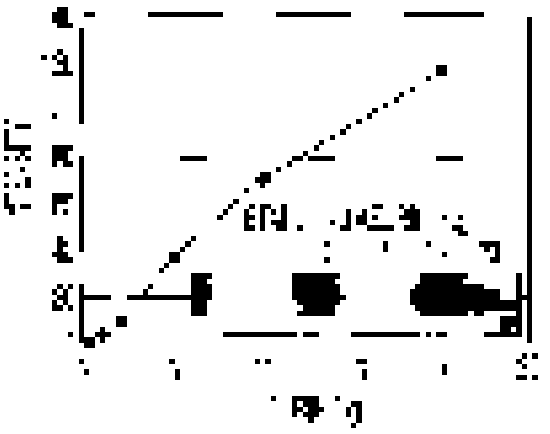

FIG. 1B

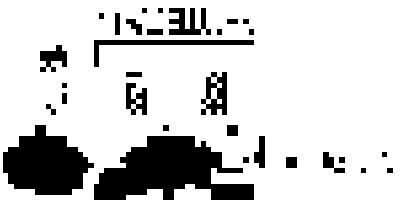

FIG. 1C

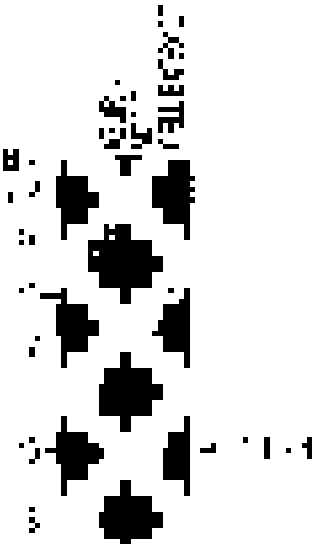

FIG. 1D

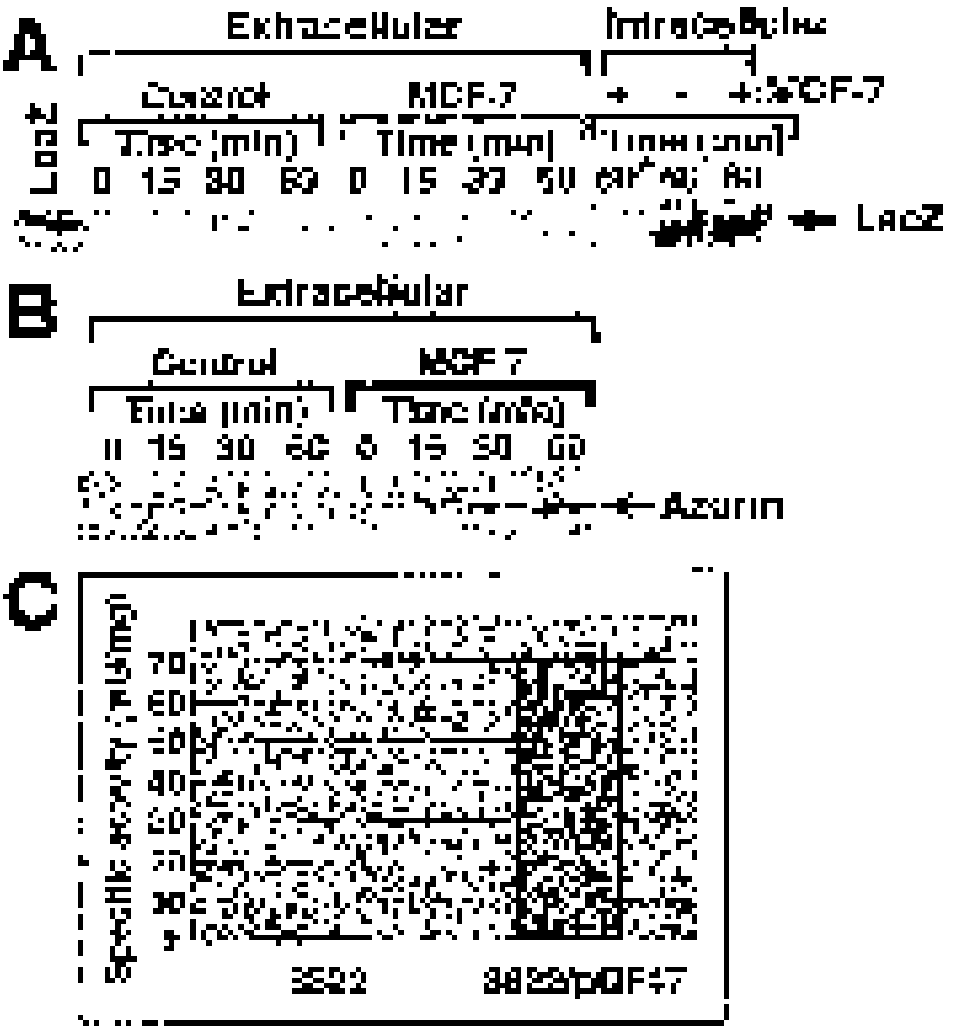
A
Extracellular
Intracellular
Control
MDF-7
+
-
+:MDF-7
Time (min)
Time (min)
Time (min)
0 15 30 60 0 15 30 60
LacZ
B
Extracellular
Control
MDF-7
Time (min)
Time (min)
0 15 30 60 0 15 30 60
Azurin
C
70
60
50
40
30
20
10
0
3522
3522/pMDF-7

Fig. 2

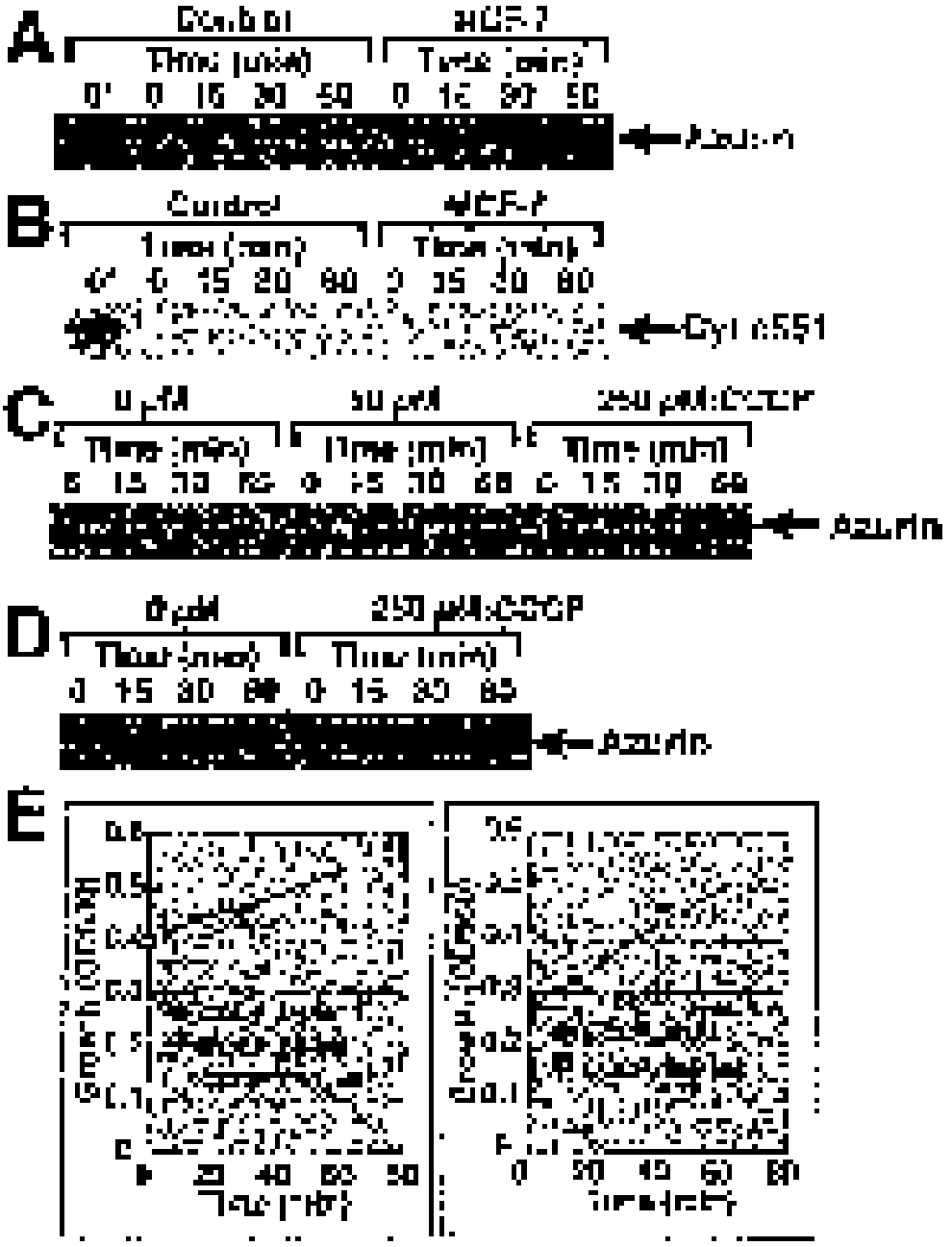
A
B
C
D
E
Control
MCF-7
Time (min)
0' 0 15 20 60 0 15 30 60
Cyt c551
0 μM
50 μM
250 μM:CCCP
Time (min)
0 15 30 60 0 15 30 60 0 15 30 60
Azurin
0 μM
250 μM:CCCP
Time (min)
0 15 30 60 0 15 30 60
Azurin
0.6
0.5
0.4
0.3
0.2
0.1
0
0 20 40 60 80
Time (min)
0.5
0.4
0.3
0.2
0.1
0
0 20 40 60 80
Time (min)

Fig. 3

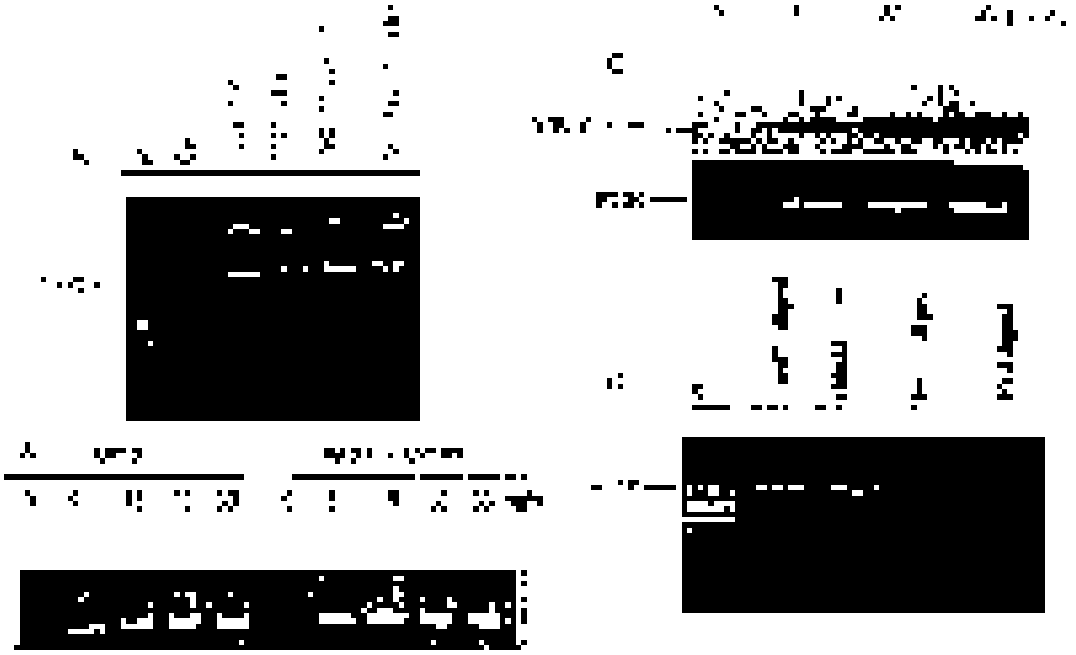

Fig. 6

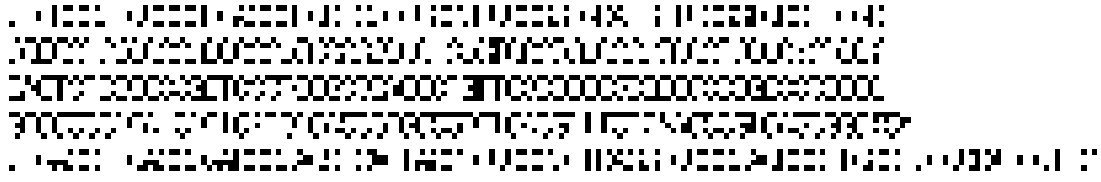

FIG. 5A

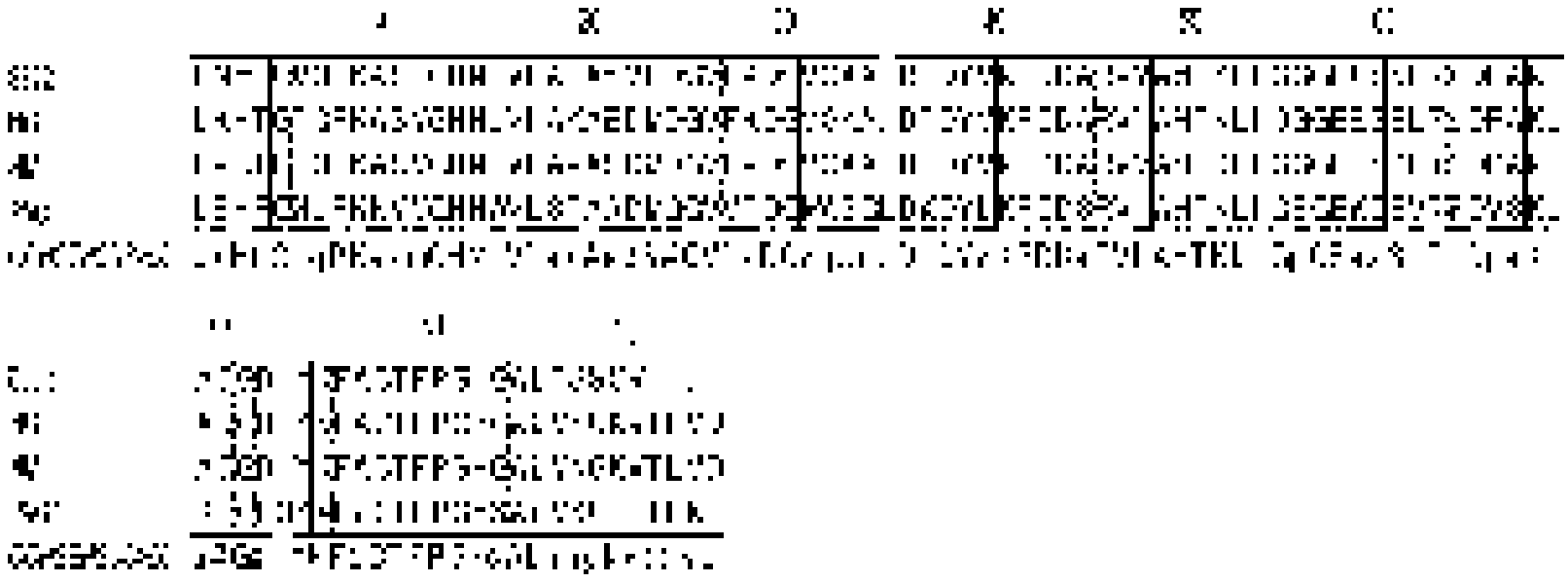

FIG. 5B

FIG. 6A

FIG. 6B

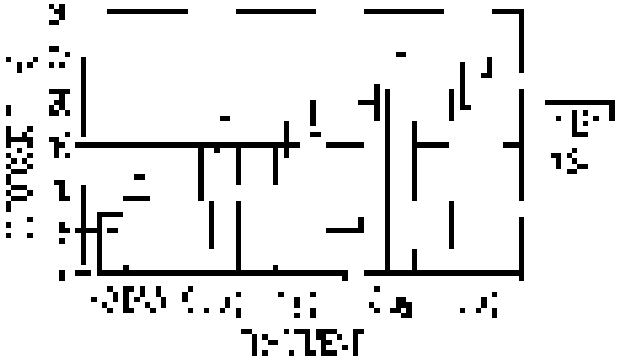

FIG. 6C

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Figure 9

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TABLE 1

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(12) United States Patent
Chakrabarty et al.

(10) Patent No.: US 7,691,383 B2
(45) Date of Patent: Apr. 6, 2010

(54) [illegible]

(75) Inventors: [illegible]

(73) Assignee: [illegible]

(*) Notice: [illegible]

(21) Appl. No.: [illegible]

(22) Filed: [illegible]

(65) Prior Publication Data

[illegible]

Related U.S. Application Data

(60) [illegible]

(51) Int. Cl.
[illegible]

(52) U.S. Cl. [illegible]

(58) Field of Classification Search [illegible]

See application file for complete search history.

(56) References Cited

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

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OTHER PUBLICATIONS

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(Continued)

Primary Examiner—[illegible]

(74) Attorney, Agent, or Firm—[illegible]

(57) ABSTRACT

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[illegible] Claims, [illegible] Drawing Sheets

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FIG.1a

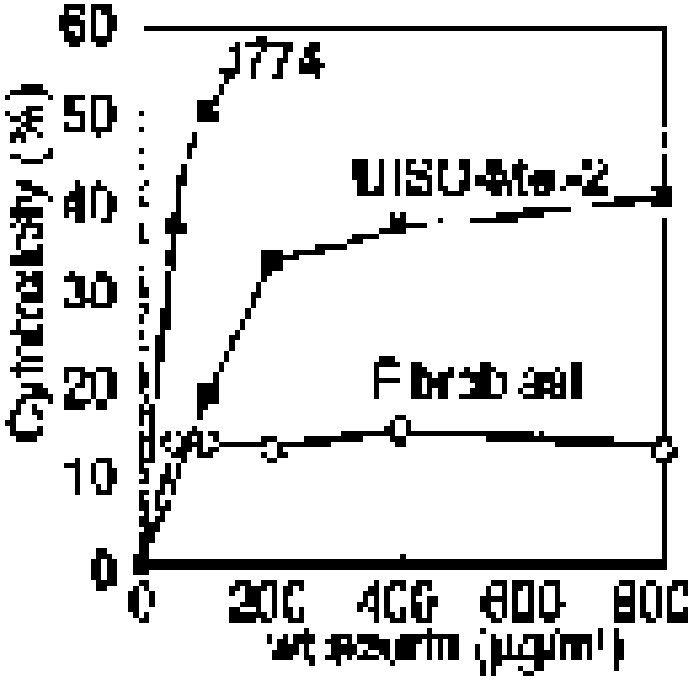

60
50
40
30
20
10
0
Cytotoxicity (%)
1774
UISU-Mte-2
Fibroblast
0
200
400
600
800

FIG.1b

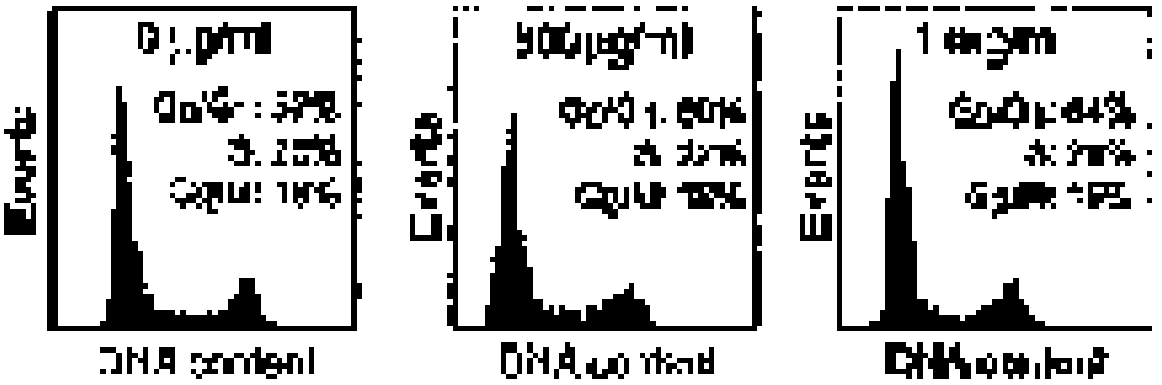

Events
DNA content
DNA content

FIG. 2a

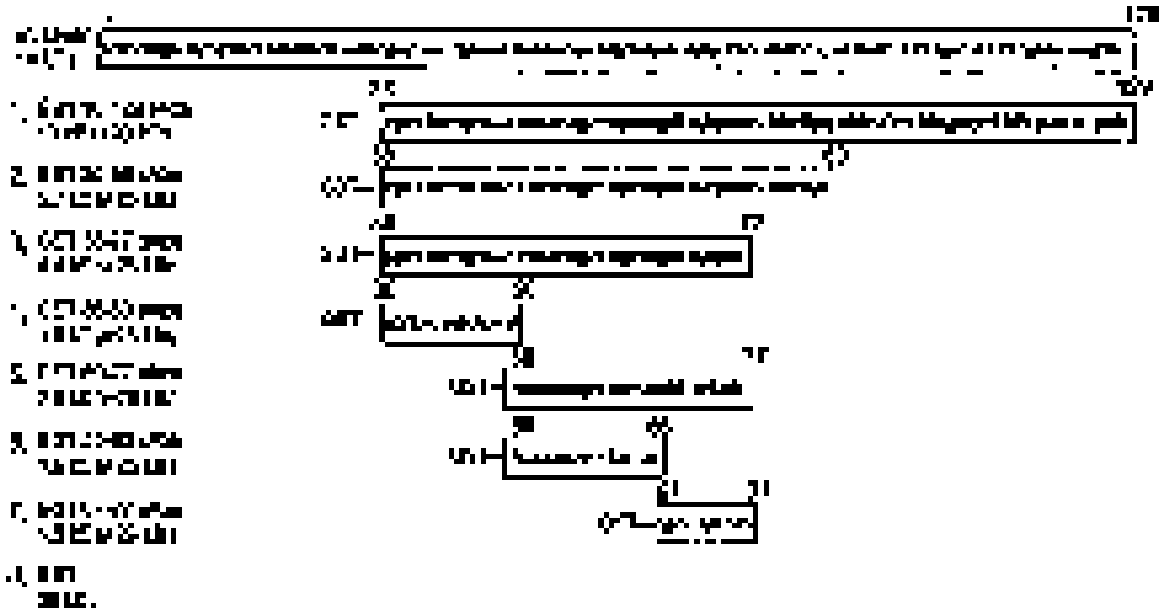


FIG. 2b

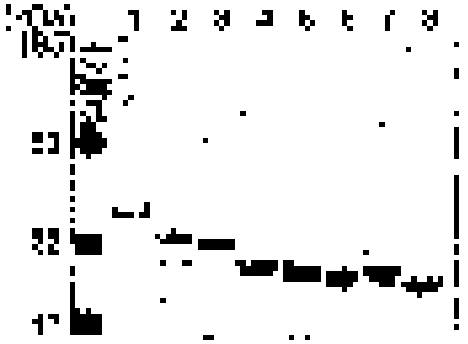
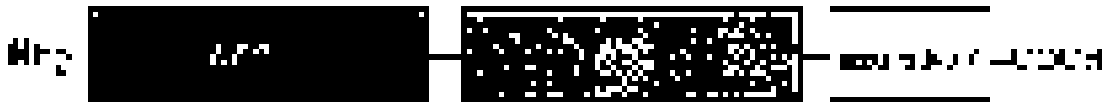


FIG. 3a



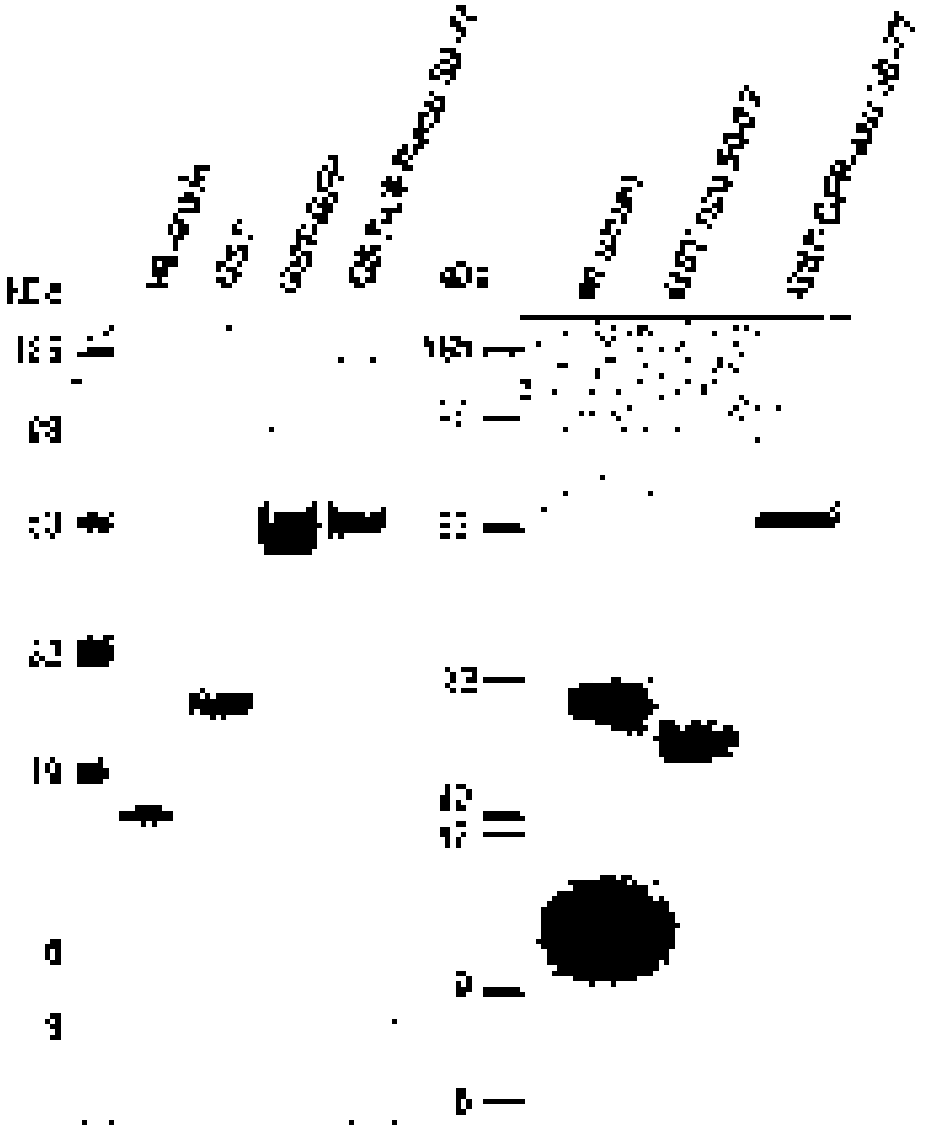


FIG. 3b

FIG. 3c

FIG. 4a

FIG. 4b

FIG. 4c

FIG. 5a

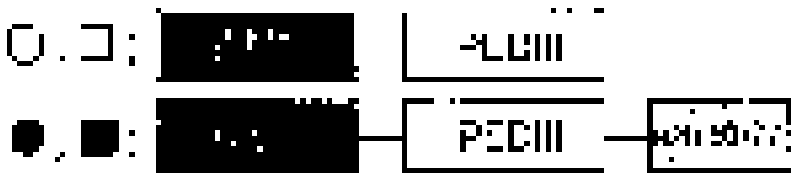


FIG. 5b

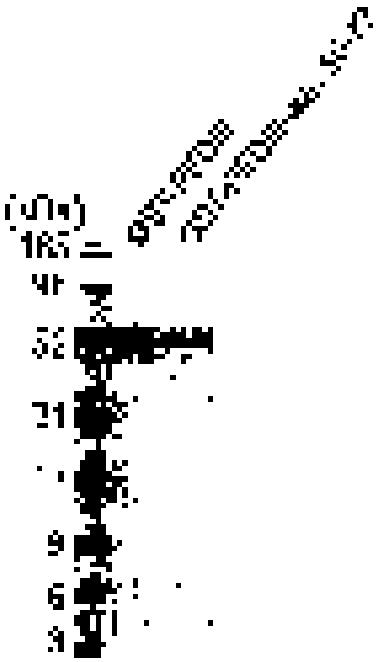
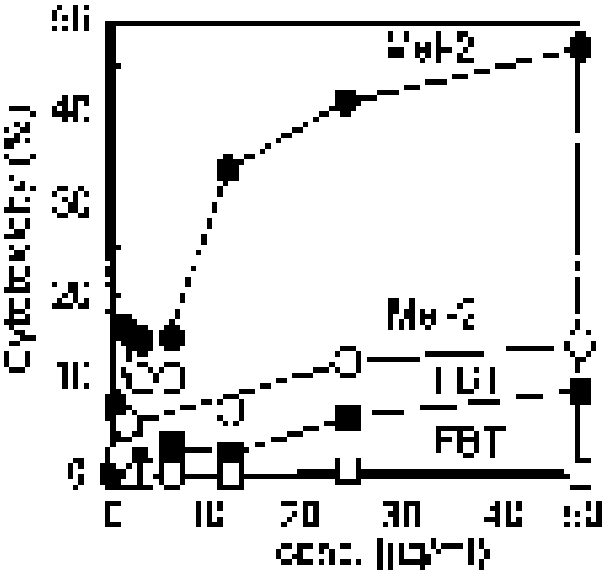


FIG. 5c



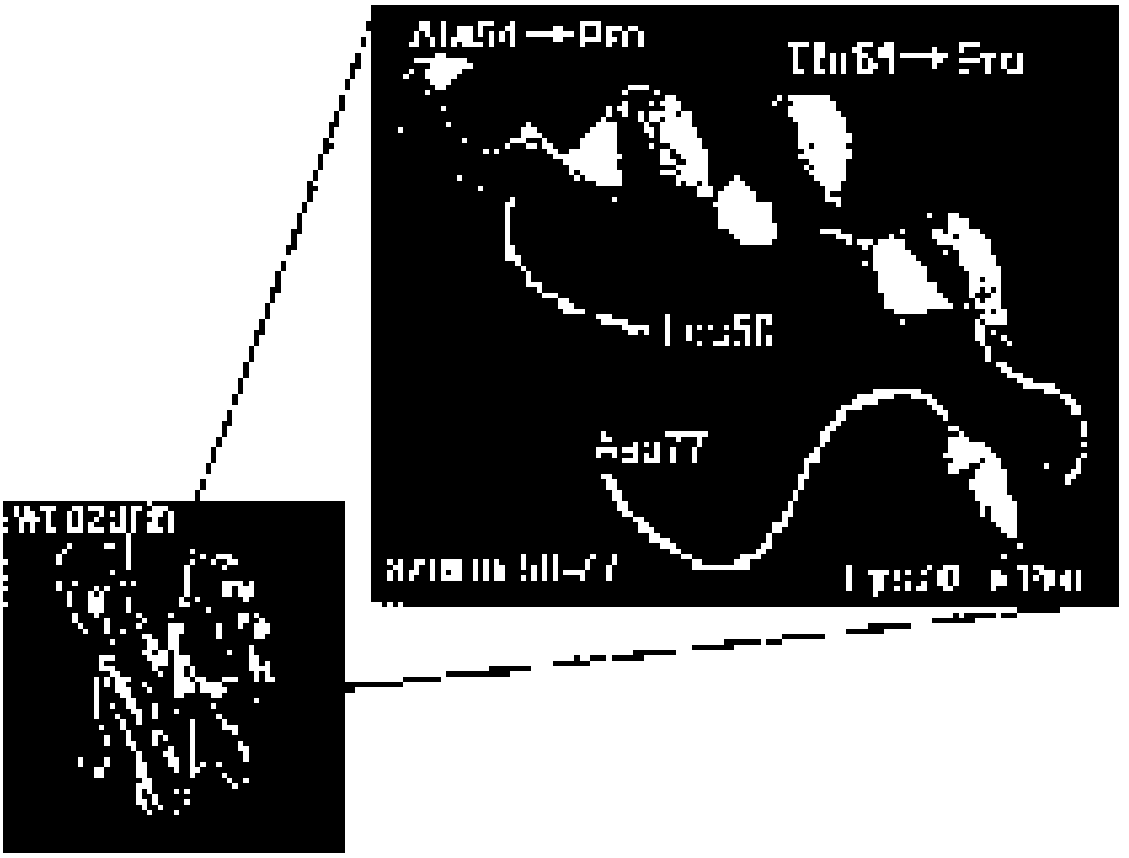


FIG. 6

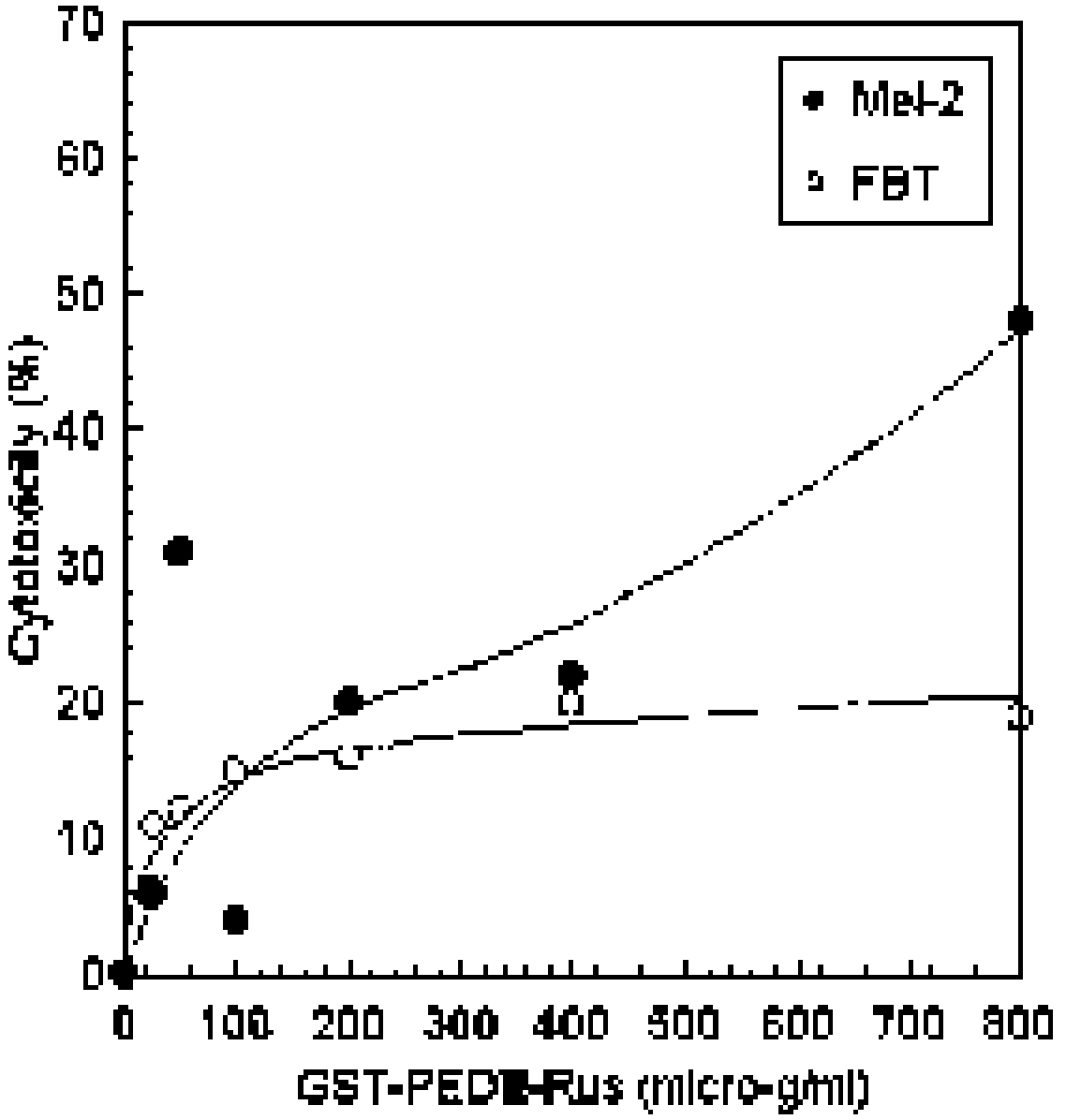

70
60
50
40
30
20
10
0
Cytotoxicity (%)
Mel-2
FBT
0
100
200
300
400
500
600
700
800
GST-PEDF-Rus (micro-g/ml)

FIG. 7

FIG. 8a

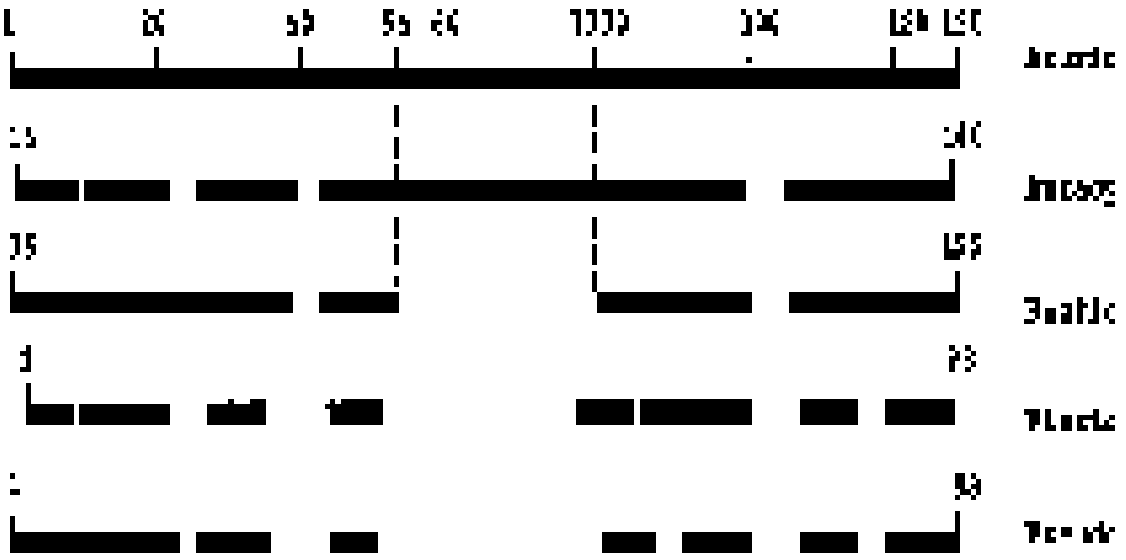
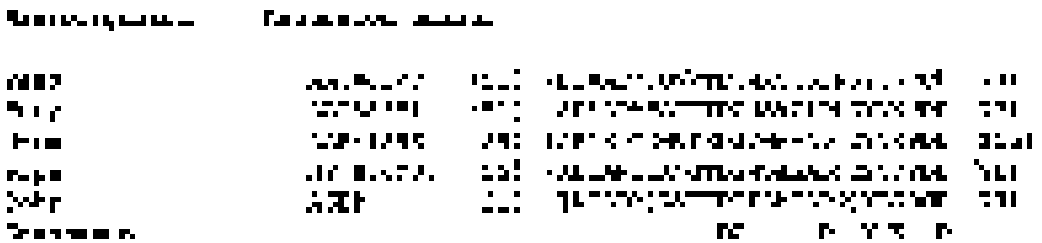


FIG. 8b



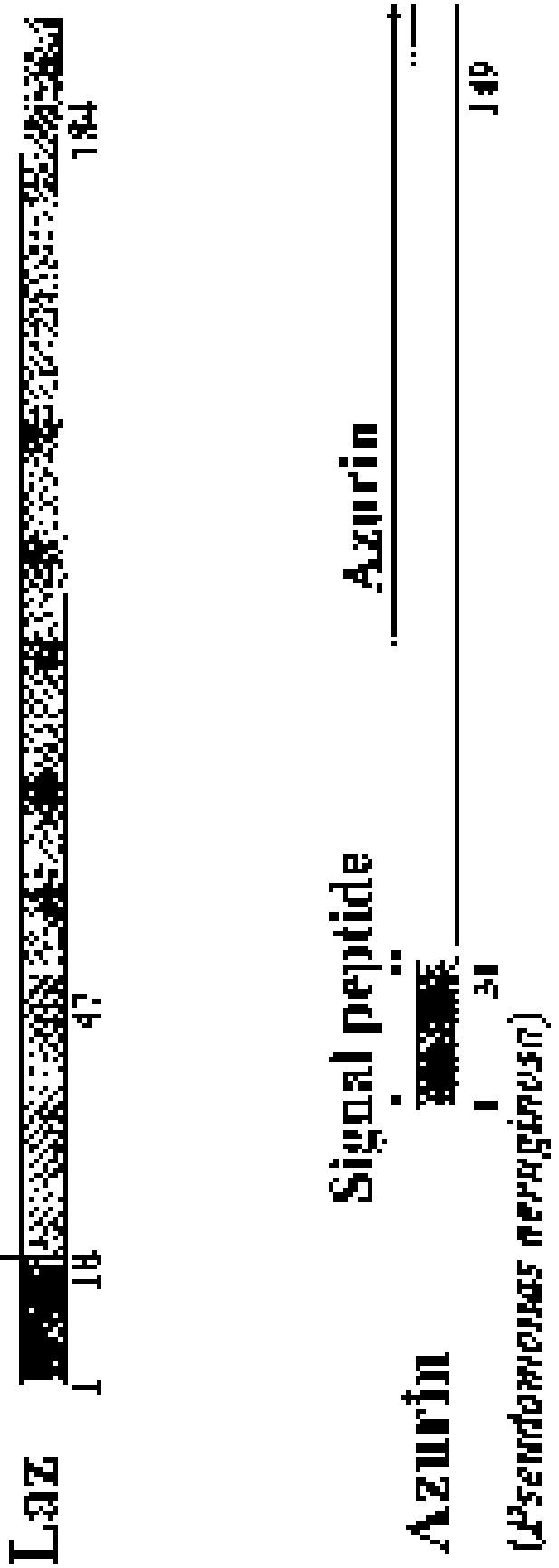


FIG. 8

PRIOR ART

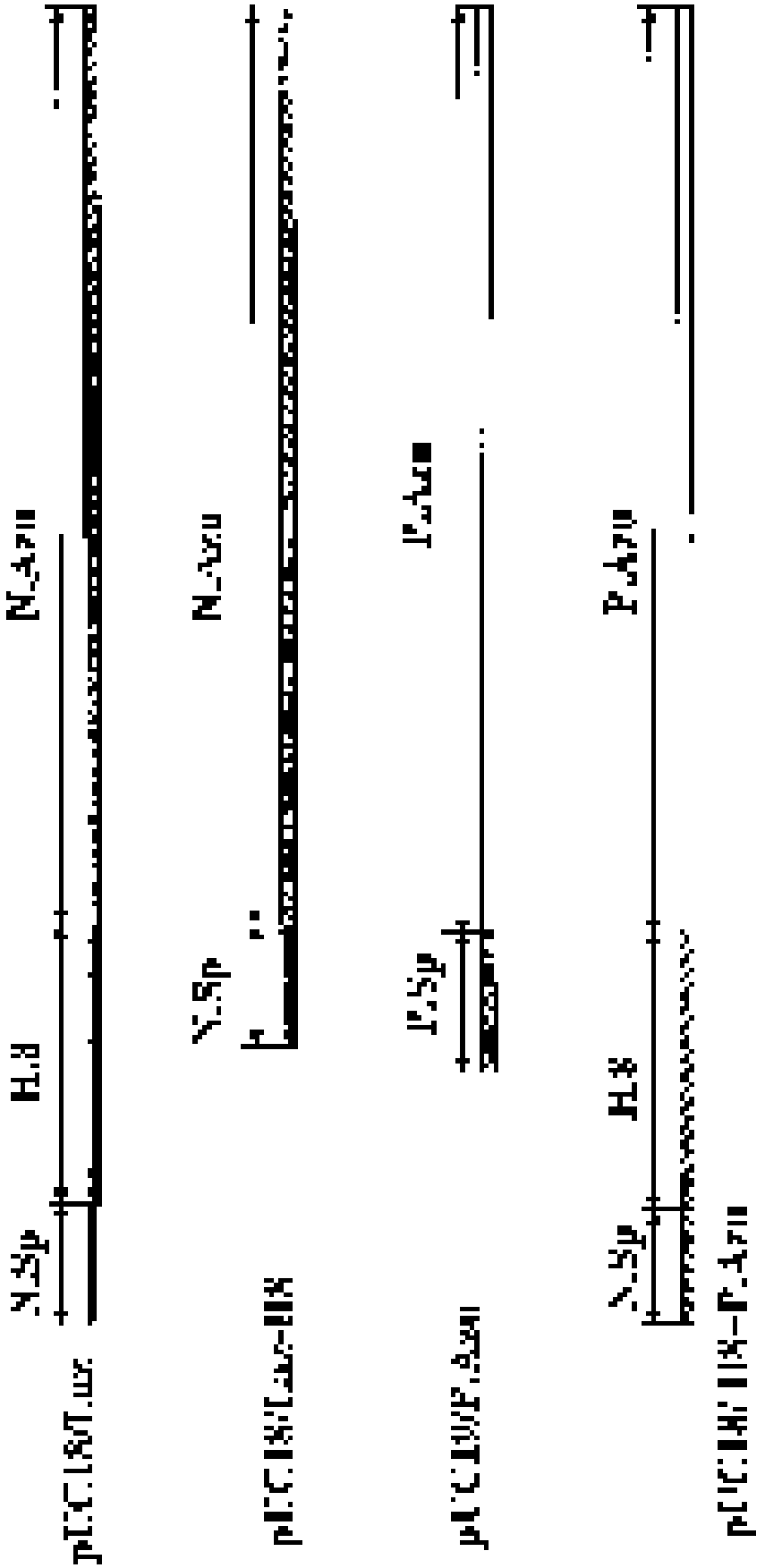


FIG. 10

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Example 11

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Seventh Day of August, 2012

David J. Kappos

Director
[illegible]

United States Patent [illegible]

[illegible] et al.

Patent No.: [illegible]

Date of Patent: [illegible]

[illegible]

ABSTRACT

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Fig. 1

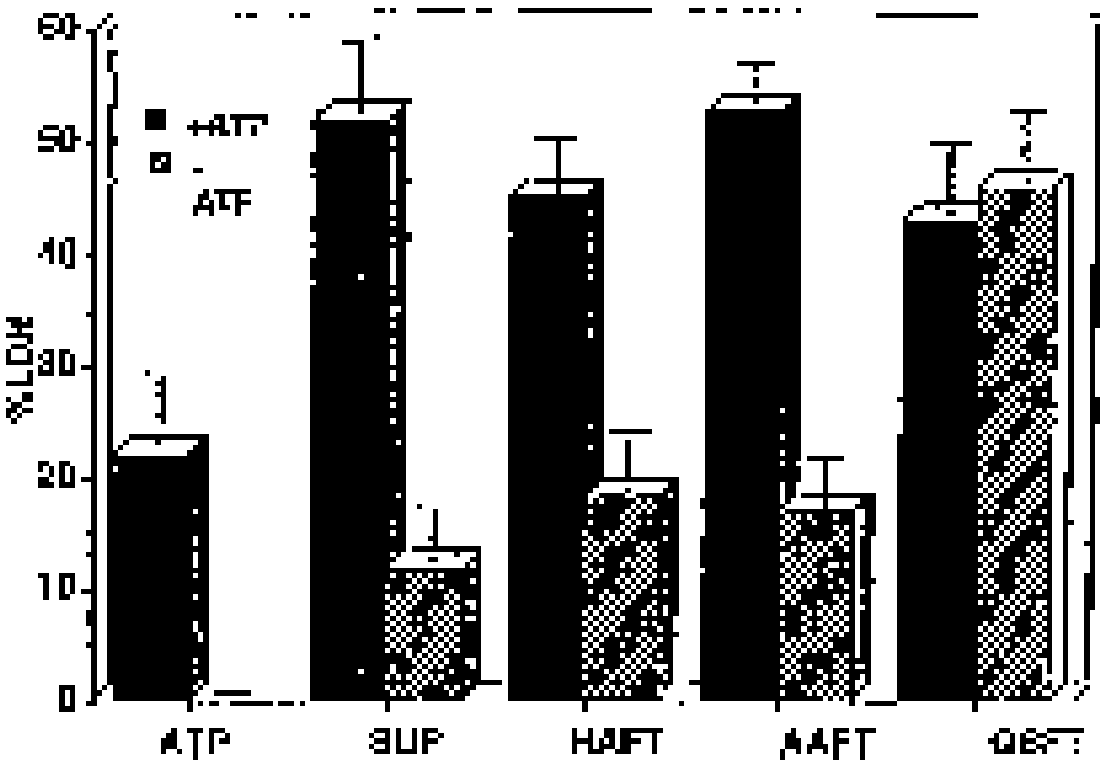


Fig. 2

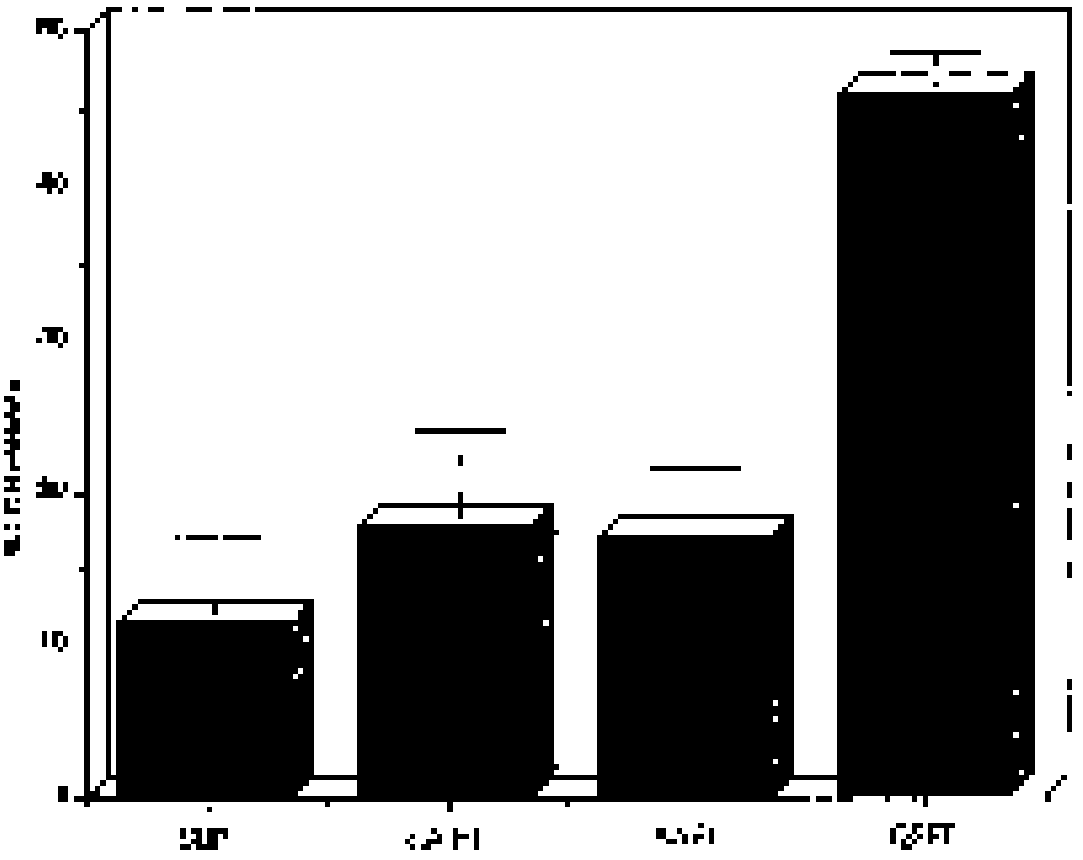


Fig. 3

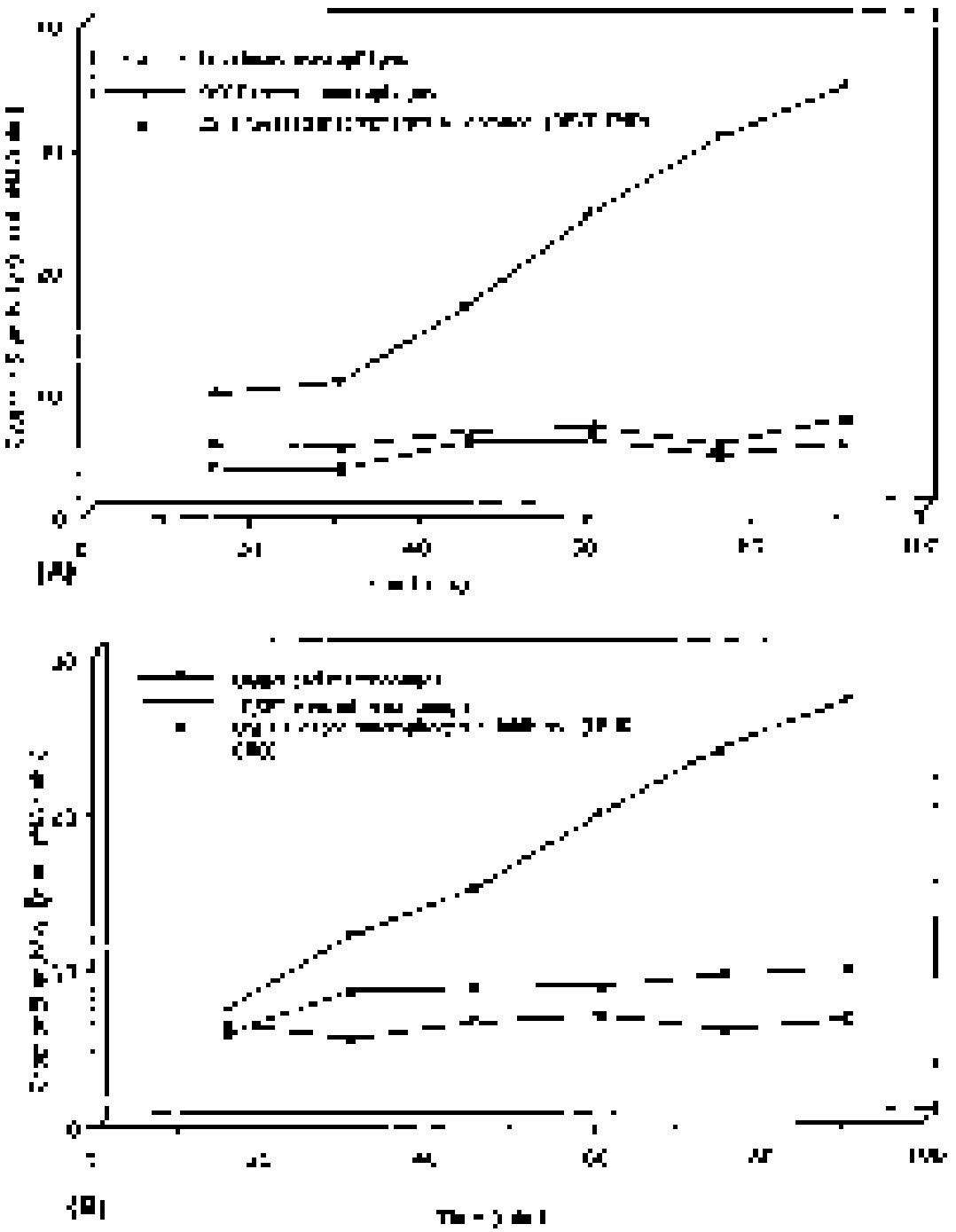
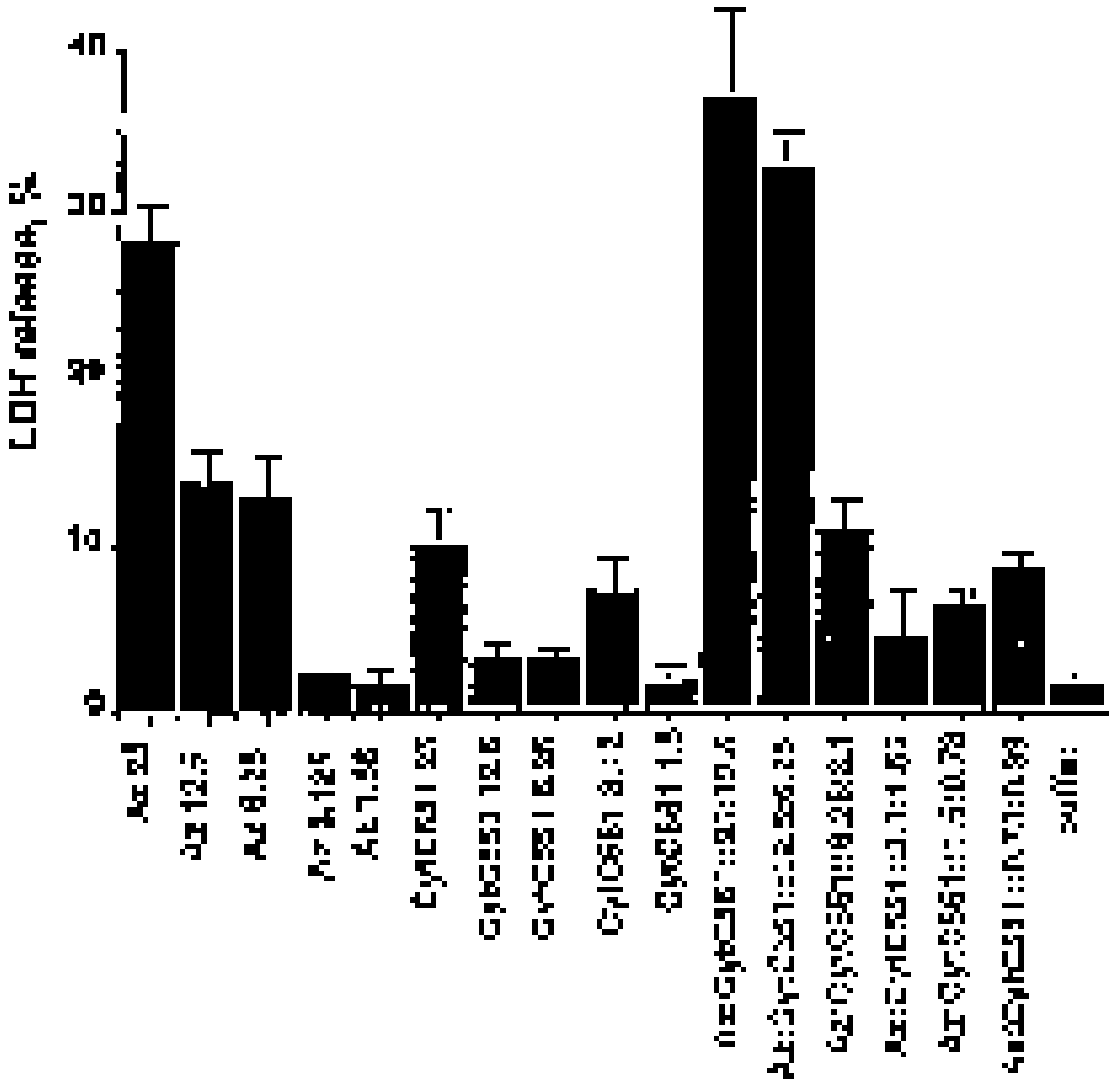


Fig. 4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40
30
20
10
0
Az 25
Az 12.5
Az 6.25
Az 3.125
Az 1.56
CytC551 25
CytC551 12.5
CytC551 6.25
CytC551 3.12
CytC551 1.5
Az:CytC551::25:19.4
Az:CytC551::12.5:6.25
Az:CytC551::6.25:3.1
Az:CytC551::3.1:1.56
Az:CytC551::1.5:0.78
Az:CytC551::0.78:0.39
buffer

Fig. 5

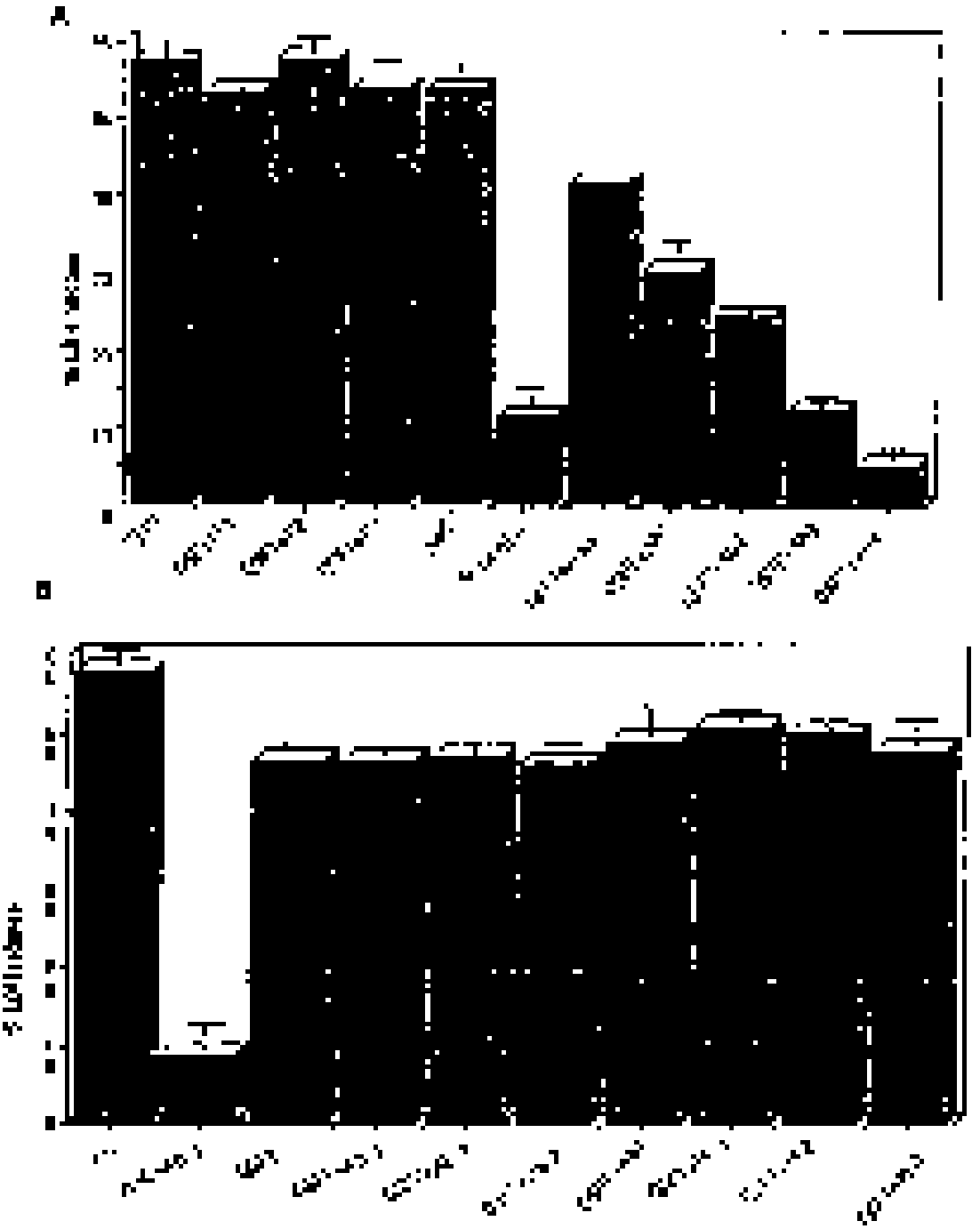


Fig. 6

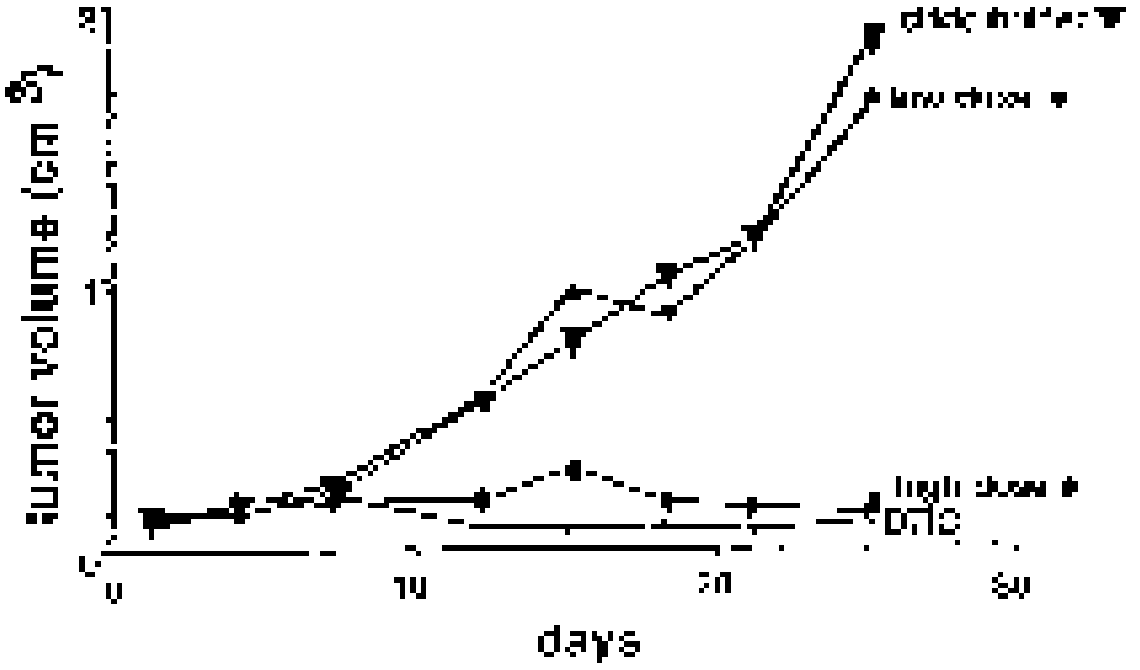
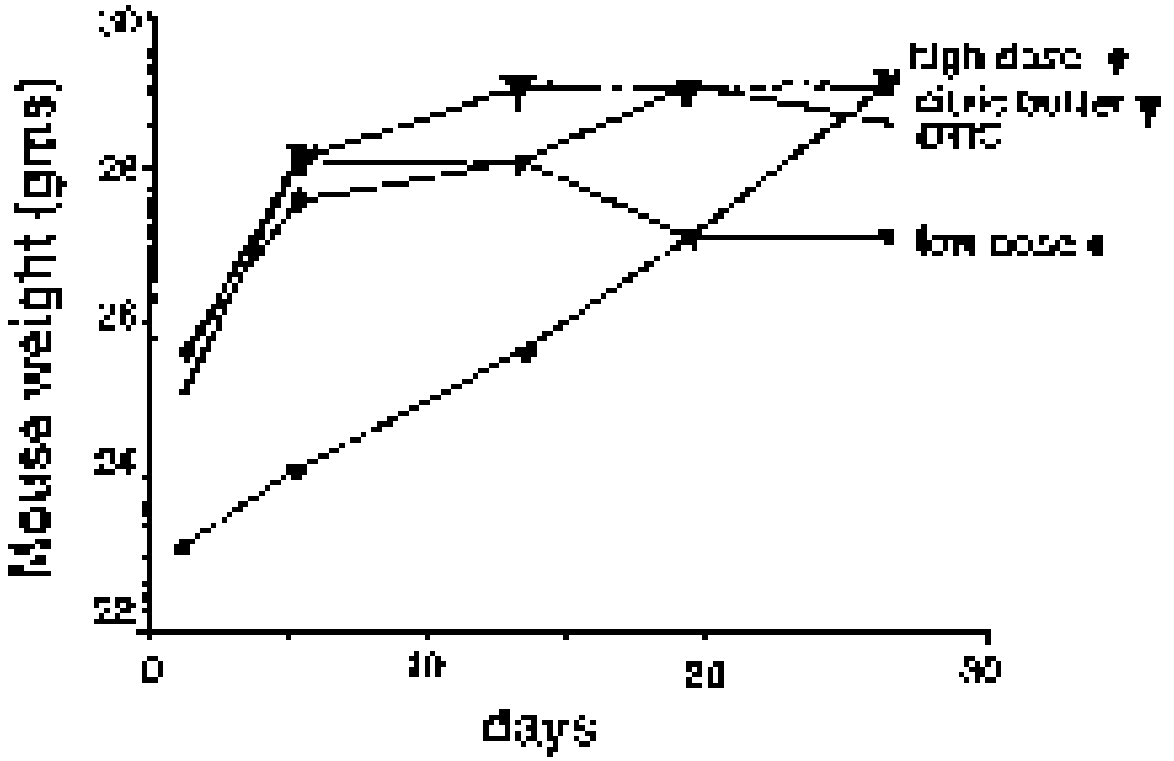


Fig. 7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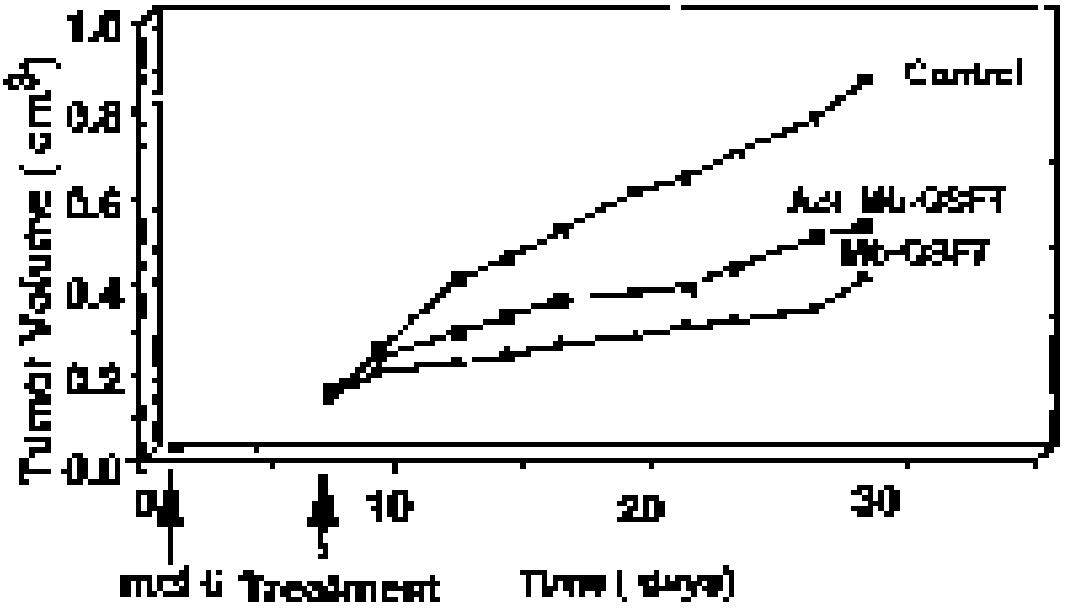
30
28
26
24
22
0
10
20
30
days
High dose
citric buffer
DMC
low dose

Fig. 8


Tumor Volume (cm³)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Control
Act MU-G3F7
MU-G3F7
mcl-1
Treatment
Time (days)

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UNITED STATES PATENT AND TRADEMARK OFFICE

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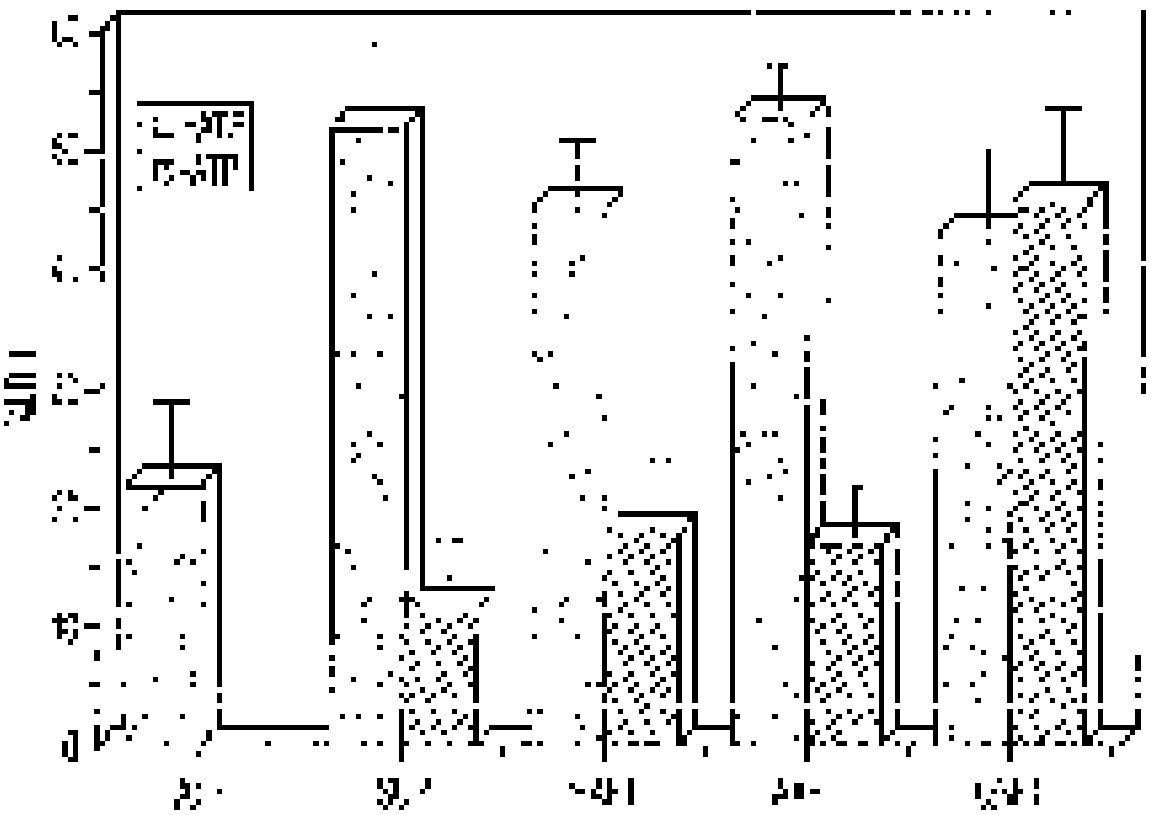
United States
Patent Application Publication
Chakraborty et al.

Pub. No. US 2009/0137468 A1
Pub. Date: May 28, 2009

Polymer Compositions



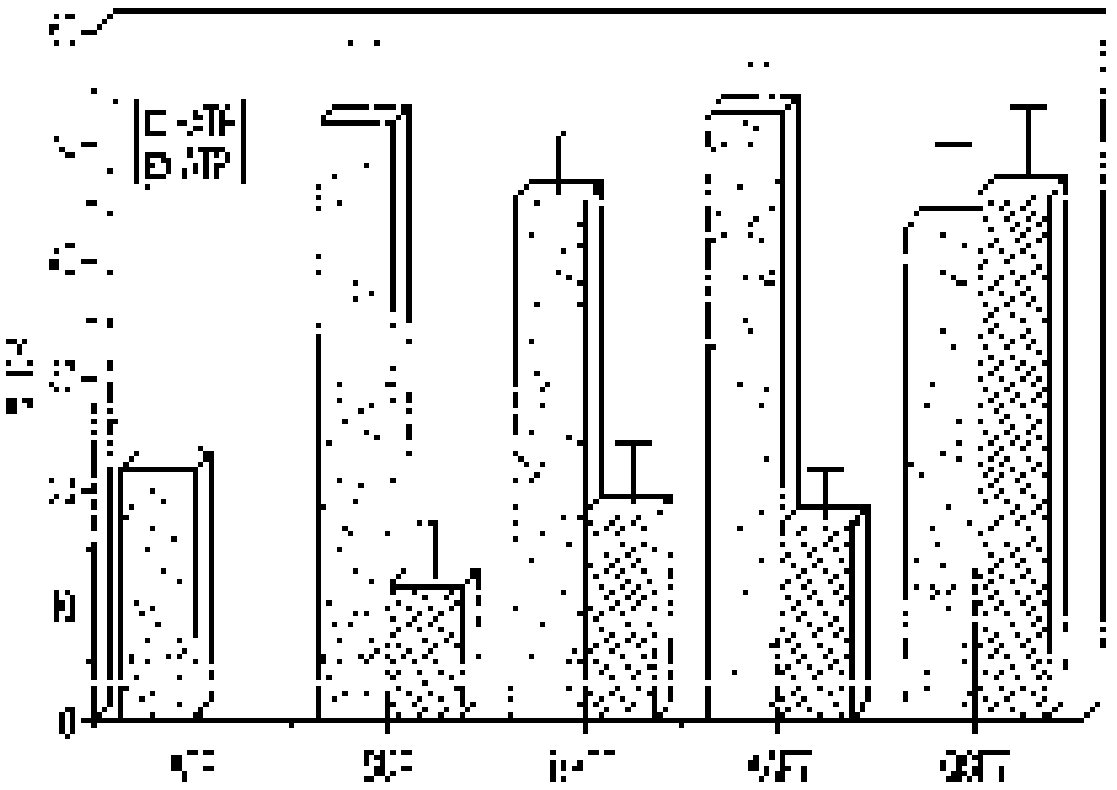


FIG. 1

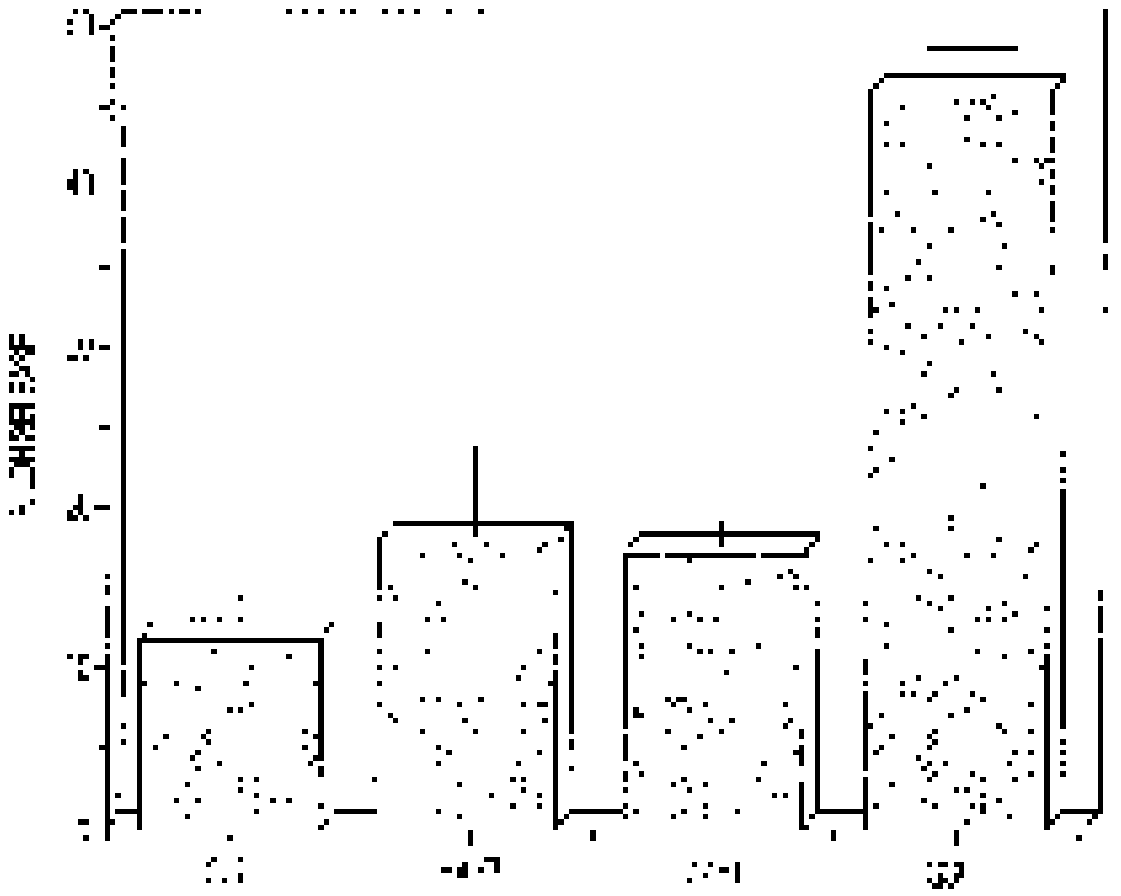


FIG. 2

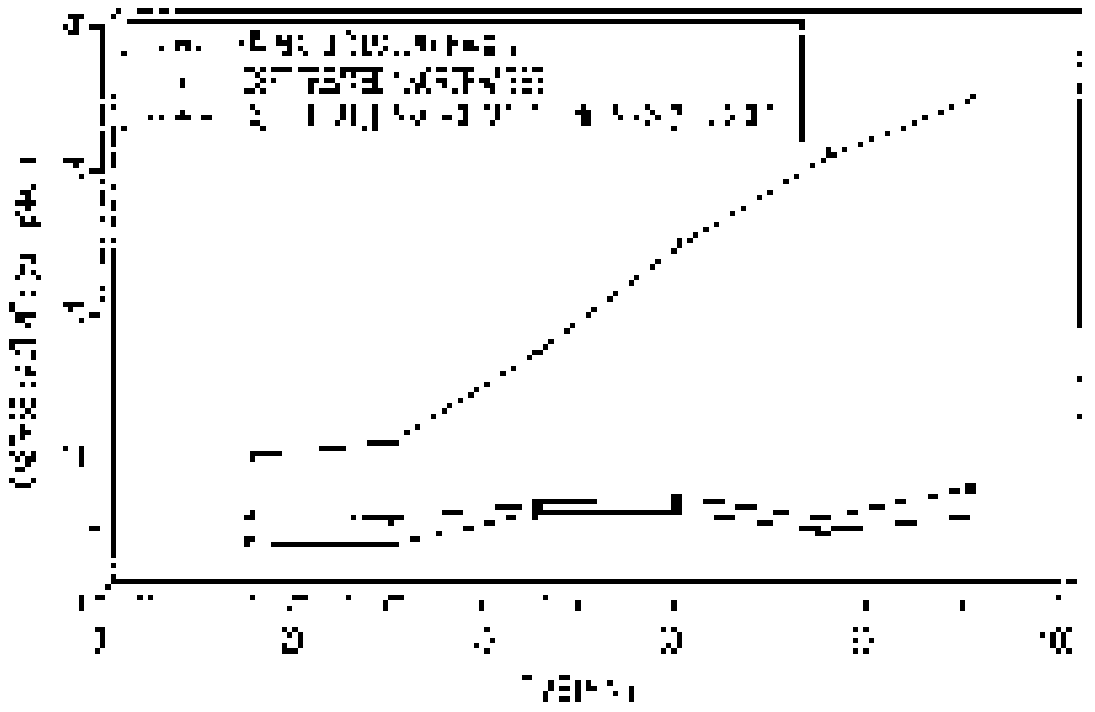


FIG. 3A

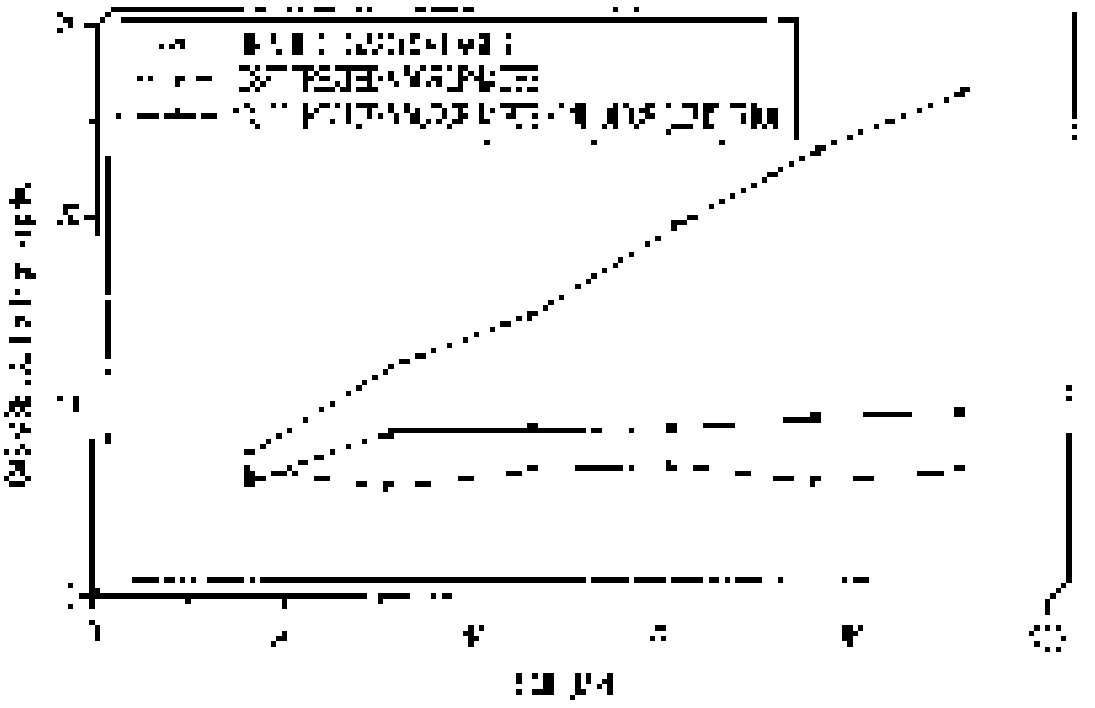


FIG. 3B

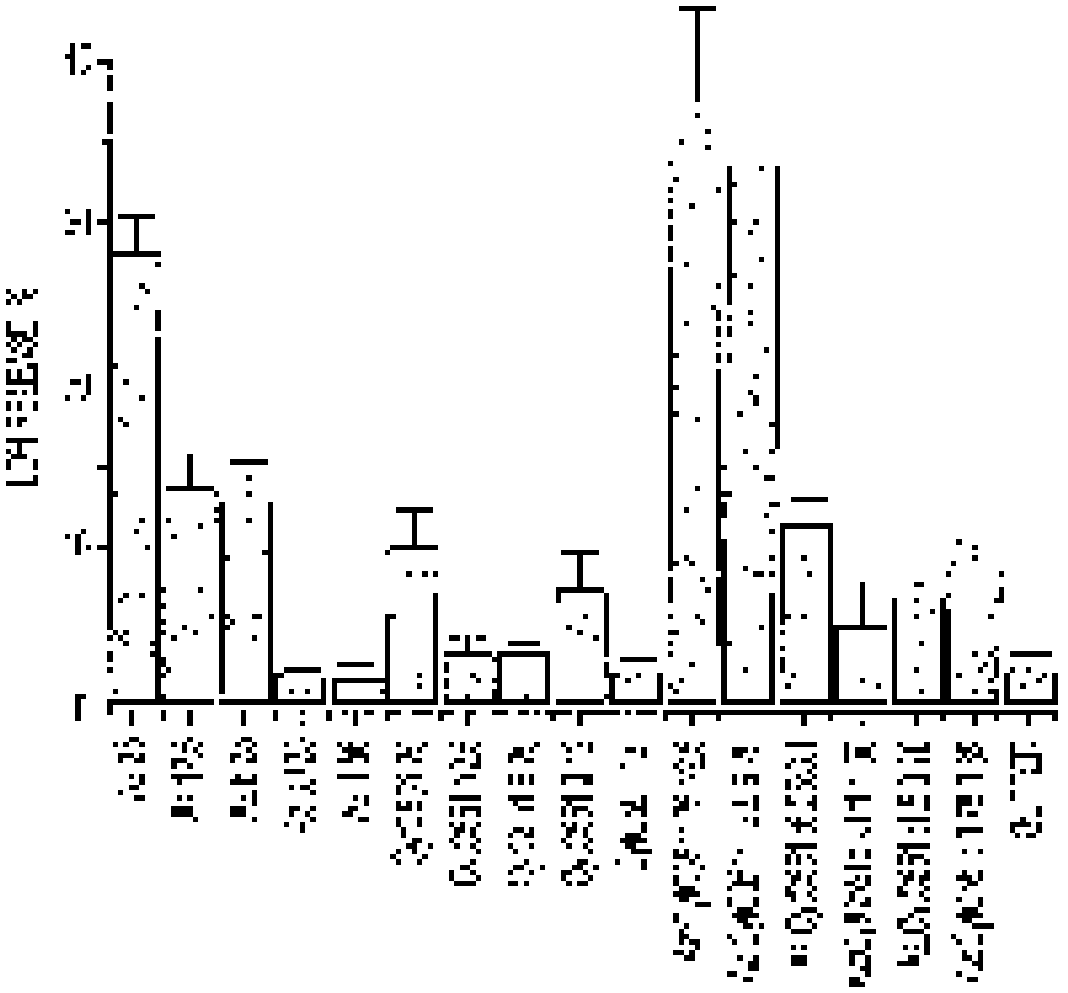


FIG. 4

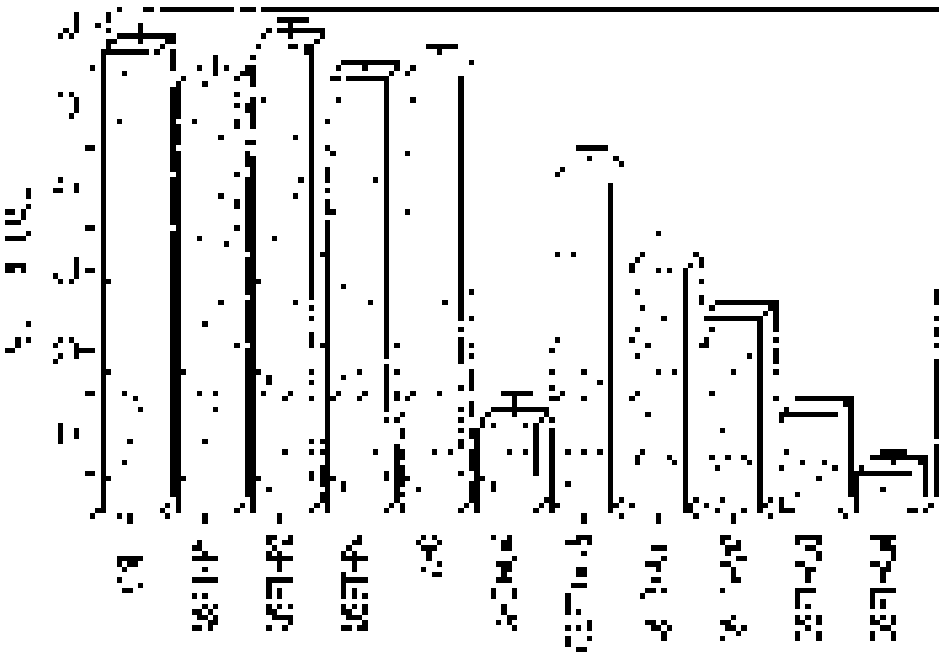


FIG. 5A

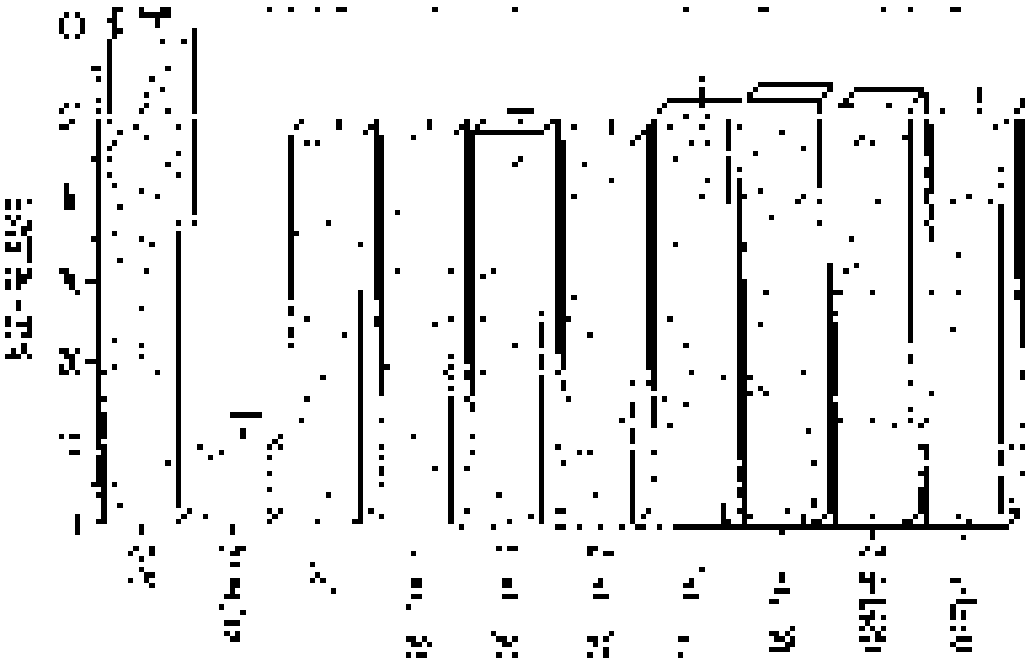


FIG. 5B

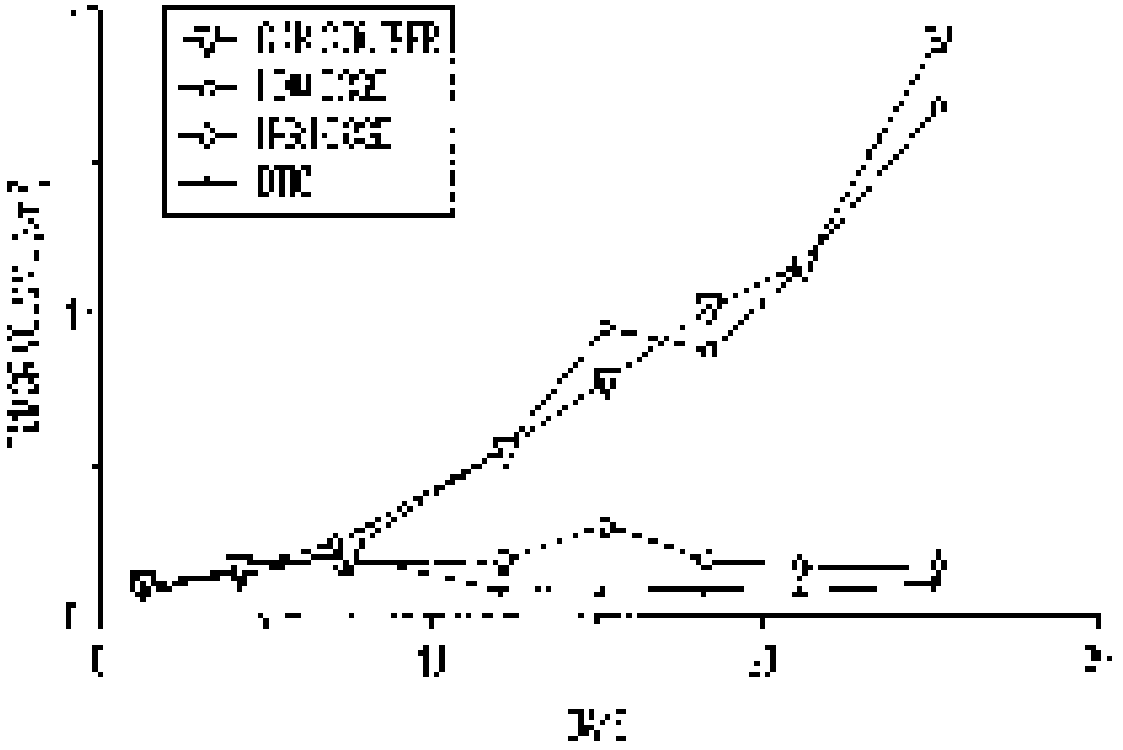


FIG. 6

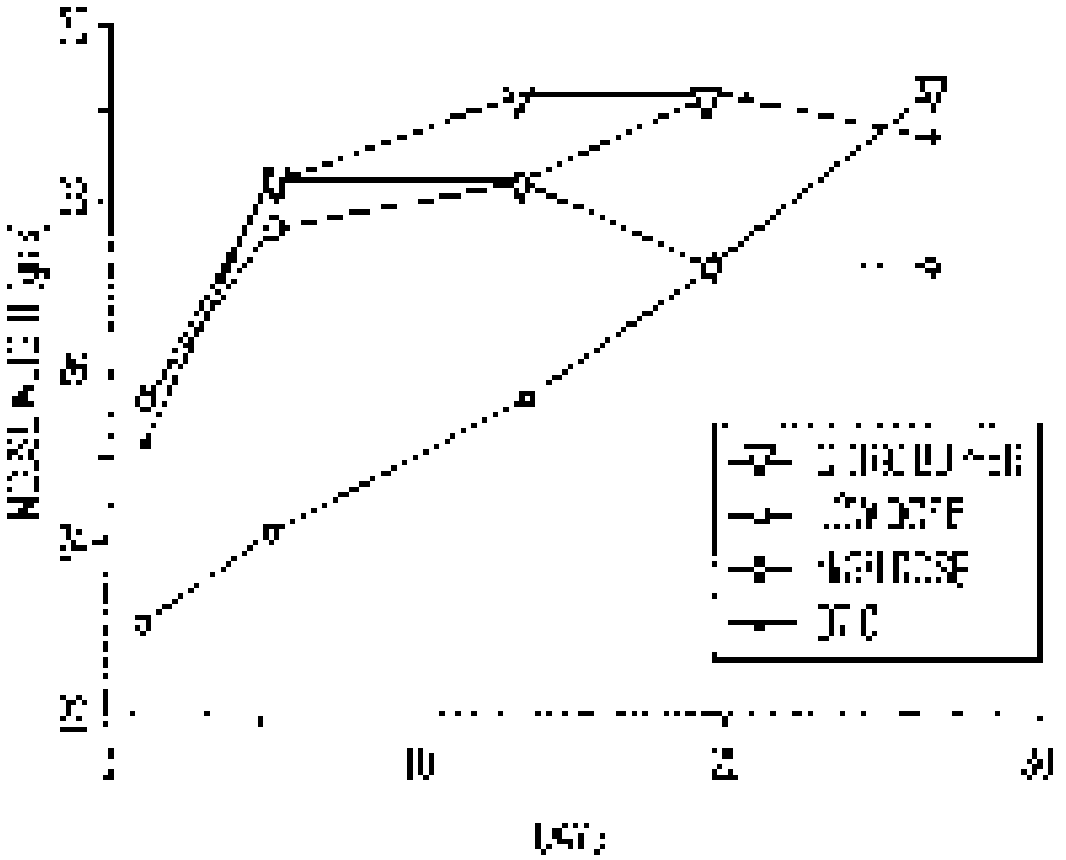


FIG. 7

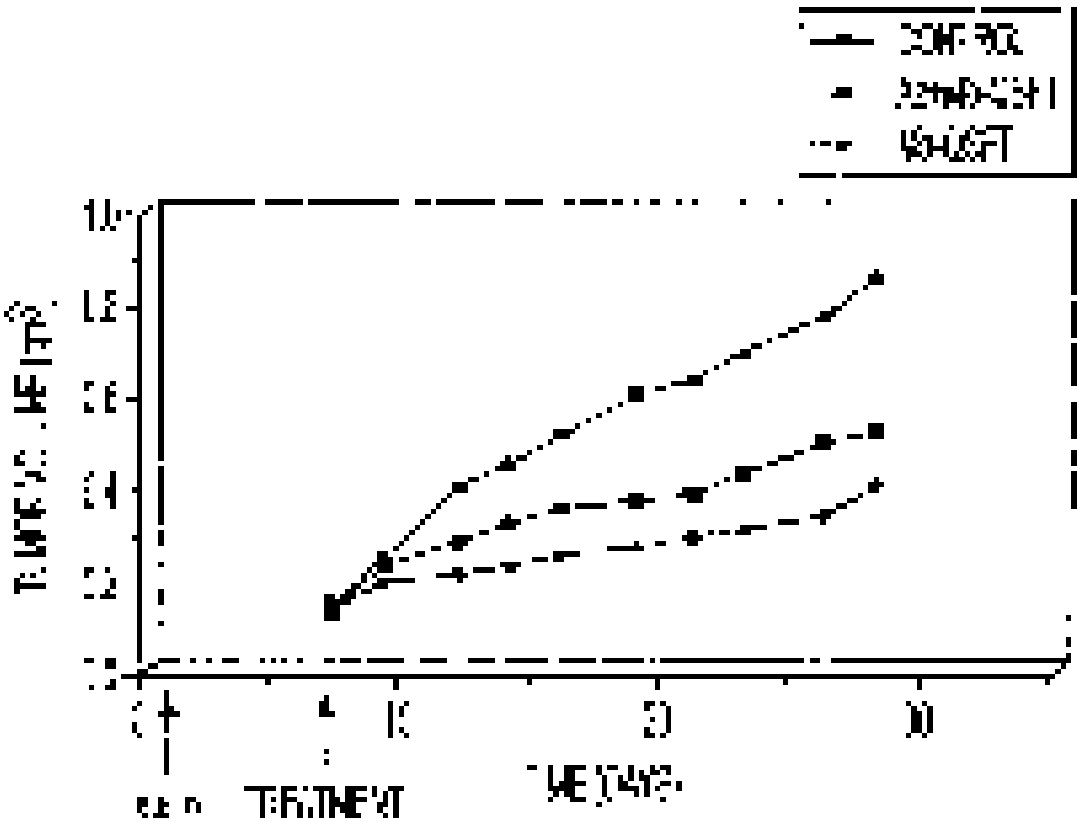


FIG. 8

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US 8,597,183 B2

United States Patent
Hingorani et al.

Patent No. 8,597,183 B2
Date of Patent: Nov. 5, 2013

(54) [illegible]

(75) Inventors: [illegible]

(73) Assignee: [illegible]

(1) Notice: [illegible]

(2) Filed: [illegible]

(3) Priority: [illegible]

(4) Int. Cl. [illegible]

(5) U.S. Cl. [illegible]

(6) Field of Classification Search [illegible]

(7) Prior Publication Data

[illegible]

Related U.S. Application Data

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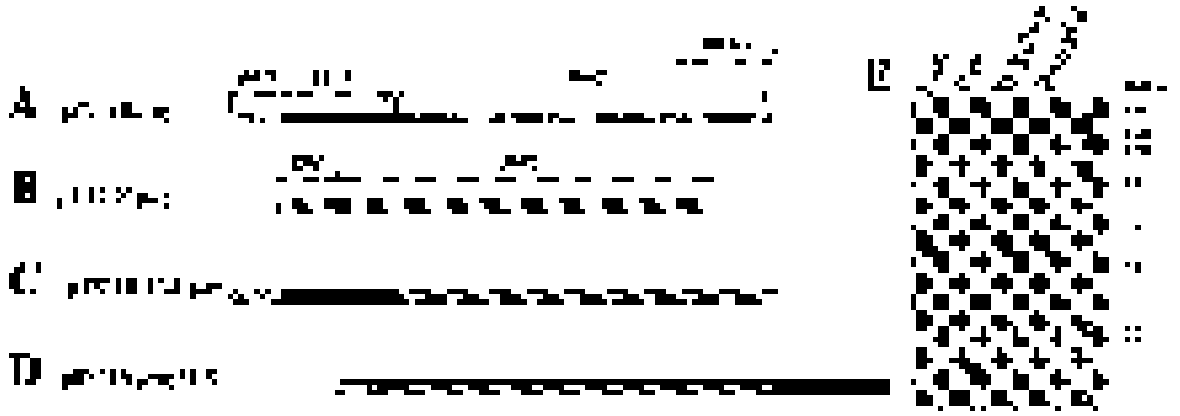

Figure 1

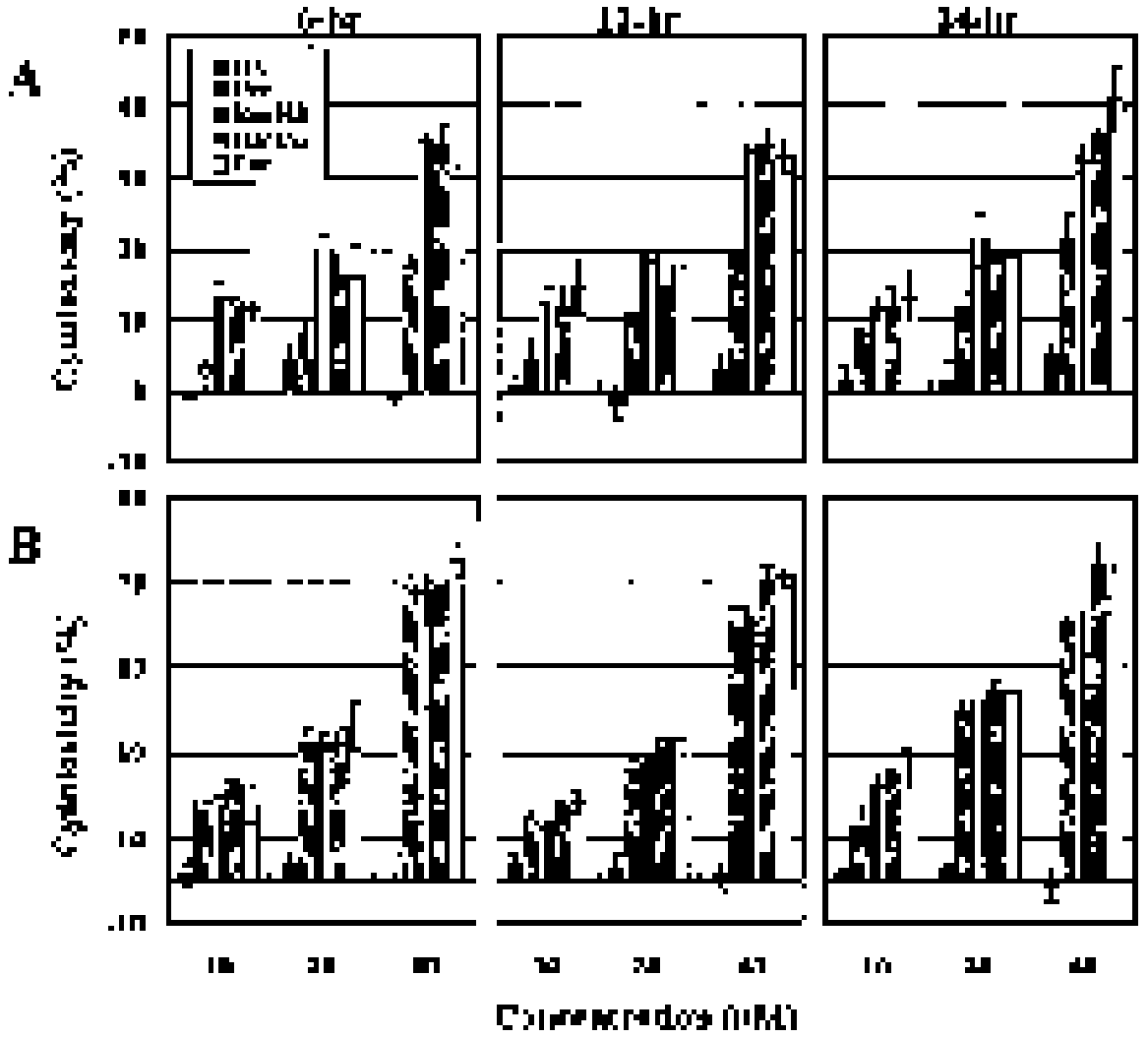

Figure 2

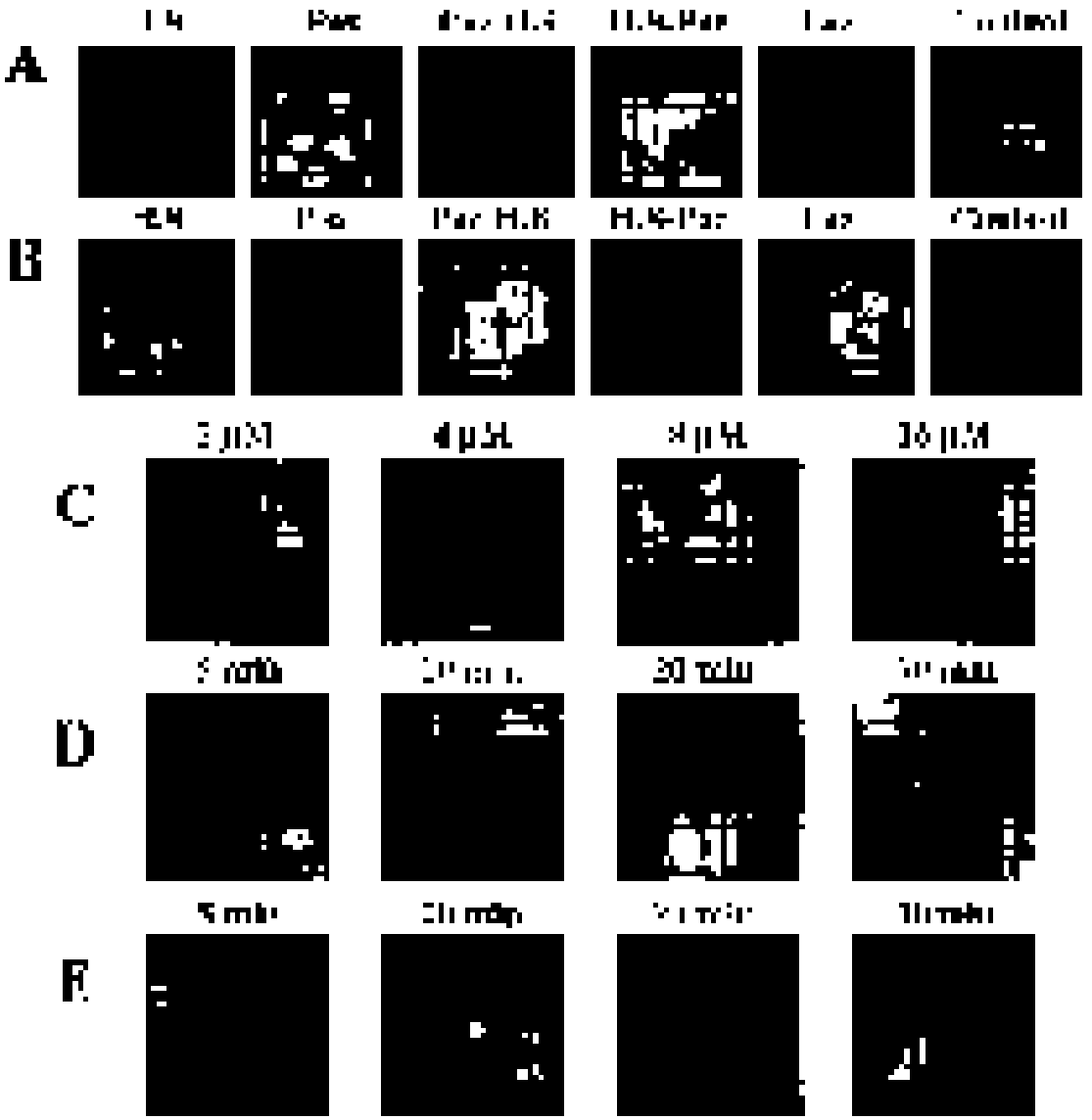

Figure 3

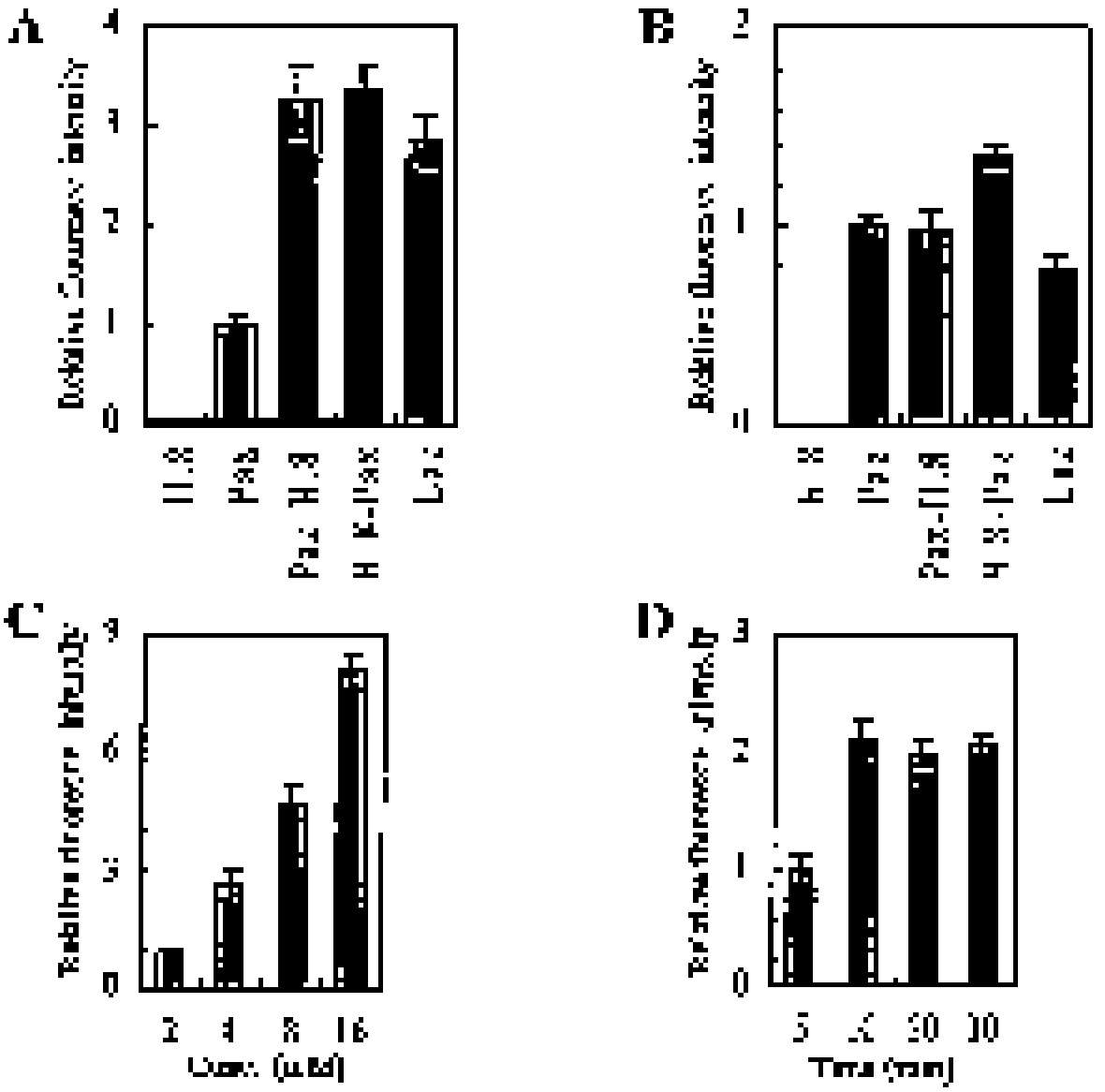

Figure 4

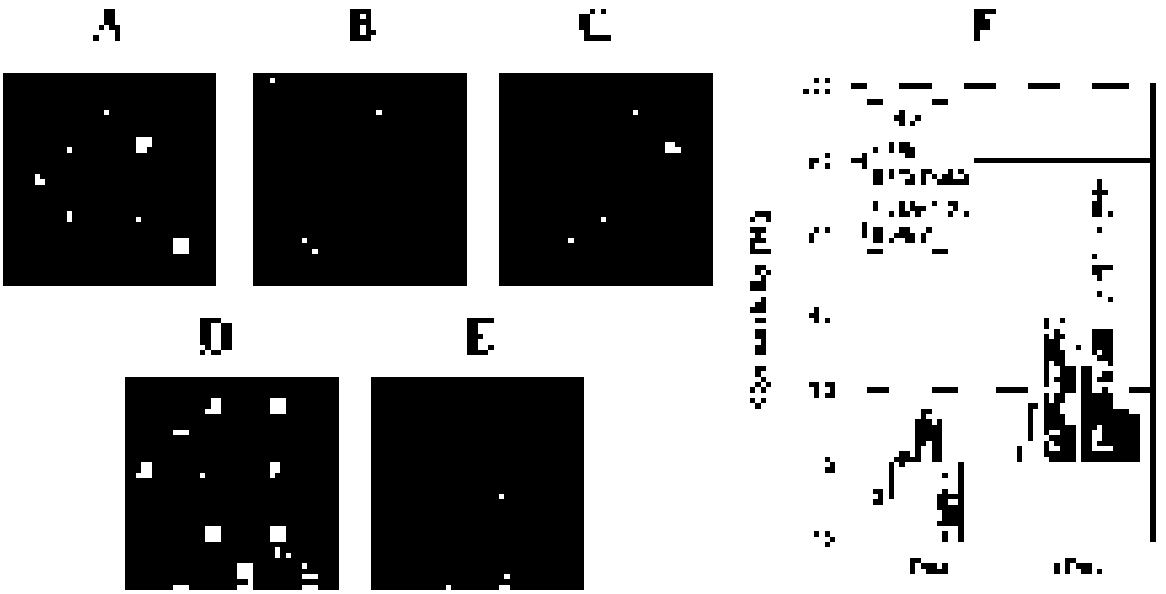

Figure 3

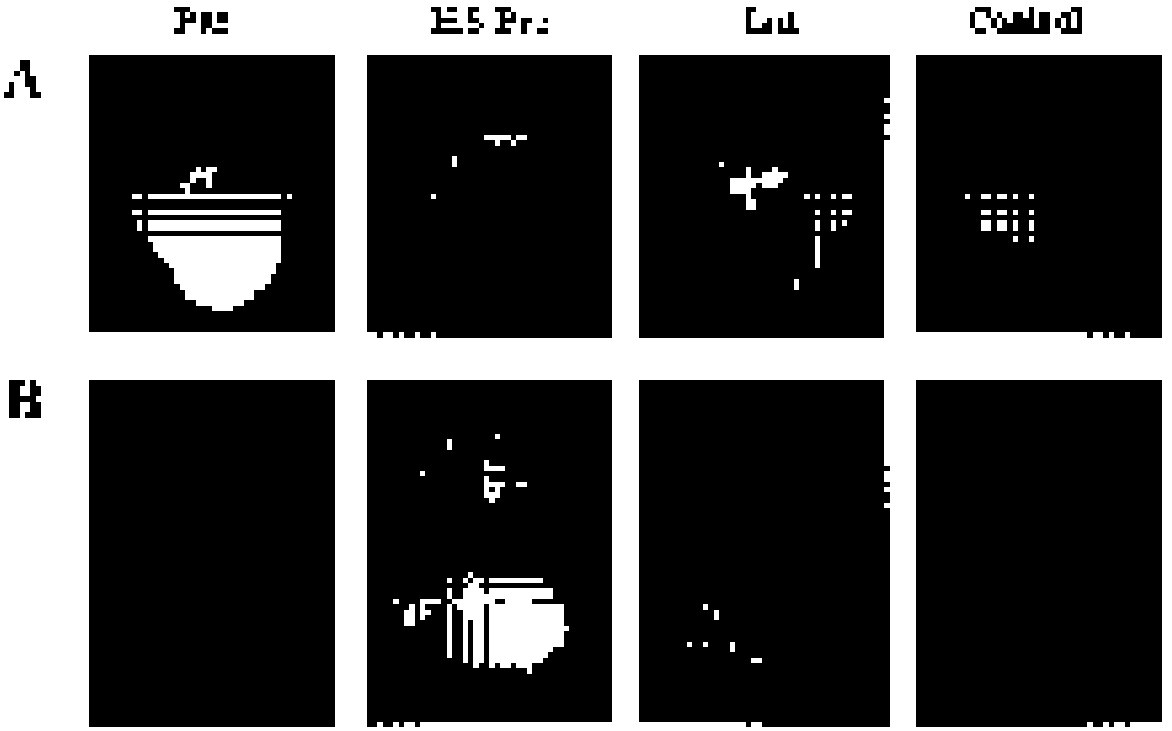

Figure 6

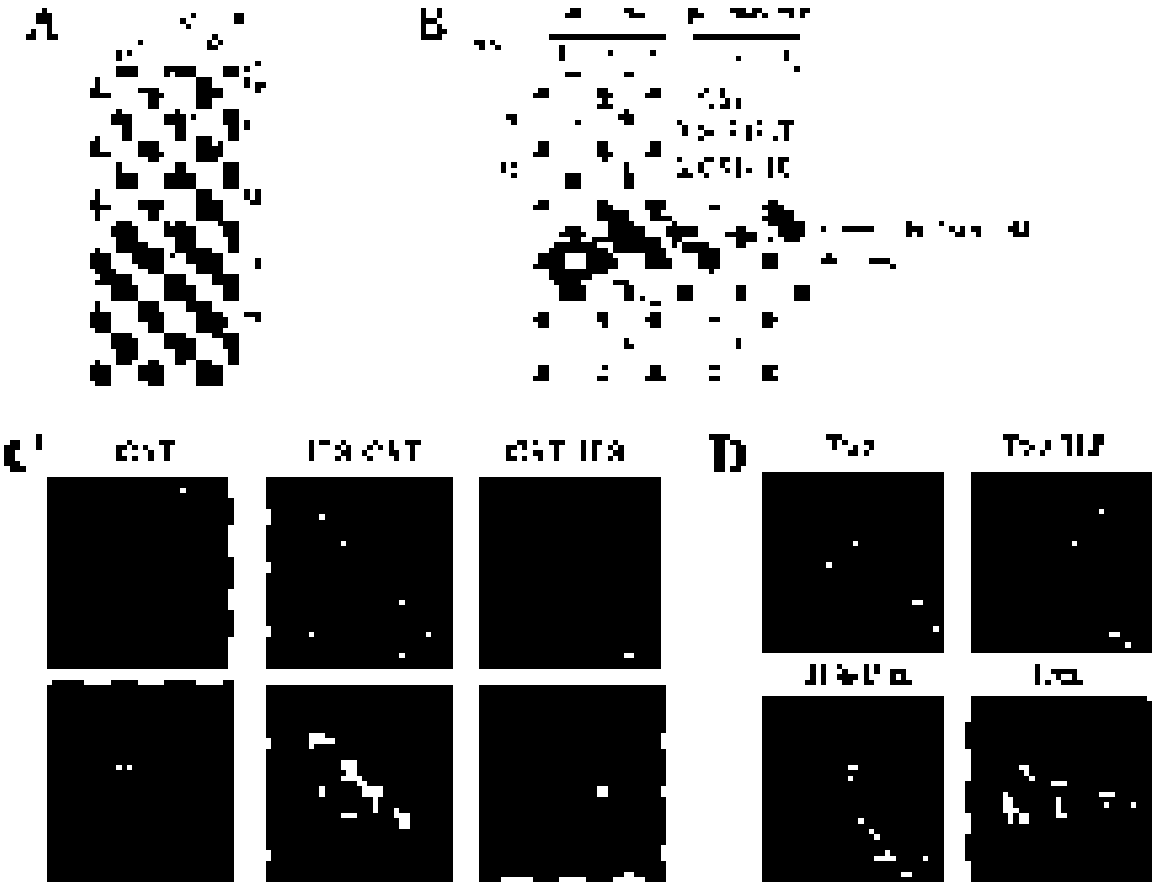

Figure 7

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DETAILED DESCRIPTION OF THE DEVICE

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